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STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF ATORVASTATIN AND CLOPIDOGREL IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A reversed phase high performance liquid chromatography (RP-HPLC) consist of simple, isocratic, and accurate method was developed for quantitative estimation of Atorvastatin and Clopidogrel in marketed capsule formulation. The chromatographic separation was achieved on a C_{18} column using a mixture of phosphate buffer (pH= 3.0): Acetonitrile (40:60) as a mobile phase and UV detection at 242nm. Atorvastatin and Clopidogrel eluted at retention time 6.90 and 10.05 respectively. Current method was validated with respect to precision, accuracy, linearity, robustness according to ICH guidelines. Atorvastatin, Clopidogrel and their combination drug product was subjected to stress conditions (acid, base, oxidation, thermal, humidity and photolysis). Major degradation was found under thermal and humidity stress conditions while only a minor degradation was observed during photolysis.

KEYWORDS

Atorvastatin, Clopidogrel, Validation, Stress Testing and ICH guidelines.

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INTRODUCTION

The development of stability indicating assays is increasing recently, and the approach to stress testing enshrined in International conference on harmonization (ICH) guideline Q1A (R2)[1] Clopidogrel bisulphate (CLP), chemically (+)-(S)-(2-chlorophenyl)-6, 7-dihydrothieno [3, 2-c] pyridine- 5 (4H) acetic acid methyl ester sulphate1(A) is a potent oral antiplatelet agent often used for the treatment of coronary artery disease, peripheral vascular disease and cerebrovascular diseases. Since it is a prodrug, it must be metabolized by CYP450 enzymes to produce the

active metabolite that inhibits platelet aggregation. This active metabolite selectively inhibits adenosine diphosphate (ADP) binding to its platelet P2Y12 receptor and subsequently the ADP- mediated activation of the glycoprotein GPIIb/IIIa complex, there by inhibiting platelet aggregation. Atorvastatin (ATV), chemically known as [R-(R, R)]-2-(4-fluorophenyl)-dihydroxy-5-(1- methyl ethyl)–3-phenyl4 [(phenylamino) carbonyl] –l H-pyrrole-1-heptanoic acid 1(B), is a potent inhibitor of the enzyme hydroxymethylglutaryl co-enzyme A-reductase (HMG-COA reductase. It also acts as a rate limiting enzyme in cholesterol synthesis in the liver[2,3]. Literature survey reveals that few analytical methods have been reported for Clopidogrel including RP-HPLC methods[5-8], HPTLC method[9,10], UV method[11], normal phase HPLC[12], GC method[13], LC-MS method[14], capillary electrophoresis method[15]. In accordance with ICH guidelines, current work includes the development and validation of a rapid, economical, precise and accurate stability-indicating isocratic reversed-phase HPLC method for analysis of Atorvastatin and Clopidogrel in the presence of its degradation products[16].

MATERIAL AND METHODS

Chemicals and reagents

Atorvastatin and Clopidogrel reference standards were obtained from Amoli Organics Pvt Ltd and MNS Laboratory respectively. Acetonitrile was procured from Fisher Scientific, Water (HPLC grade) of Lab-Q Ultra, Orthophosphoric acid and phosphate buffer was procured from Thomas Baker and Lab Cheme respectively.

Atorvastatin and Clopidogrel (100 mg each) were weighed and transferred into 100 ml volumetric flasks A and B respectively. Stock solution was prepared by dissolving in mobile phase (Acetonitrile: buffer) pH-3.0. 1ml solution was taken from flask A and diluted upto 10 ml with same mobile phase to get final concentration of 10 µg/ml. 7.5ml solution was taken from flask B and diluted up to 10ml using same mobile phase to get final concentration of and 75 µg/ml.

Instrumentation and chromatographic conditions

An isocratic HPLC (Shimadzo) with software Clarity and pump p-5000, uv/visible detector was used along with an Injector of Rhyodyne manual injector with syringe of Hamilton 25 µL loop. The analysis was carried on Inertsil ODS C_{18} (4.6X 250mm) column.

A freshly prepared mobile phase of Acetonitrile and potassium dihydrogen phosphate buffer of 0.04M with pH 3.0 (adjusted by ortho phosphoric acid) was taken in a ratio of 60:40v/v respectively. These were filtered by 0.45 µM Whatman filter paper and sonicated before use. The flow rate of mobile phase was 1.2 ml/min. The column was maintained at ambient temperature (25±5ºC). The detection was carried out at 242nm and run time was around 12 minutes.

