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A Stability Indicating Simultaneous Estimation of Rosuvastatin and Clopidogrel Bisulphate in Combined Dosage Formulations by Reverse Phase High Performance Liquid Chromatography

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ABSTRACT

An accurate, rapid, selective and specific reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Rosuvastatin and Clopidogrel in a combined formulation. Chromatographic separation was achieved on Inertsil ODS 3 C-18 (250mm x 4.6mm,5µm) column by isocratic elution mode with three mobile phase components, 0.05M potassium phosphate buffer (pH4.2): methanol: acetonitrile (60: 30:10 v/v/v) at a flow rate of 1.0 mL/min and quantified at 238 nm. The average retention times for Rosuvastatin and Clopidogrel were 4.57 and 2.96 min, respectively. The method offers excellent separation of two drugs with resolution > 2.0 and tailing < 1.0 and with no interferences from the excipients. The method is linear over the concentration range of 3.1-18.6 µg/mL for Rosuvastatin and 22.68-136.08 µg/mL for Clopidogrel with a correlation not less than 0.999. The method is accurate with recoveries of both the drugs in between of 98.0 -101.0% and precise with %RSD value less than 2.0% for the Assay of both the drugs. This method is simple, rapid, accurate and specific for the assay of commercial capsules.

Keywords: Clopidogrel (CD), Rosuvastatin (RSV), Validation, Capsules and Reverse phase High performance liquid chromatography (RP-HPLC)

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INTRODUCTION

Rosuvastatin[1], chemically (I3R, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(N-methyl methane sulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3, 5-dihydroxyhept-6–enoicacid reduces the LDL cholesterol by inhibiting the key enzyme HMG-CoA reductase involved in the synthesis of cholesterol. Clopidogrel[1]is chemically methyl (2S)-2-(2-chlorophenyl)-2-{4H, 5H, 6H, 7H- thieno [3, 2-c] pyridin-5-yl} acetate an anti-platelet drug exhibits its mechanism of action by prevents binding of adenosine diphosphate to its platelet receptor. The Chemical structures of Rosuvastatin and Clopidogrel are given in Figure.1 (a) and (b).

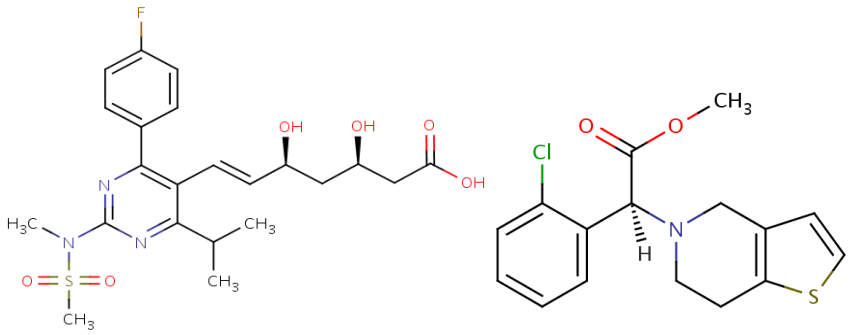

Figure1 Chemical structure of (a) Rosuvastatin and (b) Clopidogrel

There are many reported methods for the estimation of Rosuvastatin[2,3]and Clopidogrel individually and in biological fluids[5-8] but only few methods are available for the determination in combined pharmaceutical dosage forms[9-12] by using Reverse phase High performance liquid chromatography. However none of the reported analytical methods describes the stability indicating method for the simultaneous determination of Rosuvastatin and Clopidogrel. The present manuscript describes a simple, rapid, precise accurate and specific isocratic reverse phase stability indicating HPLC method for the simultaneous determination of Rosuvastatinand Clopidogrel in capsule dosage forms.

MATERIAL AND METHODS:

Waters HPLC e2695 separation module equipped with Waters 2998 photodiode array detector was used for the study with data acquisition software EMPOWER version 2.0. The chromatographic separation was achieved on Inertsil ODS 3 C-18 (250mm x 4.6mm,5µm) column by isocratic elution mode with three mobile phase components, A 0.05M potassium phosphate buffer (pH4.2): methanol: acetonitrile (60: 30:10v/v/v). Mobile phase was pumped at a flow rate of 1mL/min and UV detector was operated at 238 nm. The column temperature was maintained at 35^{o}C and an

injection volume of 20µL was used. The mobile phase was filtered through 0.45µm membrane filter prior to use.

