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Simultaneous RP-HPLC estimation and validation of ramipril and atorvastatin in bulk and combined tablet formulation

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ABSTRACT

A simple, precise, accurate and reproducible RP-HPLC method was developed and validated for simultaneous estimation of ramipril and atorvastatin in bulk and combined pharmaceutical formulations. Separation of ramipril and atorvastatin was successfully achieved on an Inertsil ODS C18 column (150mm x 4.6mm x 5 µm). The mobile phase consisted of 0.1% ortho phosphoric acid and methanol (60:40, v/v) at a flow rate of 1.2 ml/min. The detection was performed at 245 nm. The method was validated according to ICH guidelines for linearity, sensitivity, accuracy, precision and robustness. The response was found to be linear in the concentration range of 2.5 µg/ml to 7.5 µg/ml for ramipril and 10 µg/ml to 30 µg/ml foratorvastatin. The LOD and LOQ for ramipril were found to be 0.009 µg/ml and 0.029 µg/ml, respectively. The LOD and LOQ for atorvastatin were 0.0269µg/ml and 0.0898 µg/ml, respectively. The percentage recovery for ramipril and atorvastatin were found to be 100% and 101%, respectively. The excellent percentage recovery values indicate the high accuracy of the proposed method. The method specifically determines the analytes in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for linearity, range, accuracy, precision, specificity and robustness.

Key words: Ramipril, atorvastatin, analysis, RP-HPLC, combined dosage form

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INTRODUCTION

Ramipril [1,2] is a 2-aza-bicyclo [3.3.0]-octane-3-carboxylic acid derivative belonging to the angiotensin-converting enzyme (ACE) inhibitor class of drugs. Chemically, ramipril is known as (2*S*,3*aS*,6*aS*)-1[(*S*)-N-[(*S*)-1-carboxy-3-phenylpropyl] alanyl] octahydrocyclopenta [*b*]pyrrole-2-carboxylic acid, 1-ethyl ester. The chemical structure is given in Figure 1. The prodrug, ramipril, is metabolized in the liver to ramiprilat. Ramiprilat is a potent competitive inhibitor of ACE. The angiotensin-converting enzyme is responsible for the formation of angiotensin II from angiotensin I. Angiotensin II involves in the regulation of blood pressure. Ramipril act by lowering the production of angiotensin II. Ramipril may be used to treat high blood pressure (hypertension) or congestive heart failure and to get better survival after a heart attack.

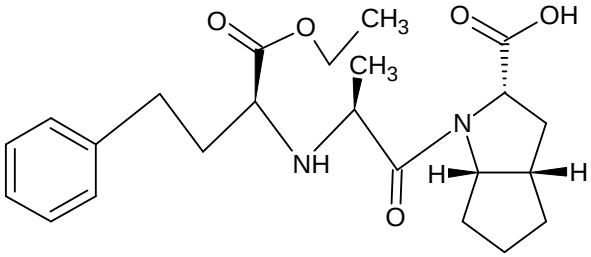

Figure 1: Chemical structure of ramipril

In the literature kinetic spectrophotometry [3], visible spectrophotometry [4,5,6], spectroflourimetry [5], atomic absorption spectrophotometry [6], liquid chromatography-mass spectrometry [7] and liquid chromatography with UV detection [8] techniques were reported for the quantification of ramipril alone in biological samples and pharmaceutical dosage forms.

Atorvastatin [9,10] is a member of the drug class known as statins. Chemically atorvastatin is called as [R-(R*, R*)]-2-(4-fluoro phenyl)-β, δ-dihydroxy-5-(1-methyl ethyl)-3-phenyl-4-[(phenyl amino) carbonyl]-1Hpyrrole-1-heptanoic acid and its chemical structure is given in Figure 2. Atorvastatin acts as an anticholesteremic agent or hydroxymethylglutaryl-CoA reductase inhibitor and is used for lowering plasma cholesterol. Atorvastatin is a competitive inhibitor of hydroxymethylglutaryl-coenzyme-A reductase, that catalyzes the conversion of HMG-CoA to mevalonate, an important step in the synthesis of cholesterol in liver. Decreased hepatic cholesterol levels increases hepatic uptake of cholesterol and reduces plasma cholesterol levels. The chief use of atorvastatin is in the treatment of dyslipidemia and the prevention of cardiovascular disease.

