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STABILITY RELATED FORCED DEGRADATION STUDIES FOR A NEWLY DEVELOPED AND VALIDATED METHOD FOR AMLODIPINE AND ROSUVASTATIN BY RP-HPLC METHOD

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ABSTRACT

A simple, specific, accurate by reversed phase high performance liquid chromatographic method was developed validated and forced degradation studies of Amlodipine and Rosuvastatin 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 (60:40 v/v) adjusted to pH 2 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 were 2.26min 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 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.

INTRODUCTION

"CHROMATOGRAPHY" is a method of separating a mixture of components into individual components through equilibrium distribution between two phases. Separation of two sample components in chromatography is based on their different distribution between two non-miscible phases. The one, the stationary phase, a liquid or solid, is fixed in the system. The other, the mobile phase, a fluid, is streaming through the chromatographic system. In gas chromatography the mobile phase is a gas, in liquid chromatography it is a liquid.

High Performance Liquid Chromatography (HPLC) is one mode of chromatography, one of the most used analytical techniques. HPLC as compared with the classical LC technique is characterised by: **,** High resolution, Small diameter (4.6 mm), stainless steel, glass or titanium columns, Column packing with very small (3, 5 and 10 µm) particles, Relatively high inlet pressures and controlled flow of the mobile phase, Continuous flow detectors capable of handling small flow rates and detecting very small amounts, Examples of such materials include amino acids, nucleic acids, hydrocarbons, carbohydrates, drugs, terpenoids, pesticides, antibiotics, and metal organic species. HPLC separation is based on interactions and differential partition of

sample between the mobile phase and stationary phase. [1-5] Reverse phase chromatography is a bond phase chromatography technique, uses water as base solvent. Separation is based on solvent strength and selectivity. Separation is affected by column temperature and pH. In general, the more polar compounds elute faster than the less polar compounds. UV detection is the most common detection technique used. According to ICH guidelines this following procedure is applicable to the development of new analytical methods by HPLC. It usually involves different steps as follows: Selection and optimization, Buffer and its strength (if any), pH of the buffer or pH of the Mobile Phase, Mobile Phase Composition, Selection of Column, Selection of solvent delivery system, Selection of Flow Rate, Selection of Column Temperature, Selection of Detector Wavelength, Selection of Diluent for Sample Preparation, Selection of sample concentration, injection volume, System suitability test, Capacity factor (k^1), Resolution (R_s), Tailing factor (T_f), Theoretical plates (N).

The simple meaning for method validation is a method which give reliable results and checking the reliability of the results in all aspects. Other definitions include "Establishing documented evidence that a system does what it purports to do."FDA defines validation as "the documented program providing high degree of assurance that specific process or equipment, will consistently produce product, meeting predetermined specification and quality attributes. [6-10] The steps involved in method validation are: Method validation protocol definition, Laboratory method validation, Validated test method generation, Validation report. USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, Limit of quantitation, Linearity and range, Ruggedness, Robustness. Forced degradation study by stressing active pharmaceutical ingredient (API) using Acid, Base, H_2O_2, Water and heat. If the molecule is known to be sensitive to light then stress the sample and sample solution with light. Subject the drug substance to stress with varied strengths of stressing agents to obtain degradation between 10% & 30%. Inject the samples into a HPLC system equipped with photo diode array(PDA) and check for separation of degradants formed under stressed conditions and the peak purity of the Active pharmaceutical ingredient(API) peak. If the purity of the peak is found to be satisfactory as per the individual software requirements, then the method can be considered as stability indicating. [11-15] The API (Amlodipine & Rosuvastatin) was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage and/or after administration to body. This is one type of accelerated stability studies that helps us determining the fate of the drug that is likely to happen after a long time storage, within a very short time as compare to the real time or long term stability testing. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, thermal degradation and oxidative degradation. The main aims and objectives of the present study are: To undertake solubility and stability studies of Rosuvastatin & Amlodipine and to develop initial U.V. and chromatographic conditions. Setting up of initial UV and chromatographic conditions for the method development in pure and pharmaceutical dosage forms. Optimization of initial chromatographic and spectrophotometric conditions. Carry out assay of Rosuvastatin & Amlodipine with developed chromatographic conditions. Analytical method validation of the developed RP- HPLC method. Quantitative determination of Rosuvastatin & Amlodipine in pharmaceutical dosage form using the method developed and validated. To perform forced degradation studies of Rosuvastatin & Amlodipine with the developed method. [16-20] To our knowledge, there is no HPLC method reported for the combination, availability of an HPLC method with high sensitivity and selectivity will be very useful for the estimation of ROSUVASTATIN and AMLODIPINE in combined pharmaceutical dosage forms. Therefore the aim of the study was to develop and validate sensitive, precise, accurate and

specific HPLC method for the determination of ROSUVASTATIN and AMLODIPINE simultaneously in formulation as per ICH guidelines . The present work describes a simple reverse phase LC method for the determination of ROSUVASTATIN and AMLODIPINE in tablets. It is necessary to find the content of each drug either in bulk or single or combined dosage forms for purity testing. It is also essential to know the concentration of the drug and it's metabolites in biological fluids after taking the dosage form for treatment. The scope of developing and validating an analytical method is to ensure a suitable method for a particular analyte more specific, accurate and precise. The main objective for that is to improve the conditions and parameters, which should be followed in the development and validation. [21-25]

Preliminary studies: As a starting point of method development, the following preliminary studies were performed for Amlodipine and Rosuvastatin.