General procedure:

A. Preparation of pH 4.2 Buffer:

0.05 M Potassium phosphate solutionsis prepared and pH is adjusted to 4.2 with orthophosphoric acid. The solution is filtered through 0.45µ filter.

B. Preparation of Mobile phase:

Mobile phase was prepared by mixing 60 volumes of buffer and 30 volumes of methanol and 10 volumes of acetonitrile. The mobile phase was ultra sonicated for 10 minutes, filtered through 0.45µm membrane filter and degassed.

C. Preparation of Standard stock solutions:

Standard stock solution was prepared by weighing 15.5 mg of Rosuvastatin and 148 mg of Clopidogrel into two separate 50mL volumetric flasks and dissolved in 20mL of methanol by sonicating for 5 minutes. The volume was made up to the mark with methanol.

D. Preparation of standard solution:

5.0 mL each of the standard stock solutions was transferred into 100 mL of volumetric flask and diluted with the mobile phase to get a final concentration of approximately 15µg/mL of Rosuvastatin and 112.5 µg/mL of Clopidogrel and individual standard solutions were also made in the same procedure. The solutions are injected and the chromatograms are shown in Figure 2A to 2C.

E. Preparation of sample solution for the assay of Capsules:

Twenty capsules of Rosuvastatin and Clopidogrel were weighed and their average weight was determined. Accurately weighed and transferred an amount of capsule powder equivalent to 15mg of Rosuvastatinand 112.5mgof Clopidogrel into a 50 mL volumetric flask, added 20mL of methanol, sonicated for about 15 minutes to dissolve, final volume was made up to the mark with the methanol, filtered through 0.45 µm membrane filter. 5mL of the filtered solution was transferred into 100mL volumetric flask and diluted with the mobile phase. The solution is injected and the chromatogram is shown in Figure.3

METHOD VALIDATION:

System suitability:

System suitability parameters were checked by injecting the standard solution 5 times into the HPLC and system suitability parameters were determined.

Specificity:

Specificity of the method was determined by injecting blank, placebo, stress degraded solutions and mobile phase into the liquid chromatograph and the chromatograms were observed. The chromatograms were shown as Figure 4A to 4G.

Method precision:

Precision of the method was determined by preparing the six individual sample solutions as per the test procedure mentioned in I.1. and injecting the solutions into the liquid chromatographic system. The %Assay values of the six determinations and the %RSD value for the assay values were calculated.

Intermediate precision:

Intermediate precision was determined by preparing the sample solutions six times on two different days by the two different analysts and injecting them into the liquid chromatograph. The %Assay values of the six determinations and the %RSD value for the assay values were calculated.

Linearity:

The Linearity was established at 6 concentration levels 20, 40, 60, 80, 100and 120% for both the drugs. The solutions for linearity were prepared by transferring 0.5, 1.0mL, 1.50mL, 2.0mL, 2.5mL, 3.0mL, of standard stock solutions of Rosuvastatin and 1.0, 2.0 mL, 3.0 mL, 4.0 mL, 5.0 mL, 6.0 mL of standard stock solutions of Clopidogrel into 50mL volumetric flask and diluted up to the mark with the mobile phase to get a concentrations of 3.1, 6.2, 9.3, 12.4, 15.5, 18.6 µg/mL of Rosuvastatin and 22.5, 45.0, 67.5, 90.0, 112.5, 135.0 µg/mL of Clopidogrel. Linearity was established by least squares regression analysis of the calibration curve.

Accuracy:

The accuracy of the method was evaluated in triplicate at three different concentration levels (80, 100, and 120%) The percentage recovery for each drug was calculated by spiking both the drugs to the placebo at the three levels.

Robustness:

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in the method parameters like flow rate, column temperature, pH and mobile phase composition. The system suitability parameters and the %Assay values were calculated.

Solution stability:

The stability of the solutions was established by injecting blank, placebo, standard and sample solutions kept at room temperature into liquid chromatograph. %Assay of each drug was determined at 3 hours, 6 hours, 12 hours, 14 hours, and 16hours.

