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Development and Validation of Novel Analytical Forced Degradation and Stability Indicating Method for the Simultaneous Estimation of Ramipril and Atorvastatin by RP HPLC

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ABSTRACT

A simple, accurate, precise stability indicating method was developed for the simultaneous estimation of Ramipril and Atorvastatin were in bulk and tablet dosage form. The chromatogram was obtained by using the mobile phase was optimized with consists of methanol: Phosphate buffer (pH-3) mixed in the ratio of 70:30 % v/ v. A Symmetry C_{18} column C18 (4.6 x 150mm, 5µm, Make: Waters) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were analyzed at a constant flow rate of 1 ml/min. the linearity range of Ramipril and Atorvastatin was found to be from 64-320 ppm, and 10-50µg/ml respectively. The linear regression coefficient was not more than 0.999, 0.999. The values of % RSD are less than 2% indicating the accuracy and precision of the method. The percentage recovery varies from 100.8-99.8% of Ramipril and Atorvastatin LOD and LOQ were found to be within the limit. Ramipril and Atorvastatin 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 Ramipril and Atorvastatin in tablet dosage forms.

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INTRODUCTION

Analytical chemistry is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials. Analytical chemistry is divided into two parts. **Qualitative analysis** is the identification of elements, species, and/or compounds present in the sample and **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species, or compounds present in the sample.

"] *Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*".

"Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which is stationary while other moves in a definite direction (IUPAC)".

The mobile phase could be either a liquid or a gas, and accordingly, we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, two other modes use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro-osmotic flow, as in the case of Capillary Electro Chromatography(CEC).

Analytical Method Development: A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally, method development should be as simple as possible and it should allow the use of sophisticated tools such as computer modeling. The important factors, which are to be taken into account to obtain reliable quantitative analysis, are careful sampling and sample preparation, Appropriate choice of the column, Choice of the operating conditions to obtain the adequate resolution of the mixture, Reliable performance of the recording and data handling systems, Suitable integration/peak height measurement technique, The mode of calculation best suited for the purpose, Validation of the developed method.

Careful sampling and sample preparation: Before beginning method development one needs to review what is known about the sample to define the goals of separation. The sample-related information that is important is summarized in the following.

Optimization of HPLC method: During the optimization stage, the initial sets of conditions that have evolved from the first stages of development are improved or maximized in terms of resolution and peak shape, plate counts, asymmetry, capacity factor, elution time, detection limits, the limit of quantitation and overall ability to quantify the specific analyte of interest.

The various parameters that include being optimized during method development are the Selection of the mode of separation, the Selection of stationary phase, the Selection of mobile phase, Selection of the detector.[1-5]

Selection of mode of separation: In reverse phase mode, the mobile phase is comparatively more polar than the stationary phase. For the separation of polar or moderately polar compounds, the most preferred mode is the reverse phase. The nature of the analyte is the primary factor in the selection of the mode of separation. A useful and practical measurement of peak shape is the peak asymmetry factor and peak tailing factor. Peak asymmetry is measured at 10% of full peak height and peak tailing factor at 5%. Reproducibility of retention times and capacity factors is important for developing a rugged and repeatable method.

Buffers and buffer capacity: Buffer and its strength play an important role in deciding the peak symmetries and separations. Some of the most commonly employed buffers are phosphate buffers.

Method Validation: Method validation can be defined as per ICH "Establishing documented evidence which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics".

ICH Method validation parameters: For chromatographic methods used in analytical applications there is more consistency in validation. Related substances are commonly present in pharmaceutical products but those are always within the limits as specified in ICH (Q2B): Linearity, Accuracy, Precision, Limit of Detection, Limit of Quantitation, Robustness, and System suitability.

The literature review reveals that there are fewer analytical methods reported for the analysis of Stability indicating forced degradation method for Ramipril and Atorvastatin by simultaneous estimation by RP-HPLC. There is a need for new analytical method

development for the simultaneous estimation of Ramipril and Atorvastatinin pharmaceutical dosage forms.

The present work is aimed to develop a new, simple, fast, rapid, accurate, efficient, and reproducible RP-HPLC method for the simultaneous analysis of Ramipril and Atorvastatin. The developed method will be validated according to ICH guidelines.

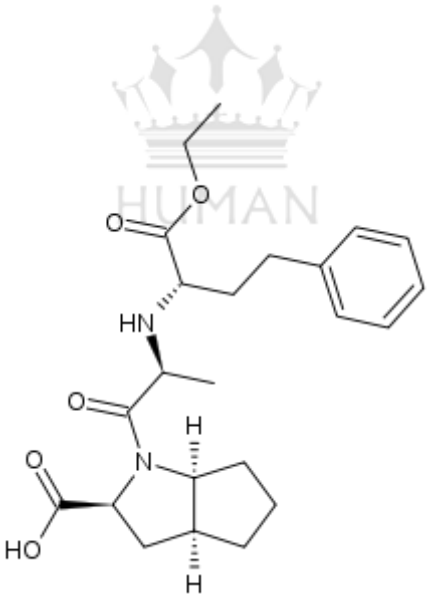
- The analytical method for the simultaneous estimation of Ramipril and Atorvastatin will be developed by the RP-HPLC method by optimizing the chromatographic conditions.

- The developed method is validated according to ICH guidelines for various parameters specified in ICH guidelines, Q2 (R1).