Accurately weighed quantity (10mg) of Amlodipineand Rosuvastatin was taken into a test tube individually and the below mentioned solvents were added and sonicated for 10 min.

- ✓ Water.
- ✓ Methanol
- ✓ Acetonitrile.
- ✓ Acetonitrile/water.

Solubility of the drugs was observed visually. Results are discussed in Table. [26-30]

Estimation of Maximum Wavelength by UV Spectroscopy: Accurately weighed quantities of the Amlodipine and Rosuvastatin (10mg each) were dissolved in methanol and the final volume was made up to 10ml, separately. The prepared solutions were scanned under UV region (200-400nm) for the estimation of maximum wavelength $\lambda_{max.}$ The results thus obtained are shown in figure 4-1 and discussed in Table 4-2.

Materials and Methods:

Table 3-1: Chemicals and Reagents

S.No	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.	Ortho phosphoric 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

Instrumentation: The following are the list of instruments/equipments, chemicals/reagents and standards to perform the HPLC Analysis of the drug Amlodipine& Rosuvastatin.

Table 3-2: 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 appraturs	--------	

Preparation of solutions
Preparation of phosphate buffer (pH-32.5)

Table 3-3: phosphate buffer solutions pH 2.5

P_H	Potassium dihydrogen orthophosphate	Dipotassium hydrogen phosphate
2.5	2.625 gm	0.2625 gm

Above quantity added to 500ml HPLC grade water. pH adjusted with orthophosphoric upto pH 2.5.

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 acid) and Acetronitrile in a ratio of 65:35.

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 has been made to get the final concentration of 100 µg/ml. [31-35]

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.

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 size 5µm i.d. where analytes elute in order of decreasing polarity was selected for separation and quantification of drug.

Selection of conditions:

Selection of Mobile phase: Mobile phase was selected based on solubility studies and on the literature survey.

Selection of organic Mobile phase: Acetonitrile was selected as the organic mobile phase as it provides good resolution for Amlodipine& Rosuvastatin.

Selection of Buffer and its pH: During the initial trials with acetate buffer (pH 4.8) it was observed that the peak symmetry was not proper, hence to improve the resolution and peak shape, phosphate buffer pH 2.5 was used to improve the peak shape. The phosphate buffer with varying pH (2.0 2.5, 3.0 and 2.5) was tried. Symmetric peaks were observed best at pH 2.5.

Selection of Mobile phase composition: Varying proportions of phosphate buffer (pH -2.5) and acetonitrile were studied for the proper selection of ratio of the mobile phases. 35:65 ACN:Buffer (% v/v) was found to be optimum since at this ratio no interference was observed with good resolution and peak purity. [36-40]

Effect of flow rate: The flow rates of 0.5, 0.8 and 1.0 mL/min were used and chromatograms were recorded. At 1.0 mL/min symmetrical peaks with acceptable tailing factor were observed.

Effect of Column temperature: Separations were performed at three different column temperature 20^0C, 25^0C, 30^0C. However no specific change was observed upon changing the column temperature. For the present study 25^0C was selected.

Method development trails:

Trail 1:

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 : 25^0C.

The initial condition gave peaks with insufficient resolution and peaks showed low retention. Chromatogram is shown in figure 3.1. [41-45]

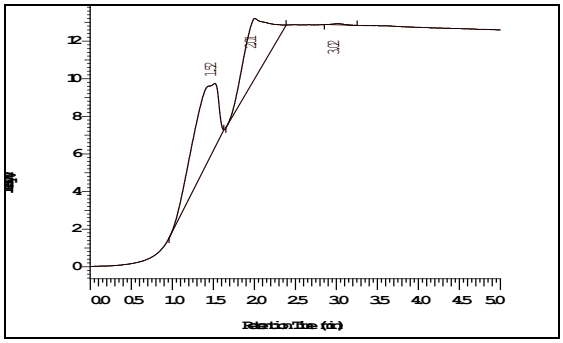


Fig.3-1. Chromatogram for Trail 1

Trail 2:

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 : 25^0C

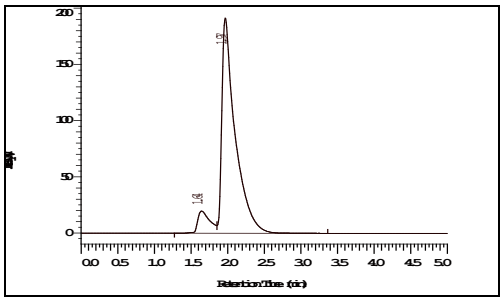


Fig.3-2. Chromatogram for Trail 2

The peaks are separated but not adequately and the retention time is also very low. Some amount of tailing was also observed. Chromatogram is shown in figure 3.2.

Trail 3:
Stationary phase : Waters C_{18}, 5µm, 25cmx4.6mm i.d.
Mobile phase : Acetonitrile : water (70:30)
Mobile phase B: Acetonitrile.
Elution mode : Isocratic
Sample concentration: 100ppm.
Injection volume : 20µL.
Run time : 10 min.
Flow rate : 1.0 ml/min.
Detection wavelength: 243 nm.
Temperature : 25^0C

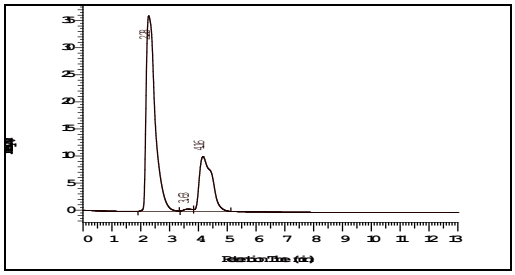


Fig.3-3. Chromatogram for Trail 3

The peaks are broken. Some amount of tailing was also observed. Chromatogram is shown in figure 3.3.