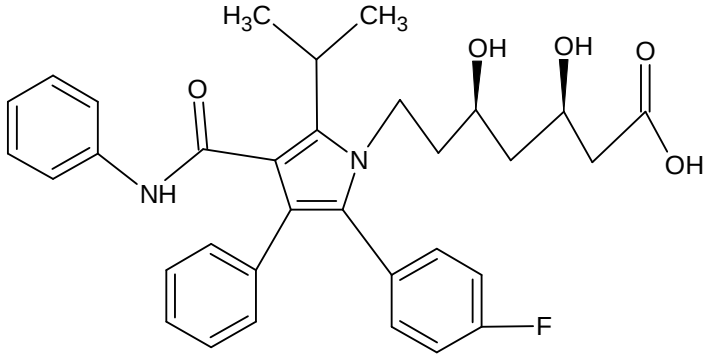

Figure 2: Chemical structure of atorvastatin

Literature survey reveals several methods for determination of atorvastatin individually in biological fluids and formulations like spectrophotometry [11], HPLC with UV detection [12,13], HPLC with fluorescence detection [14], TLC-densitometry [15], LC-MS/MS [16] and capillary electrophoresis [17] and microchip electrophoresis [17].

Atorvastatin and ramipril as a fixed dose combination are used in the treatment of patients with both essential hypertension and hypercholesterolemia. Spectroscopy [18], HPLC [18,19] and HPTLC [19] methods were reported in the literature for determination of atorvastatin and ramipril in combination. This paper describes a simple, rapid, accurate, reproducible and robust method for simultaneous determination of atorvastatin and ramipril in tablet formulation using RP-HPLC method.

EXPERIMENTAL SECTION

Apparatus:

During the method development and validation, Waters Alliance HPLC system equipped with 1525 separation modules having 2487 ultraviolet detector was used. Data acquisition, analysis and processing were done using

Empower II software. The analytical column used for the separation was 150 mm × 4.6 mm I.D., 5 μm particle size, Inertsil ODS C18 column.

Chemicals
All chemicals and solvents used were of analytical and HPLC grade quality, respectively. HPLC grade water was obtained from Milli Q water. Methanol of HPLC grade was purchased from Merck (India) and ortho phosphoric acid of analytical grade was obtained from Sd Fine Chemicals Ltd., Mumbai.

Mobile Phase
The mobile phase consisted of 0.1% ortho phosphoric acid and methanol (60:40, *v/v*).

Stock standard solution:
Accurately weighed quantity, 2.5 mg of ramipril and 10 mg of atorvastatin was transferred into 100 ml of volumetric flask and add 20 ml of mobile phase and sonicate for 15 min. Make up the volume with mobile phase.

Working standard solutions for calibration curve:
From the stock solutions, aliquot volumes containing ramipril and atorvastatin were quantitatively transferred into a series of 25 ml volumetric flasks, so that the final concentration were in the range of 2.5–7.5 μg/ml (i.e., 2.5, 3.75, 5.0, 6.25 and 7.5 μg/ml) for ramipril and 10–30 μg/ml (i.e., 10, 15, 20, 25 and 30 μg/ml) for atorvastatin. The solutions were completed to the volume with the mobile phase.

Preparation of sample solution:
Tablets containing ramipril and atorvastatin were weighed and their average weight was determined. The tablets were finely powdered into homogenous mixture. An accurately weighed 102.50 mg of tablet powder was transferred into a conical flask containing 30 ml of mobile and sonicated for 10 min with continuous shaking. The resulting solution was filtered through a 0.45 μm membrane filter into 100 ml volumetric flask and completed to volume with same solvent. This solution was appropriately diluted with the same solvent for the analysis by the proposed method.

