

Development and Validation of RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium and Ramipril in Tablet Dosage Forms

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ABSTRACT

Objective: A New method was established for simultaneous estimation of atorvastatin calcium and ramipril by RP-HPLC method.

Methods: Chromatographic separations were carried using phenomenex luna C18 (250 × 4.6 mm, 5µm) column with a mobile phase composition of methanol in addition to phosphate buffer (0.1% v/v triethylamine pH 4.5 well balanced with 0.1% v/v orthophosphoric acid) have been delivered at a flow rate of 1 ml/min and the detection was carried out using waters HPLC auto sampler, separation module 2695 HPLC system with PDA detector at wavelength 254 nm. The running time 12 min.

Results: The retention time for atorvastatin and ramipril were 3.02 and 6.10 minute respectively. The correlation coefficient values in linearity were found to be 0.999 and concentration range 20-70 µg/ml for atorvastatin and 20-70 µg/ml for ramipril respectively. For accuracy, the total recovery was found to be 99.8% and 99.8% for atorvastatin and ramipril. LOD and LOQ for atorvastatin 2.95 and 9.96. LOD and LOQ for ramipril 3.34 and 10.05

Conclusion: The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of atorvastatin calcium and ramipril in tablet dosage form.

INTRODUCTION

Atorvastatin (Lipitor®) is a lipid-lowering drug included in the statin class of medications. By inhibiting the endogenous production of cholesterol in the liver, statins lower abnormal cholesterol and lipid levels, and ultimately reduce the risk of cardiovascular disease. More specifically, statin medications competitively inhibit the enzyme Hydroxy Methyl Glutaryl-Coenzyme A (HMG-CoA) Reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid [1]. This conversion is a critical metabolic reaction involved in the production of several compounds involved in lipid metabolism and transport, including cholesterol, Low-Density Lipoprotein (LDL) (sometimes referred to as "bad cholesterol"), and Very-Low-Density Lipoprotein (VLDL). IUPAC name calcium bis((3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoate). Chemical formula C₆₆H₆₈CaF₂N₄O₁₀. Molecular weight 1155.34. Atorvastatin (calcium salt hydrate) is soluble in organic solvents such as ethanol, DMSO, and Dimethyl Formamide (DMF), which should be purged with an inert gas. The solubility of atorvastatin (calcium salt hydrate) in these solvents is approximately 0.5, 15, and 25 mg/ml, respectively.

Ramipril is a prodrug belonging to the Angiotensin-Converting Enzyme (ACE) inhibitor class of medications. It is metabolized to ramiprilat in the liver and, to a lesser extent, kidneys. Ramiprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of Angiotensin I (ATI) to Angiotensin II (ATII). ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events [2]. IUPAC name (2S,3aS,6aS)-1-[(2S)-2-[[[(2S)-1-ethoxy-1-oxo-4-phenylbutan-2-yl]amino]propanoyl]-octahydrocyclopenta[b]pyrrole-2-carboxylicacid. Chemical formula $C_{23}H_{32}N_2O_5$. Molecular weight 416.58. Ramipril is soluble in organic solvents such as ethanol, DMSO, and Dimethyl Formamide (DMF), which should be purged with an inert gas. The solubility of ramipril in ethanol is approximately 25 mg/ml and approximately 30 mg/ml in DMSO and DMF (Figures 1 and 2).

Figure 1. Structure of Atorvastatin calcium.

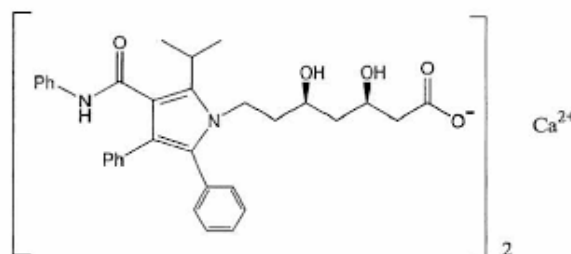
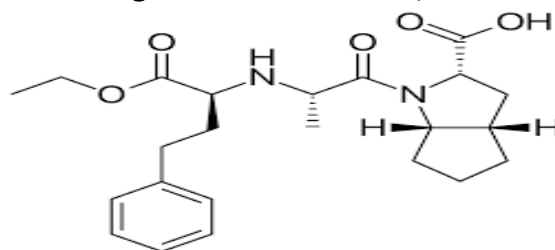


Figure 2. Structure of Ramipril.



