WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 7, 1612-1625. Research Article ISSN 2277– 7105

# DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF RAMIPRIL, ATORVASTATIN CALCIUM AND ASPIRIN IN A COMBINED CAPSULE DOSAGE FORM

**Kshipra S. Karnik*[1], Dr. Ashok P. Pingle[1], Dr. Aarya Jadhav[2]**

[1]University of Pune, Department of Pharmaceutical Chemistry, MVP Samaj's College of Pharmacy, Nashik-422002, India.

[2]University of Pune, Department of Pharmacology, MVP Samaj's College of Pharmacy, Nashik-422002, India.

Article Received on
30 April 2015,

Revised on 25 May 2015,
Accepted on 20 June 2015

***Correspondence for Author**
**Kshipra S. Karnik**
University of Pune, Department of Pharmaceutical Chemistry, MVP Samaj's College of Pharmacy, Nashik-422002, India.

## ABSTRACT

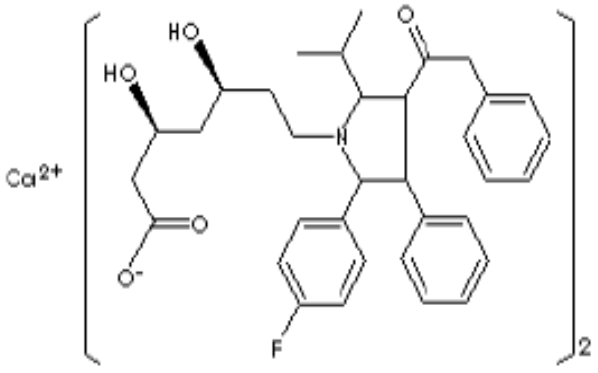
A simple, sensitive, accurate and rapid reverse phase high performance liquid chromatographic method is developed for the simultaneous estimation of ramipril, aspirin and atorvastatin calcium in a combined capsule dosage form. The chromatography was performed on a 4.6ID x 250mm, Particle size: 5 micron, C18 Grace column with a mobile phase consisting of methanol:potassium dihydrogen phosphate buffer 10mM adjusted to pH 3 (70:30v/v), at a flow rate of 0.9ml/min. The detection was carried out at a wavelength of 215nm. Total run time was 10min; the retention times were about 3.751 for Aspirin, 4.077 for Ramipril and 6.448 for Atorvastatin Calcium respectively. The developed method was validated according to ICH guidelines for linearity, accuracy, precision, robustness, limit of detection and limit of quantitation. Limits of detection were 0.023, 0.048 and 0.35 ng $mL^{-1}$ and limits of quantitation were 0.070, 0.147 and 1.060 ng $mL^{-1}$ for ramipril, atorvastatin and aspirin respectively. All the three drugs were subjected to acid and alkali hydrolysis and oxidation degradation. As the method could effectively separate the drug from its degradation products, it can be employed as a stability- indicating one. The validated method was successfully used for quantitative analysis of marketed preparations.

**KEYWORDS:** RP-HPLC, Ramipril, Aspirin, Atorvastatin Calcium, Stability-indicating.

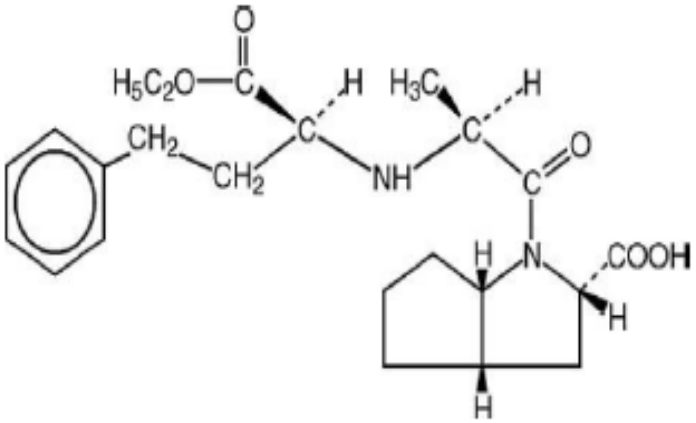
## INTRODUCTION

Atorvastatin calcium is chemically known as (β*R*,8R)-2-(4-fluorophenyl)-α,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]1H-pyrrole-1-heptanoic acid trihydrate,[1,2] is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase inhibitor used in Hyperlipoproteinemia. Ramipril (2s,3aS,6aS)-1-[(S)-2-[[(S)-1-(ethoxycarbonyl)-3phenylpropyl]amino] propanoyl]octahydrocyclopenta[b]pyrrole-2-carboxylic acid,[2] is an angiotensin converting enzyme inhibitor used in the treatment of hypertension and congestive heart failure. Aspirin which is chemically known as Acetyl salicylic acid is a non-steroidal anti-inflammatory drug which comes under the class of Non-selective COX inhibitors. [1, 2, 3]

