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# Simultaneous estimation of rosuvastatin and aspirin by RP-HPLC in pharmaceutical formulations

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## ABSTRACT

We intends to develop RP-HPLC method by simultaneous determination with simple, rapid, greater sensitivity and faster elution. The work is to establish a stability-indicating HPLC method for simultaneous determination of Rosuvastatin and Aspirin in combined dosage form. The validatedmethod would be applicable in both formulation development and routine quality control analysis. The estimation of Aspirin and Rosuvastatin was done by RP-HPLC. The assay of Aspirin and Rosuvastatin was performed with tablets and the % assay was found to be 99.11 and 100.76 which shows that the method is useful for routine analysis. The linearity of Aspirin and Rosuvastatin was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity.
The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.5 for Aspirin and Rosuvastatin which shows that the method is precise.
The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.5 for Aspirin and Rosuvastatin which shows that the method is repeatable when performed in different days also.
The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.34% and 100.22% for Aspirin and Rosuvastatin. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility.
The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Aspirinwas found to be 3.00 and 9.98 and LOD and LOQ for Rosuvastatin was found to be 3.02 and 10.00.

**Keywords:** Rosuvastatin calcium; Aspirin; Simultaneous estimation; HPTLC.

## INTRODUCTION

Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials. Chemical composition is the entire picture (composition) of the material at the chemical scale and includes geometric features such as molecular morphologies and distributions of species within a sample as well as single dimensional features such as percent composition and species identity.[1]

To be effective and efficient, analyzing samples requires expertise in

1. The chemistry that can occur in a sample.
2. Analysis and sample handling methods for a wide variety of problems (the tools-of-the-trade).
3. Accuracy and precision of the method.
4. Proper data analysis and record keeping.

The pharmaceutical analysis comprises the procedures necessary to determine the “identity, strength, quality and purity” of such compounds. It also includes the analysis of raw material and intermediates during manufacturing process

of drugs.

### *High performance liquid chromatography*

High performance liquid chromatography is a very sensitive analytical technique most widely used for quantitative and qualitative analysis of pharmaceuticals. The principle advantage of HPLC compared to classical column chromatography is improved resolution of the separated substance, faster separation times and the increased accuracy, precision and sensitivity[8].

### *Analytical parameters for validation*

Validation may be defined as a process involving confirmation or establishing by laboratory studies that a method/ system/ analyst gives accurate and reproducible result for intended analytical application in a proven and established range [10,11].

### *Validation parameters*

The parameters for method validation as defined by the ICH guidelines are summarized below.

### *Linearity*

It is the ability of the method to elicit test result that is directly proportional to analytic concentration within a given range. It is generally reported as variance of slope of regression line. It is determined by series of three to six injections of five of more standards.

### *Precision*

It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as % of relative standard deviation (% RSD). This involves

a) Repeatability
b) Reproducibility
c) Intermediate precision

**% RSD = 100 S/X**

Where,
S = Standard deviationX = Mean
It is determined at three levels.

### *Repeatability*

Precision of the method when repeated by the same analysts, same test method and under same set of laboratory conditions (reagent, equipments), within a short interval of time, the only difference being the sample.

### *Reproducibility*

When the subject method is carried out by different analysts in different laboratoriesusing different equipments, reagents and laboratory settings and on different days of variability of analytical results as function of analyst, day to day, laboratory to laboratory, equipment to equipment etc., using the samples from same homogenous batch.

### *Intermediate precision*

It is determined by comparing the results of a method within the same laboratory butdifferent days, analysts, equipments and reagents.

### *Accuracy*

Defined as the closeness of agreement between the actual (true) value and mean analytical value obtained by applying the test method a number of times. Accuracy is acceptable if the difference between the true value and mean measured value does not exceed the RSD values obtained for repeatability of the method.
One can design experiments for recovery of known or spiked samples in presence of expected matrix, keeping the matrix constant. Accuracy can also be determined by comparing the results with those obtained using an alternative method which has already been validated.