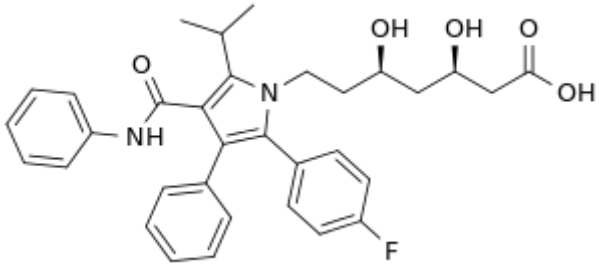
- Development of Stability related forced degradation studies as per the ICH guidelines by using Acid, Base, Photolytic, Thermal, and Peroxide Degradation.[6-10]

MATERIALS AND METHODS

Ramipril Structure



Atorvastatin Structure



Instruments used

SL.No	Instrument	Model
1	HPLC	WATERS, software: Empower 2, 2695 separation module. 996 PDA detector.
2	UV/VIS spectrophotometer	LABINDIA UV
3	pH meter	LabIndia
4	Weighing machine	Sartorius
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Enertech

Chemicals used:

S.No	Chemical	Brand names
1	Ramipril	Sura labs
2	Atorvastatin	Sura labs
4	Water and Methanol for HPLC	LICHROSOLV (MERCK)
5	Acetonitrile for HPLC	Merck
6.	Anhydrous dihydrogen phosphate	Finar chemicals
7.	Triethylamine Buffer	Finar chemicals
8.	Citric Acid	Finar chemicals

HPLC Method development:

Mobile Phase Optimization: Initially the mobile phase tried was TEA buffer Methanol and TEA buffer: ACN with varying proportions. Finally, the mobile phase was optimized to phosphate buffer (pH 3), Methanol in proportion 30:70 v/v respectively.

Optimization of Column: The method was performed with various columns like the C18 column, X- bridge column, Xterra, and C8 column. Symmetry C18 (4.6 x 150mm, 5µm, Make: Waters) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS:

The instrument used: Waters HPLC with an auto sampler and PDA detector 996 model.

Temperature : Ambient

Column : Symmetry C18 (4.6 x 150mm, 5µm, Make: Waters) or equivalent

Buffer : Phosphate buffer(pH-3)-Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000mL. Adjust the pH 3 by using ortho phosphoric acid.

pH : 3

Mobile phase: Phosphate buffer (pH 3), Methanol in proportion 30:70 v/v respectively.

Flow rate : 1 ml per min

Wavelength : 260 nm

Injection volume : 10 µl

Run time : 10 min.

Optimized chromatogram, blank, and System suitability parameters is shown in the figure and the results are shown in Table. [11-15]

Preparation of buffer and mobile phase:

Preparation of Phosphate buffer (pH-3): Dissolve 0.9g of anhydrous dihydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000mL.Adjust the p H 3 by using orthophosphoric acid.

Preparation of mobile phase: Accurately measured 700 ml (70%) of HPLC Methanol and 300 ml of Phosphate buffer (30%) were mixed and degassed in a digital ultrasonication for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation: The Mobile phase was used as the diluent.

Validation parameters:

Method Precision:

Preparation of Standard Solution: Accurately weigh and transfer 10 mg of Ramipril and 10mg of Atorvastatin working standard into a 10 mL and 10 ml of clean dry volumetric flasks add about 10mL and 10 ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3 ml and 0.192 ml of the above Ramipril, and Atorvastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation of Sample Solution: Accurately weigh 10 combination tablets crush them in a mortar and pestle and transfer equivalent to 10 mg of ramipril, sample into a 10mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.192 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

The standard and sample solutions of 192 µg/ml of Atorvastatin, and 30 µg/ml of Ramipril were injected five times and the peak areas were recorded.

The mean and percentage relative standard deviation were calculated from the peak areas.

Intermediate Precision/Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on a different day by using different make columns of the same dimensions.

Preparation of stock solution: Accurately weigh 10 combination tablets crush them in a mortar and pestle and transfer equivalent to 10 mg of ramipril, Atorvastatin sample into a 10mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.192 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

The standard and sample solutions containing concentrations were 192 µg/ml of Atorvastatin and 30 µg/ml of Ramipril.

Procedure: The standard solution was injected five times and measured the area for all five injections in HPLC was. The %RSD for the area of five replicate injections was found to be within the specified limits.

Accuracy:

Preparation of Standard stock solution: Accurately weigh and transfer 10 mg of Ramipril and 10mg of Atorvastatin working standard into a 10 mL and 10 ml of clean dry volumetric flasks add about 7mL and 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.196 ml and 0.3 ml of the above Ramipril, Atorvastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration): Accurately weigh 10 combination tablets crush in mortar and pestle and transfer equivalent to 5 mg of Ramipril, Atorvastatin sample into a 10mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.196 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

The standard and sample solutions of containing concentrations were 50%.

For preparation of 100% solution (With respect to target Assay concentration):
Accurately weigh 10 combination tablets crush in mortar and pestle and transfer equivalent to 10 mg of Ramipril, Atorvastatin sample into a 10mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.192 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

The standard and sample solutions of containing concentrations were 100%.

For preparation of 150% solution (With respect to target Assay concentration): Accurately weigh 10 combination tablets crush in mortar and pestle and transfer equivalent to 15 mg of Ramipril , Atorvastatin (marketed formulation-dose of Atorvastatin medoxomil is 80 mg, Dose of Ramipril is 12.5 mg in combination tablet) sample into a 10mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.192 ml of above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

The standard and sample solutions of containing concentrations were 150%.

Procedure: Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions.