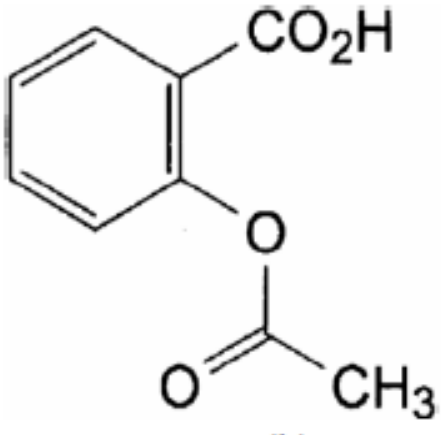
Literature survey revealed that stability-indicating RP-HPLC methods are determined for Atorvastatin calcium, Ramipril as well as Aspirin as a single dosage form.[4,5,6,7] These drugs are also determined in the combination dosage form simultaneously by RP-HPLC.[8,9,10] Also the above three drugs are successfully determined individually or in combination by HPTLC,[11,12,13,14,15] UV.[16] and Spectrophotometry,[17,18] respectively. Referring to the literature survey, there is no published stability-indicating RP-HPLC method for the simultaneous estimation of Atorvastatin calcium, Ramipril and Aspirin respectively in a capsule dosage form. The present paper describes a simple, sensitive, accurate and rapid reverse phase high performance liquid chromatographic method is developed for the simultaneous estimation of ramipril, aspirin and atorvastatin calcium in a combined capsule dosage form. Also the above developed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines.[19]




**(a)**



**(b)**



**(c)**

**Figure.1 Chemical structures of (a) Atorvastatin calcium (b) Ramipril, (c) Aspirin.**

## MATERIALS AND METHODS

### Instrument

The chromatographic separation was done on Analytical Technologies Ltd. HPLC Binary Gradient System (HPLC 3000 series) equipped with P-3000-M (40MPa) Reciprocating pump and UV 3000-M detector with 20µl loop volume.

### Chemicals and Reagents

Methanol (HPLC grade)

Potassium dihydrogen phosphate (AR grade)

The above reagents and chemicals were purchased from Spectrochem, Mumbai, India.

Pure working standards of Atorvastatin calcium, Ramipril and Aspirin were obtained as gift samples. The marketed formulation Ramitorva (Atorvastatin-10mg/capsule, Ramipril-5mg/capsule and Aspirin-75mg/capsule) manufactured by Zydus Medica, Ltd. was purchased from local market.

**Method**

**Chromatographic conditions**

Chromatographic separation was achieved on Grace C18 column (250mm x 4.6mm) at an ambient temperature using mobile phase as Methanol: Potassium dihydrogen phosphate buffer 10mM (70:30 v/v); pH was adjusted to 3 using dil. ortho phosphoric acid. The flow rate was kept at 0.9ml/min with the detection wavelength at 215nm. The injection volume was 20µl. The mobile phase was filtered through a 0.45µ membrane filter and was sonicated for 5 min.

**Preparation of standard stock solution**

10mg each of Ramipril, Aspirin and Atorvastatin calcium was weighed and transferred into a 10ml volumetric flask. The diluent methanol was used to dissolve the drug and the final volume was made with the same diluents to obtain a concentration of 1000µg/ml of each drug. Further the stock solution was diluted to obtain a concentration of 5-25µg/ml of Ramipril, 10-50µg/ml of Atorvastatin calcium and 75-375µg/ml of Aspirin.

**Method Development**

The concentration of the mobile phase buffer was changed keeping the other chromatographic conditions constant with a flow rate of 1ml/min. Initially 20mM buffer was taken but that resulted in the splitting of peak. Then 10mM buffer was selected with decreasing the flow rate to 0.9ml/min which resulted in good resolution with acceptable peak area alongwith other system suitability parameters.

**Analysis of Marketed Formulation**

Ten capsules were weighed individually and their average weight was determined. The powder sample equivalent to 10mg of Atorvastatin calcium, 5mg of Ramipril and 75mg of Aspirin was weighed accurately and transferred to a 20ml volumetric flask containing 5ml of methanol and was sonicated for 15mins. Then the volume was made up to the mark with methanol. From the above prepared stock, 1ml was transferred to 10ml volumetric flask and the volume was made up to the mark with the diluent methanol to obtain 50ppm of final

concentration. The sample was injected and the peak area of each drug along with the amount of drug present per capsule was calculated.