### *Limit of Detection (LOD)*

It is defined as the lowest concentration of an analyte in a sample that can be detected but not quantified. LOD is expressed as a concentration at a specified signal to noise ratio. The LOD will not only depend on the procedure of analysis but also on the type of instrument.
In chromatography, detection limit is the injected amount that results in a peak with a height at least twice or thrice as high as baseline noise level.

**S/N = 2/1 or 3/1**

### *Limit of Quantification (LOQ)*

It is defined as lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy and reliability by a given method under stated experimental conditions. The procedure usually followed is to analyze samples containing decreasing known quantity of the analyte and determine the Lowest level at which acceptable level of accuracy is attained.
LOQ is expressed as a concentration at a specified signal to noise ratio. In chromatography, limit of quantification is the injected amount that results in a peak with a height, ten times as high as base line noise level.

**S/N = 10/1**

### *Ruggedness*

Degree of reproducibility of test results obtained by analyzing the same sample under variety of normal test conditions such as different analysts, instruments, days, reagents, column and TLC plates.

### *Robustness*

It is the measure of the capacity of the analytical method to remain unaffected by small but deliberate variation in procedure. It provides an indication about variability of the method during normal laboratory conditions.

## *Drug profile*

### *Rosuvastatin*

Rosuvastatin is an antilipemic agent that competitively inhibits hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase. HMG-CoA reducuase catalyzes theconversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis. Rosuvastatin belongs to a class of medications called statins and is used to reduce plasma cholesterol levels and prevent cardiovascular disease.

## MATERIALS AND METHODS

**Table 1: Instruments used**

| SL.No | Instrument | Model |
|---|---|---|
| 1 | HPLC | WATERS, software: Empower, 2695 separation module.2487 UV detector. |
| 2 | UV/VIS spectrophotometer | LABINDIA UV 3000$^+$ |
| 3 | pH meter | Adwa – AD 1020 |
| 4 | Weighing machine | Afcoset ER-200A |
| 5 | Pipettes and Burettes | Borosil |
| 6 | Beakers | Borosil |

**Table 2: Chemicals used**

| SL.No | Chemical | Brand |
|---|---|---|
| 1 | Aspirin | Supplied by Pharmatrain |
| 2 | Rosuvastatin | Supplied by Pharmatrain |
| 3 | $KH_2PO_4$ | FINAR chemical LTD |
| 3 | Ortho phosphoric acid | FINAR chemical LTD |
| 4 | Water and Methanol for HPLC | Standard solutions Ltd |
| 5 | Acetonitrile for HPLC | Standard solutions Ltd |
| 6 | HCl, $H_2O_2$, NaOH | MERCK |

### *HPLC method development*

### *Wave length selection*

UV spectrum of 10µg/ml Aspirin and Rosuvastin diluents (mobile phase composition) was recorded by scanning in the range of 200nm to 400nm. From the UV spectrum wavelength selected as 240nm. At this wavelength, both the drugs show good absorbed.

### *Optimized chromatographic conditions*

Equipment High performance liquid chromatography equipped withAuto Sampler and uv Detector

Mobile phase : ACN: OPA 3 (80:20)
Column : Inertsil ODS (4.6 x250 mm, 5□m)
Flow rate : 1mL per min
Wavelength : 240 nm Injection volume: 20□l Column oven: Ambient
Run time : 10min

### *Preparation of buffer and mobile phase*

### *Preparation of OPA Buffer*

Take accurately 1ml of ortho phosphoric Acid is dissolved in 1000ml of Hplc water thenadjust the pH 3 with NAOH.

### *Preparation of mobile phase*

Mix a mixture of above buffer 200 mL (20%) and 800 mL of Acetonitrile HPLC (80%) degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

### *Diluent Preparation*

Use the Mobile phase as Diluent.

