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SEPARATION AND DETERMINATION OF PROCESS-RELATED IMPURITIES OF CLOPIDOGREL BISULPHATE BY RP-HPLC

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ABSTRACT

Clopidogrel bisulfate is a potent oral antiplatelet agent. Pro-drug which belongs to the chemical family of Thienopyridine adenosine diphosphate(ADP)-receptor antagonists.[1-2] It is mainly used to reduce atherosclerotic events like myocardial infarction, stroke and vascular death in patients who had a recent stroke, myocardial infarction or have established peripheral vascular disease. The main objective of the present study is to separate Clopidogrel bisulphate and its process related impurities in the drug substance. In order to develop a suitable and robust RP-HPLC method for the determination of Clopidogrel bisulphate and its process related impurities, an attempt was made with a C18 column using mobile phase composed of acetonitrile and water in the ratio 50:50% v/v. Experiments were conducted by using different columns, different buffers and different concentrations of organic modifier in order to optimize the chromatographic conditions. Reasonable separation between all compounds was observed in case of Clopidogrel bisulphate. It was felt necessary to carry out further optimization studies and hence attempts were made in this direction. Finally, a mobile phase composed of potassium dihydrogen phosphate buffer (pH 3.5) and acetonitrile in the ratio 78:22% v/v with a flow rate of 1.0 ml/min gave sharp peaks with minimum tailing and good resolution for both the drug and its impurities.

KEYWORDS: Clopidogrel bisulphate, Process related impurities, Potassium dihydrogen phosphate.

INTRODUCTION

Clopidogrel bisulfate is a potent oral antiplatelet agent. It is a pro-drug which belongs to the chemical family of Thienopyridine adenosine diphosphate (ADP)-receptor antagonists.[1-2] Clopidogrel is structurally and pharmacologically similar to Ticlopidine.[3] However, it is mainly used to reduce atherosclerotic events like myocardial infarction, stroke and vascular death in patients who had a recent stroke, myocardial infarction or have established peripheral vascular disease. It is also used to treat acute coronary syndrome patients with non-ST-segment elevation or unstable angina. It is contraindicated in patients with hypersensitivity and active bleeding.[4]

Adenosine diphosphate (ADP) plays an important role in homeostasis and thrombosis, where its receptors are potential targets for antithrombotic drugs. Two G-protein-coupled P2 receptors contribute to platelet aggregation P2Y1 and P2Y.[5] Initiation of platelet aggregation through the mobilization of calcium stores is achieved by P2Y1 receptor and the further process along with stabilization of the formed platelet aggregates is promoted by P2Y receptor which is coupled to adenylyl cyclase inhibition.[6] The latter is the molecular target of the ADP-selective anti aggregating drugs Ticlopidine and Clopidogrel. The active metabolite of Clopidogrel inhibits binding of adenosine diphosphate (ADP) to its platelet receptor there by preventing the ADP-mediated activation of the glycoprotein GPIIb/IIIa complex. Since this complex is the major receptor for fibrinogen, its impaired activation results in the inhibition of platelet aggregation.[7] Recent study indicated that the concomitant use of Clopidogrel and PPI (proton pump inhibitor) for ACS (acute coronary syndrome) resulted in increased risk of adverse effects than use of Clopidogrel without PPI. This is due to inhibition of CYP2C19 which is required for activation of Clopidogrel, which is a pro-drug.[8]

LITERATURE REVIEW

Literature survey revealed that only a few analytical methods have been reported for determining Clopidogrel bisulphate in dosage forms as well as biological fluids.

Lagorce et al. have developed an assay method for the carboxylic acid metabolite of Clopidogrel in human plasma by GC-MS.[9] Mitakos and Panderi reported a HPLC method

for the determination of Clopidogrel in pharmaceutical preparations. In this method separation was achieved on a BDS C8 (250 x 2.1 mm) column, detected by UV at 235nm. Mobile phase consisted of acetonitrile–Sodium dihydrogen phosphate buffer (0.01 M, 65:35% v/v) pH adjusted to 3.0 with dilute orthophosphoric acid and flow rate was 0.3ml/min.[10] Singh et al. proposed a HPLC method for the estimation of carboxylic acid metabolite in wistar rat plasma and its application to pharmacokinetic study. In this method chromatographic separation was achieved with gradient elution on Kromasil ODS (250x4.6mm) column, buffer (0.05% Trifluoroacetic acid in water), acetonitrile as a mobile phase and the eluents were monitored at 220 nm.[11] Ksycinska et al. studied a LC-MS method for the determination of Clopidogrel metabolite in human plasma.[12] Bahrami et al. have reported a HPLC method for the determination of inactive metabolite of Clopidogrel in human plasma. In this method CLC-ODS (150 x 4.6 mm) column was used for the separation using a mixture of 0.05M Sodium phosphate buffer (pH 5.7) and acetonitrile in the ratio (56:44% v/v) with a flow rate of 1.7 ml/min.[13] Mohan et al. have studied the identification and characterization of a principal oxidation impurity in Clopidogrel drug substance and drug product.[14] Dermis and Aydogan have developed a spectrophotometric and chromatographic method for the determination of Clopidogrel in tablets. In this method Nova-Pak C18 column and a mobile phase composed of phosphate buffer (pH 8.0) and acetonitrile in the ratio 30:70% v/v at a flow rate of 0.8ml/min was used for the chromatographic separation using UV detection at 210 nm.[15] Karazniewicz-Lada et al. have studied a CE method for the determination of (+)-S Clopidogrel carboxylic acid metabolite in human plasma and urine.[16] Aboul-Enein et al. have developed a RP-LC method for the high-throughput analysis of Clopidogrel in pharmaceutical formulations . Rao et al. have studied a stability-indicating normal phase LC method for Clopidogrel bisulphate and its impurities in bulk drug and pharmaceutical dosage forms. Serra et al. have reported a capillary electrophoresis method for the simultaneous determination of Clopidogrel and its carboxylic acid metabolite.

PRESENT STUDY

In the present study determination of process-related impurities by RP-HPLC method was carried out. The developed method was subjected for studies on stability indicating factors as per ICH guidelines for acid hydrolysis, alkali hydrolysis, oxidation, thermal, photolytic and humidity conditions. A comprehensive study was undertaken to characterize the process impurities by FT-IR, MS and ^{1}H NMR spectroscopy.

MATERIALS AND METHODS

Instrumentation

High performance liquid chromatography

An integrated HPLC system with computer based chromatography software (empower) was used. The Waters alliance system with 2695 quaternary low pressure gradient system, auto sampler, and column thermostat and photodiode array detector were used for this experiment.