Calculate the Amount found and Amount added for Ramipril and Atorvastatin and calculate the individual recovery and mean recovery values. These solutions were filtered through a 0.45µ membrane and then each concentration; three replicate injections were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. [16-20]

Linearity:

Preparation of stock solution: Accurately weigh 10 combination tablets crush them in mortar and pestle and transfer equivalent to 10 mg of ramipril, Atorvastatin sample into a 10mL clean dry volumetric flask add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock I solution 1000 ppm)

Further dilutions from the above stock I solution take 1ml in 10ml of volumetric flask and add diluent and make up to the mark with diluents (Stock II solution 100ppm).

Preparation of Level – I (10 ppm of Ramipril & 64ppm of Atorvastatin): 6.4 ml of stock II solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (20 ppm of Ramipril & 128ppm of Atorvastatin): 1.28 ml of stock I solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (30 ppm of Ramipril & 192ppm of Atorvastatin): 1.92ml of stock I solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (40 ppm of Ramipril & 256ppm of Atorvastatin): 2.56 ml of stock I solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (50 ppm of Ramipril & 320ppm of Atorvastatin): 3.20 ml of stock I solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Limit of detection:

Preparation of 10 µg/ml solution: Accurately weigh and transfer 10 mg equivalent weight of Ramipril and Atorvastatin combination tablet powder into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution-1000 ppm)

Further pipette 0.1 ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. (10 ppm)

Preparation of 0.003 µg/ml solution): Further pipette 0.003ml of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Limit of quantification:

Preparation of 10 µg/ml solution: Accurately weigh and transfer 10 mg of equivalent weight of Ramipril and Atorvastatin combination tablet powder working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1 ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of 0.01 µg/ml solution): Further pipette 0.01ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

ROBUSTNESS: The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation in results.

Preparation of sample solution (30µg/ml of Ramipril & 192 µg/ml of Atorvastatin): Accurately weigh and transfer 10 mg of Ramipril and 10mg of Atorvastatin working standard into a 10 mL and 10 ml of clean dry volumetric flasks add about 7mL and 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 0.3 ml and 0.192 ml of the above Ramipril, and Atorvastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Effect of Variation of flow: The sample was analyzed at 0.8 ml/min and 1.0 ml/min instead of 0.9 ml/min, the remaining conditions are the same. 10µl of the above sample was injected twice and chromatograms were recorded.

Effect of Variation of mobile phase organic composition: The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate buffer pH3 was taken in the ratio and 60:40, 80:20 instead 70:30, remaining conditions are same. 10µl of the above sample was injected twice and chromatograms were recorded.[21-23]

RESULTS AND DISCUSSION

Optimized Method:

Mobile phase : Methanol: pH 3Phosphate Buffer (70:30%v/v)

Column : symmetry C18 5µm (4.6*150mm) 5 µ

Flow rate : 1 ml/min

Wavelength : 260 nm

Column temp : Ambient

Sample Temp : Ambient

Injection Volume : 10 µl

Run time : 10minutes

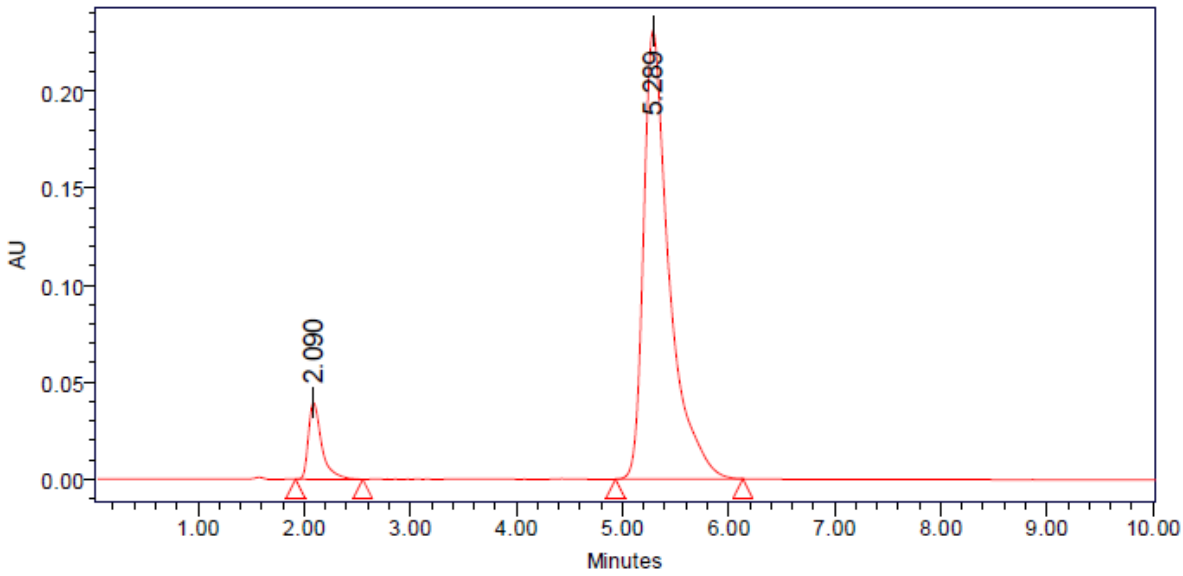


Figure: chromatogram for trial 5

Table: - peak results for trial 5

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Ramipril	2.090	342126	39690		1.70	2587
2	Atorvastatin	5.289	3864998	231194	9.80	1.77	2698

Observation:

From the above chromatogram, it was observed that the Ramipril and Atorvastatin peaks are well separated.