### *Validation parameters*

### *Assay*

### *Standard Solution Preparation*

Accurately weigh and transfer 75 & 10mg of Aspirin & Rosuvastin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6 ml of Aspirin & Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

### *Sample Solution Preparation*

Accurately weigh and transfer equivalent to 75 & 10mg of Aspirin & Rosuvastin sample (Tablet powder) into a 10ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock solution). Further pipette 0.6ml of Aspirin & Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

### *Procedure*

Inject 20 □L of the standard, sample into the chromatographic system and measure the areas for the Aspirin & Rosuvastin peaks and calculate the %Assay by using the formulae.

### *Linearity*

### *Preparation of stock solution*

Accurately weigh and transfer 75 & 10mg of Aspirin & Rosuvastin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution) Further pipette 0.6 ml of Aspirin & Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Preparation of Level – I (150ppm & 20ppm of Aspirin & Rosuvastatin):**

0.2ml of stock solution has taken in 10ml of volumetric flask dilute up to the markwith Diluents.

**Preparation of Level – II (300ppm & 40ppm of Aspirin &**

**Rosuvastatin):**
0.4ml of stock solution has taken in 10ml of volumetric flask dilute up to the markwith Diluents.
**Preparation of Level – III (450ppm & 60ppm of Aspirin & Rosuvastatin):**
0.6ml of stock solution has taken in 10ml of volumetric flask dilute up to the markwith Diluents.
**Preparation of Level – IV (600ppm & 80ppm of Aspirin & Rosuvastatin):**
0.8ml of stock solution has taken in 10ml of volumetric flask dilute up to the markwith Diluents.
**Preparation of Level – V (750ppm & 100ppm of Aspirin & Rosuvastatin):**
1.0ml of stock solution has taken in 10ml of volumetric flask dilute up to the markwith Diluents.

### *Procedure*
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.

### *Precision*
### *Preparation of stock solution*
Accurately weigh and transfer 75 & 10mg of Aspirin & Rosuvastin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Aspirin & Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

### *Procedure*
The standard solution was injected for five times and measured the area for all five injectionsin HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

### *Intermediate precision/ruggedness*
To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day within the laboratory.

### *Preparation of stock solution*
Accurately weigh and transfer 75 & 10mg of Aspirin & Rosuvastin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Aspirin & Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

### *Procedure*
The standard solution was injected for six times and measured the area for all six injections inHPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

### *Accuracy*
For accuracy determination, three different concentrations were prepared separately i.e. 50%, 100% and 150% for the analyte and chromatograms are recorded for the same.

### *Preparation of Standard stock solution*
Accurately weigh and transfer 75 & 10mg of Aspirin & Rosuvastin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Aspirin & Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

### *Preparation Sample solutions*
**For preparation of 50% solution (With respect to target Assay concentration):** Accurately weigh and transfer 37.5 & 5mg of Aspirin & Rosuvastin working standardinto a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completelyand make volume up to the mark with the same solvent. (Stock solution). Further pipette 0.6 ml of Aspirin & Rosuvastin of the above stock solution into a 10mlvolumetric flask and dilute up to the mark with diluent.
**For preparation of 100% solution (With respect to target Assay concentration):** Accurately weigh and transfer 75 & 10mg of Aspirin & Rosuvastin working standard intoa 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely andmake volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Aspirin & Rosuvastin of the above stock solution into a 10mlvolumetric flask and dilute up to the mark with diluent.
**For preparation of 150% solution (With respect to target Assay concentration):** Accurately weigh and transfer 112.5 & 15mg of Aspirin & Rosuvastin working standardinto a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completelyand make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Aspirin & Rosuvastin of the above stock solution into a 10mlvolumetric flask and dilute up to the mark with diluent.

### *Procedure*
Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150%solutions. Calculate the Amount found and Amount added for Aspirin & Rosuvastin and calculatethe individual recovery and mean recovery values.

### *Limit of detection*
### *Preparation of Aspirin solution: Preparation of 450µg/ml solution*
Accurately weigh and transfer 75mg of Aspirin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Aspirin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

### *Preparation of 0.69 µg/ml solution*

Accurately weigh and transfer 75 mg of Aspirin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Aspirin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 0.24ml of the above stock solution into a 100ml volumetric flask anddilute up to the mark with diluent.
Further pipette 0.65ml of the above stock solution into a 100ml volumetric flask anddilute up to the mark with diluent.