**Method Validation**

The proposed method was validated as per ICH guidelines for specificity, linearity, accuracy, precision, robustness, limit of detection and limit of quantitation.

**1) Specificity**

The specificity of the proposed method was checked by comparing the chromatograms obtained from standard and sample.

**2) Linearity**

Linearity of response for Aspirin, Ramipril as well as Atorvastatin calcium was performed using standard solution in a range of 75-375µg/ml, 5-25µg/ml and 10-50µg/ml respectively.

**3) Accuracy (%Recovery)**

For the analysis if accuracy, recovery studies were carried out by adding known amount of standard drug solution to the pre-analyzed sample solutions at three different levels 50%, 100% and 150%. Amount of Atorvastatin calcium, Ramipril and Aspirin were quantified and % recovery was calculated from amount found and actual amount added.

**4) Method precision (Repeatability)**

Samples of a single batch of sample was prepared six times and analyzed as per the test method and the relative standard deviation is thus measured.

**5) Robustness**

Robustness was evaluated following small deliberate variations were made in the method and samples were analyzed in triplicate. The flow rate of the method was changed and the effect was analysed by determining the linearity and coefficient of correlation from the results obtained.

**6) Limit of Detection**

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

$$LOD = \frac{3.3\ X\ SD}{S}$$

Where, **SD =** Standard deviation,

**S =** Slope of the curve

**7) Limit of Quantification**

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

It may be calculated based on standard deviation (SD) of the response and slope of the curve(S).

$$LOQ = \frac{10\ X\ SD}{S}$$

Where, SD = Standard deviation,

**S =** Slope of the curve.

**Forced degradation studies**

**a) Acid degradation**

The powder sample equivalent to 10mg of Atorvastatin calcium, 5mg of Ramipril and 75mg of Aspirin was weighed accurately and transferred to a 100ml volumetric flask. To this add 10ml of diluent methanol and sonicate for 15min with intermittent shaking. To it add about 5ml of 1N HCl and heat on a water bath for 30min, cool to room temperature. To the sample add 1mL of 1N NaOH, mix sonicate for about 10 minutes with intermittent shaking, cool the solution and make up the volume with diluent methanol and mix well.

**(b) Alkali degradation**

The powder sample equivalent to 10mg of Atorvastatin calcium, 5mg of Ramipril and 75mg of Aspirin was weighed accurately and transferred to a 100ml volumetric flask. To this add 10ml of diluent methanol and sonicate for 15min with intermittent shaking. To it add about 5ml of 1N NaOH and heat on a water bath for 30min, cool to room temperature. To the sample add 1mL of 1N HCl, mix sonicate for about 10 minutes with intermittent shaking, cool the solution and make up the volume with diluent methanol and mix well.

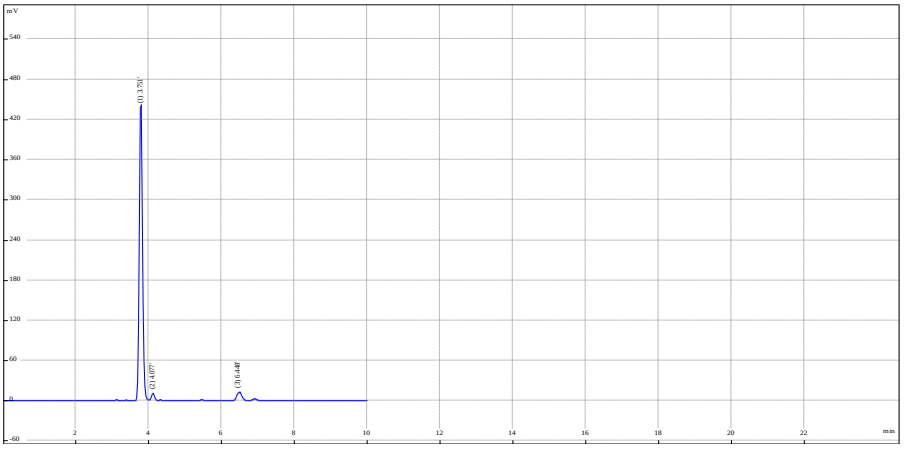
**(c) Peroxide degradation**

The powder sample equivalent to 10mg of Atorvastatin calcium, 5mg of Ramipril and 75mg of Aspirin was weighed accurately and transferred to a 100ml volumetric flask. To this add 10ml of diluent methanol and sonicate for 15min with intermittent shaking. To it add about 30% hydrogen peroxide and reflux on a water bath for 30min, cool to room temperature and make up the volume with diluent methanol and mix well.

