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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF CLOPIDOGREL BISULPHATE IN TABLET DOSAGE FORM

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ABSTRACT

An isocratic RP-HPLC method was developed and validated for the quantitation of Clopidogrel bisulphate in tablet dosage form. Quantification was achieved by using a reversed-phase C18 column (Inertsil ODS 3V, 5µ, 250 mm × 4.6 mm) at ambient temperature with mobile phase consisting of Acetonitrile: 0.1% Trifluro acetic acid aqueous solution (70: 30, v/v)). The flow rate was 1.0 ml/min. Measurements were made at a wavelength of 220 nm. The average retention time for Clopidogrel bisulphate was found to be 2.95 min. The proposed method was validated for selectivity, precision, linearity and accuracy. The assay method was found to be linear from 60-140 µg/ml for Clopidogrel bisulphate. All validation parameters were within the acceptable range. The developed method was successfully applied to estimate the amount of Clopidogrel bisulphate in tablet dosage form.

KEYWORDS

Clopidogrel bisulphate, RP-HPLC method, Inertsil ODS 3V, Trifluro acetic acid, Acetonitrile and Validation.

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INTRODUCTION

Clopidogrel bisulphate (Figure No.1) is an inhibitor of adenosine diphosphate (ADP) induced platelet aggregation. It acts by direct inhibition of ADP binding to its receptor and of subsequent ADP mediated activation of glycoprotein GPIIb/IIIa complex. It is used as an effective drug for reducing the incidence of ischemic strokes, heart attacks or claudicating due to vascular diseases such as atherosclerosis. Some pharmacopeias mentioned that the clopidogrel bisulphate acts as an antidepressant drug[1-3].

Contraindications

Active pathological bleeding, such as peptic ulcer or intracranial hemorrhage. Hypersensitivity to clopidogrel or any component of the product was observed.

Adverse Reactions

Fatal bleeding and Life threatening.

Literature review revealed that analysis of Clopidogrel bisulphate carried out by UV-Spectrophotometry and HPLC. There are some UV-spectrophotometric methods[4, 5] and one HPLC method[6] have been reported for estimation of Clopidogrel bisulphate in tablet dosage form. The present work describes simple, specific, rapid, accurate, precise chromatographic method based on RP-HPLC mechanism for estimation of drug in tablet dosage form.

MATERIAL AND METHOD

Instruments

The chromatographic technique performed on a Shimadzu LC20-AT Liquid chromatograph with SPD-20A prominence UV-visible detector and Spinchrom software , reversed phase C18 column (Inertsil ODS 3V C18, 5µ, 250 mm × 4.6 mm) as stationary phase. Thermo Electron Corporation double beam UV-visible spectrophotometer (vision pro-software), Ultrasonic cleaner, Shimadzu analytical balance AY-220, Vaccum micro filtration unit with 0.45µ membrane filter was used in the study.

Materials

Pharmaceutically pure sample of Clopidogrel bisulphate was obtained as gift sample from Chandra laboratories pvt ltd, Prashanthinagar, Kukatpally, Hyderabad, India. The purity of the drug was evaluated by obtaining its melting point, ultraviolet (UV) and infrared (IR) spectra. No impurities were found. The drug was used without further purification. HPLC-grade acetonitrile was obtained from standard reagents pvt ltd. Trifluro acetic acid (AR grade) was from Merck. A tablet formulation of Clopidogrel bisulphate (75 mg (75 mg label claim) was procured from local market (Clopilet, Sun Pharma, India)

Determination of Working Standard Wavelength (λmax)

10 mg of the Clopidogrel bisulphate standard drug is taken in a 10 ml volumetric flask and dissolved in methanol and volume made up to the mark, from this solution 0.1ml is pipetted into 10 ml volumetric flask and made upto the mark with the methanol to give a concentration of 10 µg/ml.

The above prepared solution is scanned in UV between 200-400 nm using methanol as blank. The λmax was found to be 220nm (Figure No.2).

Preparation of mobile phase

Buffer: 0.1 ml of tri fluro acetic acid was pipetted into a 100 ml of HPLC grade water to give a 0.1% aqueous solution.