### *Preparation of Rosuvastatin solution*
### *Preparation of 60 µg/ml solution*

Accurately weigh and transfer 10mg of Rosuvastin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

### *Preparation of 0.63 µg/ml solution*

Accurately weigh and transfer 10mg of Rosuvastin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 1ml of the above stock solution into a 10ml volumetric flask and dilute upto the mark with diluents
Further pipette 1.1ml of the above stock solution into a 100ml volumetric flask and dilute up to the mark with diluent.

### *Limit of quantification*
### *Preparation of Aspirin solution: Preparation of 450 µg/ml solution*

Accurately weigh and transfer 75 of Aspirin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Aspirin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

### *Preparation of 2.31 µg/ml solution*

Accurately weigh and transfer 75 of Aspirin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 0.6 ml of Aspirin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 1 ml of the above stock solution into a 100ml volumetric flask and dilute up to the mark with diluent.

### *Preparation of Rosuvastatin solution*
### *Preparation of 60 µg/ml solution*

Accurately weigh and transfer 10mg of Rosuvastin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

### *Preparation of 2.10 µg/ml solution*

Accurately weigh and transfer 10mg of Rosuvastin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 4ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
Further pipette 0.88ml of the above stock solution into a 100ml volumetric flask anddilute up to the mark with diluent.

### *Robustness*

As part of the Robustness, deliberate change in the Flow rate, Mobile Phasecomposition, Temperature Variation was made to evaluate the impact on the method.

a. The flow rate was varied at 0.9 ml/min to 1.1ml/min. Standard solution 450 ppm & 60 ppm of Aspirin & Rosuvastin prepared and analysed using the varied flow rates along with method flow rate.

b. Standard solution 450 µg/ml & 60µg/ml of Aspirin & Rosuvastin was prepared andanalysed using the varied Mobile phase composition along with the actual mobile phase composition in the method.

### *Degradation studies*

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Aspirin and Rosuvastin using the proposed method.

### *Preparation of stock*

Accurately weigh and transfer 75 & 10mg of Aspirin & Rosuvastin working standard into a 10mL clean dry volumetric flask add Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.6 ml of Aspirin & Rosuvastin of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

### *Hydrolytic degradation under acidic condition*

Pipette 0.6 ml of above solution into a 10ml volumetric flask

and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 micronssyringe filters and place in vials.

### *Hydrolytic degradation under alkaline condition*

Pipette 0.6 ml of above solution into a 10ml volumetric flask into and add 3 ml of 0.1N NaOH was added in 10 ml of volumetric flask. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and place in vials.

### *Thermal induced degradation*

Aspirin and Rosuvastin sample was taken in petridish and kept in Hot air oven at $110^0$ C for 24 hours. Then the sample was taken and diluted with diluents and injected into UPLC and analysed.

### *Oxidative degradation*

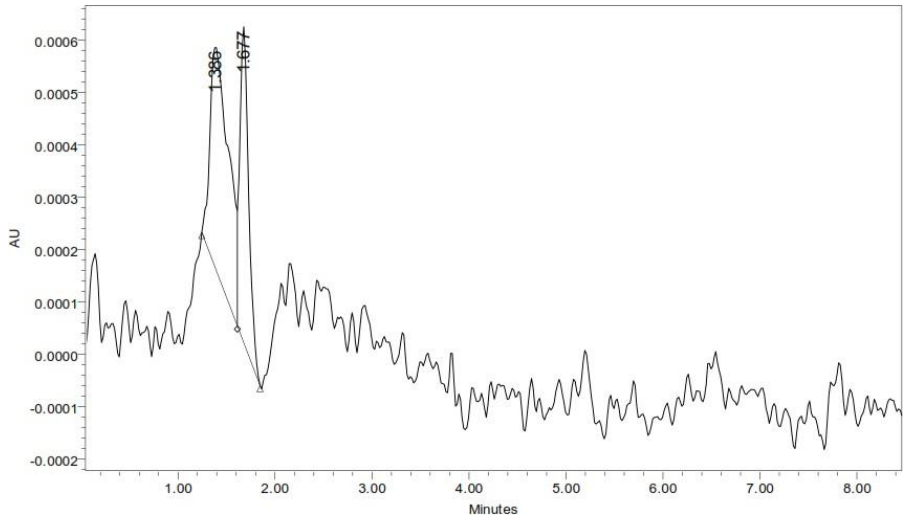
Pipette 0.6 ml above stock solution-2 into a 10ml volumetric flask, 1 ml of 3% w/v of hydrogen peroxide added and the volume was made up to the mark with diluent . The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and place in vials.