Chemicals and reagents

Clopidogrel bisulphate working standard and its process related impurities were synthesized at Pharmazell Research Centre, Visakhapatnam (India) and obtained as gift samples. HPLC grade acetonitrile was obtained from Merck. Analytical grade Potassium dihydroen phosphate, orthophosphoric acid were used. HPLC grade water was deionized with Milli-Q Elix and then using Milli-Q academic purification system(Millipore).

Preparation of Buffer

1.36 g of Potassium dihydrogen phosphate was weighed and dissolved in 1000 ml of water and pH was adjusted to 4.5 ± 0.05 with orthophosphoric acid. Then the solution was filtered through 0.45µ filter paper and degassed it in an ultrasonic bath.

Preparation of mobile phase and diluent

A mixture of the buffer and acetonitrile in the ratio 78:22% v/v was prepared and degassed. Mobile phase was used as diluent.

Preparation of Standard solution

Standard solution was prepared by accurately weighing 50 mg of Clopidogrel bisulphate and transferring into a 100 ml volumetric flask, containing 60 ml of diluent. This flask was sonicated for 5 min. to dissolve the drug completely and the volume was made up with the diluent (0.5 mg/ml).

Preparation of test solution

About 50 mg of sample was accurately weighed and transferred into 100 ml volumetric flask, 60 ml of diluent was added, sonicated for 5 min. to dissolve the drug completely and the volume made up with the diluent (0.5 mg/ml).

Preparation of impurity mixture

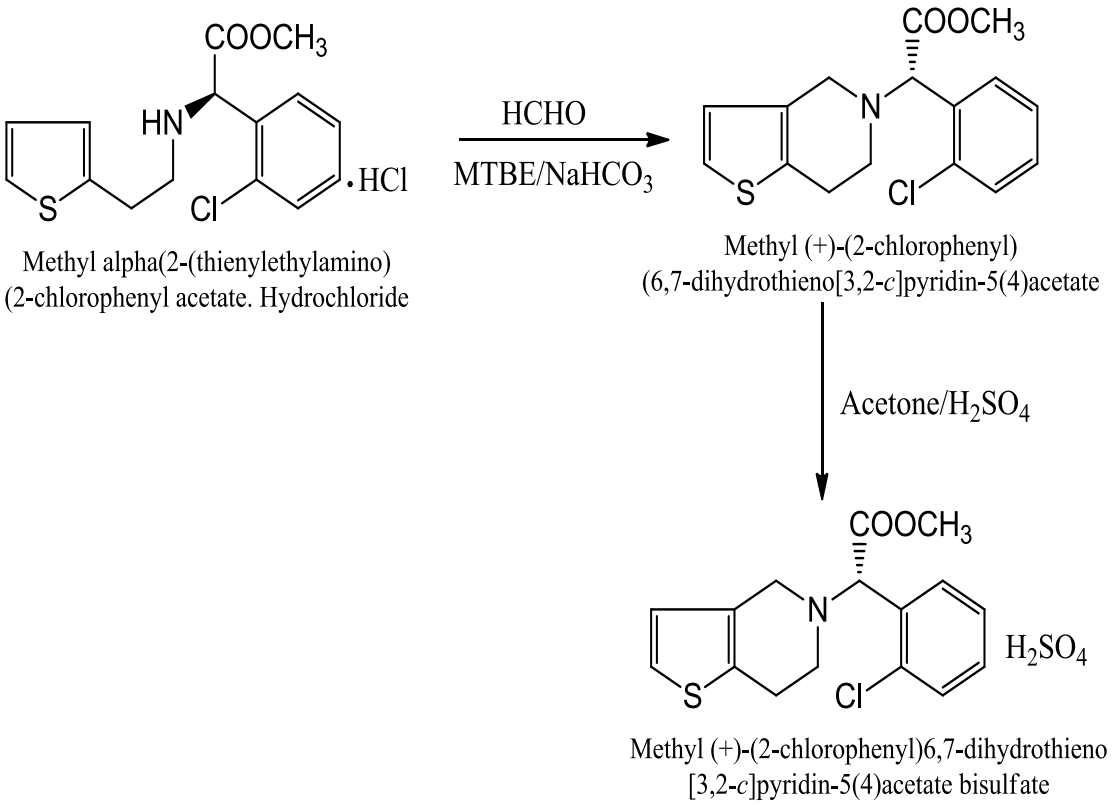
About 10, 20, 60 and 100 mg each of Clopidogrel and its process related impurities (impurity A, B, and C) were weighed accurately and transferred into a 50 ml volumetric flask,30 ml of diluent was added, sonicated for 5 min. to dissolve the drug and its impurities and volume made up with the diluent. 5 ml of this solution was made upto 100 ml with the diluent. This impurity stock solution was adequately diluted to study accuracy, precision, linearity, limit of detection and quantization.

Method development and optimization of Clopidogrel bisulphate by RP-HPLC

The main objective of the chromatographic method is to separate Clopidogrel bisulphate and its process related impurities in the drug substance. In order to develop a suitable and robust RP-HPLC method for the determination of Clopidogrel bisulphate and its process related impurities, an attempt was made with a C18 column using mobile phase composed of acetonitrile and water in the ratio 50:50% v/v. In this condition elution was very broad for Clopidogrel and its impurities. Primary attempts for elution with a little separation was observed for all compounds with the mobile phase consisting of aqueous potassium dihydrogen phosphate and acetonitrile (50:50% v/v) in the pH range 3-6. These efforts were not successful. Later on many experiments were conducted by using different columns, different buffers and different concentrations of organic modifier in order to optimize the chromatographic conditions. It gave a reasonable separation between all compounds, however, a broad peak shape was observed in case of Clopidogrel bisulphate. It was felt necessary to carry out further optimization studies and hence attempts were made in this direction. Finally, a mobile phase composed of potassium dihydrogen phosphate buffer (pH 3.5) and acetonitrile in the ratio 78:22% v/v with a flow rate of 1.0 ml/min gave sharp peaks with minimum tailing and good resolution for both the drug and its impurities. The final optimized chromatographic conditions are shown in Table-1.

Table 1: Optimized chromatographic conditions.