Mobile phase: 70 volumes of Acetonitrile and 30 volumes of buffer were mixed and sonicated for 15 min and filtered through a 0.45 µ membrane filter.

Analysis of formulation

Preparation of standard solution

A 50mg of standard Clopidogrel bisulphate was weighed and transferred to 50 ml of volumetric flask and dissolved in mobile phase. The flask was shaken and volume was made up to mark with mobile phase to give a primary stock solution containing 1000µg/ml Clopidogrel bisulphate. From the above solution 5ml of solution is pipetted out into a 50 ml volumetric flask and volume was made up to mark with mobile phase to give a solution containing 100µg/ml of Clopidogrel bisulphate.

Preparation of sample solution

For the estimation of the drug in tablet formulation, twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 50 mg of Clopidogrel bisulphate was accurately weighed and the powder was transferred to 50 ml volumetric flask and shaken vigorously with mobile phase and sonicated for 15 min and volume made up to the mark with mobile phase. The solution was shaken vigorously and filtered using whatmann filter no.41. From the above filtered clear solution 5ml of sample pipetted out into a 50 ml volumetric flask and volume made up to the

mark with mobile phase to give a solution containing 100µg/ml of Clopidogrel bisulphate.

Calculation

5 replicates of each of sample and standard solutions were injected and their average peak areas were taken.

The amount of Clopidogrel bisulphate present in the formulation by using the formula given below, and results shown in above Table No.6.

$$\% \text{ Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation
AT: Peak area due to assay preparation
WS: Weight of standard drug taken
WT: Weight of sample in assay preparation
DT: Dilution of assay preparation
DS: Dilution of standard preparation
AW: Average weight of 20 tablets
LC: Label claim
P: Purity of standard drug

METHOD VALIDATION

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 60-140µg/ml for the drug. From the primary stock solution containing 1000 µg/ml concentration of Clopidogrel bisulphate 0.6ml, 0.8ml, 1.0ml, 1.2ml, 1.4 ml of aliquots are pipetted into 10 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 60µg/mL, 80 µg/mL, 100 µg/mL, 120 µg/mL, 140 µg/mL. Calibration curve (Figure No.3) with concentration verses peak areas was plotted by injecting the above prepared solutions and the obtained data were subjected to regression analysis using the least squares method (Table No.1).

Method precision (repeatability)

The precision of the instrument was checked by repeated injections and measurement of peak areas and retention times of solutions (*n* = 6) for, 100 µg/ml of Clopidogrel bisulphate without changing the parameter of the proposed chromatographic method (Figure No.4).

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 different days over a period of 1 week for 100 µg/mL concentration of standard solutions of Clopidogrel bisulphate (Figure No.5). The result was reported in terms of relative standard deviation (% RSD).

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve by using the equations (2) and (3), respectively (Table No.2).

LOD = 3.3 δ/S ……. (2)
LOQ =10 δ/S …….. (3)

Where,

ʒ= the standard deviation of the response
S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of Clopidogrel bisulphate by the standard addition method (Table No.3). Known amounts of standard solutions of Clopidogrel bisulphate were added at 20% concentration to pre quantified sample solutions of Clopidogrel bisulphate (80,100,120µg/ml) (Figure No.6). The amount of Clopidogrel bisulphate recovered was estimated by using the following formulas.

$$\% \text{ Recovery} = \frac{\text{amount found}}{\text{Amount added}} \times 100$$

$$\text{Amount Found (mcg/ml)} = \frac{\text{Mean test area}}{\text{Mean standard area}} \times \text{Std. con}$$

Specificity

In an assay, demonstration of specificity requires it can be shown that the procedure is unaffected by the presence of impurities or excipients. In practice, this can be done by spiking the drug substance or product with appropriate levels of impurities or excipients and demonstrating that the assay results are unaffected by the presence of these extraneous materials.

There should be no interference of the diluents, placebo at retention time of drug substances (Figure No.7).

Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters like flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied ±2nm and flow rate was varied ±0.2 ml/min. The results were shown in Table No.4.

Ruggedness

The ruggedness of the method was studied by analyzing the sample and standard preparations by two analysts. The %RSD assay values between two analysts was calculated i.e. (limit <2%). This indicates the method was rugged. The results were shown in Table No.5 and 6.

RESULTS AND DISCUSSION

In RP HPLC method, the primary requirement for developing a method for analysis is that by using different solvents and buffers and columns to get better retention time, less cost and time saving method than the previously developed methods. The λmax of the clopidogrel bisulphate in methanol was found to be 220nm (Figure No.2) by scanning in UV region.The chromatographic method was optimised with mobile phase consisting of Acetonitrile: 0.1% Trifluro acetic acid aqueous solution (70: 30, v/v) and inertsil ODS column.

All the validation parameters were studied at a wavelength 220nm. Accuracy was determined by calculating the recovery (Table No.3) and the results were in acceptable range (limit 98-102%). The method was successfully used to determine the amount of clopidogrel bisulphate present in the Tablet. The results obtained were in good agreement with the corresponding labelled amount (Table No.3). The method was linear in the concentration range of 60-140 µg/ml (Figure No.3, Table No.1). Precision was calculated as repeatability and intra and inter day variations (% RSD) for the drug (Table No.7 and 8).

Robustness and ruggedness results were in acceptable range (Table No4 and Table No5). Summary of all validation parameters for method is given in Table No.9. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of clopidogrel bisulphate in tablet dosage form.

Table No.1: Linearity

S.No	Concentration (µg/ml)	Peak Area
1	60	2067.010
2	80	2703.052
3	100	3201.245
4	120	3865.510
5	140	4451.644

Table No.2: LOD and LOQ values from calibration curve

S.No	Clopidogrel bisulphate	
	Concentration (µg/ml)	**Peak Area**
1	60	2067.01
2	80	2703.052
3	100	3201.245
4	120	3865.510
5	140	4451.644
SD	31.62	938.55
Slope	29.650	-

S.No	Concentration (µg/ml)	Peak Area
LOD	3.52	104.46
LOQ	10.67	316.54

Table No.3: Recovery data of MCA and DULO by Spectrophotometric Method

S.No	Level	Drug	Amount of Sample Taken (µG/ML)	Amount of Standard Spiked (%)	% Recovery
1	I	MCA	80	20 %	100.98%
			80	20 %	
			80	20 %	
2	II	DULO	100	20 %	98.89%
			100	20 %	
			100	20 %	
3	III	-	120	20 %	99.71%
			120	20 %	
			120	20 %	

Table No.4: Results of Robustness study

S.No	Parameter	Clopidogrel bisulphate			
		Retention time(min)	Tailing factor	Peak area	% Assay
1	Flow Rate				
	0.8 ml/mi	2.480	1.762	3417.689	99.12
	1.0 ml/min	2.950	1.710	3427.780	99.91
	1.2 ml/min	3.693	1.820	3510.689	100.12
2	Wavelength				
	218nm	2.977	1.720	3561.435	100.31
	220nm	2.950	1.710	3427.780	99.91
	222nm	2.967	1.680	3321.466	98.01

Table No.5: Ruggedness results

S.No	Analyst	Sample area	Standard area	% Assay	%RSD
1	Analyst 1	3449.245	3428.094	100.61	**0.59**
2	Analyst 2	3420.002	3427.591	99.77	

Table No.6: Assay Results

S.No	Clopidogrel bisulphate		
1	Standard Area	1	3435.24
		2	3441.826
		3	3425.172
		4	3435.948
		5	3400.253
		Average	3434.079
2	Sample area	1	3438.119
		2	3423.326
		3	3438.149
		4	3447.016
		5	3444.107
		Average	3438.143

3	Standard weight	-	50
4	Sample weight	-	197.8
5	Average Wt.	-	296.8
6	Label claim	-	75
7	Std.purity	-	99.8
8	Assay in mg	-	74.96
9	%Assay	-	99.95