Preparation of solution of marketed formulation

Twenty capsules were emptied and weighed and its contents were finely triturated. A quantity equivalent to 10 mg of powdered Atorvastatin and 75 mg of Clopidogrel were transferred to a 100ml volumetric flask and dissolved in mobile phase. The solution then was filtered by using 0.45µM Whatman filter paper. From the above solution, 1 ml was pipetted out and diluted upto 10ml using mobile phase to give test solution containing 10µg/ml of Atorvastatin and 75 µg/ml of Clopidogrel.

RESULTS AND DISCUSSION

Optimization and chromatographic conditions

The primary objective in developing the present stability-indicating HPLC method was to achieve resolution amongst Atorvastatin, Clopidogrel and its degradation products. The choice of chromatographic conditions selected was based on symmetry of peak shape and reduction of chromatographic analysis. The mobile phase of acetonitrile and potassium dihydrogen phosphate buffer of 0.04M (with pH adjusted till 3.0 by ortho phosphoric acid) in a ratio of 60: 40 v/v was selected. In mobile phase, organic solvent was used due to its favourable UV transmittance, low viscosity and good resolution of two drugs. Water

consisting of buffer helps in obtaining sharp peaks and produces good resolution with retention time 6.90 and 10.05 for Atorvastatin and Clopidogrel respectively. The detection was carried out at 242 nm in UV where both drugs showed good absorbance. Resolution of two components was around 9.14 with clear baseline separation.

Method validation

The test method was validated for specificity, linearity, precision, accuracy, range, stability of sample solution and robustness and was found to be in accordance with the acceptance criteria. The validated method was found to be specific, linear, precise, accurate and robust for the assay of Atorvastatin and Clopidogrel in the capsule dosage form. Following parameters were performed for method validation'.

Linearity

From the experimental conditions described above, linear calibration curves of Clopidogrel and Atorvastatin were obtained at five different concentration. The linearity range for Clopidogrel and Atorvastatin were found to be (6 to 14 µg/ml) and (45 to 105µg/ml) respectively. Linear correlations were found between peak area of Clopidogrel and Atorvastatin concentration and are described by the regression equation having r^2= 0.991and 0.994. A calibration curve for each sample was obtained by plotting area response versus concentration which gave a straight line corresponding to the equation: y = mx + c.

Accuracy

The accuracy of the method was studied by measuring recovery after standard addition method of Atorvastatin and Clopidogrel at 80%, 100%, 120% level. Known amounts of standard solutions of Atorvastatin (9, 10 and 11 µg/ml) and Clopidogrel (67.5, 75 and 82.5 µg/ml) was used. The % recovery of both the drugs was found in the range of 98% to 102%.

Precision

Precision, evaluated as % RSD from the area of Atorvastatin and Clopidogrel peaks from six replicated injections of standard solution indicated that the measurement system was precise for the determination of Atorvastatin and Clopidogrel in capsules.

Robustness

As recommended in the ICH guidelines, a robustness assessment was performed during the development of the analytical procedure. The robustness of the method was investigated under a variety of conditions including slight changes of flow rate (1.2 ± 0.2 ml/min), change in detection wavelength (242 ± 2.0 nm). % RSD obtained was not more than 2%.

System suitability

In the system suitability test, solution containing 10 µg/ml of Atorvastatin and 75 µg/ml of Clopidogrel were prepared and injected five times (n=5). The standard and sample solutions were stored at room temperature and analysed over the time period of 0 hrs, 12 hrs and 24 hrs. Different parameters such as retention time, theoretical plates, tailing factor and resolution were calculated from the obtained chromatograms.

Forced Degradation Studies

In literature, there exists many reports where stability-indicating assay has been established by carrying out stress tests directly on pharmaceutical formulations[16,17]. In the present study, the stress testing was carried out on pharmaceutical formulation containing Atorvastatin and Clopidogrel and the degradation was observed when the analyte was subjected to acid, base, oxidation, photolytic, thermal and humidity stress conditions.