Trail 4:

Stationary phase : C_{18} Develosil ODS HG-5 (150mm x 4.6mm i.d, 5µm)
Mobile phase: Acetonitrile: Acetate buffer (6:4)
Elution mode : Isocratic
Sample concentration: 100ppm.
Injection volume : 20µL.
Run time : 10 min.
Flow rate : 1.0 ml/min.
Detection wavelength: 243 nm.
Temperature : 25^0C

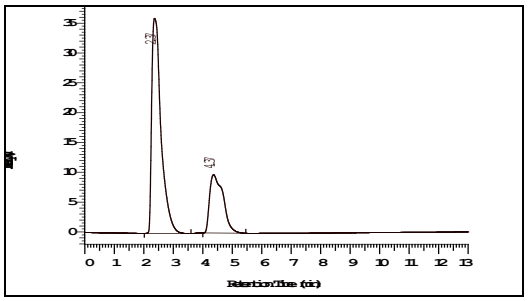


Fig.3-4. Chromatogram for Trail 4

These conditions results in good peaks when compared with the initial conditions, but broad peaks are obtained. However, some amount of tailing was still observed. Chromatogram is shown in figure 3.4.

Trail 5: 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	**:**	25^0C

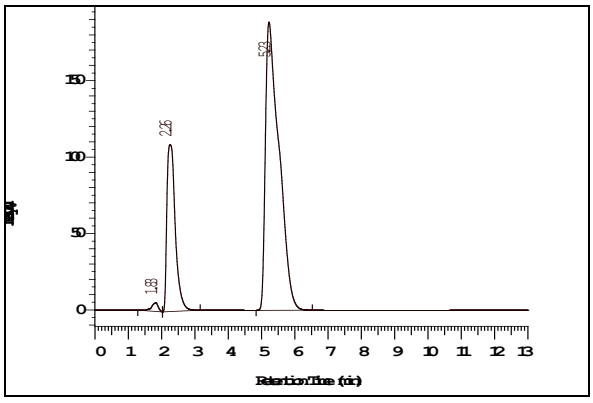


Fig. 3-5 Chromatogram for Trail 5

The peaks showing good responce. Chromatogram is shown in figure 3.5

Method validation:

1. Accuracy: For accuracy determination, three quality control samples were prepared i.e., 10 ppm, 25ppm and 50ppm of Amlodipine and Rosuvastatin injected in five replicate volumes of 20µL each. Accuracy is reported as the percent recovery of the known, added amount. Results are given in table 4-7 and 4-8.

Acceptance criteria: The percentage recovery should be in the range of 85 to 115%

2. Precision: Precision was determined by replicate processing. Precision was reported as Percent Relative Standard Deviation. 10 ppm, 25ppm and 50ppm of Amlodipineand Rosuvastatin was selected to determine precision of the method. The Percentage Relative Standard Deviation for the areas were calculated (should not be more than 15%). Results are given in table 4-11 4-12 4-13& 4-14.

Acceptance criteria: The precision determined at each concentration level should not exceed 15% of the coefficient of variation (CV) except for the LLOQ, where it should not exceed 20% of the CV.

Linearity: Linearity of the developed method was demonstrated with Amlodipineand Rosuvastatin at six different concentrations from 1-100 ppm. Calibration QC standards were prepared fresh on the day of analysis by diluting the appropriate working solutions with mobile phase and injected into chromatographic system. The data were subjected to statistical analysis using a linear-regression model. The calibration curves were obtained by weighted linear regression (weighing factor $1/x^2$) using Microsoft Excel 2007 software. A graph was plotted with concentration versus peak area by covering six points as shown in Fig. 4-8 4-9.

Acceptance criteria: The plot for concentration versus peak area should be linear with a regression coefficient not less than 0.9990.

LOD and LOQ: LOD and LOQ was calculated according to ICH guidelines. The LOD and LOQ are shown in table 4-15.

System suitability: System suitability was demonstrated using 50ppm Amlodipineand Rosuvastatin and 10µL volume of this solution was injected six times into the chromatographic system and the chromatogram was recorded. Results are shown in table 4-16. System suitability was determined with the following mention parameters: Resolution., Capacity factor., Retention Time.

Forced Degradation Studies:

Acid hydrolysis: An accurately weighed 25 mg. of pure drugs were transferred to a clean & dry 25 ml of two separate volumetric flasks. To which 0.1 N Hydrochloric acids was added & make up to the mark & kept for 24 hrs. from both the volumetric flask 0.3 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of HCl (after all optimized conditions)

Basic hydrolysis: An accurately weighed 25 mg. of pure drugs were transferred to a clean & dry 25 ml of two separate volumetric flasks. To which 0.1 N Sodium hydroxide was added & make up to the mark & kept for 24 hrs. from both 0.3 ml was taken in to a 10 ml volumetric flask & make up to the mark with mobile phase, then injected into the HPLC system against a blank of . NaOH (after all optimized conditions)

Thermal degradation: An accurately weighed 25 mg. of pure drugs were transferred to a clean & dry 25 ml of two separate volumetric flasks, make up to the

mark with mobile phase. From this solution take 0.3 ml make up to the volume 10 ml & was maintained at 50 ^{0}C. for 24 hrs. then injected into the HPLC system against a blank of mobile phase (after all optimized conditions)

Photolytic Degradation: Approximately 10 mg. of pure drugs were taken in different clean & dry Petridis. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 0.3 mg. of each UV exposed drugs were transferred to a clean & dry 10 ml. volumetric flask. First the UV exposed drug was dissolved in mobile phase & make up to the mark then injected into the HPLC system against a blank of mobile phase (after all optimized conditions)

Oxidation with (3%) H_2O_2: Accurately weighed 10 mg. of pure drug was taken in a clean & dry 100 ml. volumetric flask. 30 ml. of 3% H2O2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 100 ppm solution. The above sample was injected into the HPLC system.