Table No.7: Method Precision (Repeatability)

Method Precision		
Clopidogrel bisulphate		
S.No	**Rt**	**Area**
1	2.967	3425.968
2	2.957	3427.591
3	2.963	3436.181
4	2.958	3420.002
5	2.960	3437.678
6	2.890	3427.78
Avg	2.9492	3429.200
Stdev	0.0292	6.637
%RSD	**0.991**	**0.19**

Table No.8: Intermediate precision

Method Precision(day1)			**Method Precision(day2)**			**Method Precision(day3)**		
Clopidogrel bisulphate			**Clopidogrel bisulphate**			**Clopidogrel bisulphate**		
S.No	**Rt**	**Area**	**S.No**	**Rt**	**Area**	**S.No**	**Rt**	**Area**
1	2.98	3419.528	1	2.967	3425.968	1	2.977	3447.016
2	2.997	3378.004	2	2.960	3435.247	2	2.983	3433.382
3	3.007	3390.177	3	2.963	3433.066	3	2.977	3400.249
4	3.013	3324.842	4	2.983	3386.336	4	2.983	3414.586
5	3.000	3417.832	5	2.960	3395.777	5	2.983	3414.304
6	3.003	3409.702	6	2.957	3389.872	6	2.960	3409.702
Avg	3.000	3390.014	**avg**	2.965	3411.044	**avg**	2.977	3420.349
Stdev	0.0113	35.845	**stdev**	0.0094	22.739	**stdev**	0.0089	17.127
%RSD	**0.38**	**1.06**	**%RSD**	**0.32**	**0.67**	**%RSD**	**0.30**	**0.50**

Table No.9: Validation parameters of evaluated method

S.No	**Parameters**	**Values obtained**	**DULO at λ- max 289**
1	A Accuracy	98.89%-100.98%	99.30 ±1.46 - 100.90 ± 0.77%
2	LOD[b] (µg/mL)	3.52	0.5164
3	LOQ[c](µg/mL)	10.67	1.5651
4	Linearity Concentration range(µg/mL) Regression coefficient (R2 value)	60-140 0.998	0.4784
5	Precision (% RSD) Method precision(Repeatability) (%RSD[d], n = 6) Intermediate Precision(%RSD)	0.19 – 0.99 %	0.44 – 1.05 % 0.20 – 1.04 %
6	Robustness(%Assay)	98.01-100.31%	-
7	Ruggedness(%RSD analyst to analyst variation)	0.59%	-