Stationary phase(column)	**ULTRON ES-OVM (150x 4.6)mm,5µm**
Mobile phase	Phosphate buffer:Acetonitrile 78:22% v/v
Flow rate(ml/min)	1.0 ml/min
Column temperature(ºC)	25ºC
Volume of injection(µl)	10 µl
Detection wavelength	220 nm
Total Run time	20 min



Methyl alpha(2-(thienylethylamino) (2-chlorophenyl acetate. Hydrochloride

Methyl (+)-(2-chlorophenyl) (6,7-dihydrothieno[3,2-*c*]pyridin-5(4)acetate

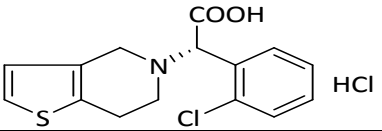
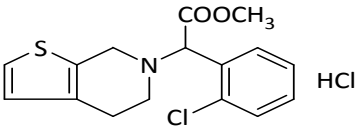
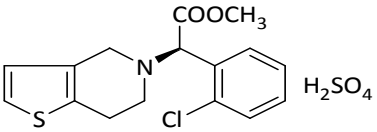
Methyl (+)-(2-chlorophenyl)6,7-dihydrothieno [3,2-*c*]pyridin-5(4)acetate bisulfate

Synthesis of Clopidogrel bisulphate

Process-related impurities

The process-related impurities that may appear in the final API of Clopidogrel bisulphate are given in Table 2.

Table 2: Process-related impurities for Clopidogrel bisulphate.

S. No	Name of the Impurity	Structure	Impurity Code
1	(*S*)-(*o*-chlorophenyl)-6,7-dihydrothieno[3,2-*c*]-pyridine-5(4*H*)- acetic acid, hydrochloride		Impurity A
2	Methyl (±)-(*o*-chlorophenyl)-4, 5-dihydrothieno [2, 3-*c*]-pyridine-6(7*H*)-acetate, hydrochloride		Impurity B
3	Methyl (-)-(*R*)-(*o*-chlorophenyl)-6, 7-dihydrothieno [3, 2-*c*]-pyridine-5(4*H*)-acetate, hydrogen sulfate		Impurity C

The process-related impurities in the API "Clopidogrel bisulphate" were identified using the reference standard provided by M/S Pharmazell R&D Centre, (India) Pvt. Ltd. These impurities were synthesized and characterized before using them for this study. The

impurities were injected in to the chromatographic system separately as well as with Clopidogrel bisulphate (spiked with sample). The impurities in the drug substance were identified based on there retention time (RT) and relative retention time (RRT) observed from the spike study. The data was given in Table 3 and their individual chromatograms are shown in Fig 1-5.

Table 3: RT and RRT for Clopidogrel bisulphate and its impurities.

S. No	Name	RT (min)	RRT(min)
1	Impurity-A	2.69	0.50
2	Impurity-B1 (First enantiomer)	4.84	0.91
3	Impurity-B2 (Second enantiomer)	6.92	1.13
4	Impurity-C	11.10	1.79
5	Clopidogrel bisulphate	5.34	1.00

Typical chromatograms for individual impurities

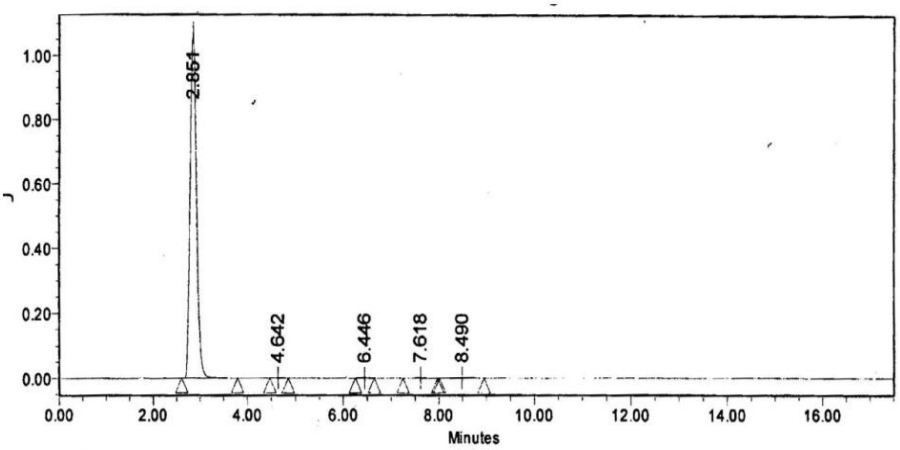


Fig 1: Typical chromatogram for impurity-A.

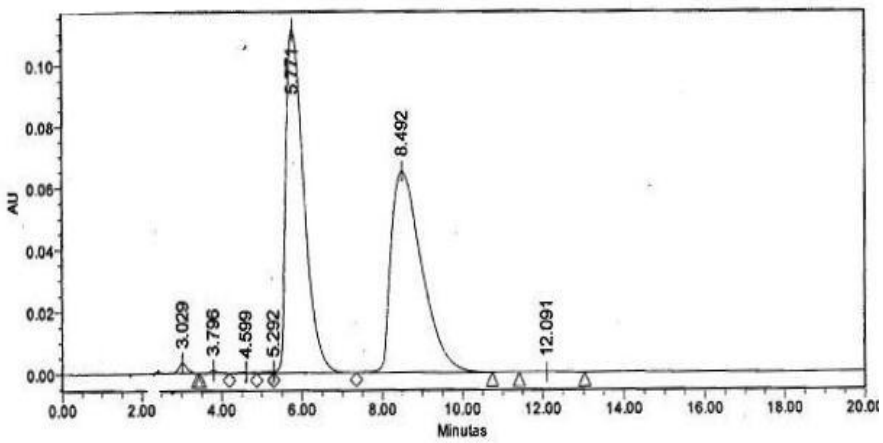


Fig 2: Typical chromatogram for impurity-B.

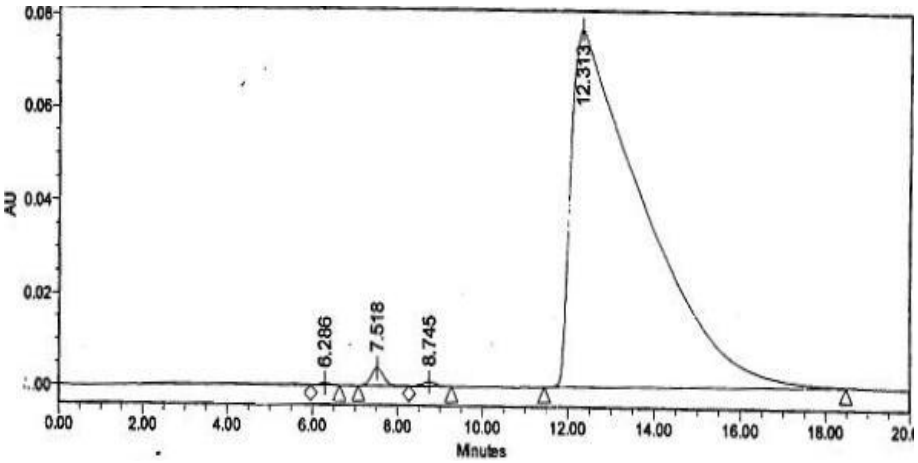


Fig 3: Typical chromatogram for impurity-C.