The literature survey revealed that There are very few methods reported in the literature for analysis of atorvastatin calcium and ramipril alone or in combination with other drugs in the pure form and pharmaceuticals formulations by RP-HPLC [2-7], RP-LC [8]. In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of simultaneous estimation of atorvastatin calcium and ramipril in tablet dosage form, attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of atorvastatin calcium and ramipril. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the Simultaneous estimation of Atorvastatin calcium and ramipril in tablet dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form. To apply the developed method for the simultaneous estimation of atorvastatin calcium and ramipril in tablet dosage form.

MATERIALS AND METHODS

Chemicals and reagents

Atorvastatin calcium and ramipril were purchased from gland pharma india limited. NaH_2PO_4 was analytical grade supplied by finerchem limited, orthophosphoric acid (Merck), and water and methanol for HPLC (Lichrosolv (Merck).

Equipment and chromatographic conditions

The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and empower 2 software. Analysis was carried out at 274 nm with column symmetry C 18 (4.6 × 150 mm, 5 μm), dimensions at 25 °C temperature. The optimized mobile phase consists of Sodium phosphate buffer 2.5 pH and acetonitrile (20:80). Flow rate was maintained at 1 ml/min and run time for 12 min.

Preparation of solutions

Preparation of buffer: Accurately weigh and dissolve 1.3 grams of potassium dihydrogen ortho phosphate in 500 ml of water and adjust the pH-2.6 with orthophosphoric acid and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration.

Preparation of mobile phase: Accurately measured 200 ml of methanol and 800 ml of water were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 μ filter under vacuum filtration.

The diluents: The mobile phase was used as the diluent.

Preparation of standard stock solution: Accurately weigh and transfer 10 mg of Atorvastatin and 10 mg of ramipril working standard into a 100 ml clean dry volumetric flask add little amount of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (stock solution). Further pipette 1.5 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample stock solution: Accurately weigh and transfer equivalent to 25 mg of atorvastatin and 25 mg of ramipril sample into a 100 ml clean dry volumetric flask add little amount 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 solutions into a 10 ml volumetric flask and dilute up to the mark with diluent.

Procedure: 20 μ L of standard and sample solutions were injected into the LC-system and measure the peak areas for atorvastatin and ramipril.

Method

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 12 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 μ L of standard into phenomenex luna C18 (250 \times 4.6 mm, 5 μ m), the mobile phase of composition methanol in addition to phosphate buffer was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in Table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Atorvastatin and Ramipril in their tablet dosage form. The result obtained for Atorvastatin and Ramipril was comparable with the corresponding labeled amounts and they were shown in Table 2.

Validation of analytical method

Linearity and range: Stock solution was prepared by dissolving the appropriate amount of atorvastatin and in 7 ml of diluent and further diluted to the required concentrations with diluent. The solution ramipril was prepared at five concentration levels ranging from 800 μ g/ml to 2400 μ g/ml of atorvastatin and 30 μ g/ml to 60 μ g/ml of ramipril. 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. The results are shown in Table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the amount found and amount added for atorvastatin and ramipril and calculate the individual recovery and mean recovery values. The results are shown in Tables 4 and 5.

Precision studies: Precision was calculated from coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in Tables 6 and 7.

Ruggedness: To evaluate the intermediate precision of the method, precision was performed on different day. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found. The results are shown in Tables 8 and 9.

Robustness: As part of the robustness, deliberate change in the flow rate, mobile phase composition, temperature variation was made to evaluate the impact on the method. The flow rate was varied \pm 0.1 ml/min. The results are shown in Tables 10 and 11.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in Table 12.

LOD=3.3 σ /S and

LOQ=10 σ /S, where

σ =Standard deviation of y intercept of regression line

S=Slope of the calibration curve

RESULTS AND DISCUSSION

Figure 3. Standard chromatogram for ramipril and atorvastatin.

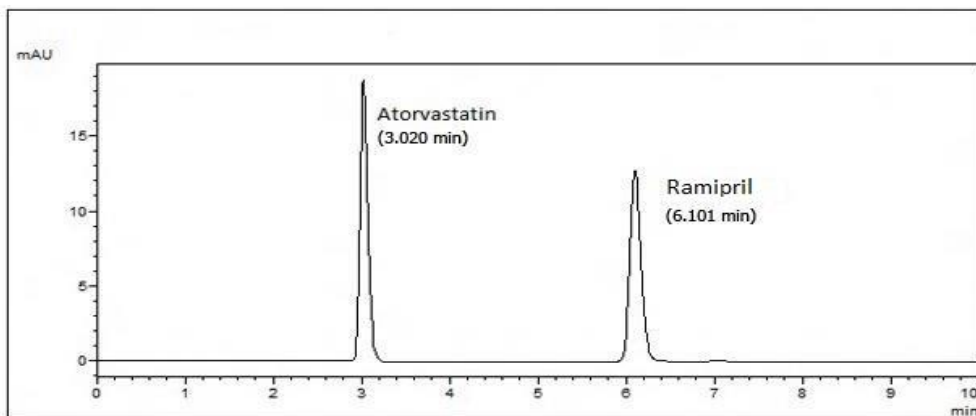


Figure 4. Sample chromatogram for ramipril and atorvastatin.