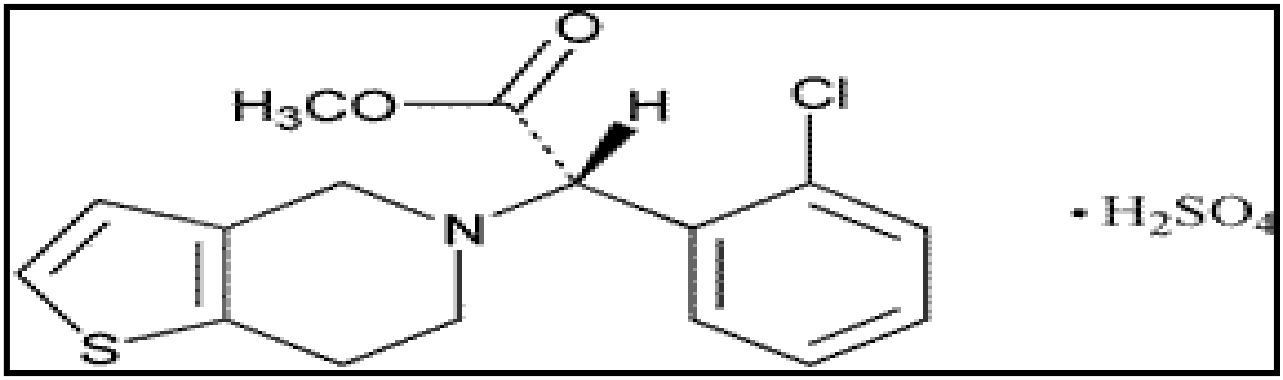

Figure No.1: Structure of Clopidogrel bisulphate

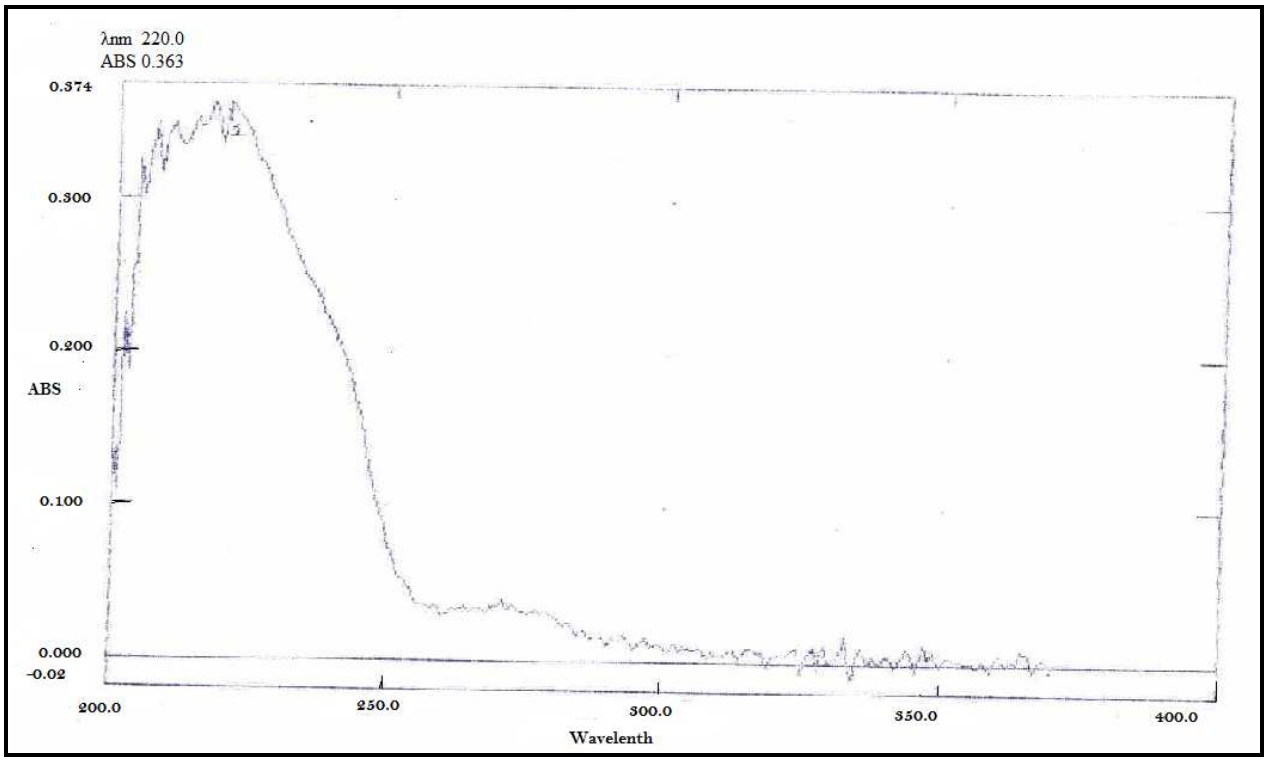

Figure No.2: Determination of Working Wavelength (λmax)

Uv scanned graph (200-400nm)

λmax was found to be 220nm with maximum absorbance of 0.363.