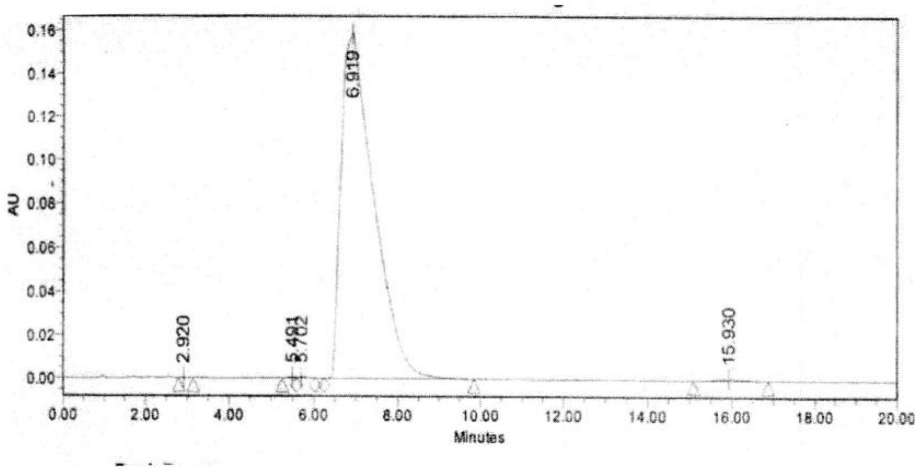


Fig 4: Typical chromatogram for Clopidogrel bisulphate.

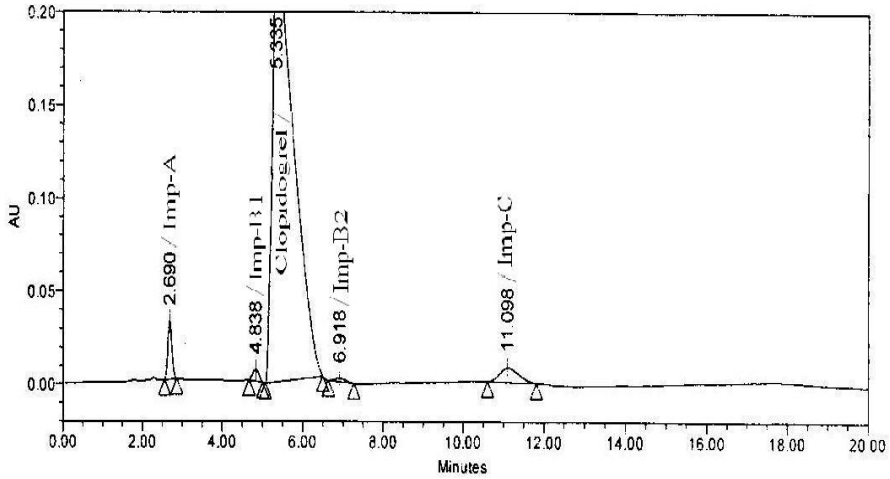


Fig 5: Typical chromatogram for Clopidogrel bisulphate spiked with impurities.

Forced degradation study

Stability testing of an active substance or finished product provide evidence on how the quality of a drug substance or drug product varies with time influenced by a variety of

environmental conditions-like temperature, humidity and light etc. Knowledge from stability studies enables understanding of the long-term effects of the environment on the drugs. Stability testing provides information about degradation mechanisms, potential degradation products, possible degradation path ways of drug as well as interaction between the drug and the excipients in drug product.

Forced degradation study was carried out by treating the sample under the following conditions

Acid degradation

50 mg of sample was weighed and transferred into a 100 ml volumetric flask and 2 ml of 0.1N HCl was added to it. The solution was warmed on water bath at 70°C for 5hr and then neutralized with 2 ml of 0.1 N NaOH. The neutralized solution was made upto the volume with the diluent.

Alkali degradation

50 mg of sample was weighed and transferred into a 100 ml volumetric flask and 2 ml of 0.1N NaOH was added to it. The solution was warmed on water bath at 70°C for 5 hrs and then neutralized with 2 ml of 0.1 N HCl. The neutralized solution was made up to the volume with the diluent.

Thermal Degradation

500 mg of the sample was taken in a watch glass and kept in an oven at 105°C for 24 hrs. From that sample 50 mg was accurately weighed and transferred into 100 ml volumetric flask, dissolved and the volume was made up with the diluent.

Photolytic Degradation

500 mg of the sample was exposed to UV light under 365 nm for 24 hrs by using photo stability chamber. From that sample 50 mg was accurately weighed and transferred into a 100 ml volumetric flask, dissolved and the volume was made up with the diluent.

Peroxide degradation

50 mg of the sample was weighed and transferred into a 100 ml volumetric flask and 2 ml of 5 % hydrogen peroxide solution was added to it. The mixture was warmed on a water bath at 70°C for 5 hrs. Then the above mixture was kept aside for few minutes and then the volume was made up with diluent.

The above stressed samples were analyzed as per the test procedure using Photo Diode Array detector. The results are summarized in Table 4 and its chromatograms are shown in Fig 6.

Table 4: Results of forced degradation study.

Stress condition	Purity angle	Purity threshold	%Assay	Degradation
Acid degradation	0.093	1.150	98.43	No degradation peak observed
Alkali degradation	0.384	1.185	85.31	Observed significant degradation of about 13.24 %. The major degradation peaks are at 6.2 min. The degradation peaks are well resolved from main peak and also from known impurity peak. This degradation peaks are not matching with any of the known peaks
Thermal degradation	0.398	1.374	96.24	No degradation peak observed
Photolytic degradation	0.749	1.174	98.38	No degradation peak observed
Peroxide degradation	0.640	1.239	96.55	No degradation peak observed

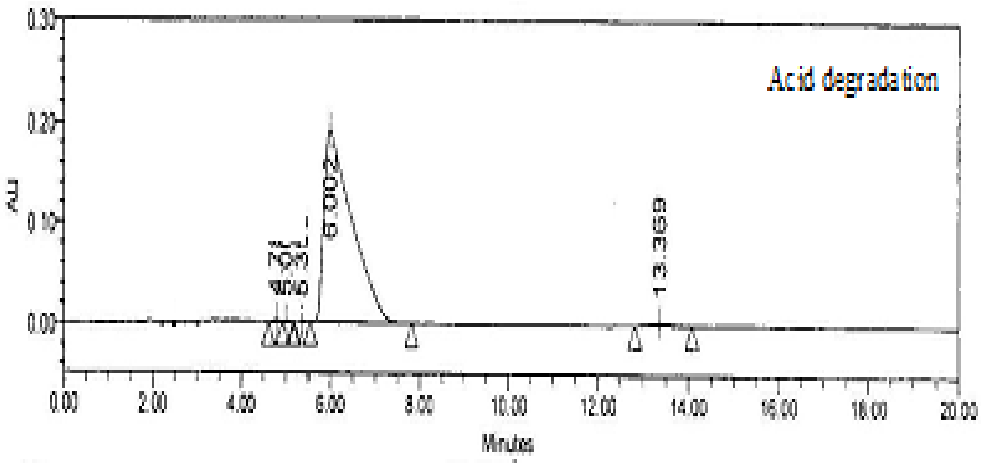
Note: If the purity angle is less than the threshold angle it is said to have passed the purity test.