Assay of the capsules:

Sample solutions were injected into the liquid chromatograph and the %Assay were calculated using standard and sample peak areas.

Forced Degradation Studies:

The standard solution of Rosuvastatin and Clopidogrel was exposed to stress conditions[13] like acid, base, peroxide and thermal. The acid and base samples were neutralized before analysis. The samples were exposed to 0.1N hydrochloric acid and 0.1N sodium hydroxide at room temperature for 90 minutes under acid and base degradation respectively. The oxidative degradation conditions applied were 3% hydrogen peroxide at ambient conditions. The sample was refluxed at 80°C for 60 minutes under thermal degradation condition and the Light Degradation is carried at UV Light at 254nm for 1Hr.

Detection Method:

Both the drugs exhibited to maximum absorbance nearer to 238nm and hence detection was carried out at UV 238 nm.

RESULTS AND DISCUSSION

HPLC method development:

The HPLC conditions were optimized with a view to develop a suitable LC method for the determination of Rosuvastatinand Clopidogrel in Capsule dosage forms. Inertsil ODS 3 C18 column was selected based on the polarity of the Rosuvastatinand Clopidogrel. The 0.05M pH 4.2 potassium phosphate buffer was selected based on the pKa of Rosuvastatin (4.6) and Clopidogrel (5.3). Initially different ratios of the buffer, methanol and acetonitrile were tried and the 60:30:10 v/v/v of buffer: methanol: acetonitrile was found to be the optimized one. The flow rate of the mobile phase was adjusted to 1.0mL/min to get resolution greater than 2.0 and theoretical plates greater than 2000. The column temperature was kept at 35°C for symmetric peaks and tailing less than 2.0.

VALIDATION:

System suitability:

The %RSD value for the peak areas and retention times for both the peaks were calculated and found to be less than 2%. The theoretical plate values were found to be greater than 2000 and the tailing factor was less than 2.0 for both the peaks. The chromatogram is shown in Figure .2 and the results were tabulated in Table 1.

Table 1: System suitability Results standard solution of Rosuvastatin and Clopidogrel

System Suitability Results		
	SV	D
USP Resolution	6.0	N/A
USP Tailing	0.95	0.97
USP Plate count	7000	6852
%RSD for the peak areas from replicate injections	0.41	0.22