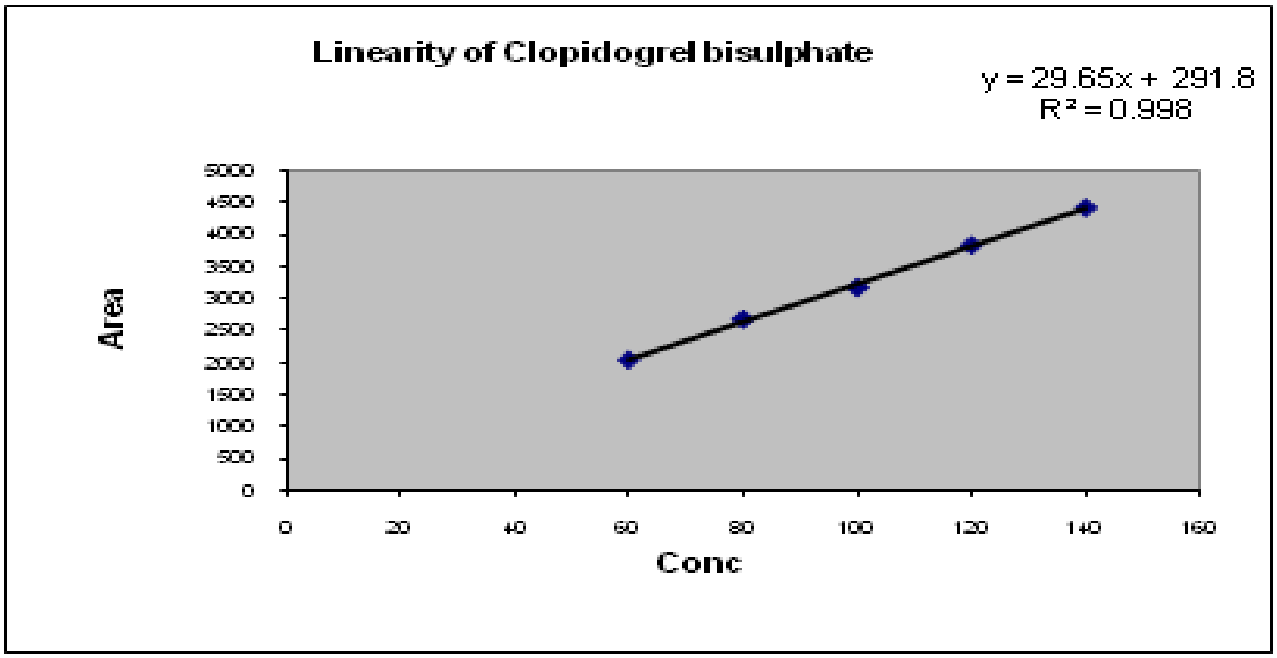

Figure No.3: Linearity (calibration) curve of Clopidogrel bisulphate

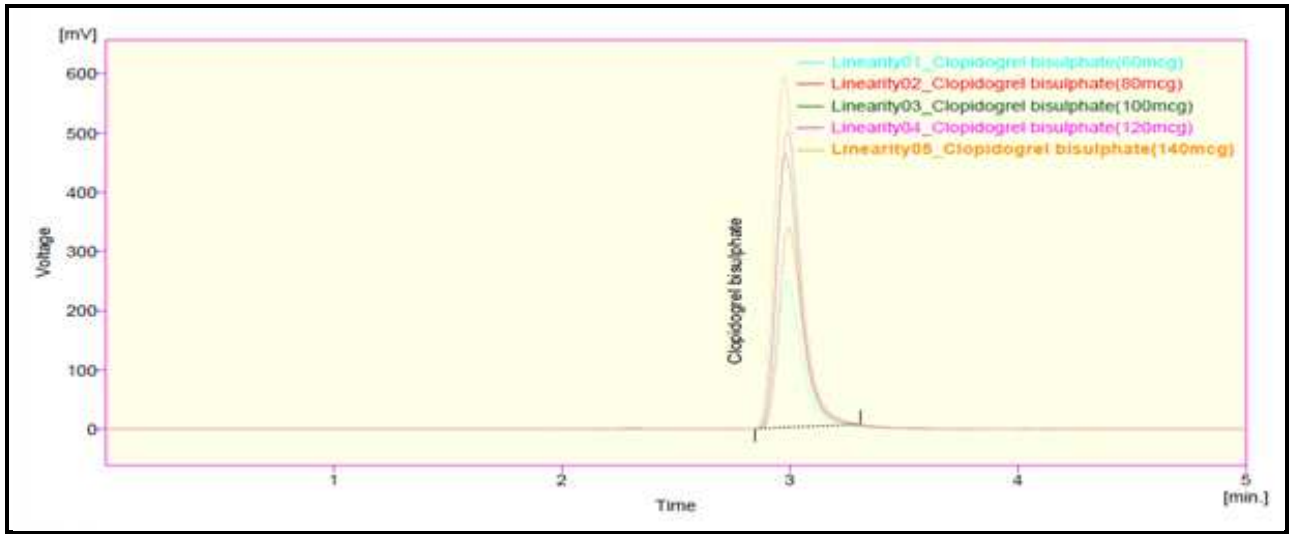

Figure No.4: Overlain chromatograms of Linearity

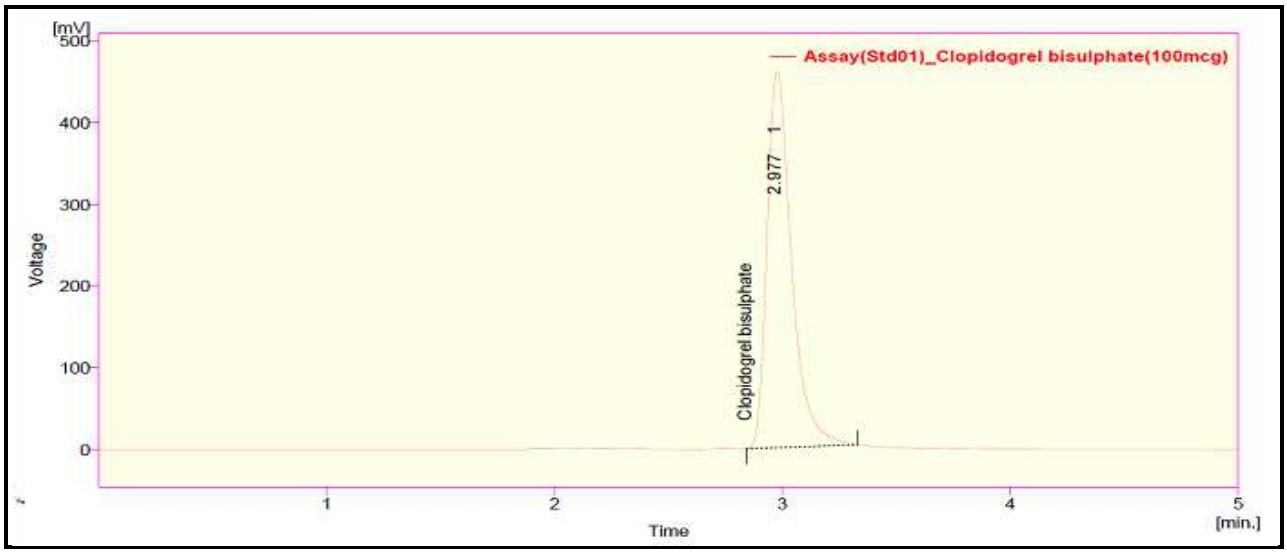

Figure No.5: Chromatogram of assay standard preparation

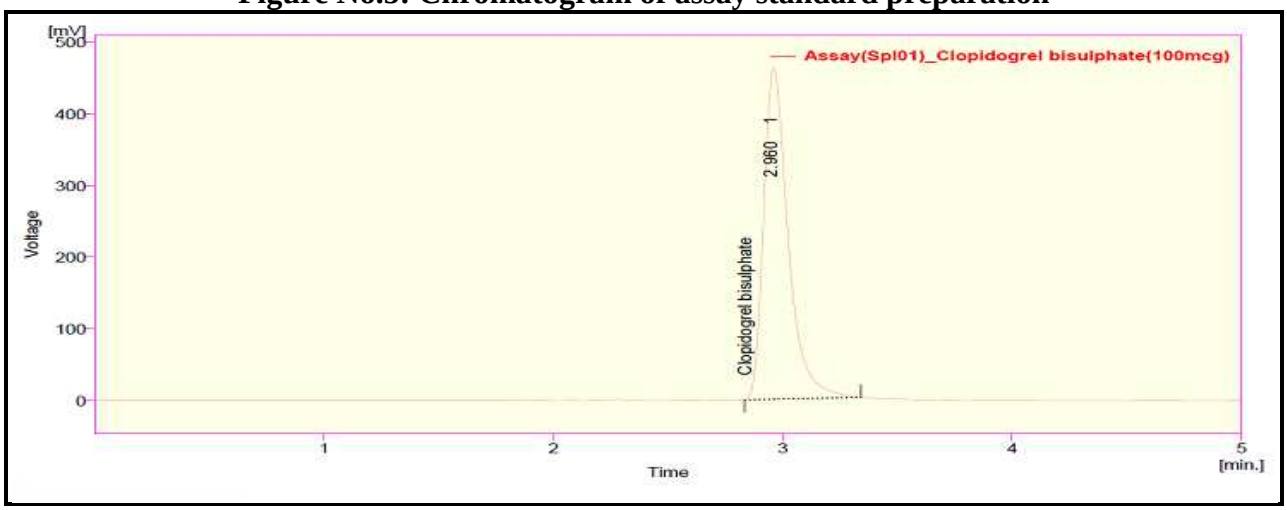

Figure No.6: Chromatogram of assay sample preparation