Acceptance criteria

In any one of the identified stress conditions, the drug product should degrade to 10-20 %.

RESULT

13.24 % degradation was observed with alkali. Examine the peak purity for Clopidogrel bisulphate. It should be spectrally homogenous and passed the purity test. (In Waters HPLC, the peak purity for Clopidogrel bisulphate was examined).



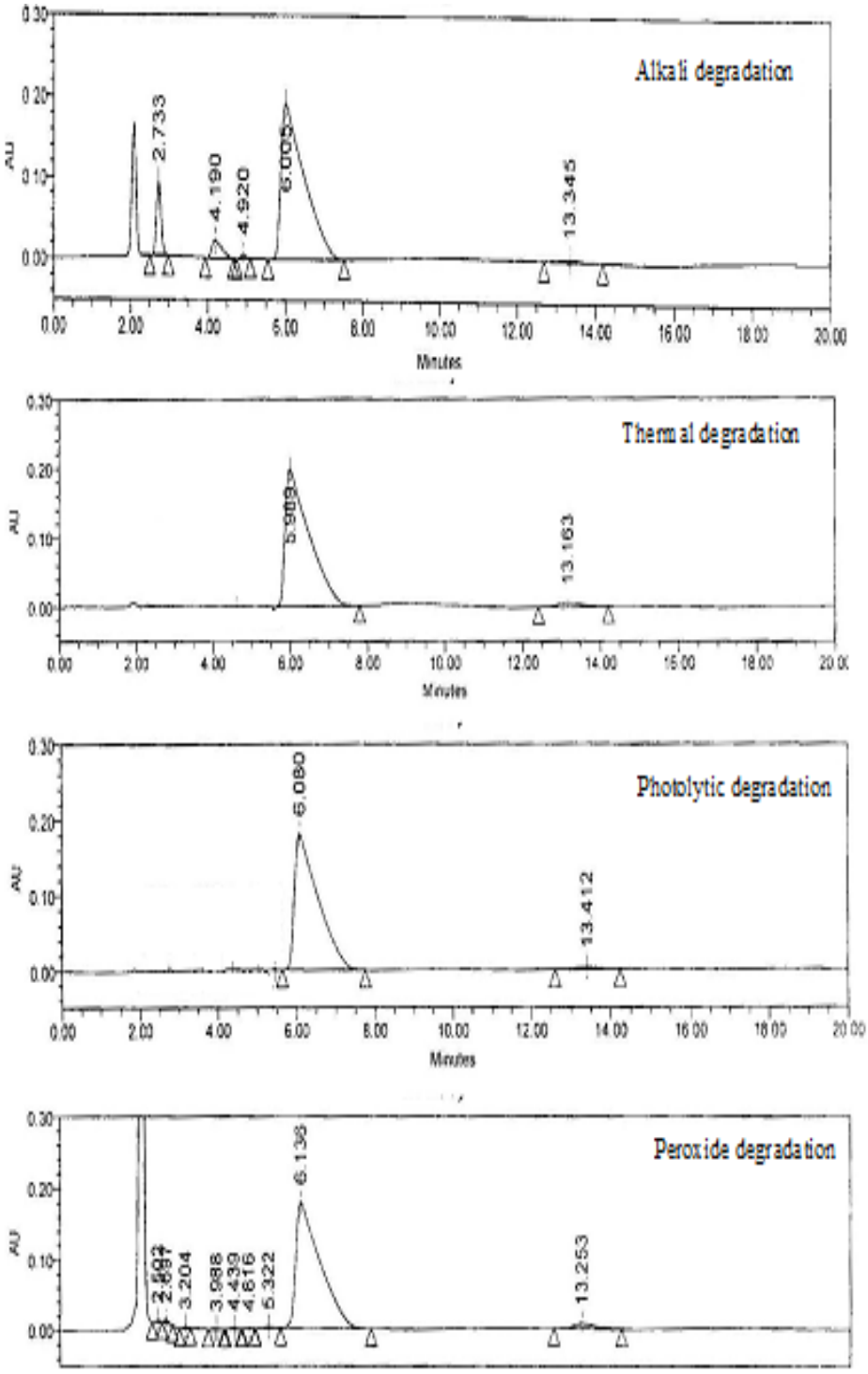


Fig 6: Forced degradation chromatograms for Clopidogrel bisulphate.

Method Validation

The validation parameters viz., specificity, accuracy, precision, linearity, limit of detection, limit of quantization, robustness, system suitability have to be evaluated as per the ICH guidelines for all analytical methods developed by HPLC.

Validation Characteristics

The following validation characteristics were verified as per the ICH guidelines.

- System suitability
- Specificity
- Linearity
- Accuracy
- Precision
- LOD & LOQ
- Robustness

System Suitability

Parameters such as plate number (N), asymmetry or tailing factors (A_s), relative retention time (RRT), resolution (R_s) and reproducibility (%R.S.D), retention time(RT) and area were determined (Table 5). These parameters were determined during the analysis of a "sample" containing the main components and related substances. System suitability terms were determined and compared with the recommended limits ($1 \geq A_s \leq 2$ and $R_s > 1.5$).

Table 5: System Suitability data.

Name	RT	RRT	Resolution	Theoretical plate	Peak symmetry
Impurity-A	2.69	0.50	-	3002.10	1.19
Impurity-B1	4.84	0.91	8.87	3100.96	1.24
Impurity-B2	6.92	1.13	1.98	3144.74	1.09
Impurity-C	11.10	1.79	6.90	3314.39	1.20
Clopidogrel bisulphate	5.34	1.00	3.33	3529.74	1.17

Specificity

The specificity of the developed HPLC method was performed by injecting blank solution and standard solution spiked with its process related impurities separately. The chromatogram of drug with impurities was compared with the blank chromatogram, to verify the blank interference. No peak was observed at the retention time of Clopidogrel bisulphate and its impurities. Hence, the method is specific for the determination of process related impurities in Clopidogrel bisulphate.