Retention time of Ramipril – 2.090 min

The retention time of Atorvastatin – 5.289 min

System suitability:

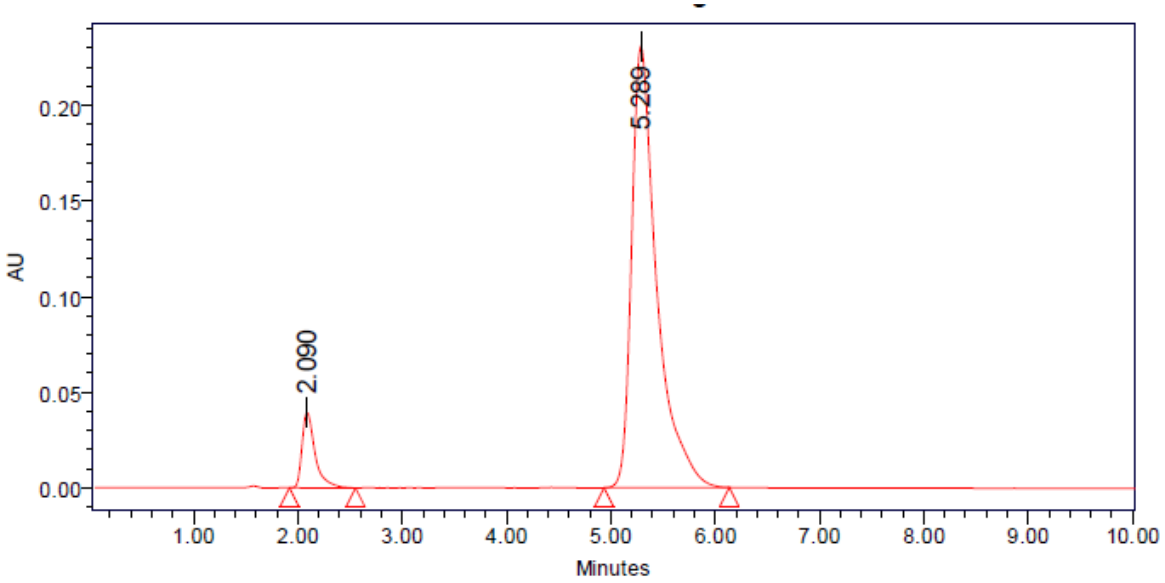


Figure: chromatogram for system suitability

Table: Results of system suitability parameters for Ramipril and Atorvastatin

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	Ramipril	2.090	342126	39690		1.70	2587
2	Atorvastatin	5.289	3864998	231194	9.80	1.77	2628

Acceptance criteria:

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- The tailing factor must be not less than 0.9 and not more than 2.
- It was found from the above data that all the system suitability parameters for the developed method were within the limit.

Validation results:

Assay (Standard):

Table: Showing assay standard results

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ramipril	2.090	342126	39690		1.70	2587	1
2	Atorvastatin	5.289	3864998	231194	9.80	1.77	2628	1
3	Ramipril	2.089	342564	39990		1.66	2571	2
4	Atorvastatin	5.338	3881443	231044	9.93	1.83	2688	2
5	Ramipril	2.089	347976	40396		1.68	2530	3
6	Atorvastatin	5.327	3896952	231969	9.91	1.86	2712	3

Assay (Sample):

Fig: Chromatogram showing assay of sample injection -3

Table: Showing assay sample results

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ramipril	2.088	352290	40269		1.69	2516	1
2	Atorvastatin	5.276	3883794	231354	9.75	1.89	2677	1
3	Ramipril	2.087	356547	41157		1.72	2557	2
4	Atorvastatin	5.268	3896493	234961	9.82	1.91	2804	2
5	Ramipril	2.085	358914	40963		1.75	2489	3
6	Atorvastatin	5.262	3900103	233541	9.78	1.95	2790	3

Table: Showing assay results

S.No	Name of compound	Label claim	Amount taken(from combination tablet)	%purity
1	Ramipril	12.5mg	12.6	100.8%
2	Atorvastatin	80mg	79.9	99.8%

The retention time of Ramipril and Atorvastatin was found to be 2.090mins and 5.289 mins respectively. The % purity of Ramipril and Atorvastatin in pharmaceutical dosage form was found to be 100.8% and 99.8% respectively.

Precision:

Precision of the method was carried out for both sample and standard solutions as described under experimental work. The corresponding chromatograms and results are shown below.

Table: Results of method precession for Ramipril:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ramipril	2.086	362266	41697	3081.3	1.8
2	Ramipril	2.083	364902	41402	3144.1	1.8
3	Ramipril	2.083	366870	41540	3118.1	1.8
4	Ramipril	2.081	367273	42256	3147.3	1.8
5	Ramipril	2.081	368101	42143	3101.8	1.8
Mean			365882.4		3118.5	1.8
Std. Dev			2338.4			
% RSD			0.6			

Table: Results of method precession for Atorvastatin:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atorvastatin	5.178	3903548	240181	2988.3	2.0	9.8
2	Atorvastatin	5.199	3905819	235523	2856.3	2.0	9.7
3	Atorvastatin	5.235	3916120	238578	2930.2	2.0	9.9
4	Atorvastatin	5.202	3916542	238814	2936.9	2.0	9.8
5	Atorvastatin	5.206	3920943	241006	3040.0	2.0	10.0
Mean			3912594.4				9.9
Std. Dev			7507.6				
% RSD			0.2				

Acceptance criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision/Ruggedness :(Day1, Analyst1)

There was no significant change in assay content and system suitability parameters at different conditions of ruggedness like day to day and system to system variation.