Results are given in table 4-18 4-19 [47-56]

Results and Discussions

Preliminary studies:

Table 4-1: Solubility study of Amlodipine:

REAGENTS	SOLUBILITY
Methanol	Sparingly soluble
Acetonitrile	Soluble
Water	Sparingly soluble

Table 4-2: Solubility study of Rosuvastatin:

REAGENTS	SOLUBILITY
Methanol	Freely soluble
Acetonitrile	Freely Soluble
Water	Moderately soluble

Rosuvastatin was found to be sparingly soluble in water & methanol. Freely soluble in acetonitrile Amlodipine was found to be moderate soluble in water and freely soluble in methanol & acetonitrile.

UV-spectrophotometer analysis:

Table 4-3: λ_{max} of Amlodipine & Rosuvastatin.

S. No.	Drug	λ_{max}
1	Amlodipine	222nm
2	Rosuvastatin	243nm

Method development:.

Optimised conditions:

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 : 25^{0}C

From the absorption spectrum of Amlodipine & Rosuvastatin, λ_{max} was found to be at two wavelengths i.e., 222nm and 243nm. For the present study wavelength of 243nm was selected.

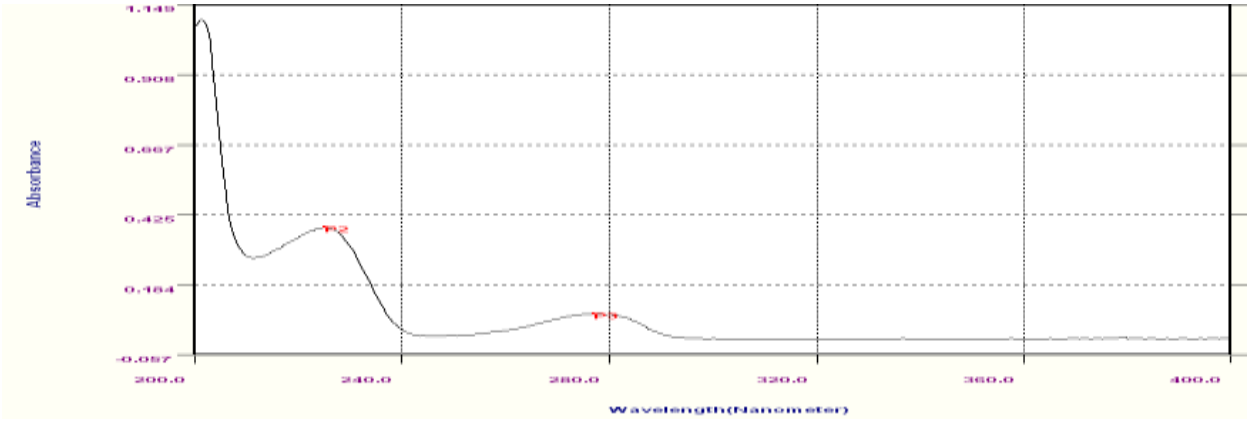


Fig. 4-1: UV-Spectrum for Amlodipine

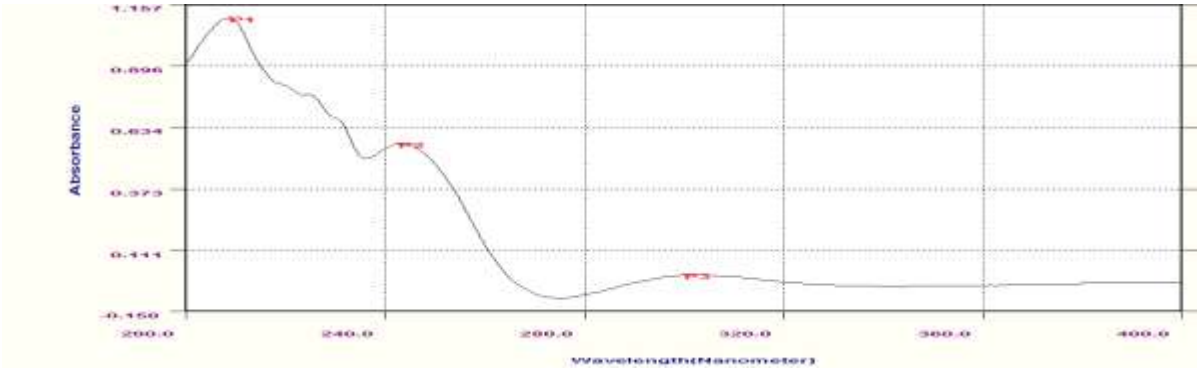


Fig. 4-2: UV-Spectrum for Rosuvastatin

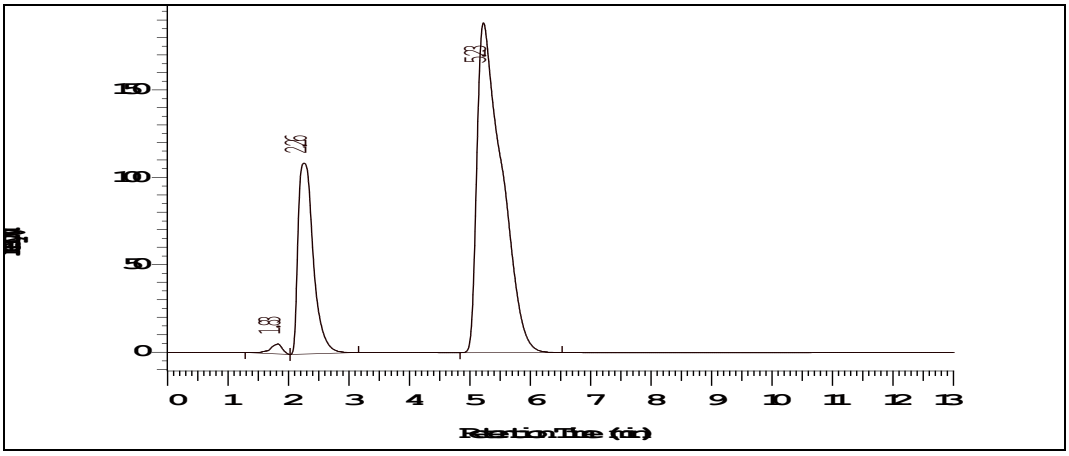


Fig. 4-3: Chromatogram for Optimised condition

Table 4-4: Peak integration data for optimised condition

Peak	Retention time(min)	PEAK CONCENTRATION
1	2.26	98.7
2	5.25	98.9

Conclusion: The peaks are well separated with good resolution and the tailing is minimised to acceptable range. Chromatogram is shown in figure 4-3.

Running the standard solution of Amlodipine

2 ml of stock solution prepared as mentioned under section 4.5.2 was pipetted out into a 10 ml volumetric flask. The volume was made up to the mark with methanol. The solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in **figure 4-4.**

