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Analytical Method Development and Validation of Amlodipine and Rosuvastatin by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, specific, accurate reversed-phase high-performance liquid chromatographic method was developed validated, and forced degradation studies of Amlodipine and Rosuvastatin were performed. C-18 Develosil ODS HG-5 (150mm X 4.6mm i.d. 5µm) column in isocratic mode, with mobile phase containing Acetonitrile:Phosphate buffer (35:65 v/v) adjusted to pH 2.5 using orthophosphoric acid was used. The flow rate was 1.0 ml/min and effluents were monitored at 243 nm. The Retention time of Amlodipine and Rosuvastatin was2.26min and 5.25min respectively. The calibration curves were linear in the concentration range of 0-150 µg/ml for Amlodipine and 0-150 µg/ ml for Rosuvastatin. Amlodipine and Rosuvastatin stock solutions were subjected to acid and alkali hydrolysis, chemical oxidation, and dry heat degradation. The degraded product peaks were well resolved from the pure drug peak with a significant difference in their retention time values. The proposed method was validated and successfully applied to the estimation of Amlodipine and Rosuvastatin in tablet dosage forms.

I. INTRODUCTION

Amlodipine is a long-acting 1,4-dihydropyridine calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting the influx of calcium in smooth muscle cells, amlodipine prevents calcium-dependent myocyte contraction and vasoconstriction. A second proposed mechanism for the drug's vasodilatory effects involves pH-dependent inhibition of calcium influx via inhibition of smooth muscle carbonic anhydrase. Some studies have shown that amlodipine also exerts inhibitory effects on voltage-gated N-type calcium channels. N-type calcium channels located in the central nervous system may be involved in nociceptive signaling and pain sensation. Amlodipine is used to treat hypertension and chronic stable angina.

Rosuvastatin is an antilipemic agent that competitively inhibits hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. *HMG-CoA reductase* catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis. Rosuvastatin belongs to a class of medications called statins and is used to reduce plasma cholesterol levels and prevent cardiovascular disease.

Literature reveals that few methods have been reported for the estimation of amlodipine and rosuvastatin,many of them suffer from one disadvantage or other,such as low sensitivity, lack of selectivity and simplicity, etc. The present attempt is made to develop a most reliable method for simultaneous estimation of amlodipine and rosuvastatin in pharmaceutical dosage forms adapting different available analytical techniques like UV spectrophotometry and RP-HPLC.