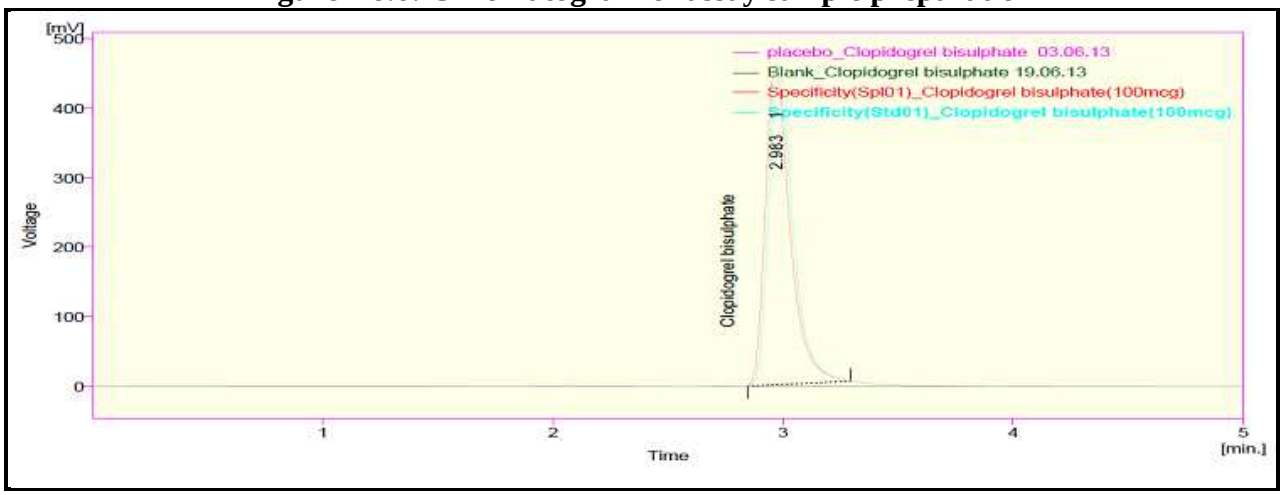

Figure No.7: Overlain chromatograms of Specificity (placebo, blank, sample and standard preparations)

CONCLUSION

The proposed RP-HPLC method was found to be simple, sensitive, accurate and precise for determination of Clopidogrel bisulphate in tablet. The method utilizes easily available and cheap solvent for analysis of Clopidogrel bisulphate hence the method was also economic for estimation of Clopidogrel bisulphate from Tablet. The common excipients and other additives are usually present in the Tablet mixture does not interfere in the analysis of Clopidogrel bisulphate; hence it can be conveniently adopted for routine quality control analysis of the drug in pharmaceutical formulation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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