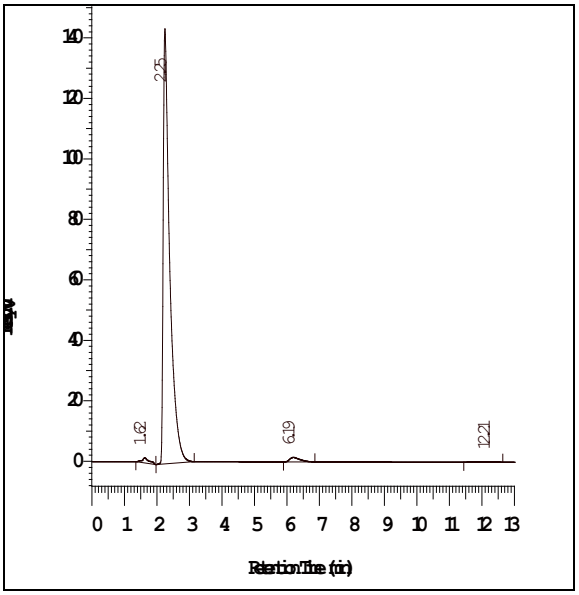


Fig. 4-4: Chromatogram of Amlodipine

Table 4-5 Peak integration data for Amlodipine

Peak	Retention time (min)	Peak concentration
Amlodipine	2.26	98.7

Retention time was found to be 2.25 min.

Running the standard solution of Rosuvastatin

5 ml of stock solution prepared as mentioned under section 4.5.3 was pipetted into a 10 ml volumetric flask. The volume was made up to the mark with methanol. The solution was filtered through the 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The solution was injected into the HPLC system. The chromatogram obtained is shown in figure 4-5.

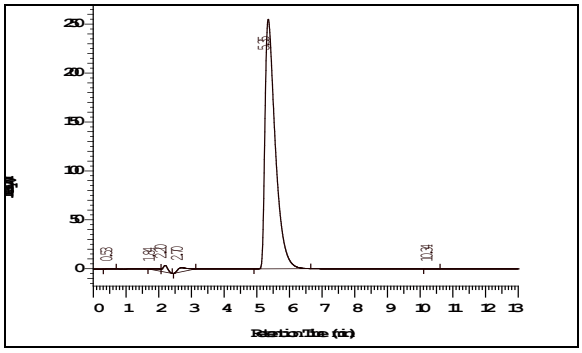


Fig. 4-5: Chromatogram of Rosuvastatin

Validation results:

Accuracy and **Recovery study:**

Table 4-7: Accuracy data for Amlodipine

HPLC Injection Replicates of Amlodipine	Area	Retention Time
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 4-6 Peak integration data for Rosuvastatin

Peak	Retention time (min)	Peak concentration
Rosuvastatin	5.25	98.9

Retention time was found to be 5.35 min

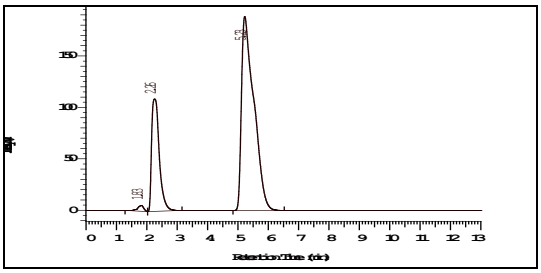


Fig. 4-7: Chromatogram for Amlodipine and Rosuvastatin

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 a RSD of 0.7684% for Amlodipine and 0.08488% for Rosuvastatin. It was concluded that the analytical technique showed good repeatability.The interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes is demonstrated in below chromatograms.There are interferences found in the analysis of the analytes. So the method is found to be specific for the given analytes.