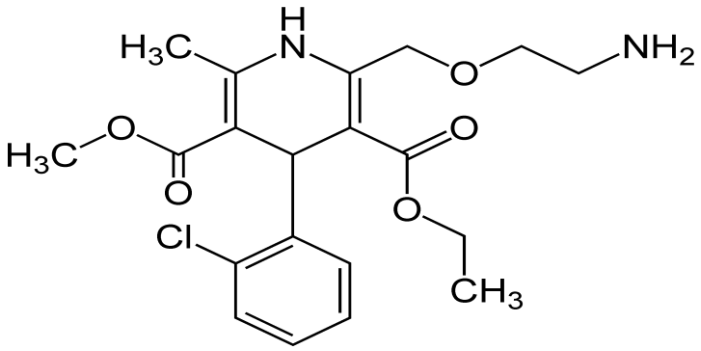

Figure No. 1: Chemical Structure of Amlodipine besylate

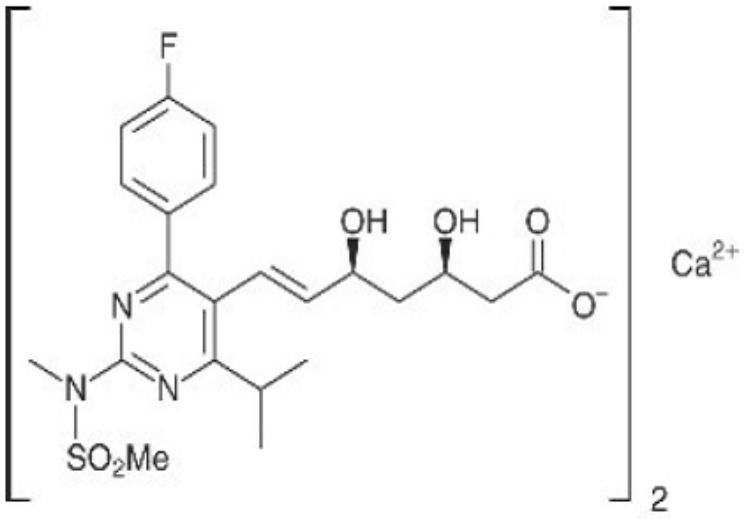

Figure No. 2: Chemical Structure of Rosuvastatin calcium

II. MATERIALS AND METHODS

A. INSTRUMENTATION

The following is the list of instruments/equipment, chemicals/reagents, and standards to perform the HPLC Analysis of the drug Amlodipine& Rosuvastatin.

Table: List of Instruments

Sr. No.	Name of Instrument	Instrument Model	Name of manufacturer
1	UV-Visible double beam spectrophotometer	UV 1800	Elico India
2	HPLC	1575	Hitachi LaChrome
3	Ultra sonicator	--------	Entrech electronics limited
4	Melting point apparatus	--------	
5	UV-Visible double beam spectrophotometer	UV 1800	Elico India
6	HPLC	1575	Hitachi LaChrome
7	Ultra sonicator	--------	Entrech electronics limited
8	Melting point apparatus	--------	

B. CHEMICALS AND REAGENTS

Table: Chemicals and Reagents

S.N.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	HPLC grade water	----	----	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	A.R.	Loba Chem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
6.	Orthophosphoric acid	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
7.	0.3% hydrogen peroxide	99.9%	L.R.	Loba Chem; Mumbai
8.	0.1 N Sodium hydroxide	99.9%	L.R.	Loba Chem; Mumbai
9.	0.1 N Hydrochloric acid	99.9%	L.R.	Sd fine-Chem ltd; Mumbai

C. METHOD DEVELOPMENT

Amlodipine& Rosuvastatin are relatively polar compounds. Preliminary attempts using reversed-phase HPLC using C_8 columns were not successful. Therefore, C_{18} Develosil ODS HG-5 RP 150mm x 4.6mm particle size5µm i.d. where analytes elute in order of decreasing polarity was selected for separation and quantification of the drug.

1. Preparation of Mobile Phase:

The mobile phase used in this analysis consists of a mixture of Phosphate Buffer (pH adjusted to 2.5 with orthophosphoric acid) and Acetronitrile in a ratio of 65:35.

2. Preparation of Standard Stock Solutions and working standards:

Accurately weighed around 25mg of Amlodipine& Rosuvastatin working standard, taken into a 25 ml volumetric flask, then dissolved and diluted to volume with the mobile phase to obtain a solution having a known concentration of about 1000 mcg/ml.

Further dilutions have been made to get the final concentration of 100 µg/ml.

3. Preparation of Test solution:

Diluted quantitatively an accurately measured volume of label claim solution with diluents to obtain a solution containing about a linear range.

4. Method development trails:

Trail 1:

In trial 1 we used ACN and water as mobile phase in the ratio of 80:20**.**

Stationary phase	**:**	Waters C_{18}, 5µm, 25cmx4.6mm i.d.
Mobile phase	**:**	ACN : water (80:20)
Elution mode	**:**	Isocratic
Sample concentration	**:**	100ppm.
Injection volume	**:**	20µL.
Run time	**:**	10 min.
Flow rate	**:**	1 ml/min.
Detection wavelength	**:**	243 nm.
Temperature	**:**	25ºC.

Trail 2:

The initial condition peaks had insufficient resolution and peaks showed low retention. So, we changed the ratio of mobile phase and flow rate to get good peaks.

Stationary phase	**:**	Waters C_{18}, 5µm, 25cmx4.6mm i.d.
Mobile phase	**:**	Acetonitrile : water (40:60)
Elution mode	**:**	Isocratic
Sample concentration	**:**	100ppm.

Injection volume	**:**	20µL.
Run time	**:**	10 min.
Flow rate	**:**	0.5 ml/min.
Detection wavelength	**:**	243 nm.
Temperature	**:**	25ºC

D. VALIDATION OF ANALYTICAL METHOD:

System suitability:

System suitability was demonstrated using 50ppm Amlodipine and Rosuvastatin and 10µL volume of this solution was injected six times into the chromatographic system and the chromatogram was recorded. System suitability was determined with the below mention parameters.