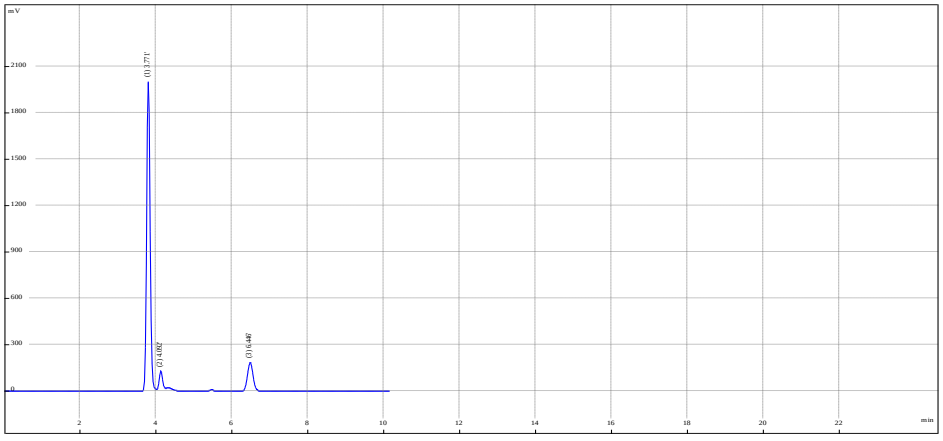
## RESULTS AND DISCUSSION

### 1) Specificity

There was no observation of any interference from the excipients or additives found to be present in the capsule (Figure 2).



**(a)**



**(b)**

**Figure.2 Chromatogram of Aspirin (3.751min), Ramipril (4.077min). and Atorvastatin calcium (6.448min) from (a) standard; and (b) capsule.**

**2) Linearity**

A linear correlation was obtained between the peak areas as well as the concentrations of all three drugs measuring the coefficient of correlation and slope of the calibration curves (Table 1).

**Table. 1 Regression analysis results of the calibration curves for aspirin, ramipril and atorvastatin calcium by the proposed RP-HPLC method.**

| Parameter | Aspirin | Rampril | Atorvastatin calcium |
|---|---|---|---|
| Slope | 46255 | 25163 | 52375 |
| Intercept | -650506 | -50650 | -431600 |
| Correlation coefficient (r) | 0.9997 | 0.9967 | 0.9977 |

**3) Accuracy (%Recovery)**

The results obtained are tabulated in the Table 2, 3 and 4 below for aspirin, ramipril and atorvastatin calcium respectively.

**Table. 2 Result and Statistical data of Accuracy (Aspirin 75mg/capsule)**

| Sr. No. | Conc. Level | Area | Conc. Found (µg/mL) | % Recovery | Average % Recovery | % RSD |
|---|---|---|---|---|---|---|
| 1. | 50% | 2888134 | 50.52 | 101.04 | 101.16 | 0.012 |
| | | 2935937 | 51.39 | 102.72 | | |
| | | 2850189 | 49.86 | 99.72 | | |
| 2. | 100% | 9363222 | 99.84 | 99.84 | 99.99 | 0.0011 |
| | | 9379238 | 100.01 | 100.01 | | |
| | | 9390577 | 100.13 | 100.13 | | |
| 3. | 150% | 13930634 | 149.13 | 99.42 | 99.46 | 0.0004 |
| | | 13934334 | 149.17 | 99.44 | | |
| | | 13945314 | 149.29 | 99.52 | | |

**Table. 3 Result and Statistical data of Accuracy (Ramipril 5mg/capsule).**

| Sr. No. | Conc. Level | Area | Conc. Found (µg/mL) | % Recovery | Average % Recovery | % RSD |
|---|---|---|---|---|---|---|
| 1. | 50% | 75734 | 49.87 | 99.74 | 99.99 | 0.0021 |
| | | 76128 | 50.13 | 100.26 | | |
| | | 75918 | 49.99 | 99.98 | | |
| 2. | 100% | 474914 | 101.07 | 101.07 | 100.70 | 0.0025 |
| | | 472240 | 100.50 | 100.50 | | |
| | | 472426 | 100.54 | 100.54 | | |
| | | 705917 | 150.70 | 100.46 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3. | 150% | 696696 | 148.73 | 99.15 | 99.99 | 0.0059 |
| | | 705286 | 150.56 | 100.37 | | |