Chromatographic conditions:
In the present study 0.1% ortho phosphoric acid and methanol in the ratio of 60:40 *v/v* is used as mobile phase. Before use the mobile phase was filtered through a 0.45 μ membrane filter and degassed for about 15 min. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.2 ml/min. the injection volume was 10 μl and the column temperature was maintained at 30 ± 1^0C.The eluents were monitored at 245 nm.

Analysis of mixture of ramipril and atorvastatin:
Ten μl aliquot of each solution prepared in the section "working standard solutions for calibration curve" was injected automatically on to the column. The chromatograms were recorded. The calibration curve was constructed by plotting the peak area against the final concentration of the drug (μg/ml). The corresponding regression equations were derived.

RESULTS AND DISCUSSION

Development of the method:
In the present study Inertsil ODS C18 analytical column (150mm × 4.6 mm I.D., 5μ particle size) was selected, because it produced good separation of the selected drugs, well shaped symmetrical peaks with high resolution. In order to achieve satisfactory peak symmetry and separation with good resolution, various combinations of methanol, orthophoshoric acid and phosphate buffer were tried systematically. Preliminary experiments indicated that use of combination of phosphate buffer with methanol was not able to separate the peaks of ramipril and atorvastatin. Then, the combination of 0.1% orthophosphoric acid and methanol in different ratios were tried. Finally, a mobile phase consisting of 0.1% ortho phosphoric acid and methanol in the ratio of 60:40 *v/v* was selected to achieve better resolution and acceptable peak symmetry. Mobile phase flow rate of 1.2 ml/min was observed to be adequate to get both the drugs eluted within less than 5 min. The column temperature was set at 30 ± 1^0C. Under the optimized chromatographic conditions, the retention times for ramipril and atorvastatin were 2.422 min and 4.340 min, respectively. No interference was found among the two peaks. A typical chromatogram is shown in Figure 3.

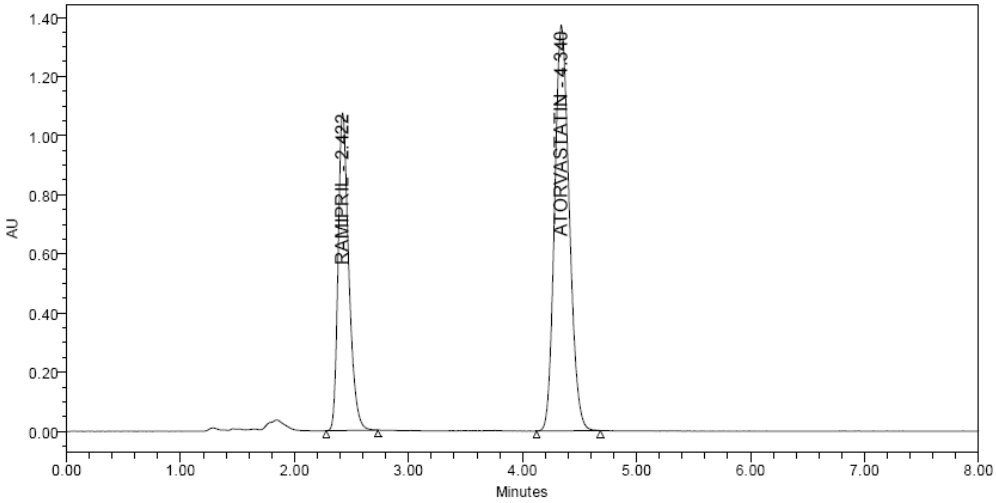

Figure 3: Chromatogram of ramipril and atorvastatin

System suitability:

In order to assess the system suitability, five replicate injections of standard solutions of ramipril and atorvastatin were injected into the HPLC system. The suitability parameters like relative standard deviation of retention time, peak area, USP plate count and USP tailing were calculated. Results are shown in Table 1. The values are within the acceptable range and are enough for the analysis to be done.