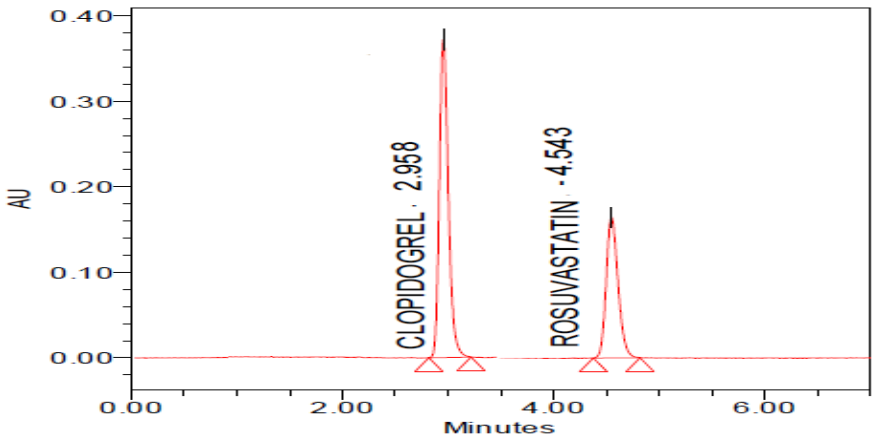

Figure 2A Chromatogram of Rosuvastatin and Clopidogrel

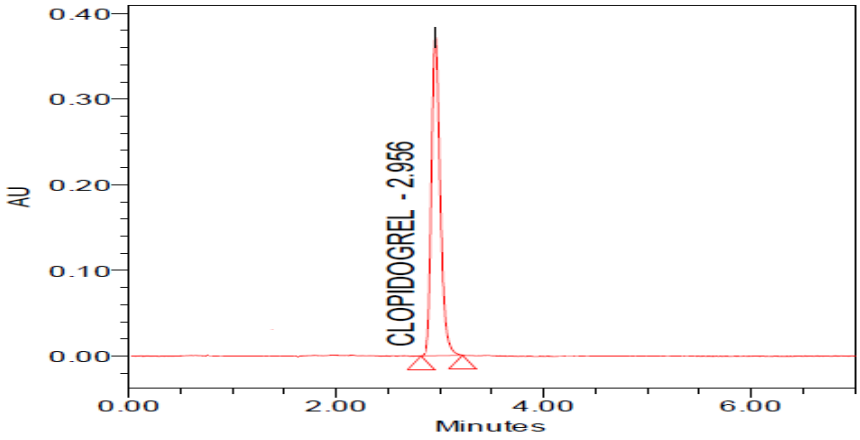

Figure 2B Chromatogram of Clopidogrel

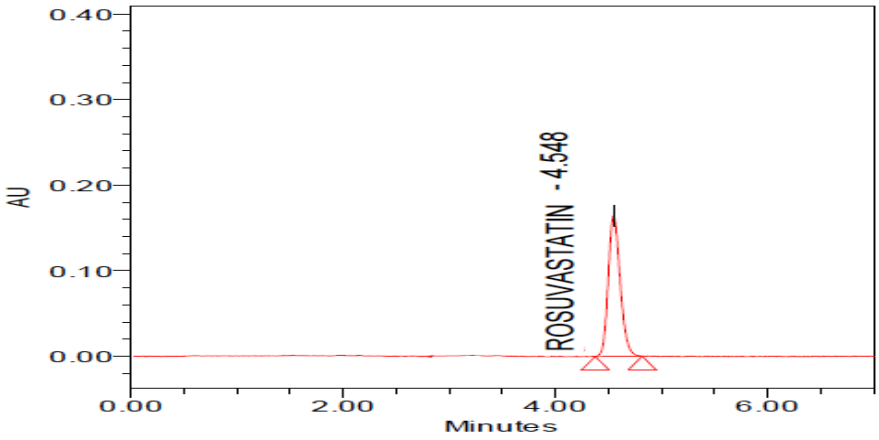

Figure 2C Chromatogram of Rosuvastatin

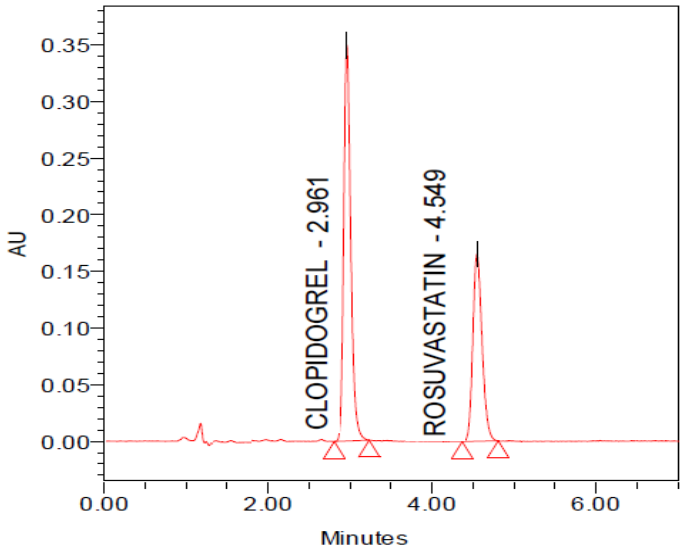

Figure 3 Chromatogram of Formulation

Method precision and Intermediate precision:

The percentage assay values for method precision and intermediate precision of 12 preparations were found to be in between 98.0% - 102.0% for both the drugs. The %RSD value for the % assay values of both the method precision and intermediate precision was less than 2.0. The results were given in Table 2A and Table 2B.

Table 2A: Precision Results for the % Assay of Rosuvastatin and Clopidogrel

Precision	RSV	CD
Method Precision	98.8	100.1
% RSD	0.55	0.53

Table 2B: Intermediate Precision Results for % Assay Rosuvastatin and Clopidogrel

Intermediate Precision	RSV	CD
Method Precision	98.6	99.6
%RSD	0.50	0.31

Linearity:

Calibration plots were constructed based on peak area versus concentration. The method was found to be linear over the concentration range of 3.1-18.6 µg/mL for Rosuvastatinand 22.5-135.0 µg/mL for CD with correlation co-efficient not less than 0.999 for both the drugs. Linearity results were summarized in the Table 3.