Acidic Degradation

A acidic media for forced degradation studies was performedin which 1 ml aliquot from stock solution was taken in 100 ml of volimetric flask, to this 2.0 ml of 0.1 N HCl was added and the solution was kept for 30 min and neutralized using same strength of 0.1 N NaOH. Appropriate aliquot was taken from the above solution and diluted with mobile phase to get a final concentration of 10.0 µg/ml and 75.0 µg/ml of Atorvastatin and Clopidogrel respectively. In acid (0.1 N HCl) degradation study no degradation of Atorvastatin and Clopidogrel was seenand retention of 6.98 and 10.20 respectively was observed.

Basic Degradation

A basic media for Forced degradation was performed in which 1 ml aliquot from stock solution was taken in 100 ml of volumetric flask, to this 2.0 ml of 0.1 N NaOH added and the solution was retained for 30 min and neutralized using same strength of 0.1 N HCl. Appropriate aliquot was taken from the above solution and diluted with mobile phase to a final concentration of 10.0 µg/ml and 75.0 µg/ml of Atorvastatin and Clopidogrel. In base (0.1N NaOH) degradation study no degradation of Atorvastatin and Clopidogrel was seen and retention of 6.99and 10.19 respectively was observed.

Oxidative Degradation

A Oxidative media for forced degradation was performed by in which 1.0 ml aliquot from stock solution was taken in 100 ml of volumetric flask, to this 2.0 ml of 5% v/v of hydrogen peroxide was added and the solution was kept for 30minutes. The solution was diluted with mobile phase to obtain a final concentration of 10.0 µg/ml and 75.0 µg/ml of Atorvastatin and Clopidogrel respectively. No degradation was found and retention time was 6.98 and 10.20 respectively.

Thermal Degradation

The thermal study was carried out by exposure of capsule contents to dry heat at 100° C for 12 hrs. Degradation of Atorvastatin and Clopidogrel was observed with retention time of 7.05 and 10.02 for the drugs and the degradation peak was found with the retention time 10.78 respectively. Thermal degradation study showed 38.12% and 2.2% degradation for Atorvastatin and Clopidogrel respectively.

Photolysis Degradation

To study the effect of UV light, the drug powder was exposed to light. The capsule content of appropriate weight was kept in a sterile glass vial and this vial was exposed to UVlight in a UVcabinet for 12hrs. Following removal of glass vial from the UV light cabinet, samples were prepared for analysis as previously described. Photolysis degradation study showed 38.7% and 3.95% degradation for Atorvastatin and Clopidogrel respectively.

Humidity Degradation

To study the degradation in presence of humidity, the capsule content of appropriate weight was kept in a sterile glass vial and kept in humidity chamber initially having wet temperature 26.2ºC and dry temperature of 20.3ºC for 12 hrs. After removal of vial the final wet and dry temperature was 25.0ºC and 19.2ºC respectively. The major degradation study showed 45.0% and 40.67% of degradation of Atorvastatin and Clopidogrel respectively. The peak of Atorvastatin and Clopidogrel showed retention time of 7.80 and 8.50 respectively. The peaks were seen at significantly close retention time.

Table No.1: Precision of Developed method

System precision			Method precision	
Peak area			%assay	
S.No	Atorvastatin	Clopidogrel	Atorvastatin	Clopidogrel
1	189.68	656.99	193.59	658.66
2	184.13	623.88	182.99	626.38
3	186.26	650.14	183.25	645.99
4	154.50	636.34	185.46	638.80
5	187.26	640.01	187.54	641.70
6	188.80	638.33	190.11	642.25
7	%RSD=1.01	%RSD=1.64	%RSD=1.1	%RSD=0.04

Table No.2: Robustness of method

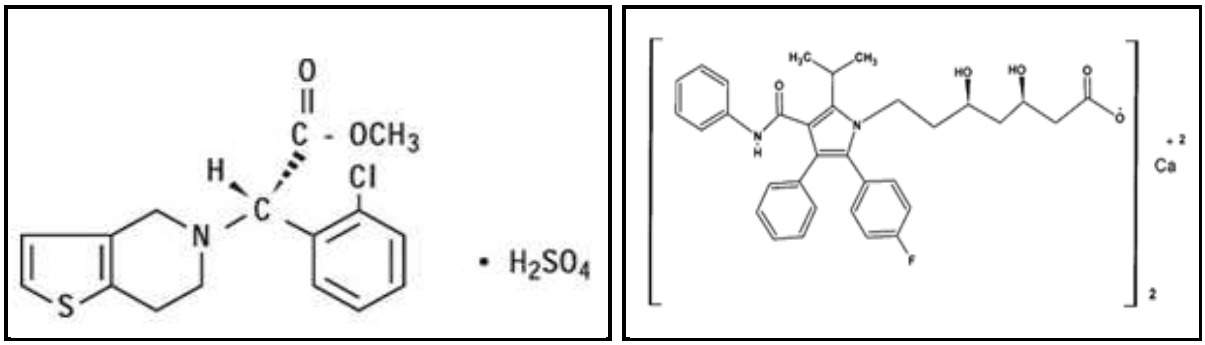
S.No	Chromatographic condition	Variation	% Assay	
			Atorvastatin	Clopidogrel
1	Flow rate	1.0	101.11	99.65
		1.4	98.75	98.48
2	wavelength	241	101.18	100.34
		243	100.52	100.37