Day1,

Table: Results of Intermediate precision for Ramipril:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ramipril	2.089	369246	42277	1537.8	1.6
2	Ramipril	2.080	370766	42708	1561.8	1.6
3	Ramipril	2.083	370840	42065	1489.3	1.6
Mean			370655.8			
Std. Dev			823.7			
% RSD			0.02			

Table: Results of Intermediate precision for Atorvastatin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atorvastatin	5.112	3743003	242955	3269.7	2.2	10.2
2	Atorvastatin	5.133	3845359	242255	3100.5	2.1	10.0
3	Atorvastatin	5.151	3885014	242854	3127.6	2.1	10.0
Mean			3864935				
Std. Dev			75905.4				
% RSD			1.2				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is rugged.

Day2, Analyst2:

Table: Results of Intermediate precision for Ramipril:

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Ramipril	2.082	371041	42568	3583.2	1.8
2	Ramipril	2.083	371386	42211	3533.2	1.8
3	Ramipril	2.078	370979	42978	3083.0	1.9
Mean			370655.8			
Std. Dev			823.7			
% RSD			0.2			

Table: Results of Intermediate precision for Atorvastatin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Atorvastatin	5.203	3922513	240346	3048.8	1.5	9.9
2	Atorvastatin	5.229	3928789	237638	2997.2	1.6	9.9
3	Atorvastatin	5.077	3841404	246818	3208.0	2.1	10.1
Mean			3542935		3728.8	1.6	
Std. Dev			65905				
% RSD			1.1				

Acceptance criteria: The % RSD of five different sample solutions should not more than 2 and the %RSD obtained is within the limit, hence the method is rugged.

Accuracy: Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Accuracy standard:

Table: Results of Accuracy standard values:

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ramipril	2.090	342126	39690		1.42	3923	1
2	Atorvastatin	5.289	3864998	231194	9.80	1.46	3149	1
3	Ramipril	2.089	342564	39990		1.46	3946	2
4	Atorvastatin	5.338	3881443	231044	9.93	1.43	3348	2
5	Ramipril	2.089	347976	40396		1.46	3946	3
6	Atorvastatin	5.327	3896952	231969	9.91	1.43	3348	3

Table accuracy (recovery) data for Ramipril

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	465654	5	4.9	98%	99.8%
100%	342564	10	10.1	101%	
150%	784620	15	15.1	100.6%	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102.0%.

Table accuracy (recovery) data for Atorvastatin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	7014143	5	4.9	98%	99.7%
100%	3881443	10	10.2	102%	
150%	9912197	15	14.9	99.3%	

Acceptance Criteria: The percentage recovery was found to be within the limit (97-103%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Accuracy 50%:

TABLE: Results of Accuracy sample 50% values:

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ramipril	2.072	465654	51853		1.91	3645	1
2	Atorvastatin	5.059	7049165	433555	9.75	2.15	2850	1
3	Ramipril	2.071	465192	51398		1.91	3685	2
4	Atorvastatin	5.017	7014143	434561	9.60	2.17	2844	2
5	Ramipril	2.072	465494	51358		1.95	3629	3
6	Atorvastatin	5.016	7018949	438533	9.67	2.19	2847	3

Accuracy 100%:

Table: Results of Accuracy sample 100% values:

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ramipril	2.090	342126	39690		1.70	3637	1
2	Atorvastatin	5.289	3864998	231194	9.80	1.77	2860	1
3	Ramipril	2.089	342564	39990		1.66	3643	2
4	Atorvastatin	5.338	3881443	231044	9.93	1.83	2833	2
5	Ramipril	2.089	347976	40396		1.68	3627	3
6	Atorvastatin	5.327	3896952	231969	9.81	1.86	2852	3

Accuracy 150%:

Table: Results of Accuracy sample 150% values:

Sno	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Ramipril	2.060	785648	78630		1.87	3623	1
2	Atorvastatin	4.991	9914398	583245	8.99	2.09	2678	1
3	Ramipril	2.063	784620	78074		1.86	3611	2
4	Atorvastatin	5.001	9912197	585314	9.05	2.09	2705	2
5	Ramipril	2.058	787406	78616		1.89	3631	3
6	Atorvastatin	5.017	9949401	584628	9.13	2.09	2694	3

Linearity:

The linearity range was found to lie from 10-50ppm of Ramipril, 64-320ppm 0f Atorvastatin and chromatograms are shown below.