Table 3: Linearity Results for standard solution of Rosuvastatin and Clopidogrel

S.No	Linearity Level (%)	RSV		CD	
		Concentration µg/mL	Response	Concentration µg/mL	Response
1	20	3.1	258691	22.5	429859
2	40	6.2	518236	45.0	858066
3	60	9.3	779828	67.5	1281572

4	80	12.4	1025613	90.0	1682075
5	100	15.5	1298948	112.5	2150845
6	120	18.6	1547893	135.0	2498124
Correlation Coefficient		0.999		0.999	

Accuracy

Accuracy of the method was determined at three different concentration levels by using recovery studies. The % recovery values were found to be in between 98.0% to 102.0%. The results were given in Table 4.

Table 4: Accuracy Results for standard solution of Rosuvastatin and Clopidogrel

% Concentration at Specification level	% Recovery	
	RSV	CD
80	98.4	98.8
100	98.9	100.2
120	99.6	99.9
Mean	99.0	99.6

Robustness

The system suitability parameters and the %assay values for both the drugs were found to be within the limits by small, deliberate changes in the method parameters and the results were tabulated in Table 5A, 5B, 5C and 5D.

Table 5A: Robustness (Flow rate) Results for Rosuvastatin and Clopidogrel

S.No	Change in Flow Rate (ml/min)	System Suitability Results					
		RSV			CD		
		USP Plate Count	USP Tailing	%Assay	USP Plate Count	USP Tailing	%Assay
1	0.8	6989	0.91	98.82	6985	0.94	100.11
2	1.0	7000	0.95	98.90	6892	0.97	100.20
3	1.2	6972	0.97	99.10	6873	0.98	99.95

Table 5B: Robustness (Mobilephase composition) Results for Rosuvastatin and Clopidogrel

S.No	Change in Organic Composition in the Mobile phase	System Suitability Results					
		RSV			CD		
		USP Plate Count	USP Tailing	%Assay	USP Plate Count	USP Tailing	%Assay
1	10% Less	6850	0.90	98.89	6817	0.95	100.01
2	Actual	7000	0.95	98.90	6892	0.97	100.20
3	10% More	6825	0.96	99.02	6845	0.94	99.89

Table 5C: Robustness (Column temperature) Results for Rosuvastatin and Clopidogrel

S.No	Change in Column temperature (°C)	System Suitability Results					
		RSV			CD		
		USP Plate Count	USP Tailing	%Assay	USP Plate Count	USP Tailing	%Assay
1	33°	6852	0.97	98.88	6617	0.95	99.81

2	Actual	7000	0.95	98.90	6892	0.97	100.20
3	37°	7102	0.91	99.12	6845	0.94	99.95

Table 5D: Robustness (pH) Results for Rosuvastatin and Clopidogrel

S.No.	Change in pH of Buffer	System Suitability Results					
		RSV			CD		
		USP Plate Count	USP Tailing	%Assay	USP Plate Count	USP Tailing	%Assay
1	4.1	6875	0.94	98.77	6802	0.94	99.77
2	Actual (4.2)	7000	0.95	98.90	6892	0.97	100.20
3	4.3	6992	0.93	99.11	6812	0.97	99.01

Solution stability:

The% assay of each drug was determined at 3, 6, 12, 14, and 16 hours and the results were tabulated in Table 6.

Table 6: Solution stability Results for Rosuvastatin and Clopidogrel

S.No.	Time (Hours)	System Suitability Results	
		RSV %Assay	CD %Assay
1	3	99.6	99.8
2	6	98.8	99.6
3	12	98.4	99.1
4	14	98.4	98.9
5	16	98.2	98.8

Forced degradation:

The degradation products were well separated from Rosuvastatinand Clopidogrel peaks in all the degradation conditions. The peak purity is checked for both the drugs and the results are summarized in Table 7.