Table 4-8: Accuracy data for Rosuvastatin

HPLC Injection Replicates of Rosuvastatin	Area	Retention Time
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

Linearity:

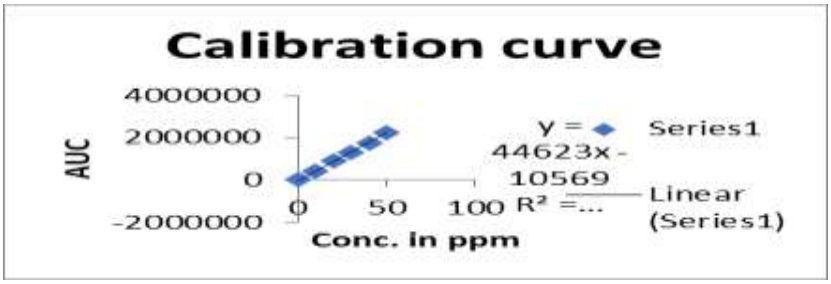


Fig. 4-8: Standard curve for Amlodipine

Table 4-9: Standard curve for Amlodipine

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

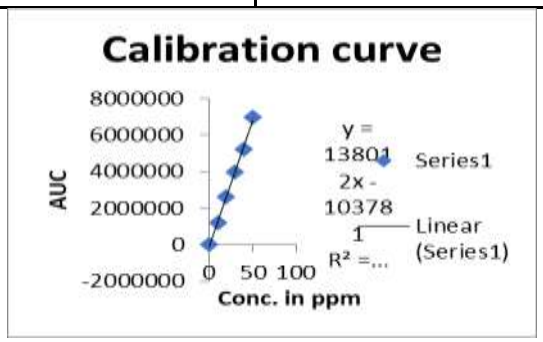
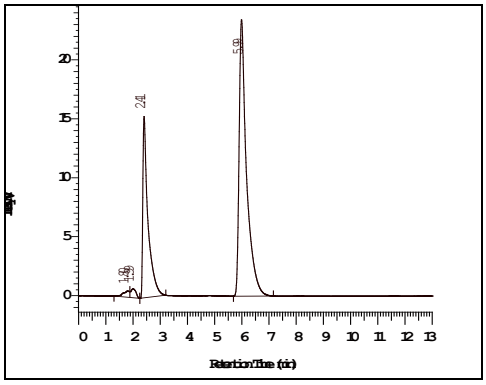


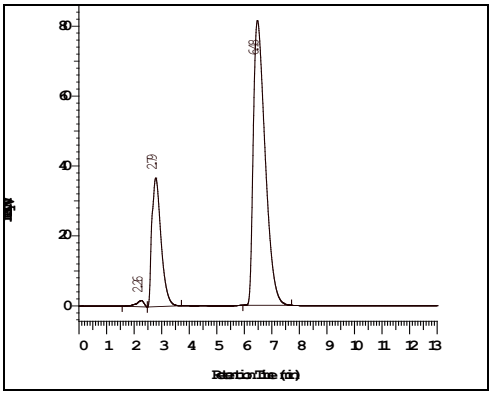
Fig. 4-9: Standard curve for Rosuvastatin

Table 4-10: Standard curve for Rosuvastatin

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



Linearity-1



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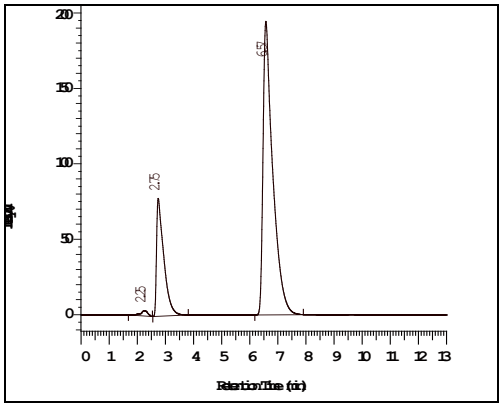

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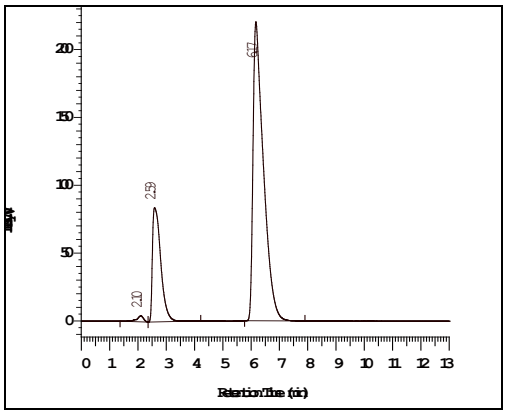

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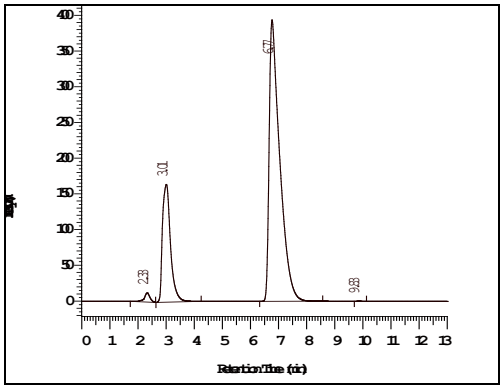


Linearity-5

Fig. 4-10 Chromatograms of Linearity Range

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 intercept were found to be 10569 & 10378 for Amlodipine and Rosuvastatin respectively.