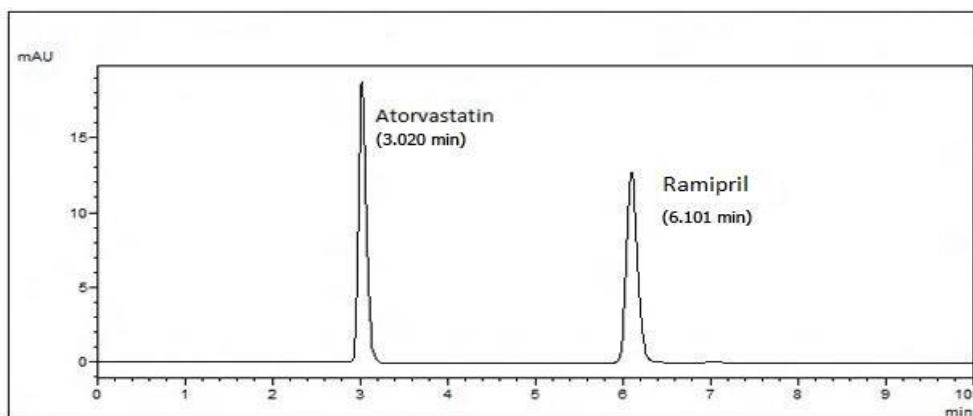


Figure 5. Blank chromatogram.

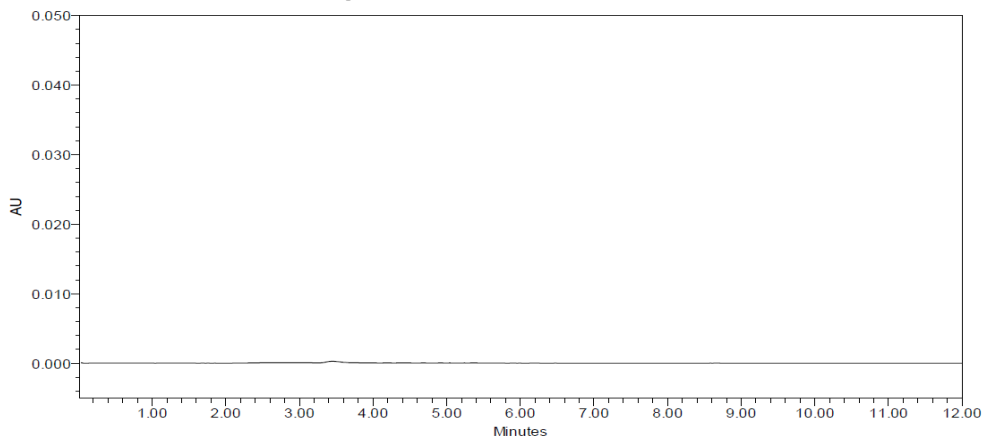


Table 1. System suitability parameters.

Parameters	Ramipril	Atorvastatin
Retention time	6.1	3
USP Plate count	2711	3428
USP Tailing	1.6	1.3

Table 2. Assay results for ramipril and atorvastatin.

	Label Claim (mg)	%Assay
Ramipril	10	100.82
Atorvastatin	10	100.91

Table 3. Linearity results for ramipril and atorvastatin.

S.No:	Concentration	Peak Area	Peak Area
	(µg/ml)	Ramipril	Atorvastatin
1	20ppm	467525	467525
2	30ppm	668668	668668
3	40ppm	899412	899412
4	50ppm	1128421	1128421
5	60ppm	1365426	1365426
6	70ppm	1594287	1594287
Mean		1131243	1131243
Co-relation Coefficient		0.999	0.999

Figure 6. Linearity graph for ramipril.