Table No.3: System suitability of suitable method

S.No	parameter	Atorvastatin	Clopidogrel
1	No of theoretical plates	8133	10884
2	Tailing factor	1.30	1.26
3	Peak area	186	676
4	Retention time	6.90	10.05

Table No.4: Forced degradation data of method

S.No	Stress condition	Atorvastatin	Atorvastatin	Clopidogrel	Clopidogrel
		% assay	%difference	% assay	% difference
1	Untreated	100.64		100.64	
2	Acid	98.36	2.28	100.77	-0.13
3	Base	97.74	2.9	99.21	1.43
4	Oxidation	98.23	2.41	99.03	1.61
5	Thermal	62.52	38.12	98.44	2.2
6	Photolysis	61.94	38.7	96.69	3.95
7	Humidity	55.59	45.05	59.97	40.67



1(A) Clopidogrel bisulphate **1(B) Atorvastatin calcium**

Figure No.1: Structures of Clopidogrel bisulphate and Atorvastatin Calcium

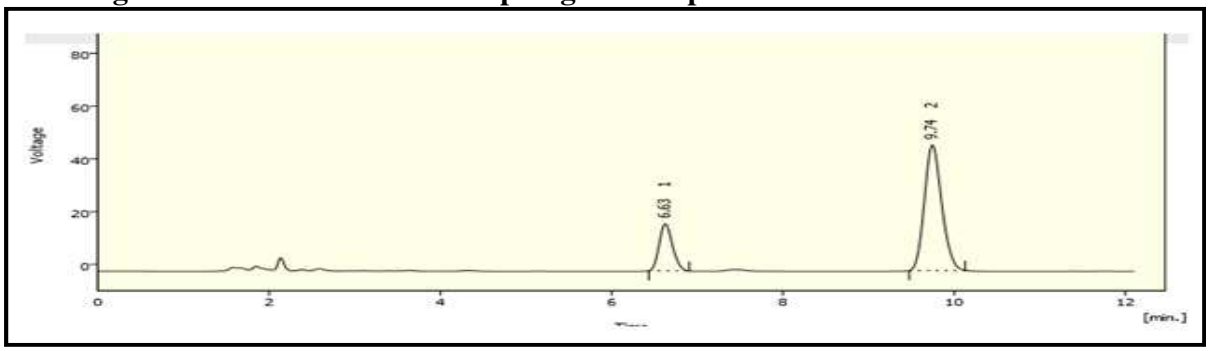


Figure No.2: Typical chromatogram obtained from Atorvastatin and Clopidogrel solution