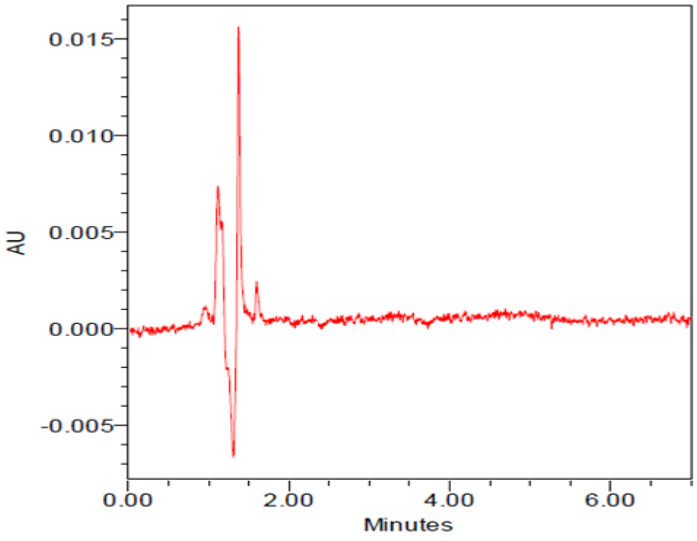

Figure 4A Chromatogram of Blank

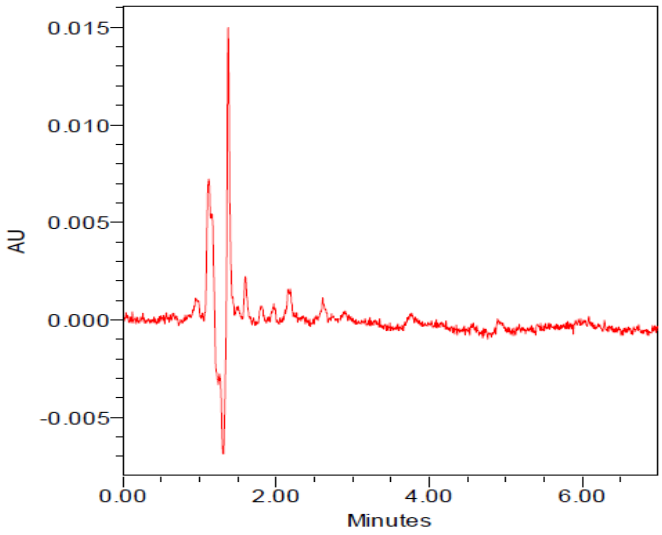

Figure 4B Chromatogram of Placebo

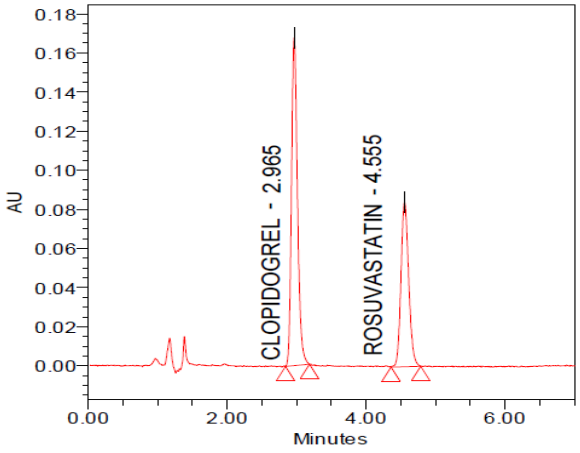

Figure4C Chromatogram of Acid Degradation

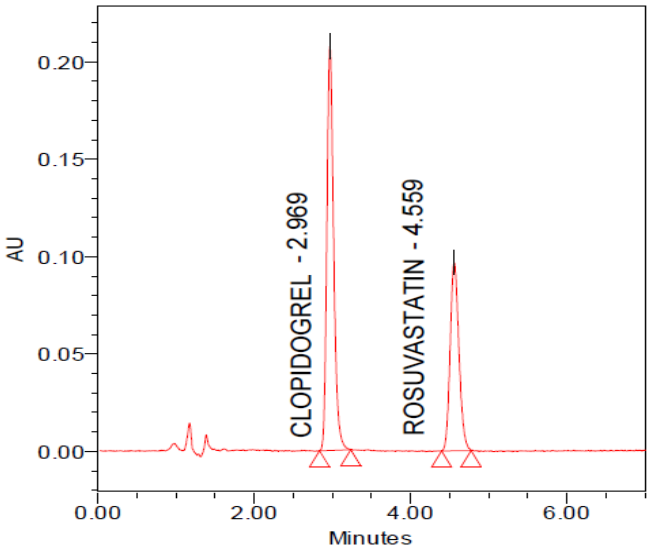

Figure 4D Chromatogram of Base Degradation

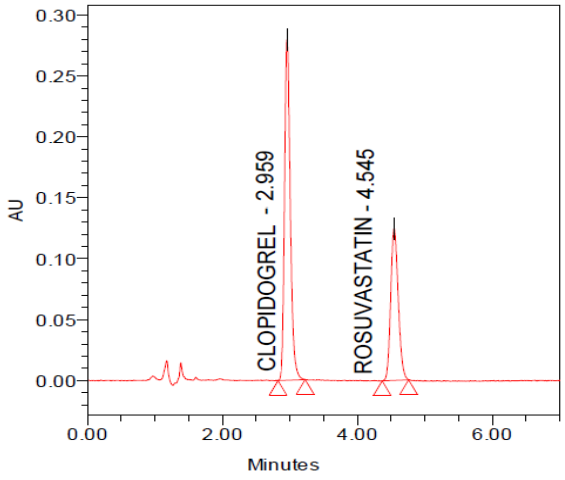

Figure 4E Chromatogram of Oxidative Degradation

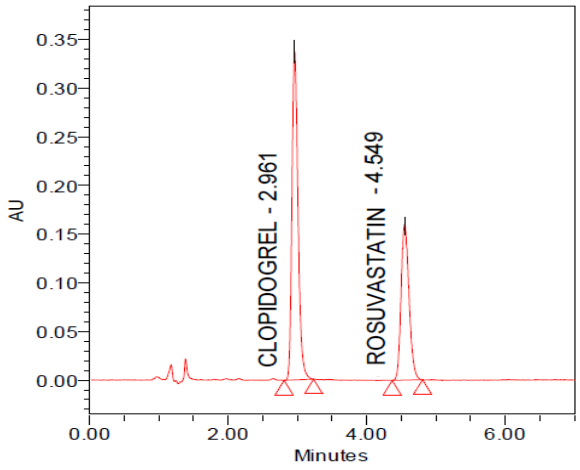

Figure 4F Chromatogram of Thermal Degradation

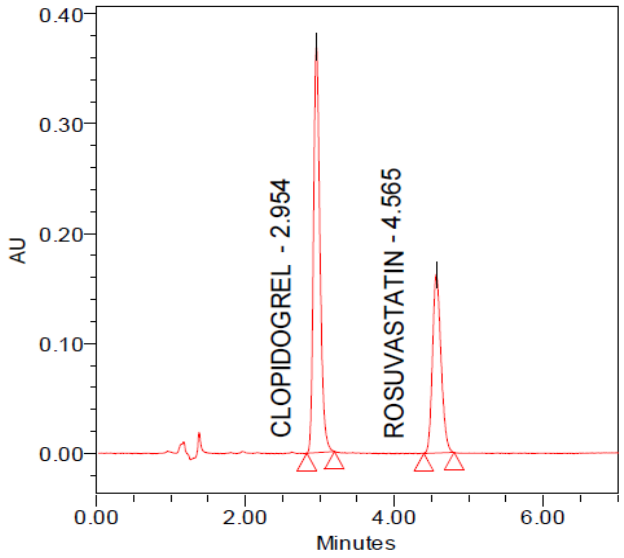

Figure 4G Chromatogram of Light Degradation

Table 7: Degradation studies Results for standard solution of Rosuvastatin and Clopidogrel

Degradation Condition	SV		D	
	Purity angle	Purity Threshold	Purity angle	Purity Threshold
Acid	0.5	0.6	0.4	0.5
Base	0.5	0.6	0.5	0.6
Oxidative	0.4	0.5	0.5	0.6
Thermal	0.4	0.5	0.5	0.6
Light Degradation	0.5	0.6	0.4	0.5

CONCLUSION:

The HPLC method proves to be simple, linear, precise and accurate. The total runtime was 7.0 minutes within which two drugs and their degradation products were well separated. The method was validated and shows satisfactory data for all the method validation parameters tested. The Developed method is stability indicating and can be used for simultaneous quantitative determination of the drugs Rosuvastatinand Clopidogrel in presence of degradation products in stability studies by the industry.

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