- Resolution.
- Capacity factor.
- Retention Time

Precision:

Precision was determined by replicate processing. Precision was reported as Percent Relative Standard Deviation. 50ppm, 100ppm, and 150ppm of Amlodipine and Rosuvastatin were selected to determine the precision of the method. The Percentage Relative Standard Deviation for the areas was calculated (should not be more than 15%).

Linearity:

Linearity of the developed method was demonstrated with Amlodipine and Rosuvastatin at six different concentrations from 0-50 ppm. Calibration QC standards were prepared fresh on the day of analysis by diluting the appropriate working solutions with mobile phase and injected into the chromatographic system.

A graph was plotted with concentration versus peak area by covering six points.

LOD and LOQ:

LOD and LOQ were calculated according to ICH guidelines. The LOD and LOQ are shown in Tables 4-15. The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

$$L.O.D. = 3.3(SD/S).$$

$$L.O.Q. = 10(SD/S)$$

Where SD = Standard deviation of the response

S = Slope of the calibration curve

Accuracy:

For accuracy determination, three quality control samples were prepared i.e., 80ppm, 100ppm, and 120ppm of Amlodipine and Rosuvastatin injected in three replicate volumes of 20µL each. Accuracy is reported as the percent recovery of the known, added amount.

Robustness:

The robustness of a method is its ability to remain unaffected by small changes in parameters such as percent organic content and pH of the mobile phase, buffer concentration, temperature, and injection volume.

Influence of small changes in chromatographic conditions such as a change in flow rate (± 0.1ml/min), Temperature (±2^0C), Wavelength of detection (±2nm) & acetonitrile content in the mobile phase (±2%) studied to determine the robustness of the method.

III. RESULTS AND DISCUSSION

A. METHOD DEVELOPMENT

Optimized conditions:

In the initial trials, the resolution was very poor. To overcome this we changed the buffer, adjusted the pH, and performed various trials by changing the mobile phase composition. The ratio of 35:65 of ACN and phosphate buffer gave the best results. The optimized conditions are listed below.

Stationary phase	**:**	C_{18} Develosil ODS HG-5 (150mm x 4.6mm i.d, 5µm)
Mobile phase	**:**	Acetonitrile : Phosphate buffer (35:65)
Elution mode	**:**	Isocratic
Sample concentration	**:**	100ppm.
Injection volume	**:**	20µL.
Run time	**:**	10 min.
Flow rate	**:**	1.0 ml/min.
Detection wavelength	**:**	243 nm.
Temperature	**:**	25ºC

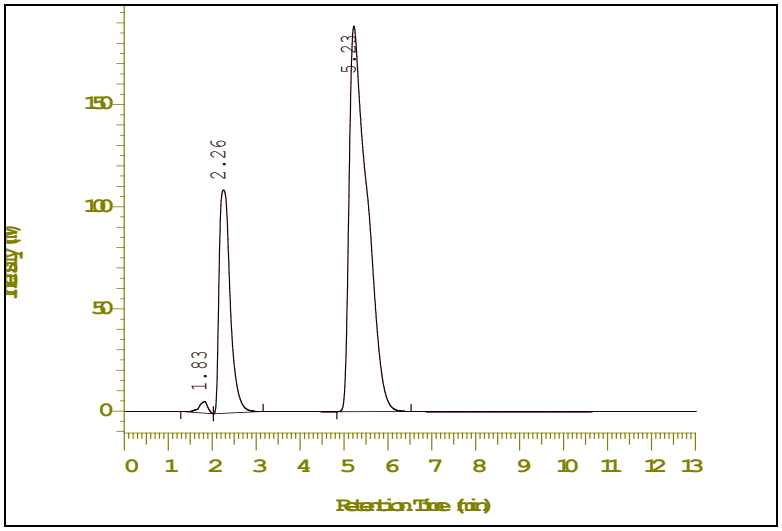

Figure no. 3: Chromatogram for Optimised condition

Table No. 1. Peak integration data for Amlodipine

Peak	Retention time (min)	Peak Concentration
Amlodipine	2.26	98.7

The retention time was found to be 2.25 min.

