EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 6
451-457
Year: 2021

# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN CALCIUM AND RAMIPRIL IN TABLET DOSAGE FORMS

**Hari Krishna* and Nihar Ranjan Das**

Jntuh, Telangana.

***Corresponding Author: Hari Krishna**
Jntuh, Telangana.

**Article Received on 14/04/2021** **Article Revised on 04/05/2021** **Article Accepted on 24/05/2021**

## 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 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 12min. **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.

**KEYWORDS:** Atorvastatin calcium, Ramipril, RP-HPLC, Simultaneous estimation.

## 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,[1] which catalyzes the conversion of HMG-CoA to mevalonic acid. 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 $C_{66}H_{68}CaF_2N_4O_{10}$. 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.

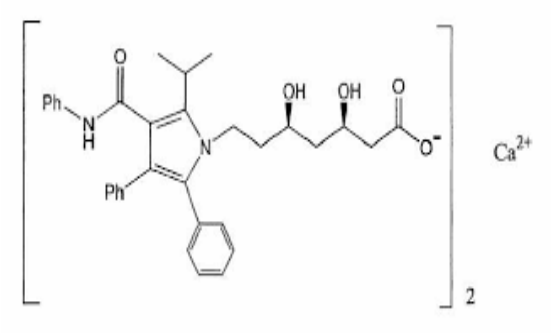

**Figure 1: Structure of atorvastatin calcium.**

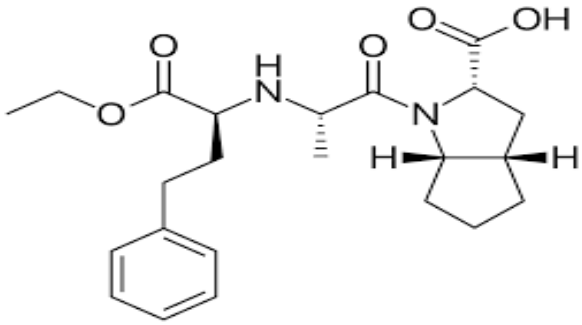

**Figure 2: Structure of ramipril.**

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,[3-7] 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 Cymmetry C 18 (4.6 x 150mm, 5µm), dimensions at $25^0$C 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.

### 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 10ml 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 10ml volumetric flask and dilute up to the mark with diluent.

**Procedure:** 20 µL 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 µL of standard into

Phenomenex Luna C18 (250 × 4.6 mm, 5µm), 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.

**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 µg/ml to 2400 µg/ml of Atorvastatin and 30 µg/ml to 60 µg/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 resulte 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 resulte are shown in table 4,5.

**Precision studies:** precision was caliculated 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 resulte are shown in table 6,7.

**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 resulte are shown in table 8,9.

**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 ± 0.1 ml/min. The resulte are shown in table 10,11.

**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 resulte are shown in table 12.

LOD = 3.3σ/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**

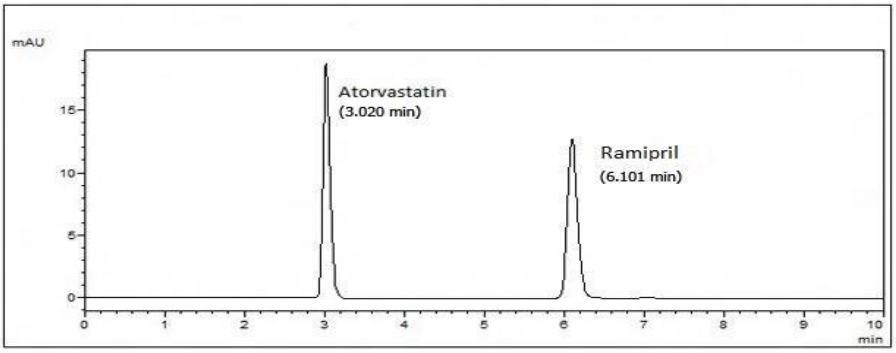


**Figure 3: Standard chromatogram**

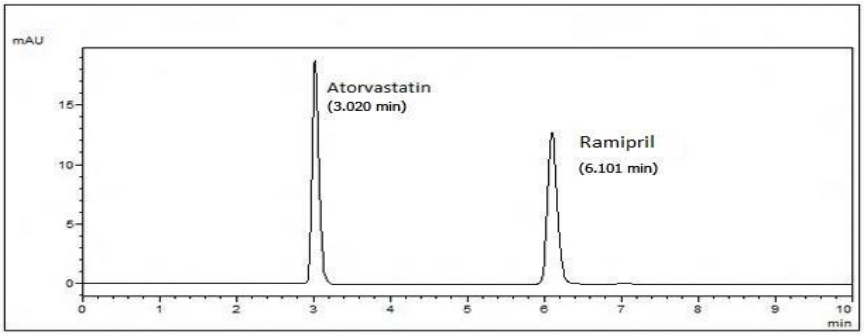


**Figure 4: Sample chromatogram.**

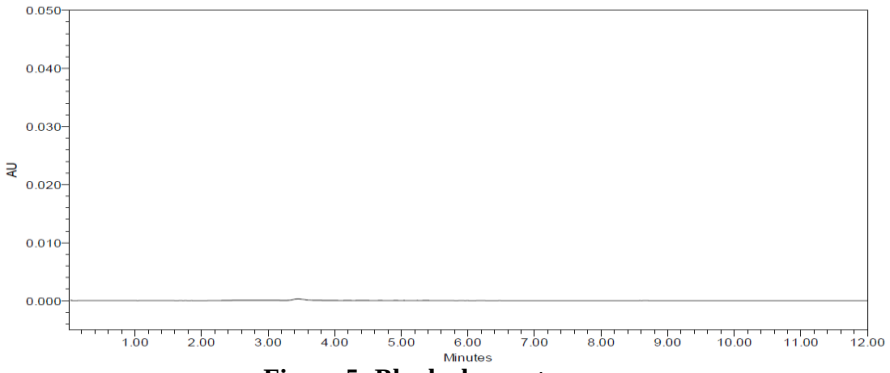


**Figure 5: Blank chromatogram.**

**Table 1: System suitability parameters.**

| Parameters | Ramipril | Atorvastatin |
|---|---|---|
| Retention time | 6.1 | 3.0 |
| 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 (µg/ml) | Peak Area Ramipril | Peak Area 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 |