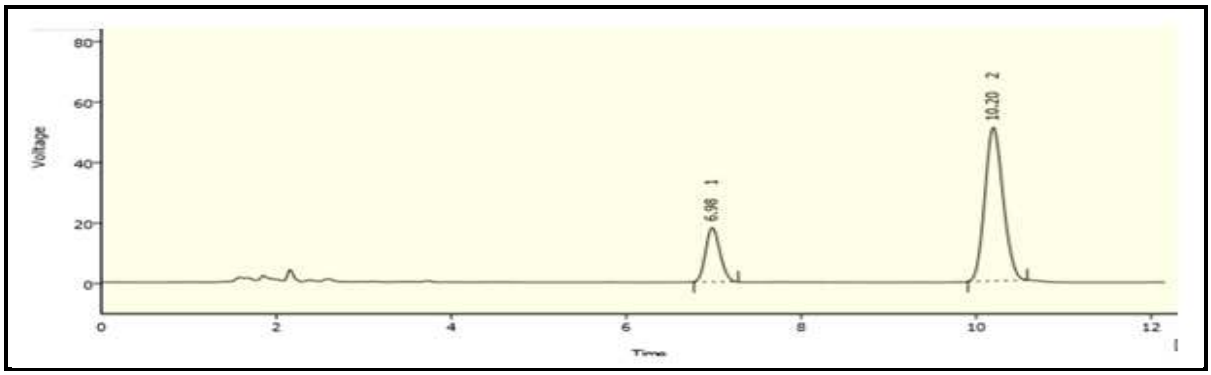


Figure No.3: Representative chromatogram obtained from Acidic degradation (0.1 N HCl) of marketed formulation

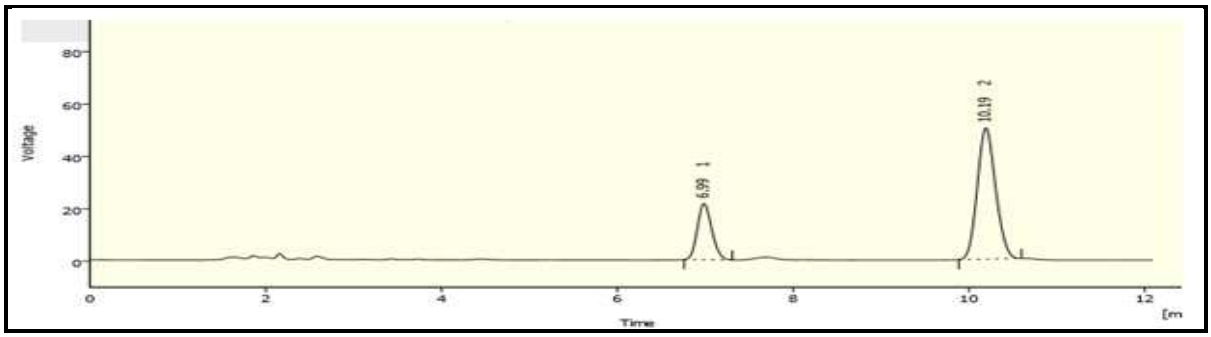


Figure No.4: Representative chromatogram obtained from Basic degradation (0.1 N NaOH) of marketed formulation

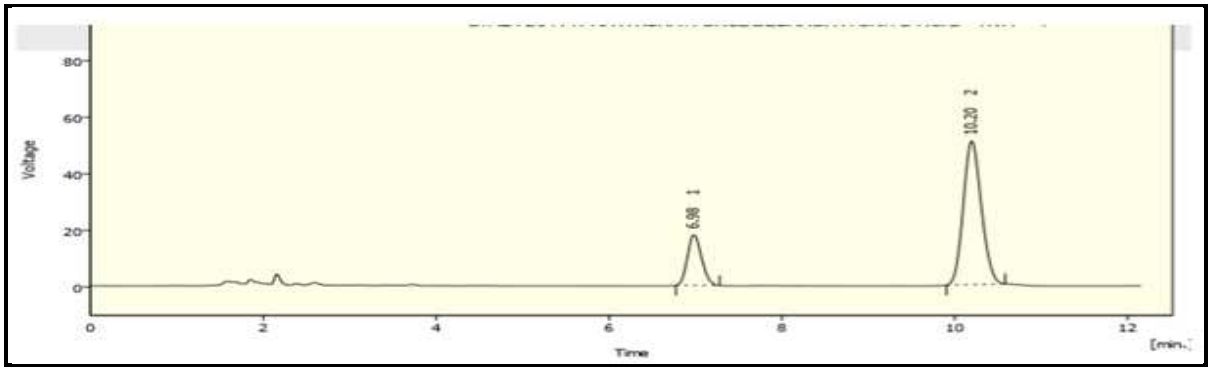


Figure No.5: Representative chromatogram obtained from oxidative degradation (5% H2O2) of marketed formulation