Precision:

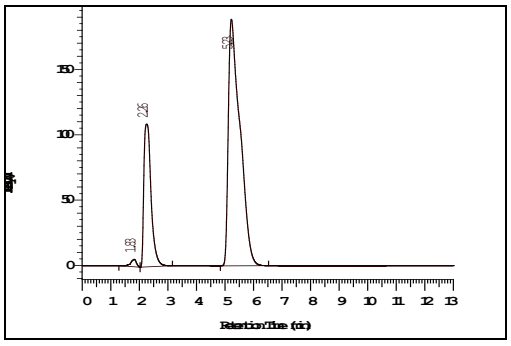
Repeatability

Table 4-11: **Data showing repeatability analysis for Amlodipine**

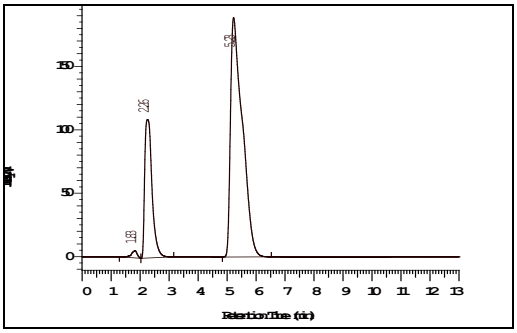
HPLC Injection Replicates of Amlodipine	Area	Retention Time
Replicate – 1	2.26	1302869
Replicate – 2	2.26	1302586
Replicate – 3	2.25	1318521
Replicate – 4	2.23	1302569
Replicate – 5	2.22	1302896
Average	2.244	1305888
Standard Deviation	0.018166	7063.605
% RSD	0.809532	0.540904

Table 4-12: **Data showing repeatability analysis for Rosuvastatin**

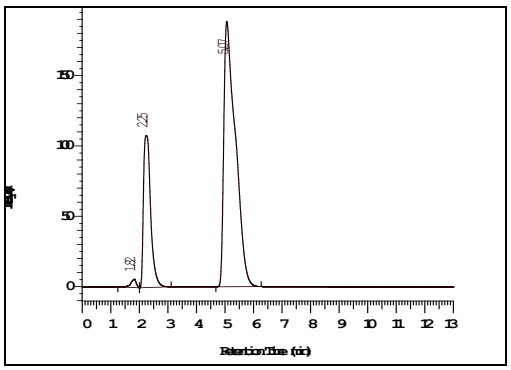
HPLC Injection Replicates of Rosuvastatin	Area	Retention Time
Replicate – 1	5.23	3983572
Replicate – 2	5.23	3985214
Replicate – 3	5.07	3990228
Replicate – 4	5.08	3985261
Replicate – 5	5.08	3996512
Average	5.138	3988157
Standard Deviation	0.084083	5295.407
% RSD	1.636498	0.132778



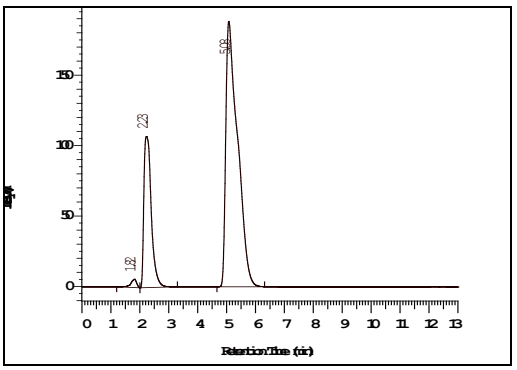
Repeatability-1



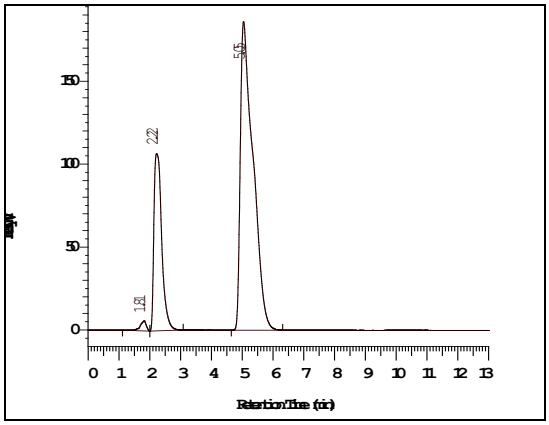
Repeatability-2



Repeatability-3



Repeatability-4



Repeatability-5

Fig. 4-11. Chromatograms of Repeatability

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 a RSD of 0.7684% for Amlodipine and 0.08488% for Rosuvastatin. It was concluded that the analytical technique showed good repeatability.

Intermediate precision:

Table 4-13: 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 4-14: 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 acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise.

LOD and LOQ:

Table 4-15: 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 1.2 µg/ml for Amlodipine and Rosuvastatin respectively which represents that sensitivity of the method is high.

System suitability parameters:

Table 4-16: 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

Recovery Data for estimation Rosuvastatin and Amlodipine

Table 4-17: Recovery Data for estimation Rosuvastatin and Amlodipine

Brand Name Of Tablets	Labelled Amount Of Drug (Mg) Amlodipine Tablet : Rosuvastatin Tablet	Mean (±Sd) Amount (Mg) Found By The Proposed Method (N=6) Amlodipine : Rosuvastatin	Assay + % Rsd Amlodipine : Rosuvastatin
Amchek –At	50 Mg 5 Mg	50.01 (±0.39) 4.91 (±0.75)	100.2 (±0.19) 98.2 (±0.35)

The amount of drugs in Amchek -AT 20 tablet was found to be 96.55 (±0.35) mg/tab for Rosuvastatin and 100.1 (±0.19) mg/tab for Amlodipine.