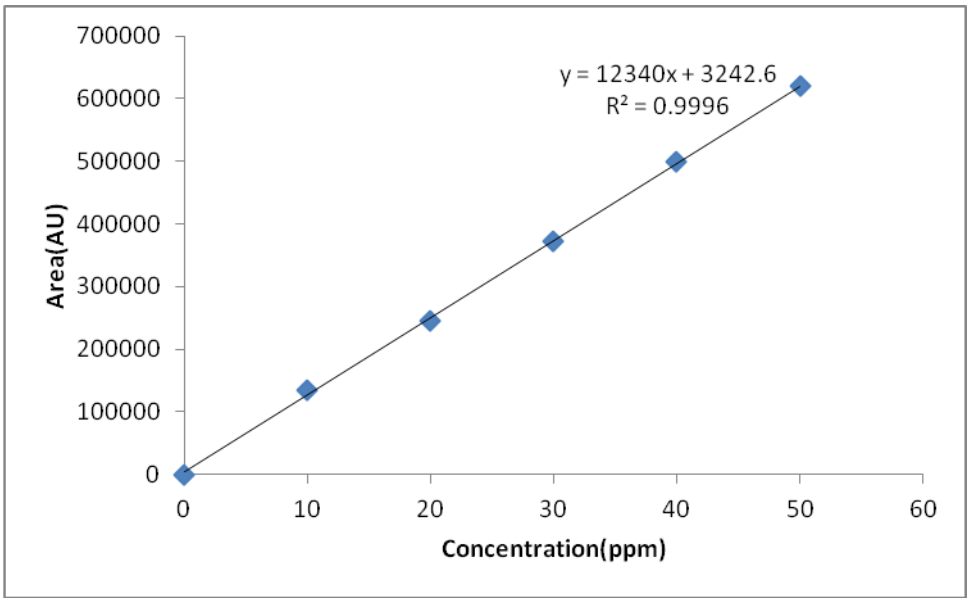


Figure calibration graph for Ramipril

Linearity Results: (for Ramipril HCl)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	10	134436
2	II	20	245571
3	III	30	371548
4	IV	40	499024
5	V	50	619830
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.99.

Linearity Results: (for Atorvastatin)

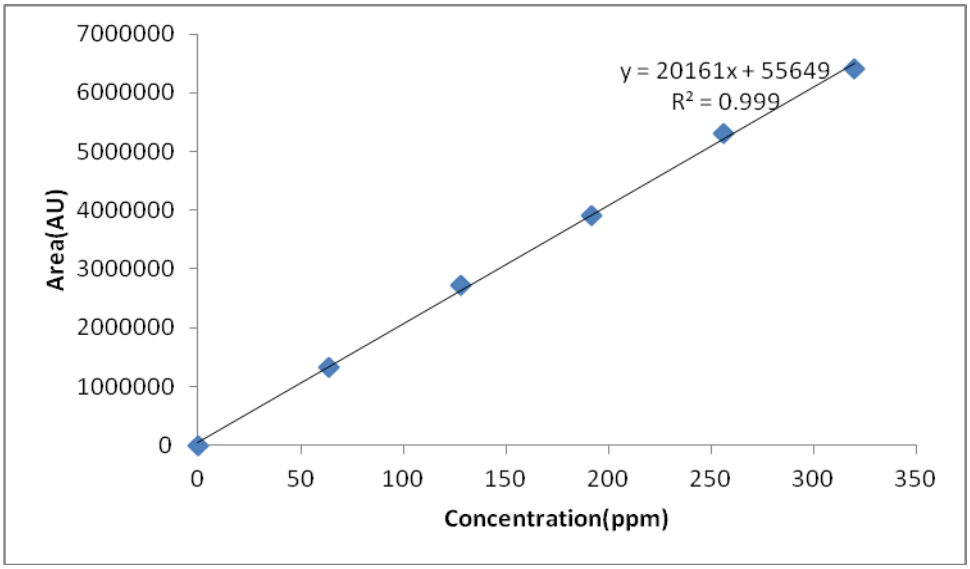


Figure calibration graph for Atorvastatin

Linearity Results: (Atorvastatin)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	64	1330054
2	II	128	2728974
3	III	192	3917063
4	IV	256	5300022
5	V	320	6412695
Correlation Coefficient			0.999

Acceptance Criteria: Correlation coefficient should be not less than 0.99.

Table- Analytical performance parameters of Ramipril and Atorvastatin

Parameters	Ramipril	Atorvastatin
Slope (m)	12340	3242
Intercept (c)	55649	55649
Correlation coefficient (R^2)	0.999	0.999

Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999.

Limit of detection for Ramipril and Atorvastatin

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

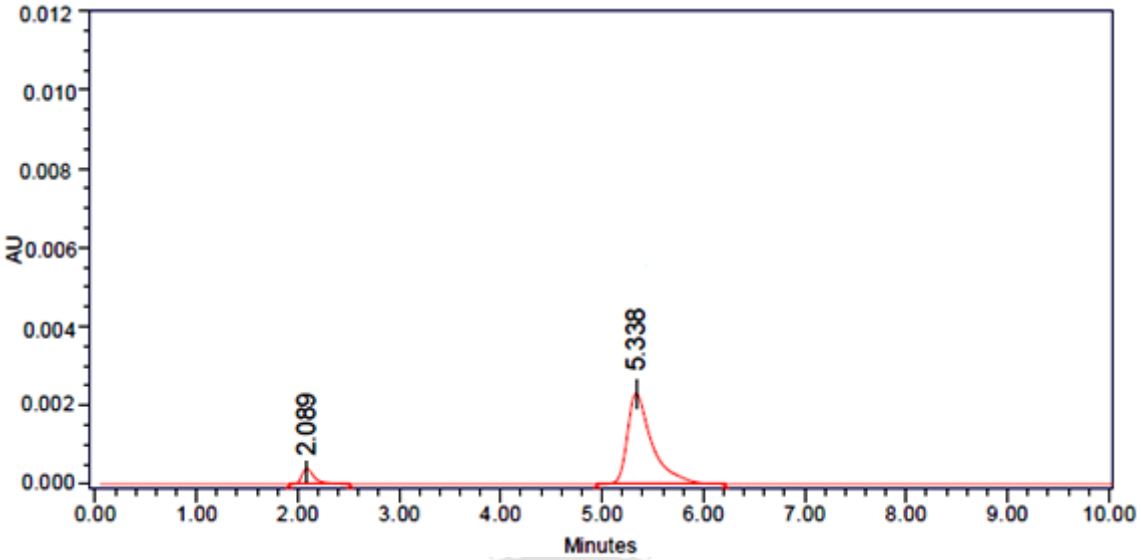


Figure: chromatogram of RAMIPRIL AND ATORVASTATIN

Showing LOD

Table Results of LOD

Drug name	**Baseline noise(µV)**	**Signal obtained (µV)**	**S/N ratio**
Ramipril	52	159	3.05
Atorvastatin	51	153	3.01

Signal to noise ratio shall be 3 for LOD solution **and** the result obtained is within the limit.