Table No. 2. Peak integration data for Rosuvastatin

Peak	Retention time (min)	Peak Concentration
Rosuvastatin	5.25	98.9

The retention time was found to be 5.35 min.

The HPLC system was set with the optimized chromatographic conditions to run the standard solution of Amlodipine & Rosuvastatin for 10 min. The retention time was found to be 2.25 min and 5.35 min respectively.

A. METHOD VALIDATION

Accuracy:

Table No. 3: Accuracy data for Amlodipine

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	8	10	99.63	Mean= 99.67667%
S_2 : 80 %	8	10	99.92	S.D. = 0.223681
S_3 : 80 %	8	10	99.48	% R.S.D.= 0.224407
S_4 : 100 %	10	10	99.19	Mean= 99.19%
S_5 : 100 %	10	10	99.25	S.D. = 0.06
S_6 : 100 %	10	10	99.13	% R.S.D.= 0.06049
S_7 : 120 %	12	10	99.25	Mean= 99.49%
S_8 : 120 %	12	10	99.54	S.D. = 0.219317
S_9 : 120 %	12	10	99.68	% R.S.D. = 0.220441

Table No. 4: Accuracy data for Rosuvastatin

Sample ID	Concentration (µg/ml)		%Recovery of Pure drug	Statistical Analysis
	Pure drug	Formulation		
S_1 : 80 %	8	10	100.23	Mean= 99.92%
S_2 : 80 %	8	10	100.15	S.D. = 0.469361268
S_3 : 80 %	8	10	99.38	% R.S.D.=0.469737058
S_4 : 100 %	10	10	99.78	Mean= 100.7266667%
S_5 : 100 %	10	10	99.86	S.D. = 1.570902076
S_6 : 100 %	10	10	102.54	% R.S.D.=1.559569207
S_7 : 120 %	12	10	99.89	Mean= 100.4066667%
S_8 : 120 %	12	10	99.34	S.D. = 1.398511113
S_9 : 120 %	12	10	101.99	% R.S.D. =1.392846869

The mean recoveries were found to be 99.67, 99.19, 99.49 % for Amlodipine and 99.92, 100.72, 100.40% for Rosuvastatin. The limit for mean % recovery is 98-102% and as both the values are within the limit, hence it can be said that the proposed method was accurate.

Specificity:

For the specificity of the method, the marketed formulations have been taken & The solution was injected into the HPLC system. The chromatograms obtained are shown in figure 4.

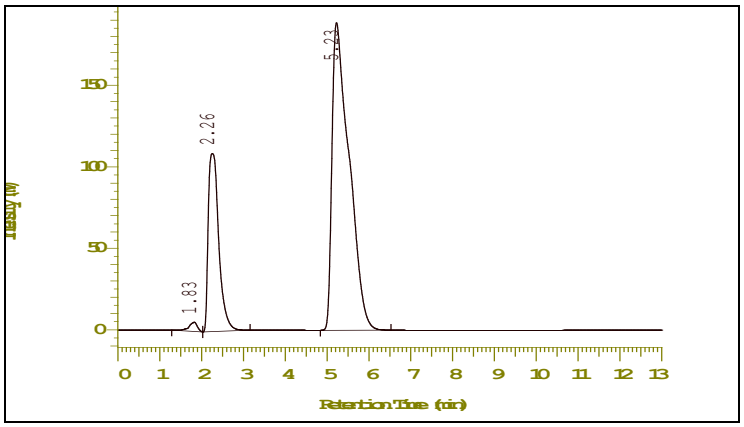

Figure No. 4: Chromatogram for Amlodipine and Rosuvastatin

No peaks were found at the retention of Rosuvastatin and Amlodipine. Specificity studies indicating that the excipients did not interfere with the analysis.

So, the method is found to be specific for the given analytes.