Forced Degradation Studies:

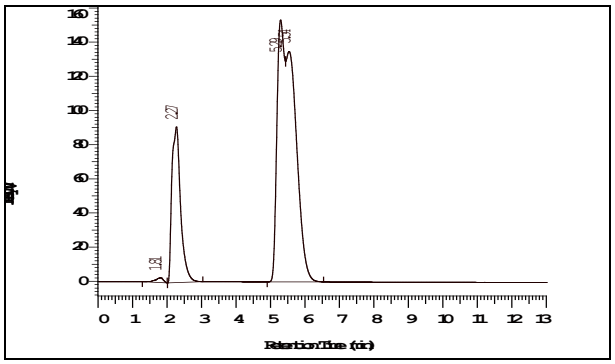


Fig. 4-12. Chromatogram showing degradation for Amlodipine & Rosuvastatin in 0.1 N HCl

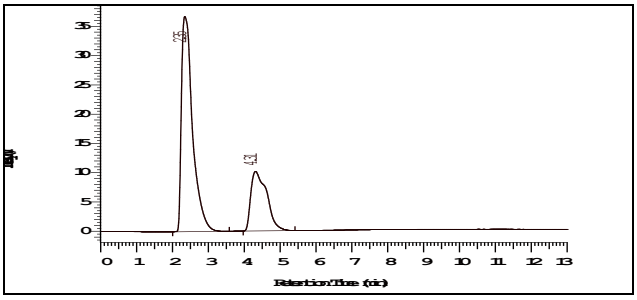


Fig. 4-13. Chromatogram showing degradation study in 0.1 N NaOH

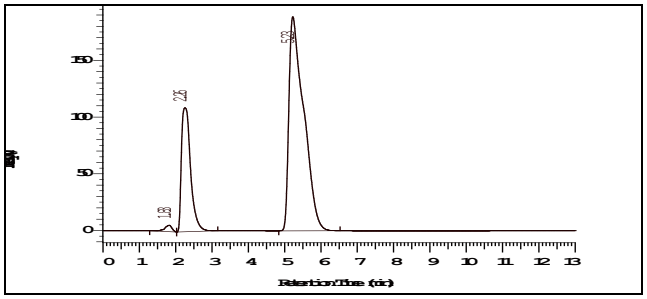


Fig. 4-14. Chromatogram showing thermal degradation studies

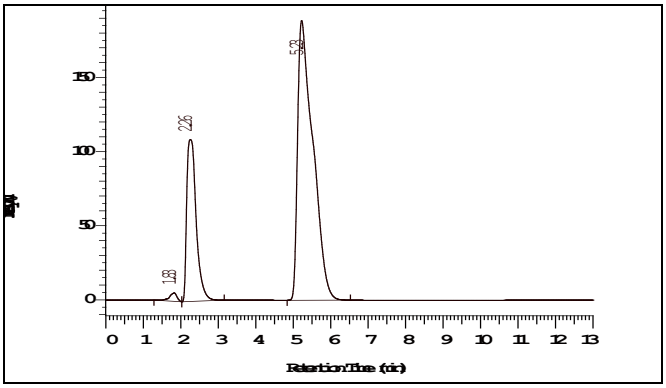


Fig. 4.15 : Chromatogram showing photolytic degradation

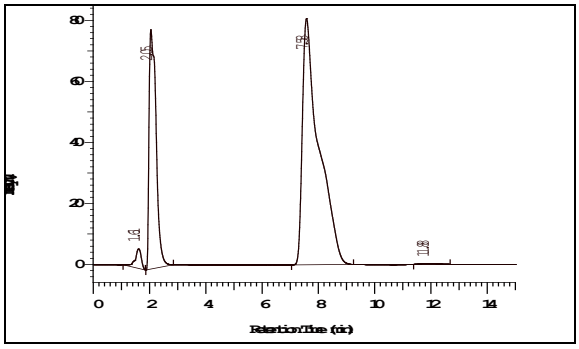


Fig. 4-16 : Chromatogram showing oxidative degradation

Table 4-18: Results of forced degradation studies of Amlodipine API.

Stress condition	**Time**	**Assay of active substance**	**Assay of degraded products**	**Mass Balance (%)**
Acid Hydrolysis (0.1 M HCl)	24Hrs.	73.75	24.61	98.36
Basic Hydrolysis (0.I M NaOH)	24Hrs.	28.57	71.02	99.59
Thermal Degradation (50 ^{0}C)	24Hrs.	97.39	----------	97.39
UV (254nm)	24Hrs.	84.95	14.36	99.31
3 % Hydrogen peroxide	24Hrs.	37.75	61.39	99.42

Table 4-19: Results of forced degradation studies of Rosuvastatin API.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	62.48	35.78	98.26
Basic Hydrolysis (0.I M NaOH)	24Hrs.	04.35	95.12	99.47

Thermal Degradation (50 ^{0}C)	24Hrs.	98.74	----------	98.74
UV (254nm)	24Hrs.	75.19	24.34	99.53
3 % Hydrogen peroxide	24Hrs.	57.14	42.28	99.42

The results of the stress studies indicated the **specificity** of the method that has been developed. Rosuvastatin & Amlodipine were degraded only in 3% H_2O_2 & basic stress conditions. The result of forced degradation studies are given in the following tables.

CONCLUSION:

A sensitive & selective stability indicting 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 described method, it can be concluded that the absence of co-eluting peak along with the main peak of Amlodipine & Rosuvastatinin 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. Even though no attempt has been made to identify the degraded products proposed method can be used as a stability indicating method for assay of Amlodipine & Rosuvastatin commercial formulations.

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