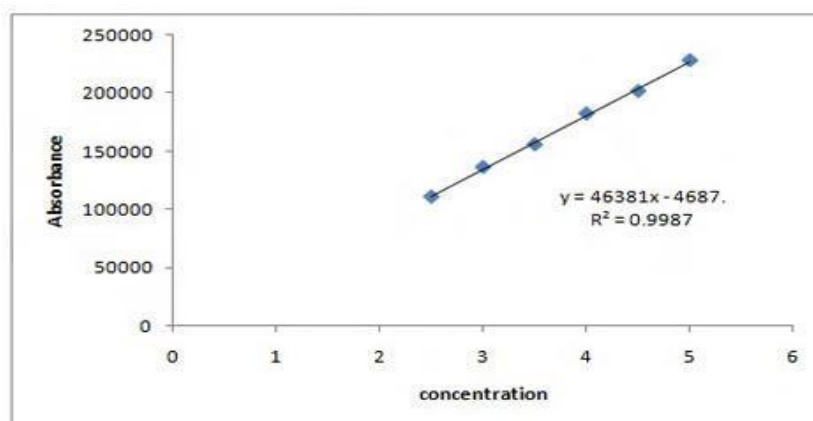


Figure 7. Linearity graph for atorvastatin.

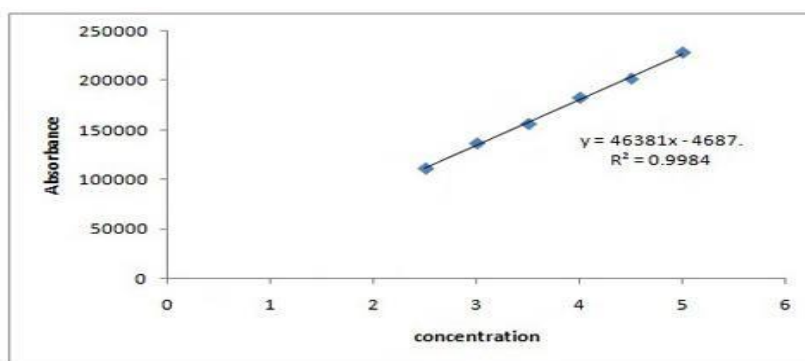


Table 4. Showing accuracy results for ramipril.

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	460216	10	10.05	99.70%	99.80%
100%	923742	20	20.05	99.90%	
150%	1386984	30	30.02	99.70%	

Table 5. Showing accuracy results for atorvastatin.

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	276897	40	40.08	99.70%	99.8
100%	556371	80	80.17	99.80%	
150%	828349	120	121.07	99.80%	

Table 6. Precision results for ramipril.

Sr. No.	Sample area	Standard area	Percentage purity
1	983375	971536	101.04
2	985049	973007	101.03
3	982956	975717	100.54
4	985219	978909	100.44
5	994145	981422	101.09
Average			100.84
%RSD			0.304

Table 7. Precision results for atorvastatin.

Sr. No.	Sample area	Standard Area	Percentage Purity
1	592403	577531	101.36
2	592352	580381	101.85
3	592357	577723	102.32
4	592323	582190	101.44
5	596525	583378	101.09

Average			101.24
%RSD			0.46

Table 8. Ruggedness results of ramipril and atorvastatin.

Sr. No	Sample area	Standard area	Percentage Purity
1	979556	984395	99.3
2	982467	984039	99.64
3	979717	983976	99.36
4	978909	984278	99.28
5	981432	973915	100.57
Average			99.63
%RSD			0.54

Table 9. Ruggedness results of ramipril and atorvastatin.

S. No	Sample area	Standard area	Percentage
1	583416	593403	99.12
2	583657	594352	99.01
3	584731	593357	99.52
4	583594	592673	99.61
5	597649	593671	99.12
Average			99.27
%RSD			0.27

Robustness results

Table 10. Flow variation results for ramipril and atorvastatin.

S. No	Peak Area for Not as much of flow rate (0.7 ml/min)		Peak Area for Additional flow rate (0.9 ml/min)	
	Ramipril	Atorvastatin	Ramipril	Atorvastatin
1	980365	574981	974803	593791
2	985334	582941	973491	594572
3	984967	582494	971234	598901
4	985907	589480	974984	593456
5	994245	584468	984542	583453
Mean	986306	582223	976755	591667
%RSD	0.45	0.8	0.53	0.8

Table 11. Result for effect of inconsistency in mobile phase configuration (organic phase).

S. No	Peak area for Less organic phase (70 %)		Peak area for More organic phase (90%)	
	Ramipril	Atorvastatin	Ramipril	Atorvastatin
1	988865	574671	980985	599871
2	987834	585781	983757	599472
3	988968	588977	989869	594561
4	989016	585762	983454	592476
5	998947	585898	997472	589633
Mean	985676	586798	984561	597487
%RSD	0.45	0.9	0.51	0.57

Table 12. LOD, LOQ of atorvastatin and ramipril.

Drug	LOD	LOQ
Ramipril	3.34	10.05
Atorvastatin	2.95	9.96

CONCLUSION

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of atorvastatin and ramipril in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Atorvastatin and Ramipril in pure and its pharmaceutical dosage forms.

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