## RESULTS AND DISCUSSION

### *Chromatographic conditions*

Column : Inspire ODS (4.6 x250 mm, 5□m)
Mobile phase ratio : ACN: Water (60:40)
Detection wavelength : 220 nm
Flow rate : 1 ml/min
Injection volume : 10µl

The trial shows no good peak separation, so more trials was required for obtaining peaks




### *System suitability*

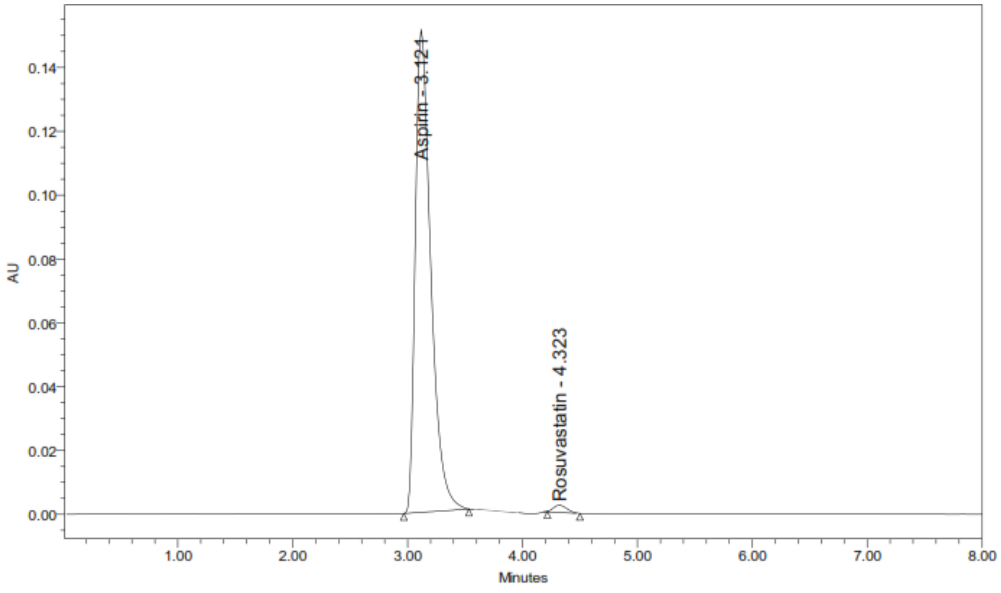



**Fig 2: Chromatogram for system suitability**

**Table 3: Results of system suitability parameters**

| S.No | Name | RT(min) | Area (µVsec) | Height(µV) | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|---|
| 1 | Aspirin | 3.121 | 1037570 | 151488 | | 1.15 | 2597.80 |
| 2 | Rosuvastatin | 4.323 | 171037 | 2178 | 5.16 | 1.27 | 6104.23 |

- Resolution between two drugs must be not less than 2.
- Theoretical plates must be not less than 2000.
- Tailing factor must be not more than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

## *Validation parameters*

### *Assay*

Standard and sample solution injected as described under experimental work. Thecorresponding chromatograms and results are shown below.