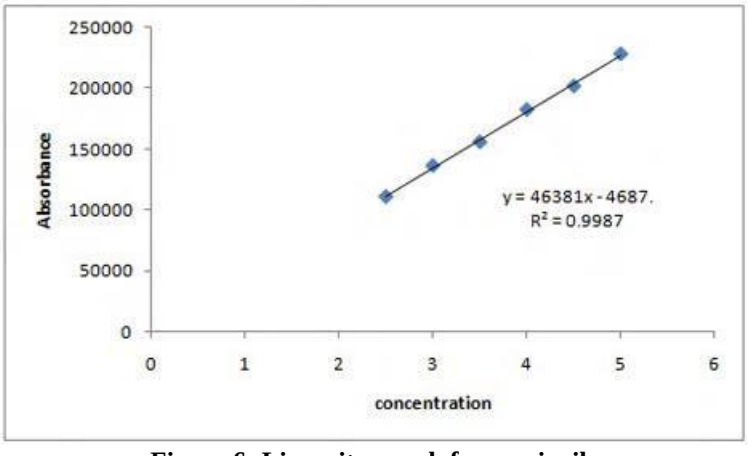


**Figure 6: Linearity graph for ramipril.**

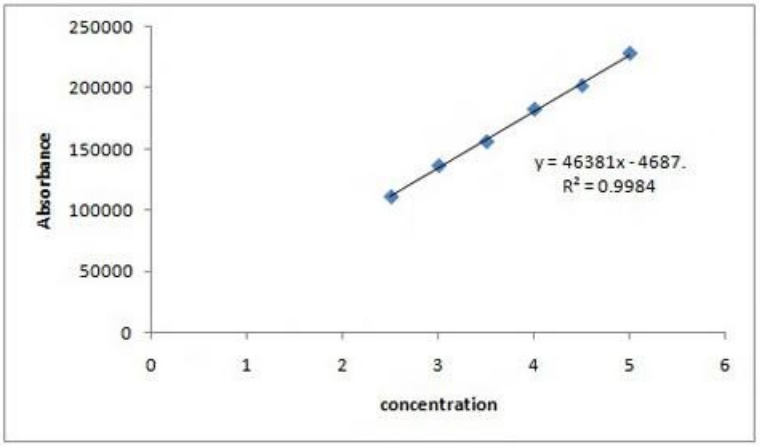


**Figure 7: Linearity graph for atorvastatin.**

**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 | 99.7% | 99.8% |
| 100% | 923742 | 20 | 20.05 | 99.9% | |
| 150% | 1386984 | 30 | 30.02 | 99.7% | |

**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 | 99.7% | 99.8 |
| 100% | 556371 | 80 | 80.17 | 99.8% | |
| 150% | 828349 | 120 | 121.07 | 99.8% | |

**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.

## REFERENCES

1. Moghadasian MH: Clinical pharmacology of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Life Sci, 1999; 65(13): 1329-37.
2. Brunton LL, Hilal-Dandan R, Knollmann BC. eds Goodman & Gilman's: The Pharmacological Basis of Therapeutics McGraw-Hill Education, 2018; 13.
3. Mishra P, Gupta A and Shah K, Simultaneous Estimation of Atorvastatin Calcium and Amlodipine Besylate from Tablets, Indian Journal of Pharmaceutical Sciences, 2007; 69(6): 831-833.
4. Jain N, Raghuwanshi R and Jain D, Development and Validation of RP-HPLC Method for Simultaneous Estimation of Atorvastatin Calcium and Fenofibrate in Tablet Dosage Forms, Indian Journal of Pharmaceutical Sciences, 2008; 70(2): 263-265.

5. Maheshwari K R, Sankar G G and Rao A L, HPLC Method for the Simultaneous Determination of Atorvastatin and Amlodipine in Tablet Dosage Forms, Indian Journal of Pharmaceutical Sciences, 2006; 68(2): 275-277.
6. Patel N M, et al., Development and Validation of HPTLC Method for the Simultaneous Estimation of Atorvastatin Calcium and Ezetimibe, Indian Journal of Pharmaceutical Sciences, 2006; 68: 793-796.
7. Thamake, S. & Jadhav, Swapnil & Pishawikar, Sachin. Development and Validation of Method for Simultaneous Estimation of Atorvastatin Calcium and Ramipril from Capsule Dosage Form by First Order Derivative Spectroscopy. Asian Journal of Research in Chemistry, 2009; 2: 52-53.
8. Panchal, Hiral & Suhagia, Bhanubhai & Patel, Natvarlal & Rathod, Ishwarsinh & Patel, Bhavesh. Simultaneous Estimation of Atorvastatin Calcium, Ramipril and Aspirin in Capsule Dosage Form by RP-LC. Chromatographia, 2008; 69: 91-95.