Limit of quantification for Ramipril and Atorvastatin

The lowest concentration of the sample was prepared with respect to the base line noise and measured the signal to noise ratio.

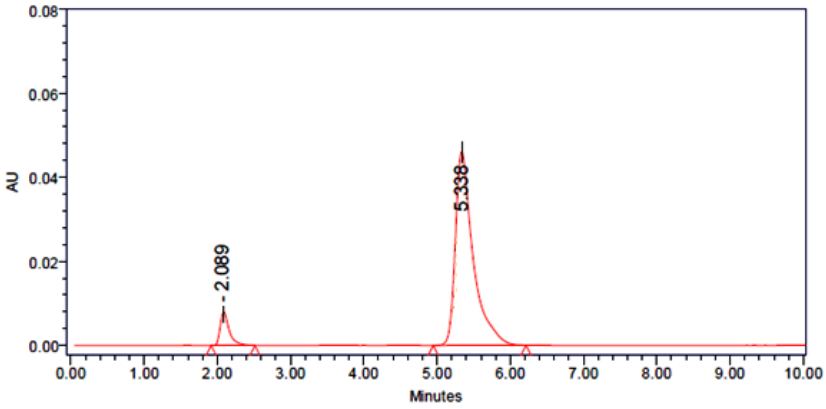


Figure no 4.3.6 chromatogram of Ramipril, Atorvastatin showing LOQ

Table: Results of LOQ

Drug name	**Baseline noise(µV)**	**Signal obtained (µV)**	**S/N ratio**
Ramipril	39.347	410	10.42
Atorvastatin	41.26	411	9.96

Signal to noise ratio shall be 10 for LOQ solution **and** The result obtained is within the limit.

Robustness:

The standard and samples of Ramipril and Atorvastatin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Variation in flow

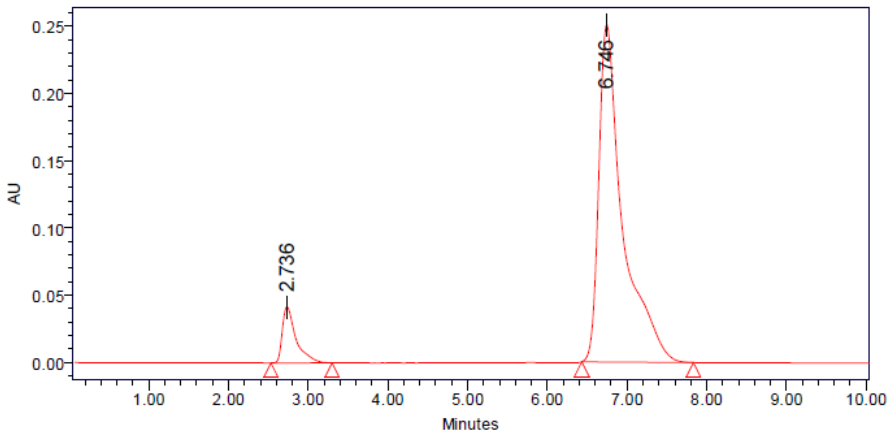


Figure: chromatogram showing less flow of 0.9ml/min

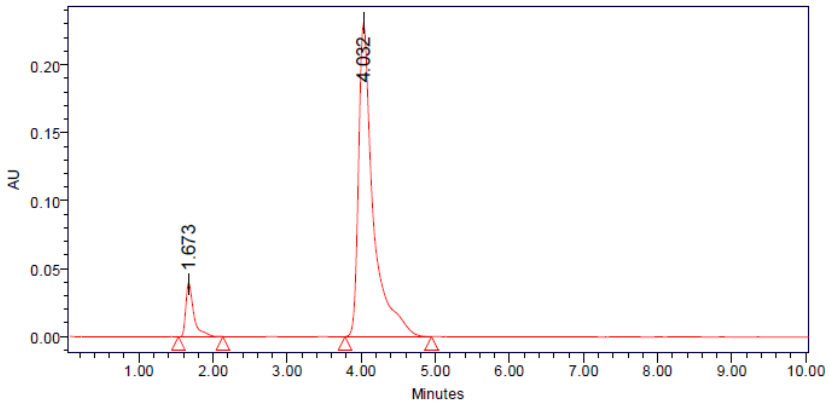


Figure: chromatogram showing more flow of 1.1ml/min

System suitability results for Ramipril:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3696	1.82
2	1.0	3923	1.42
3	1.1	3032	1.91

***** Results for actual flow (1.0 ml/min) have been considered from Assay standard.

System suitability results for Atorvastatin:

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	3108	1.88
2	1.0	3149	1.46
3	1.1	3032	1.91

***** Results for actual flow (1.0ml/min) have been considered from Assay standard.

Variation of mobile phase organic composition:

