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

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Research Article

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ABSTRACT

A New method was established for simultaneous estimation of Atorvastatin calcium and Ramipril by RP-HPLC method. Chromatographic separations were carried using Phenomenex Luna C18 (250 × 4.6 mm, 5 µm) column with a mobile phase composition of methanol in addition to phosphate cradle (0.1% v/v triethylamine pH 4.5 well balanced with 0.1% v/v orthophosphoric harsh) 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. 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. 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 hydroxymethylglutaryl-coenzyme A (HMG-CoA) Reductase, which catalyzes the conversion of HMG-CoA to mevalonic acid. This conversion is a critical metabolic reaction involved in the production of several

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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). 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 [1]. 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 [2]. 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.

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. 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, RP-LC. 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 [3]. 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.

MATERIALS AND METHODS

Chemicals and reagents

Atorvastatin calcium and Ramipril were Purchased from Gland Pharma India Limited. NaH2PO4 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 Cymmetry C 18 (4.6 × 150 mm, 5 m), dimensions at 250C temperature. The optimized mobile phase consists of Sodium Phospahte buffer 2.5 pH and Acetonitrile (20:80). Flow rate was maintained at 1 ml/min and run time for 12 min. 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. 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.

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. Further pipette 1.5 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent.

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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. Further pipette 0.1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

RESULTS AND DISCUSSION

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 [4]. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Phenomenex Luna C18 (250 × 4.6 mm, 5 µm), the mobile phase of composition methanol in addition to phosphate cradle 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).

Table 1. Linearity results for Ramipril and Atorvastatin.

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

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).

Table 2. Showing accuracy results for Ramipril.

% Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
0.5	460216	10	10.05	0.997	0.998
1	923742	20	20.05	0.999	
1.5	1386984	30	30.02	0.997	

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.
LOD=3.3 σ/S
LOQ=10 σ/S, where
σ=Standard deviation of y intercept of regression line,
S=Slope of the calibration curve

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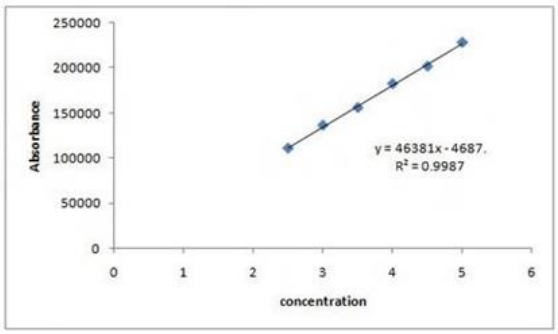
Figure 1. Linearity graph for Ramipril.



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 [5]. The flow rate was varied ± 0.1 ml/min. 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.

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. 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.

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