Table 1: System suitability parameters

Parameters	Ramipril	Atorvastatin
Retention time (min)	2.416	4.339
Peak area	7300934	12254080
USP Plate Count	3365	5349
USP Tailing	1.33	1.13

Linearity:

A linear relationship was established by plotting the peak area against the drug concentration. The relationship was found to be linear over the range 2.5–7.5 µg/ml and 10–30 µg/ml for ramipril and atorvastatin, respectively. The results are summarized in Table 2. The results show that good correlation existed between the peak area and concentration of the studied drugs.

Table 2: linearity of the method

Ramipril		Atorvastatin	
Area	**Amount of drug (ug/ml)**	**Area**	**Amount of drug (ug/ml)**
3664650	2.5	6147512	10
5420897	3.75	9199644	15
7281773	5.00	12209977	20
9083229	6.25	15262471	25
10922660	7.5	18314966	30
Regression equation: y = 72743 x R^2 = 0.9999		Regression equation: y = 122194 x R^2 = 1	

y= peak area; x= concentration of drug in µg/ml; R^2= regression coefficient

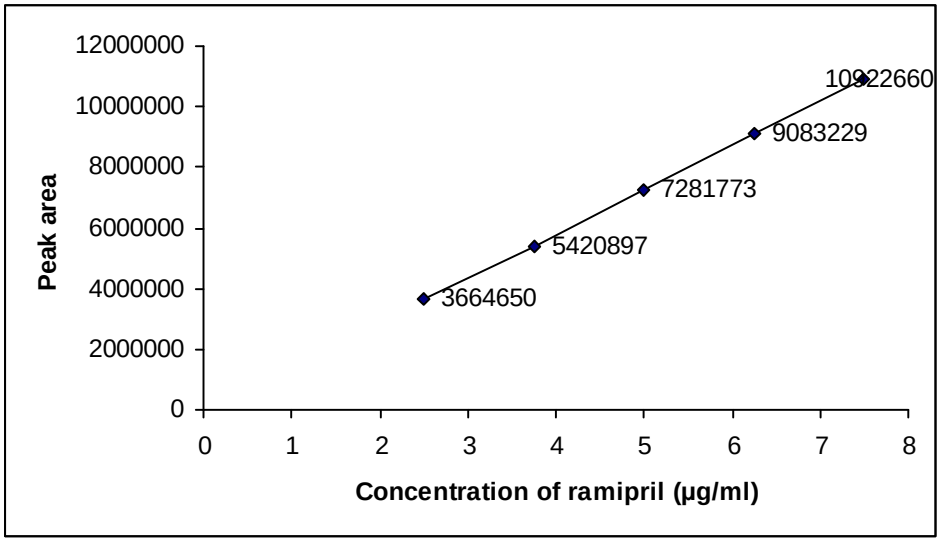

Figure 4: Linearity curve for ramipril

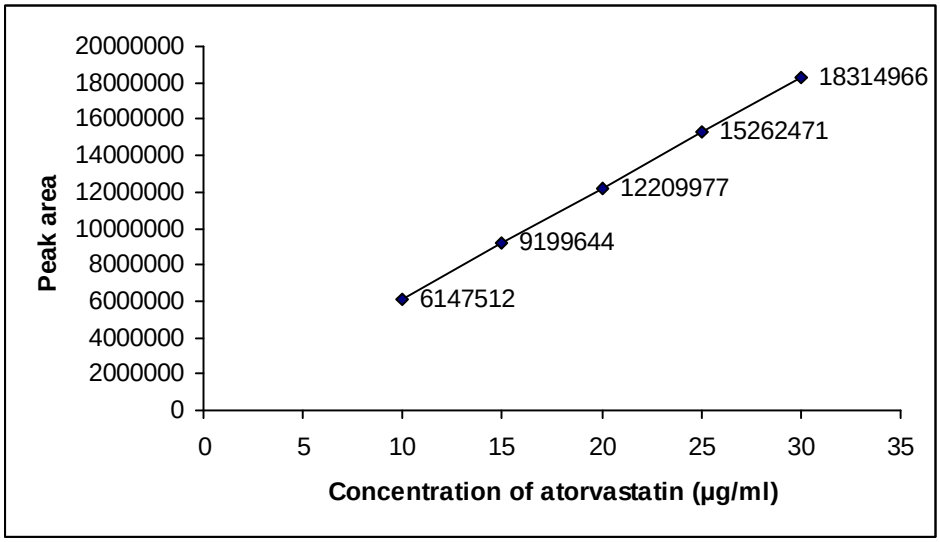

Figure 5: Linearity curve for atorvastatin

Sensitivity:

The sensitivity of the method was expressed in terms of limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were determined according to 3:1 and 10:1 signal/noise ratios, respectively. The limit of detection of ramipril and atorvastatin was 0.009 and 0.0269 µg/ml, respectively. The limit of quantification of ramipril and atorvastatin was 0.029 and 0.0898 µg/ml, respectively.