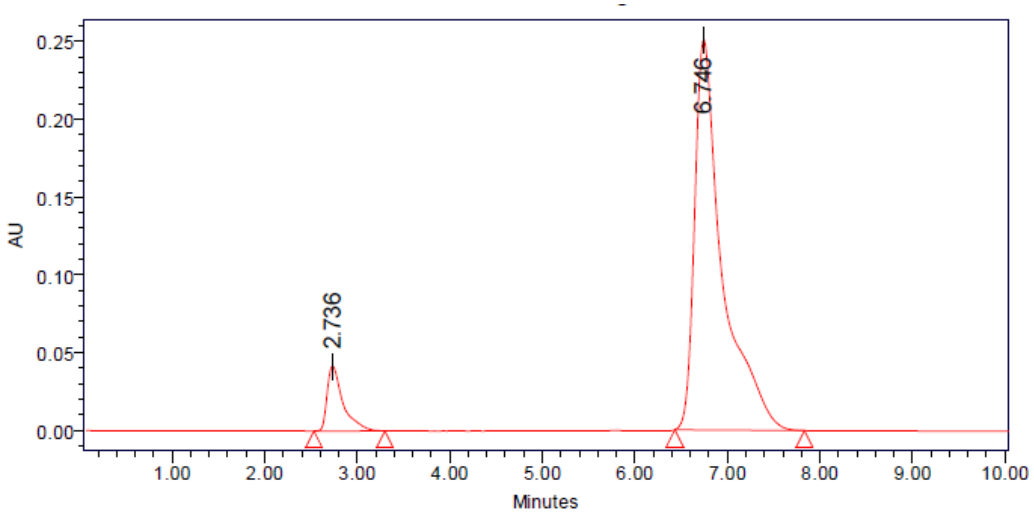


Figure chromatogram showing less organic composition

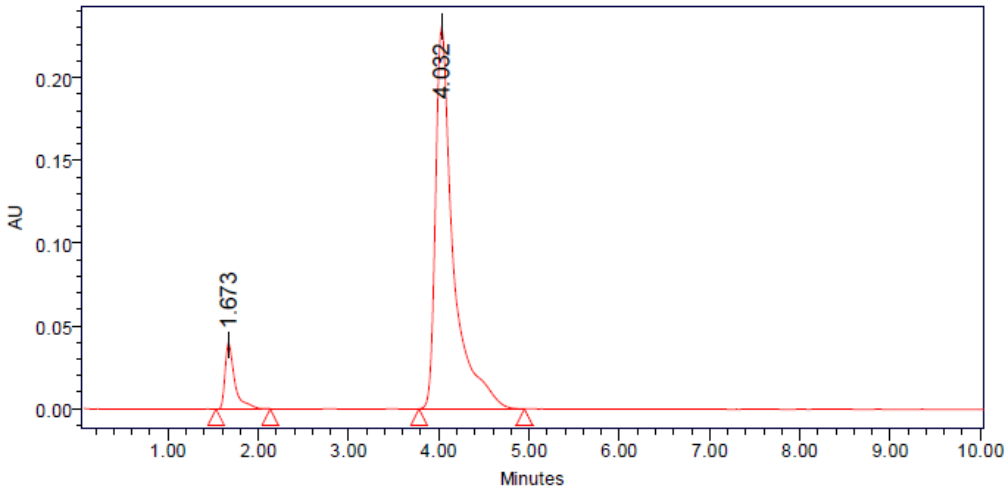


Figure chromatogram showing more organic composition

System suitability results for Ramipril

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3706	1.75
2	*Actual	3923	1.42
3	10% more	3627	1.86

System suitability results for Atorvastatin:

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	3309	1.86
2	*Actual	3149	1.46
3	10% more	3220	1.93

***** Results for actual mobile phase have been considered from Assay standard.

Table: Results of stability studies of RamiprilAPI

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	87.12	12.7	99.82
Basic Hydrolysis (0.I M NaOH)	24Hrs.	72.18	26.01	98.19
Thermal Degradation (50 ^{0}C)	24Hrs.	99.16	----------	99.16
UV (254nm)	24Hrs.	98.97	----------	98.97
3 % Hydrogen peroxide	24Hrs.	73.22	27.36	100.58

Table: Results of stability studies of AtorvastatinAPI

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Acid Hydrolysis (0.1 M HCl)	24Hrs.	68.33	32.45	100.78
Basic Hydrolysis (0.I M NaOH)	24Hrs.	85.63	14.17	99.8
Thermal Degradation (50 ^{0}C)	24Hrs.	97.95	----------	97.95
UV (254nm)	24Hrs.	99.24	----------	99.24
3 % Hydrogen peroxide	24Hrs.	83.72	16.15	99.87

The results of the stress studies indicated the **specificity** of the method that has been developed. **Ramipril** & **Atorvastatin** were almost stable in all stress conditions & areas reduced in acid, 3% H_2O_2& basic stress conditions. We did not find any impurity peaks related to forced degradation or stability studies.

CONCLUSION

High-performance liquid chromatography is at present one of the most sophisticated tools of the analysis. The estimation of Ramipril and Atorvastatin was done by RP-HPLC. The Phosphate buffer was p^H3 and the mobile phase was optimized with consists of methanol: Phosphate buffer(pH-3) mixed in the ratio of 70:30 % v/ v. A Symmetry C_{18} column C18 (4.6 x 150mm, 5µm, Make: Waters) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1 ml/min. the linearity range of Ramipril and Atorvastatin was found to be from 64-320 ppm, and 10-50µg/ml respectively. The linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating the accuracy and precision of the method. The percentage recovery varies from 100.8-99.8% of Ramipril and Atorvastatin LOD and LOQ were found to be within the limit.

Even though no attempt has been made to identify the degraded products proposed method can be used as a stability-indicating method for the assay of Ramipril and Atorvastatin commercial formulations.

The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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