**Table 4. Result and Statistical data of Accuracy (Atorvastatin 10mg/capsule)**

| Sr. No. | Conc. Level | Area | Conc. Found (µg/mL) | % Recovery | Average % Recovery | % RSD |
|---|---|---|---|---|---|---|
| 1. | 50% | 156179 | 50.00 | 100 | 99.99 | 0.0035 |
| | | 155481 | 49.78 | 99.56 | | |
| | | 156840 | 50.21 | 100.42 | | |
| 2. | 100% | 1096041 | 99.52 | 99.52 | 99.99 | 0.061 |
| | | 1096976 | 99.60 | 99.60 | | |
| | | 1110883 | 100.87 | 100.87 | | |
| 3. | 150% | 2226357 | 150.14 | 100.09 | 99.99 | 0.034 |
| | | 2214013 | 149.31 | 99.54 | | |
| | | 2232362 | 150.54 | 100.36 | | |

**4) Method Precision**

Relative standard deviation of the areas of six replicate injections are measured which indicates the developed method is precise.

**Table. 5 Results for Precision of Aspirin, Ramipril and Atorvastatin calcium.**

| Injection no. | Aspirin | | Ramipril | | Atorvastatin | |
|---|---|---|---|---|---|---|
| | Retention Time (min ) | Area | Retention Time ( min ) | Area | Retention Time ( min ) | Area |
| 1 | 3.751 | 2788134 | 4.077 | 62734 | 6.448 | 134179 |
| 2 | 3.748 | 2935937 | 4.073 | 63852 | 6.418 | 151481 |
| 3 | 3.753 | 2850189 | 4.078 | 75918 | 6.427 | 156840 |
| 4 | 3.771 | 2794384 | 4.075 | 69572 | 6.443 | 153472 |
| 5 | 3.756 | 2735973 | 4.077 | 68384 | 6.422 | 154333 |
| 6 | 3.787 | 2843741 | 4.072 | 69745 | 6.483 | 153785 |
| **Mean** | | 2824726 | | 68367.5 | | 150681.7 |
| **S.D.** | | 62602.62 | | 4329.83 | | 7545.98 |
| **%RSD** | | 0.022 | | 0.063 | | 0.05 |

**5) Limit of Detection and Limit of Quantification**

The LODs as well as LOQs are quantitated in Table 6 below:

**Table. 6 Results of LOD and LOQ of Rampril, Atorvastatin calcium and Aspirin.**

| | Ramipril (ng $ml^{-1}$) | Atorvastatin (ng $ml^{-1}$) | Aspirin (ng $ml^{-1}$) |
|---|---|---|---|
| **LOD** | 0.023 | 0.048 | 0.35 |
| **LOQ** | 0.070 | 0.147 | 1.060 |

### 6) Robustness

The proposed method was found to robust as the results were not significantly affected by the small and deliberate change in the flow rate.