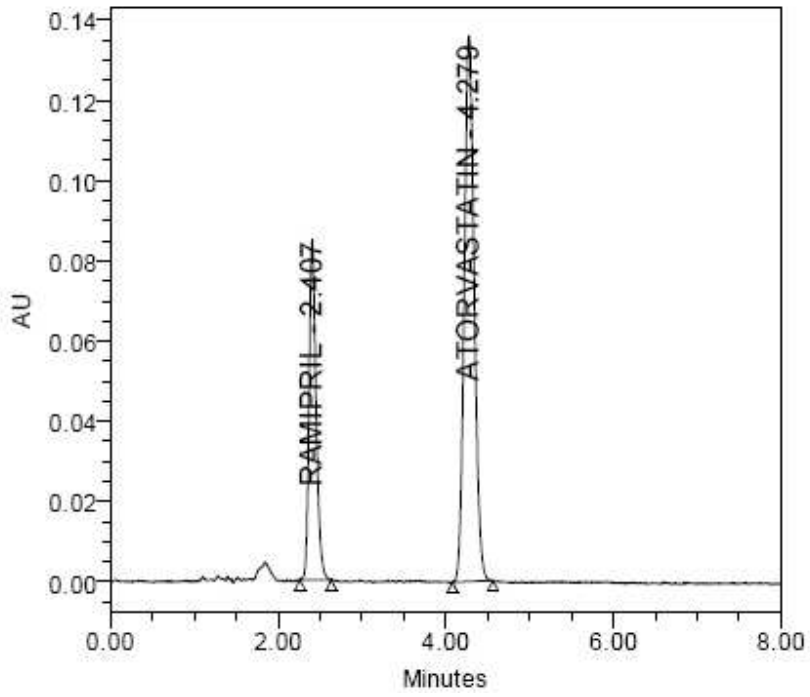

Figure 6: Chromatogram of ramipril and atorvastatin at LOD level

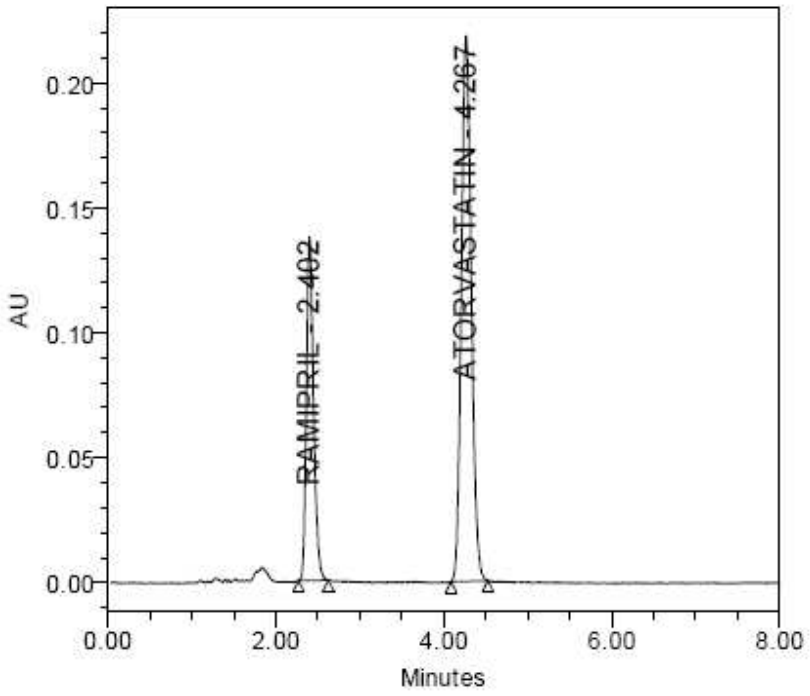

Figure 7: Chromatogram of ramipril and atorvastatin at LOQ level

Precision:

Repeatability of the method was assessed by determination of intra-day precision. Intra-day precision was assessed by injecting five standard solutions of known concentration (within linearity range) on the same day. Relative standard deviation of the peak area was then calculated to represent precision. The results of intra-day precision are

shown in Table 3. The relative standard deviation was found to be <0.5, which proves that the method is adequately precise.

Table 3: Intra-day precision

Sample Weight (mg)	Peak area of ramipril (5 µg/ml)	Peak area of atorvastatin (20 µg/ml)
102.50	7237505	12171516
102.50	7280987	12200277
102.50	7228871	12204517
102.50	7203176	12203579
102.50	7257067	12228440
102.50	7292396	12210478
	%RSD – 0.46	**%RSD** – 0.15

Accuracy:

Accuracy of the method was assessed by recovery studies through standard addition method. In the standard addition method known quantities of ramipril and atorvastatin at three different concentration levels (50, 100 and 150% of the labeled claim) were supplemented to the tablet sample solution previously analyzed. The solutions were once again analyzed by the proposed method. The percentage recoveries for ramipril and atorvastatin were calculated and are given in Table 4 & 5. Recovery studies showed the method to be adequately accurate and suitable for the simultaneous determination of ramipril and atorvastatin.

Table 4: Recovery studies for ramipril

Spiked Level	Concentration of ramipril (µg/ml)		% Recovery	% Mean
	Added	Found		
50%	2.475	2.51	101	101
	2.475	2.50	101	
	2.475	2.51	101	
100%	4.950	4.92	99	100
	4.950	4.93	100	
	4.950	4.95	100	
150%	7.425	7.44	100	100
	7.425	7.44	100	
	7.425	7.45	100	