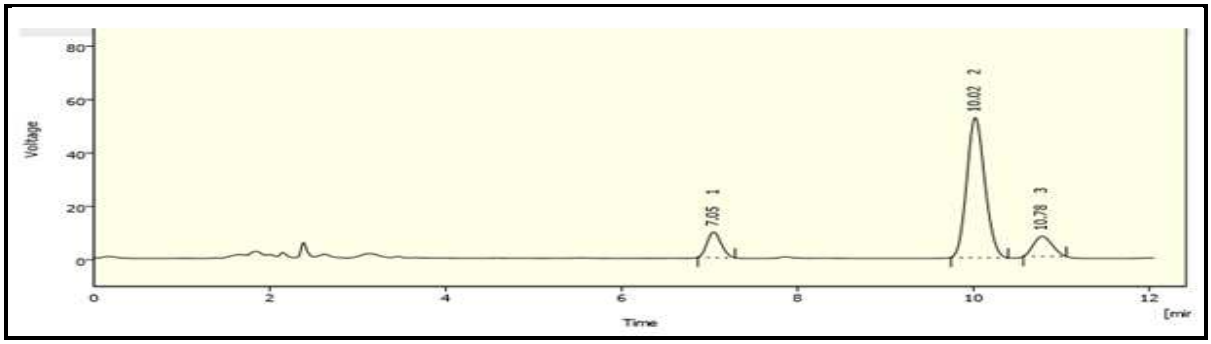


Figure No.6: Representative chromatogram obtained from thermal degradation at 100°C of marketed formulation

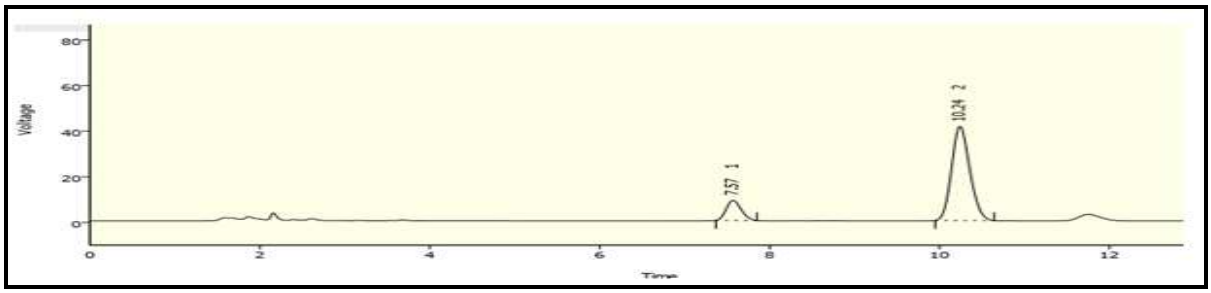


Figure No.7: Representative chromatogram obtained from UV radiation in UV light cabinet of marketed formulation

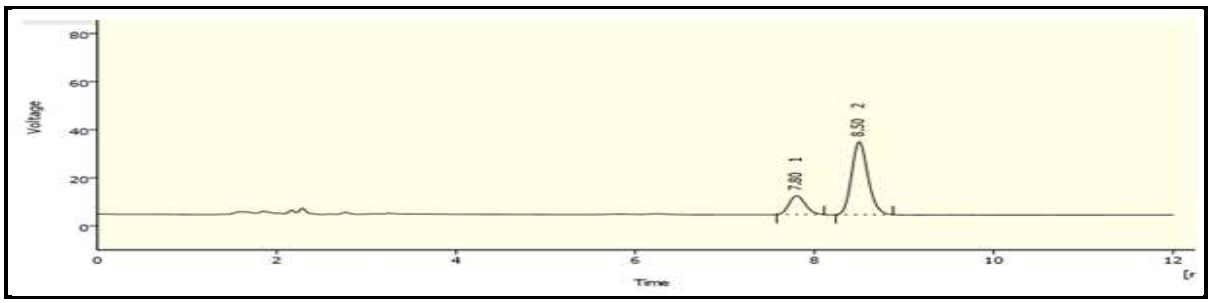


Figure No.8: Representative chromatogram obtained from exposure to humidity chamber of marketed formulation

CONCLUSION

The method developed for quantitative analysis of Atorvastatin and Clopidogrel is rapid, accurate, precise, selective and reproducible. The method was completely validated and satisfactory results were obtained for all the parameters tested. The major degradation was obtained underhumid and thermal testing conditions while only a minor degradation was observed underphotolytic stress conditions. The forced degradation studies prove the stability indicating power of the method and can be used to assess the stability of Atorvastatin and Clopidogrelin the bulk drug and in pharmaceutical dosage forms. The method can be conveniently used for assay of the pharmaceutical dosage forms containing Atorvastatin and Clopidogrel in quality control.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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