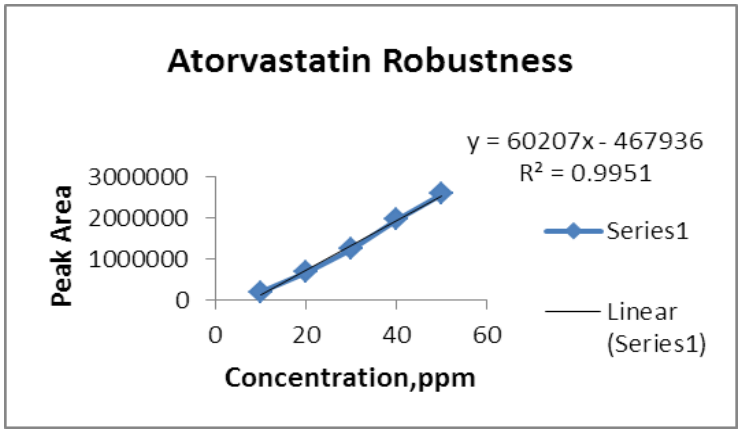



**Figure. 3 Robustness of Atorvastatin Calcium.**

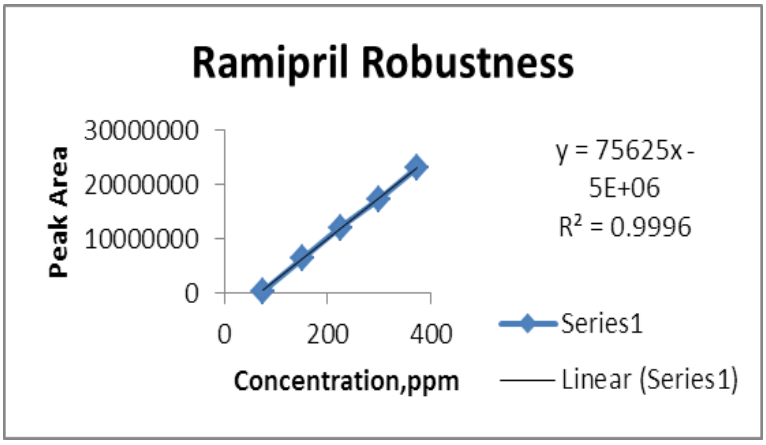



**Figure. 4 Robustness of Ramipril.**

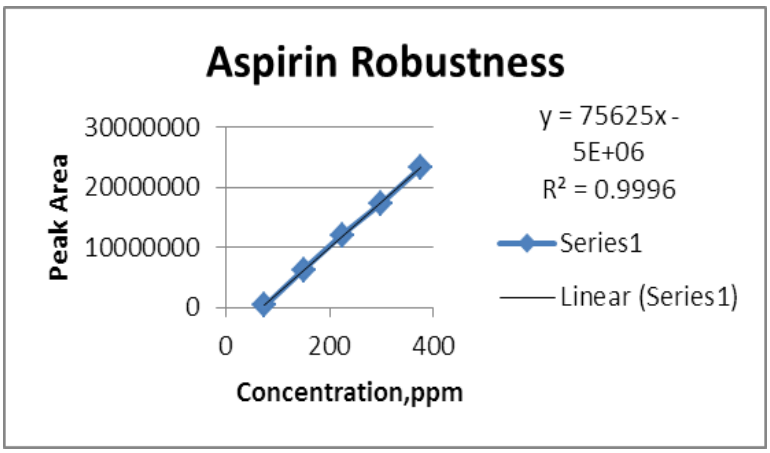



**Figure. 5 Robustness of Aspirin.**

### 7) System suitability test

The %RSD for Aspirin, Ramipril and Atorvastatin calcium was found to be 0.024, 0.068 and 0.054, respectively which was found under the given ICH limit.

### Stability-indicating or Forced degradation studies

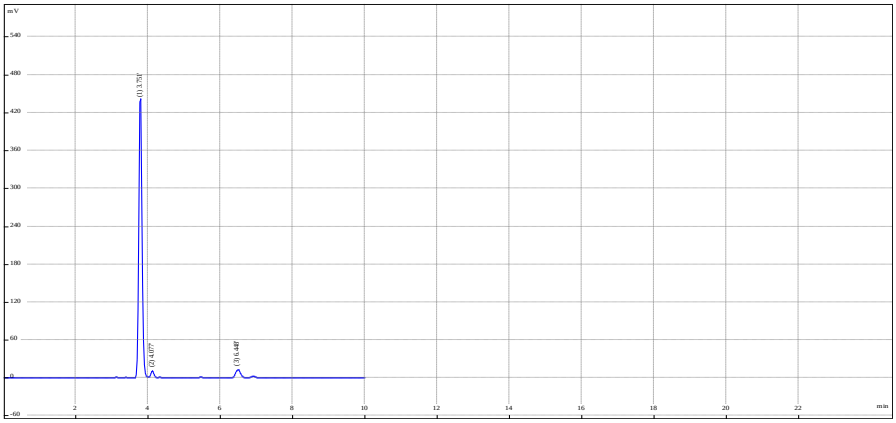


**Figure. 6 Chromatogram of an untreated sample; Aspirin (3.751min), Ramipril (4.077min). and Atorvastatin calcium (6.448min).**

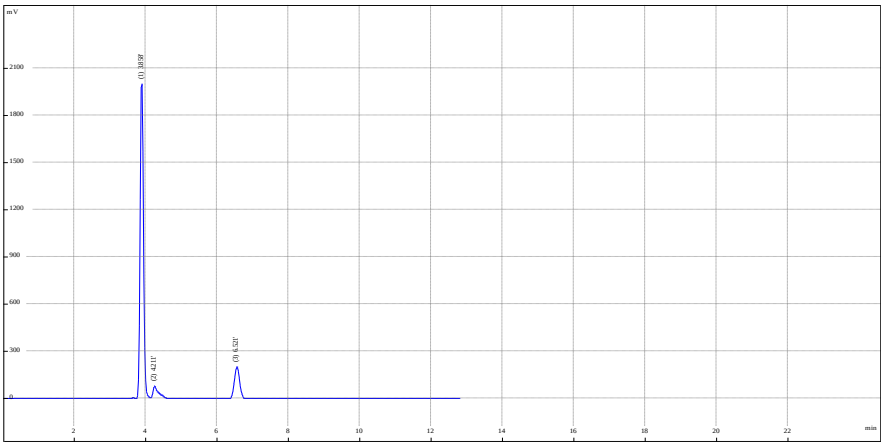


**Figure. 7 Chromatogram of acid treated sample; Aspirin (3.858min), Ramipril (4.211min). and Atorvastatin calcium (6.521min).**