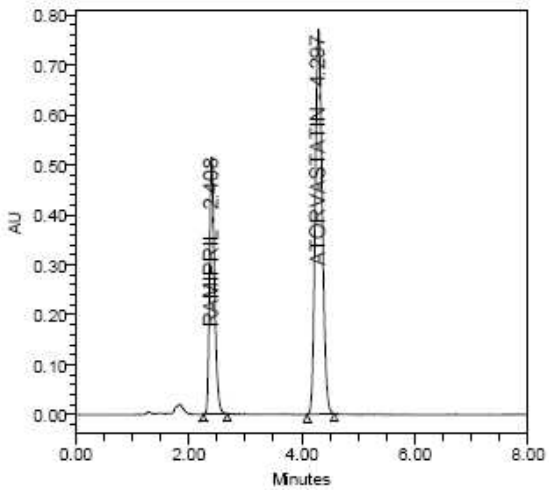

Figure 8: Chromatogram of ramipril and atorvastatin at 50% level

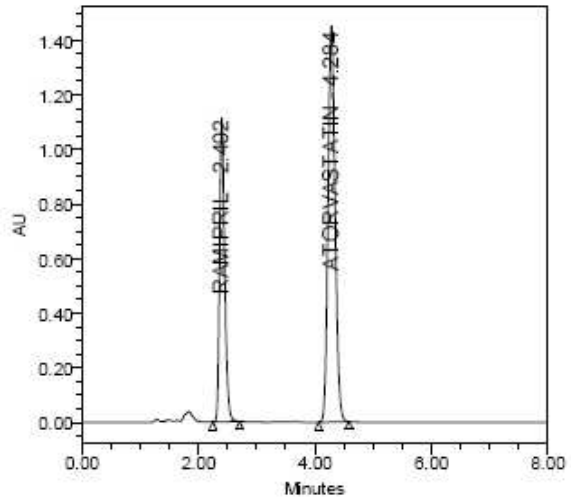

Figure 9: Chromatogram of ramipril and atorvastatin at 100% level

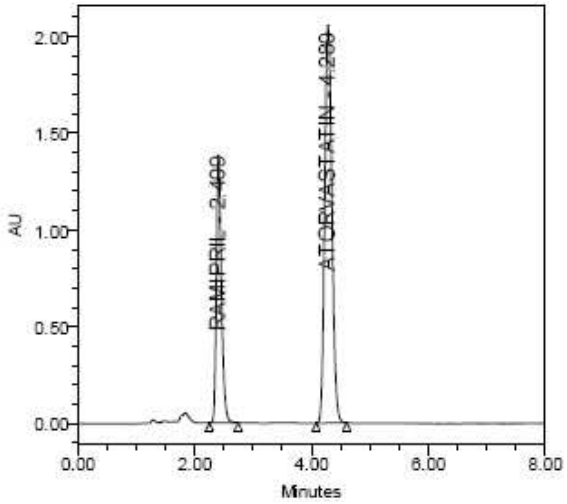

Figure 10: Chromatogram of ramipril and atorvastatin at 150% level

Table 5: Recovery studies for atorvastatin

Spiked Level	Concentration of atorvastatin (µg/ml)		% Recovery	% Mean
	Added	Found		
50%	9.900	10.01	101	101
	9.900	10.04	101	
	9.900	9.98	101	
100%	19.800	19.91	101	101
	19.800	19.87	100	
	19.800	19.93	101	
150%	29.700	30.01	101	101
	29.700	30.02	101	
	29.700	30.06	101	

Robustness:

Robustness of the method was assessed by deliberately varying chromatographic parameters. In these experiments, one parameter was changed while the others were kept unchanged. The peak area, retention time, USP plate count and USP tailing was calculated each time. The results are summarized in Table 6. The results indicated that small variation in the experimental variables did not significantly affect the analytical performance of the method.

Table 6: Robustness of the method

Sample name	Sample Name	Retention time	Peak area	Theoretical plates	USP Tailing
Ramipril	Temp-1	2.413	7231559	4021	1.27
Ramipril	Temp-2	2.412	7294380	4007	1.26
Ramipril	Flow-1	2.403	7216072	4079	1.26
Ramipril	Flow-2	2.413	7187758	4117	1.26
Atorvastatin	Temp-1	4.286	12225895	5981	1.12
Atorvastatin	Temp-2	4.276	12238228	5995	1.12
Atorvastatin	Flow-1	4.259	12263198	6423	1.13
Atorvastatin	Flow-2	4.280	12178530	6217	1.12

CONCLUSION

An isocratic HPLC with ultra violet detection method was successfully developed for the simultaneous analysis of ramipril and atorvastatin. The method validation results have proven that the method is adequately sensitive, precise, accurate, linear and robust. The method is selective for the ramipril and atorvastatin and free from the interference of the placebo and components of mobile phase. The short run time (8 min) enables rapid determination of the ramipril and atorvastatin. Further more, the method was suitable for the routine quality control of ramipril and atorvastatin in combined dosage forms.

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