Linearity

The linearity of detector response to different concentrations of Clopidogrel bisulphate and its process related impurities was studied in the range from Imp-A (0.04-2.49 µg/ml), Imp-B

(0.04-3.73 µg/ml), Imp-C (0.199-12.46 µg/ml) and Clopidogrel bisulphate(0.077-1.255µg/ml). Each of these standard solutions were injected in triplicate. A linearity plot was drawn taking the concentration on X-axis and the mean peak area on Y-axis. The data was subjected to statistical analysis using a linear-regression model. The regression equations and correlation coefficients (r^2) are given in Table 6-10 and their linearity graphs are shown in Fig7-11.

Acceptance criteria

The Correlation Coefficient should not be less than 0.99.

Table 6: Linearity data for impurity A.

S.No	Concentration (µg/ml)	Mean peak area
1	0.040	356
2	0.299	5882
3	0.498	10587
4	0.996	21834
5	1.195	26157
6	1.992	42341
7	2.490	50684
Correlation coefficient($r^{2)}$		0.9976
Slope		20791
Intercept		242.53

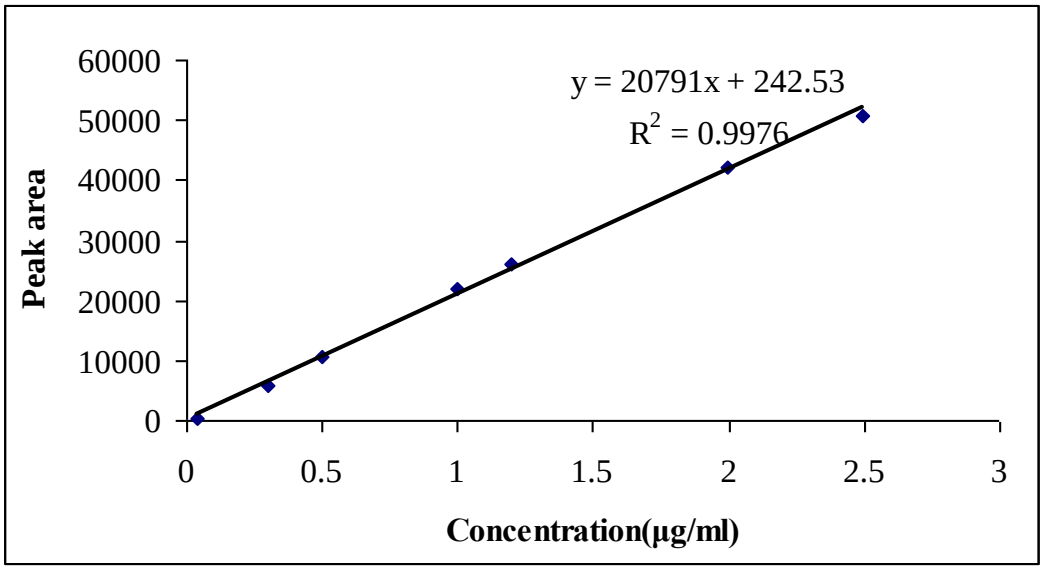


Fig 7: Linearity plot for impurity A.

Table 7: Linearity data for impurity B1 (First Enantiomer)

S.No	Concentration (µg/ml)	Mean peak area
1	0.048	488
2	0.449	7703
3	0.748	13443
4	1.495	26921
5	1.794	32863
6	2.990	53515
7	3.738	64566
Correlation coefficient($r^{2)}$		0.9998
Slope		17463
Intercept		-128.44

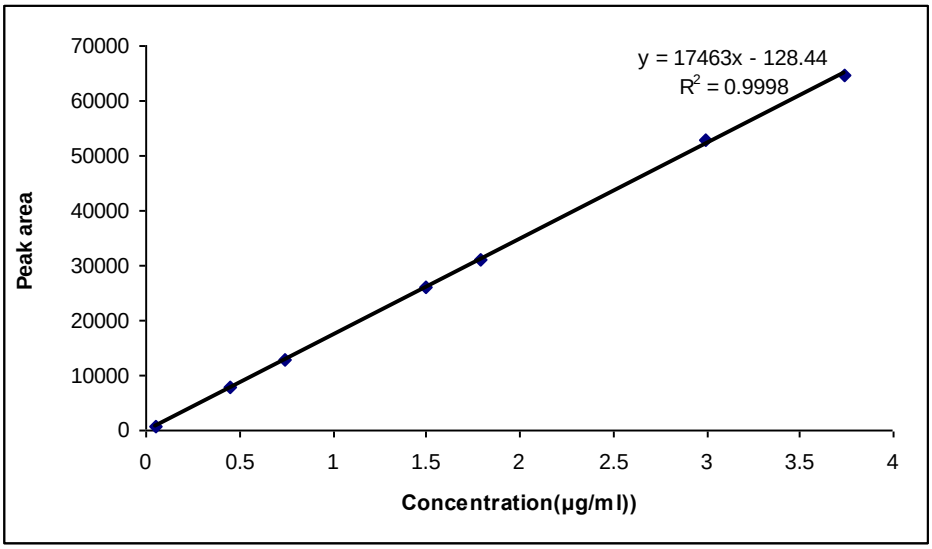


Fig 8: Linearity plot for impurity B1 (First Enantiomer)

Table 8: Linearity data for impurity B2 (Second Enantiomer)

S.No	Concentration (µg/ml)	Mean peak area
1	0.048	488
2	0.449	7703
3	0.748	13443
4	1.495	26921
5	1.794	32863
6	2.99	53515
7	3.738	64566
Correlation coefficient(r^2)		0.9987
Slope		17541
Intercept		279.68