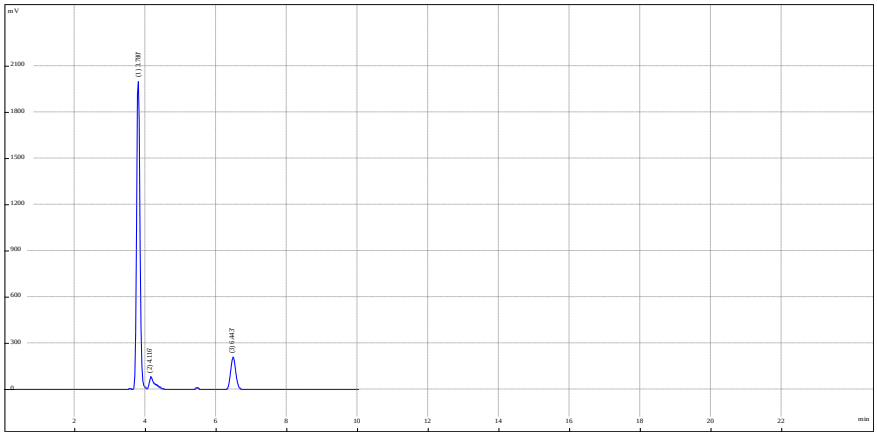


**Figure. 8 Chromatogram of Alkali treated sample; Aspirin (3.780min), Ramipril (4.116min). and Atorvastatin calcium (6.443min).**

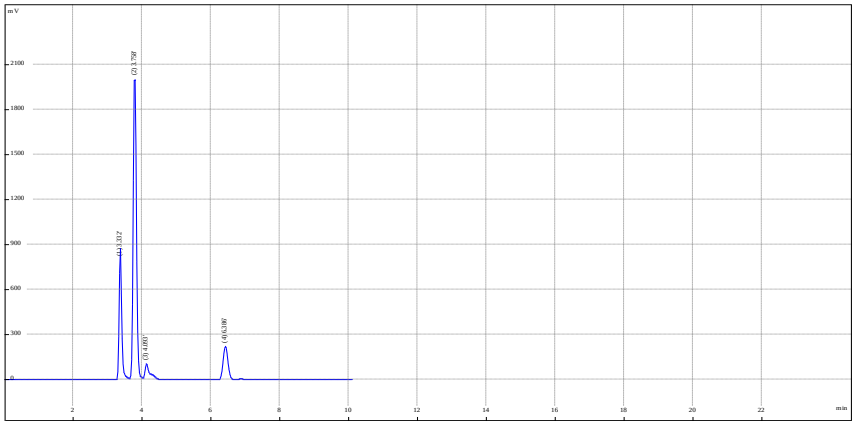


**Figure. 9 Chromatogram of Peroxide treated sample; Aspirin (3.758min), Ramipril (4.093min). and Atorvastatin calcium (6.386min).**

The chromatogram of peroxide treated sample showed an additional peak at RT of 3.332. The % degradation with respect to the untreated sample is calculated in Table 7 below:

**Table. 7 %degradation with respect to the untreated sample.**

| Name of the drug | Peroxide (H2O2) | Acid (HCl) | Base (NaOH) |
|---|---|---|---|
| Aspirin | 4.87 | 4.76 | 4.86 |
| Ramipril | 14.70 | 12.19 | 12.31 |
| Atorvastatin calcium | 16.29 | 15.63 | 16.21 |

As the % degradation of all the three drugs is between 5-30% hence the method is termed to be stability-indicating.

## CONCLUSION

The developed RP-HPLC method is simple, sensitive, accurate, robust and stability-indicating. It can be used for the successful determination of Atorvastatin calcium, Ramipril and Aspirin simultaneously in a combined dosage form without any interference from the excipients and additives present in the dosage form. As the developed method has less flow rate as well as short run time of approximately 10min, the method proves to be economical and cheap in the sense of usage of mobile phase. The time of analysis is also reduced due to relatively short run time. The drugs are resistant to degradation, hence the method is termed to be a stability-indicating one.

## ACKNOWLEDGEMENT

It is with immense gratitude that expresses my benevolent thanks to my reverent teacher and guide, Dr. Ashok. P. Pingle, Director & Professor, Dept. of Pharmaceutical Chemistry, M.V.P. Samaj's College of Pharmacy, Nashik.