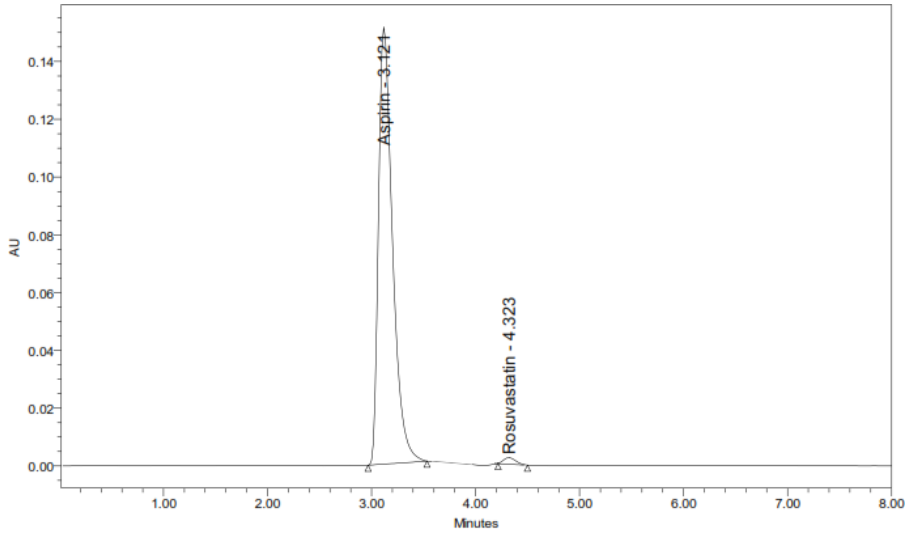



**Fig 3: Chromatogram for Standard**

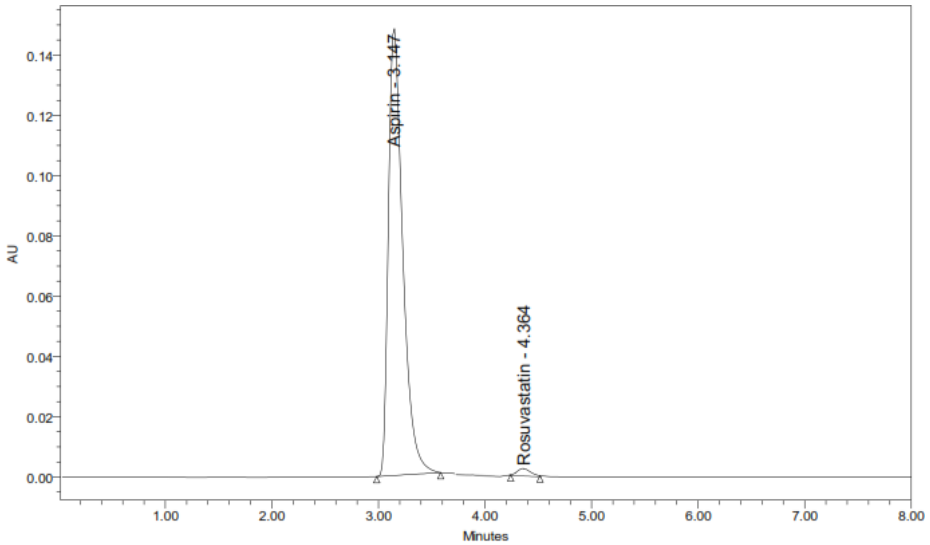



**Fig 4: Chromatogram for Sample**

**Table 4: Results of Assay for Aspirin and Rosuvastatin**

| | Label Claim (mg) | % Assay |
|---|---|---|
| **Aspirin** | 75 | 99.11 |
| **Rosuvastatin** | 10 | 100.76 |

## *Linearity*

The linearity range was found to lie from 150µg/ml to 750µg/ml of Aspirin, 20µg/ ml to 100µg/ml 0f Rosuvastatin and chromatograms are shown below.