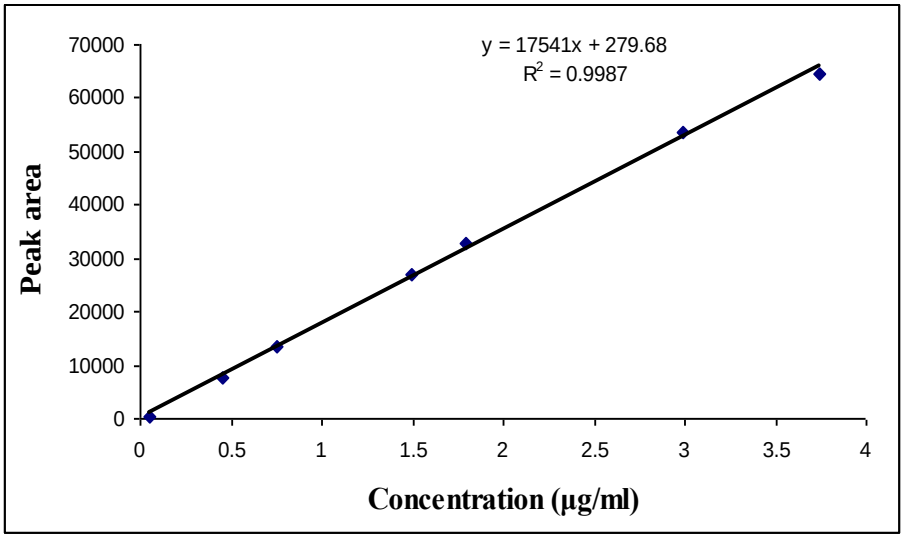


Fig 9: Linearity plot for impurity B2 (Second Enantiomer)

Table 9: Linearity data for impurity C

S.No	Concentration (µg/ml)	Mean peak area
1	0.199	4414
2	1.495	23869
3	2.492	42290
4	4.985	85699
5	5.982	103304
6	9.97	166852
7	12.462	202285
Correlation coefficient(r^2)		0.9988
Slope		16357
Intercept		1993.3

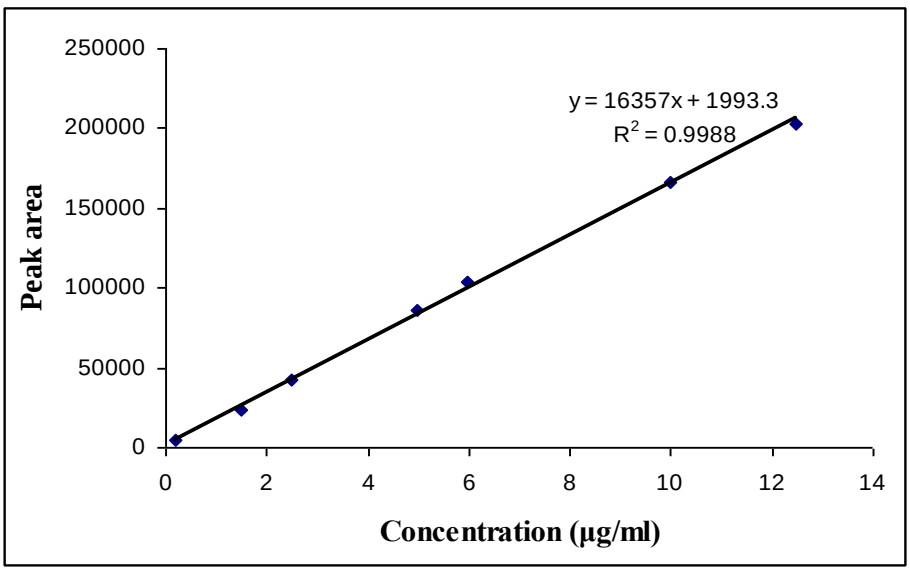


Fig 10: Linearity plot for impurity C.

Table 10: Linearity data for Clopidogrel bisulphate.

S.No	Concentration (µg/ml)	Mean peak area
1	0.077	1067
2	0.151	2620
3	0.251	4568
4	0.502	9953
5	0.602	11217
6	1.004	18382
7	1.255	22172
Correlation coefficient(r^2)		0.9967
Slope		17998
Intercept		118.58

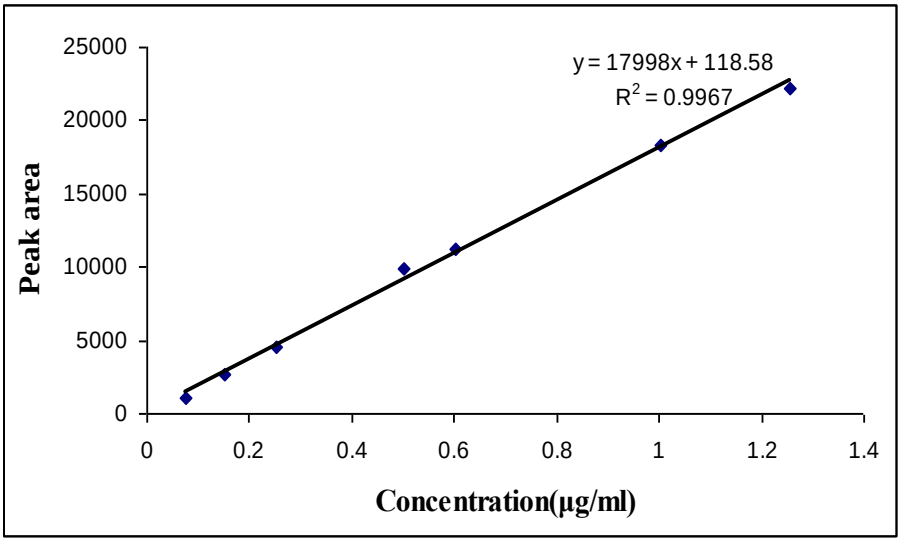


Fig 11: Linearity plot for Clopidogrel bisulphate.

Accuracy/Recovery

Accuracy of the test method was determined by analyzing Clopidogrel bisulphate drug substance spiked with impurities at four different concentration levels of 50 %, 100 %, 150 % and 250% of each in triplicate at the specified limit. The mean recoveries of all the impurities were calculated (Table 11).

Table 11: Recovery studies for impurities of Clopidogrel bisulphate,

Name	Spike level (%)	Concentration spiked (µg/ml)	Concentration recovered(µg/ml)	% Recovery[a]
	50	0.498	0.517	103.81
Impurity-A	100	0.996	1.016	102.21
	150	1.195	1.192	100.39
	250	2.49	2.42	99.06
	50	0.749	0.741	98.93
Impurity-B1	100	1.498	1.446	96.55

	150	1.797	1.754	97.64
	250	3.744	3.61	96.42
	50	0.749	0.759	101.4
Impurity-B2	100	1.498	1.457	97.3
	150	1.797	1.844	102.6
	250	3.744	3.977	101.4
	50	2.492	2.388	95.85
Impurity-C	100	4.985	4.852	97.34
	150	5.982	5.875	98.22
	250	12.462	12.046	96.67

a; average of three determinations

Acceptance criteria

The mean recovery of the impurities at each level should be not less than 85.0 % and not more than 115.0 %.