**REFERENCES**


1. Indian Pharmacopoeia 1996, Published by the Controller of Publications, Delhi, Government of India, Ministry of Health and Family Welfare: Volume I & II, pp. 69, 486,749, 1574.
2. Indian Pharmacopoeia 2007, Published by the Government of India, Ministry of Health and Family Welfare, the Indian Pharmacopoeia commission, New Delhi: Volume I & II, pp. 131,1036.
3. British Pharmacopoeia 2007, Published by the Stationary office, on behalf of the Medicine and Healthcare products.
4. Zaheer Z, Farooqui MN, Mangle AA, Nikalje AG. Stability-indicating high performance liquid chromatographic determination of Atorvastatin Calcium in Pharmaceutical Dosage form. Afr J Pharm Pharmacol, 2008; 2(10): 204-210.
5. Abdelattef HE. Spectrophotometric and Spectrofluorometric methods for the determination of Ramipril in its pure and dosage form. Spectrochim Acta A, 2001; 66: 701-706.
6. Krishnaiah V, Rami Reddy YV. Development and validation of HPLC method for the determination of Aspirin. J Chem Pharm Res, 2012; 4(3): 2349-2353.
7. Bernard S, Mathew M, Senthilkumar KL, Girija KN. A Validated Stability-indicating RP-HPLC method for the determination of Atorvastatin Calcium. International Journal of Drug Formulation and Research, 2013; 4(1): 9-16.
8. Panchal HJ, Suhagia BN, Patel NJ, Rathod IS, Patel BH. Simultaneous estimation of Atorvastatin Calcium, Ramipril and Aspirin in Capsule Dosage form by RP-LC, Chromatographia, 2009; 69: 91-95.
9. Patole SM, Potale LV, Khodke AS, Damle MC. A Validated HPLC method for analysis of Atorvastatin Calcium, Ramipril and Aspirin as the Bulk drug and in Combined Dosage forms. Int J Pharm Sci Rev Res, 2010; 4(3): 40-45.
10. Sharma R, Khanna S, Mishra GP. Development and Validation of RP-HPLC method for Simultaneous estimation of Ramipril, Aspirin and Atorvastatin in Pharmaceutical Preparations. E-Journal of Chemistry, 2012; 9(4): 2177-2184.
11. Dhaneshwar SS, Dhaneshwar SR, Deshpande P, Patil M. Development and Validation of a method for simultaneous densitometric estimation of Atorvastatin Calcium and Ezetimibe as the bulk drug and in tablet dosage form. Acta Chromatographica, 2007; 19: 141-148.

12. Deshpande PB, Shridharan G, Anandi L, Jadhav D, Damle MC, Gandhi SV. Validated method development for estimation of Atorvastatin Calcium and Fenofibrate in fixed dose combination by HPTLC. Pharma Review, 2009; 151-153.
13. Chaudhari BG, Patel NM, Shah PB, Modi KP. Development and Validation of a HPTLC method for the simultaneous estimation of Atorvastatin Calcium and Ezetimibe. Indian J Pharm Sci, 2006; 793-796.
14. Kowalczuk D, Pietra R, Hopka H. Development and Validation of an HPTLC – Densitometric method for the determination of ACE inhibitors. Chromatographia, 60: 245-249.
15. Panchal HJ, Suhagia BN, Patel NJ. Simultaneous HPTLC analysis of Atorvastatin Calcium, Ramipril and Aspirin in capsule dosage form. J Planar Chromatogr, 22: 265-271.
16. Patil PR, Rakesh SU, Dhabale PN.Simultaneous Estimation of Ramipril and Amlodipine by UV Spectrophotometric method. Research J Pharm Tech, 2009; 2(2): 304-307.
17. Baldha RG, Patel VB, Bapna M. Simultaneous spectrophotometric determination of Atorvastatin Calcium and Ezetimibe in tablet dosage form. Int J ChemTech Res, 2009; 1(2): 233-236.
18. Zambare YB, Karajgi SR, Simpi CC. Simultaneous estimation of Atorvastatin and Ramipril by First Derivative Spectrophotometric method. J Pharm Res, 2009; 2(5): 874-877.
19. ICH-Q2 (R1): Validation of Analytical Procedures: Text and Methodology, FDA, Vol 60, 1March; 1995; 11260.
20. ICH-Q1A (R2): Stability Testing of New Drug Substances and Products (Second Revision), FDA, Vol. 68; No. 225, 21 November 2003; 65717-18.