Linearity:

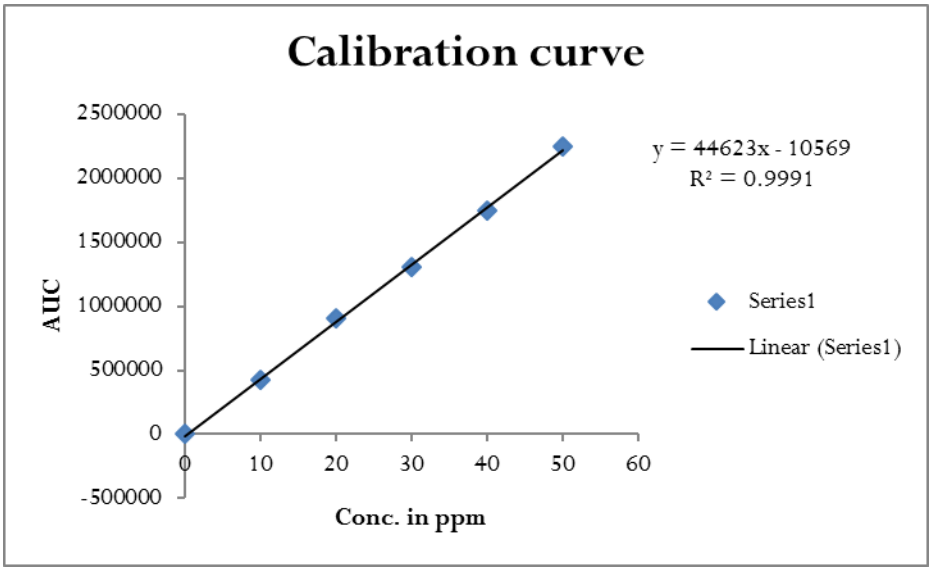

Figure No. 5: Standard curve for Amlodipine

Table No. 5: Standard curve for Amlodipine

CONC.(µg/ml)	MEAN AUC (n=6)
0	0
10	424838
20	904737
30	1302869
40	1746831
50	2250813

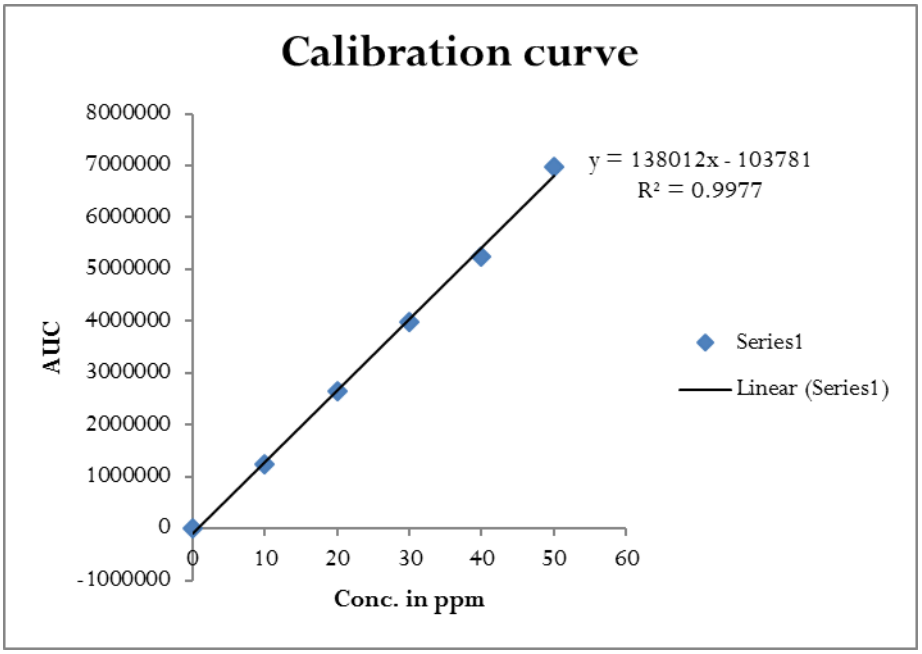

Figure No. 6: Standard curve for Rosuvastatin

Table No. 6: Standard curve for Rosuvastatin

CONC.	AUC
0	**0**
10	**1228747**
20	**2638031**
30	**3983572**
40	**5249436**
50	**6979310**

The linearity range was found to be 0-50 µg/ml for Rosuvastatin and 0-50 µg/ml for Amlodipine. The correlation coefficients were found to be 0.999 & 0.997, the slopes were found to be 44623 & 13801, and the intercept was found to be 10569 & 10378 for Amlodipine and Rosuvastatin respectively.