RESULT

The % recovery obtained is well within the limit of 85 % - 115 %. This indicated that the method is accurate to determine the impurities in Clopidogrel bisulphate.

PRECISION

System precision of the method was evaluated by injecting the Clopidogrel bisulphate standard solution six times and percent relative standard deviation (% R.S.D) for area of Clopidogrel bisulphate peak was calculated. It was found to be less than 2.0 % (R.S.D).The precision of the method for the determination of impurities related to Clopidogrel bisulphate was studied for repeatability and intermediate precision at 100 % level. Repeatability was demonstrated by analyzing the standard solution spiked with impurities for six times. The % R.S.D for peak area of each impurity was calculated. Intermediate precision was demonstrated by analyzing same sample of Clopidogrel bisulphate by two different analysts on two different days (Inter-day). Intra-day variations of impurities related to Clopidogrel bisulphate are expressed in terms of % R.S.D.Values. Repeatability and intermediate precision for the process-related impurities in Clopidogrel bisulphate were found to be less than 2.0 % R.S.D. The results are given in Table12. This confirmed good precision of the method.

Table 12: Precision studies for Clopidogrel bisulphate

Name	Method precision % R.S.D(n=6)	Intermediate precision	
		Intra day % RSD(n=6)	Inter day % RSD(n=6)
Clopidogrel bisulphate	0.43	0.4±0.08	0.43±0.06
Impurity-A	0.41	0.49±0.05	0.42±0.05
Impurity-B1	0.95	0.88±0.08	0.95±0.0
Impurity-B2	1.16	0.93±0.04	0.97±0.08
Impurity-C	0.44	0.47±0.07	0.44±0.05

Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) and limit of quantitation (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by blank samples and calculating the signal-to-noise ratio for each compound by injecting a series of solutions until *S/N* ratio 3 for LOD and 10 for LOQ. The LOQ and LOD values shown in Table 13 &, 14 and its chromatograms are shown in Fig 12 & 13.

Table 13 Results for limit of quantization

S.No	Name	Concentration (µg/ml)	Observed signal to noise ratio
1	Impurity-A	0.040	10.6
2	Impurity-B1	0.048	10.4
3	Impurity-B2	0.048	10.4
4	Impurity-C	0.199	10.8
5	Clopidogrel bisulphate	0.77	9.8

Table 14 Results for limit of detection

S.No	Name	Concentration (µg/ml)	Observed signal to noise ratio
1	Impurity-A	0.013	3.3
2	Impurity-B1	0.016	3.6
3	Impurity-B2	0.016	3.6
4	Impurity-C	0.066	3.2
5	Clopidogrel bisulphate	0.256	3.1

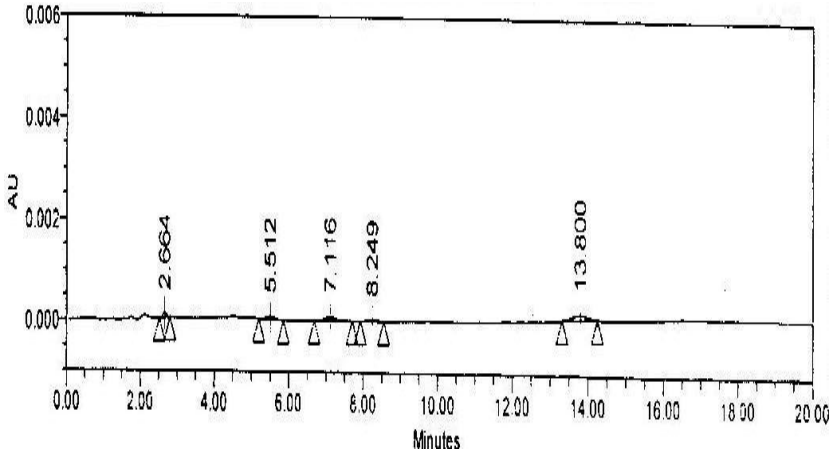


Fig 12: Typical chromatogram for LOQ.

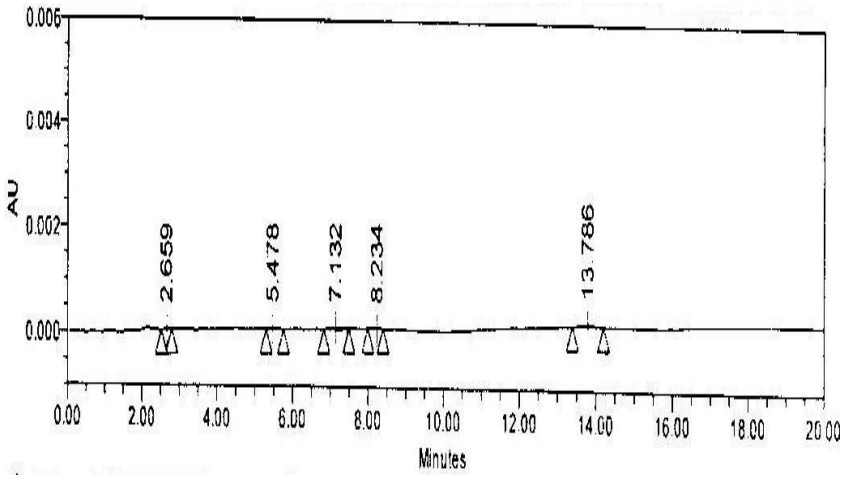


Fig 13 Typical chromatogram for LOD

Robustness

To determine the robustness of the developed method, chromatographic conditions were deliberately altered. The parameters selected were change in flow rate (± 0.2 ml/min), change in pH of the buffer (± 0.2), change in the ratio of mobile phase (± 4%) and change in the column temperature (± 5ºC), the rest of the chromatographic conditions for each alteration study was kept constant.

RESULT

In all the deliberate varied chromatographic conditions, no significant change was observed, which confirmed the robustness of the developed method**.**

CONCLUSION

A new isocratic RP-HPLC method was proposed for the separation and determination of process related impurities in Clopidogrel bisulphate and validated as per ICH guidelines. The

method was found to be simple, selective, precise, robust, sensitive and accurate. Therefore, this method can be used for routine testing as well as stability analysis of Clopidogrel bisulphate. All statistical results (Mean, % RSD, and % Recovery) were within the acceptance criteria.

CONCLUSION

The above forced degradation study showed that Clopidogrel bisulphate shows significant degradation only in the presence of alkali hydrolysis .The alkali degradation peaks were separated well from the main peak. Peak separation, peak purity results showed that the method is specific and capable of picking up all the degradation peaks. Hence, it was concluded that the method was very selective and stability indicative and it is suitable for the determination of impurities in the pure drug.

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