Precision:

Repeatability

Table No. 7: **Data showing repeatability analysis for Amlodipine**

HPLC Injection Replicates of Amlodipine	Retention Time (min)	Area
Replicate – 1	2.26	1302869
Replicate – 2	2.26	1302586
Replicate – 3	2.25	1318521
Replicate – 4	2.23	1302569
Replicate – 5	2.22	1302896
Average	2.244	1305888
Standard Deviation	0.018166	7063.605
% RSD	0.809532	0.540904

Table no 8: **Data showing repeatability analysis for Rosuvastatin**

HPLC Injection Replicates of Rosuvastatin	Retention Time (min)	Area
Replicate – 1	5.23	3983572
Replicate – 2	5.23	3985214
Replicate – 3	5.07	3990228
Replicate – 4	5.08	3985261
Replicate – 5	5.08	3996512
Average	5.138	3988157
Standard Deviation	0.084083	5295.407
% RSD	1.636498	0.132778

The repeatability study which was conducted on the solution having the concentration of about 100 µg/ml for Amlodipine and 100 µg/ml for Rosuvastatin (n =5) showed an RSD of 0.7684% for Amlodipine and 0.08488% for Rosuvastatin. It was concluded that the analytical technique showed good repeatability.

Intermediate precision:

Table No. 9: Data for Rosuvastatin analysis

Conc. Of Rosuvastatin (API) (µg/ml)	Observed Conc. Of Rosuvastatin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	10.09	1.54	10.13	0.46
30	30.03	0.75	30.84	0.82
100	99.94	0.48	99.37	0.91

Table No. 10: Data for Amlodipine analysis

Conc. Of Amlodipine (API) (µg/ml)	Observed Conc. Of Amlodipine (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
10	9.94	0.96	10.43	0.97
30	30.04	0.40	30.93	0.96
100	100.91	0.93	99.15	0.19

Intraday and interday studies show that the mean RSD (%) was found to be within the acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within a day and between days. Hence, the method at the selected wavelength was found to be precise.

LOD and LOQ:

The detection limit (LOD) and quantitation limit (LOQ) may be expressed as:

$$L.O.D. = 3.3(SD/S).$$

$$L.O.Q. = 10(SD/S)$$

Where SD = Standard deviation of the response

S = Slope of the calibration curve

Table No. 11: Data of LOD and LOQ

S. No.	Parameter	Amlodipine	Rosuvastatin
1	**LOD**	0.02	0.06
2	**LOQ**	0.04	1.12

The LOD was found to be 0.02 µg/ml and 0.06 µg/ml and LOQ was found to be 0.04 µg/ml and 1.12 µg/ml for Amlodipine and Rosuvastatin respectively which represents that sensitivity of the method is high.

System suitability parameters:

Table No. 12: Data of System Suitability Parameter

S. No.	Parameter	Limit	Result
1	Resolution	Rs > 2	9.15
2	Asymmetry	T ≤ 2	Rosuvastatin=0.12 Amlodipine =0.5
3	Theoretical plate	N > 2000	Rosuvastatin=3246 Amlodipine= 4693

Method Robustness:

Table No. 13: Result of method Robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.05
Flow (0.9 ml/min)	0.03
Temperature (27ºC)	0.07
Temperature (23ºC)	0.05
Wavelength of Detection (244 nm)	0.05
The wavelength of detection (240 nm)	0.07

Influence of small changes in chromatographic conditions such as a change in flow rate (± 0.1ml/min), Temperature (± 2^0C), Wavelength of detection (±2nm) studied to determine the robustness of the method are also in favor of (Table-4-17, % RSD < 2%) the developed RP-HPLC method for the analysis of rosuvastatin & amlodipine(API).

IV. CONCLUSION

Sensitive & selective stability-indicating RP-HPLC method has been developed & validated for the analysis of Amlodipine & Rosuvastatin API.

Based on peak purity results, obtained from the analysis of samples using the described method, it can be concluded that the absence of co-eluting peak along with the main peak of Amlodipine & Rosuvastatin in dictated that the developed method is specific for the estimation of Amlodipine & Rosuvastatin.

Further, the proposed RP-HPLC method has excellent sensitivity, precision, and reproducibility.

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