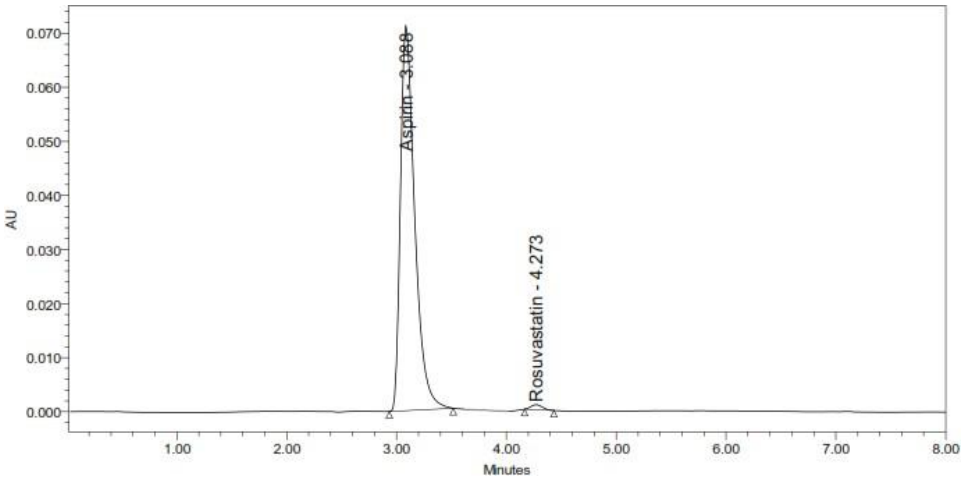



**Fig 5: Chromatogram for linearity**

**Table 5: Area of different concentration of Aspirin and Rosuvastatin**

| S. No | Aspirin | | Rosuvastatin | |
|---|---|---|---|---|
| | Concentration (µg/ml) | Area | Concentration (µg/ml) | Area |
| 1 | 150 | 360303 | 20 | 59045 |
| 2 | 300 | 692178 | 40 | 114337 |
| 3 | 450 | 1019720 | 60 | 168147 |
| 4 | 600 | 1333531 | 80 | 220495 |
| 5 | 750 | 1679118 | 100 | 276005 |

**Table 6: Analytical performance parameters of Aspirin and Rosuvastatin**

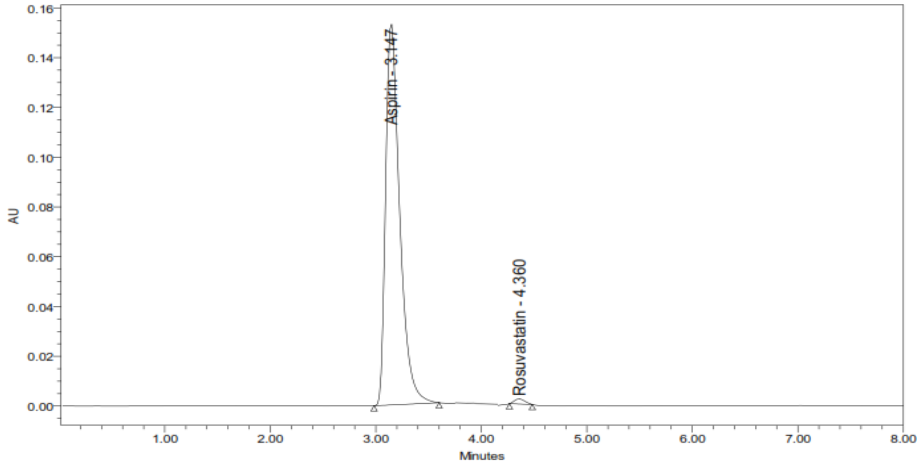
| Parameters | Aspirin | Rosuvastatin |
|---|---|---|
| Slope (m) | 2168 | 2700.4 |
| Intercept (c) | 33275 | 5582.4 |
| Correlation coefficient ($R^2$) | 0.999 | 0.999 |

*Correlation coefficient ($R^2$) should not be less than 0.999*

*The correlation coefficient obtained was 0.999 which is in the acceptance limit.*

## *Precision*

Precision of the method was carried out for both sample solutions as described underexperimental work. The corresponding chromatograms and results are shown below.



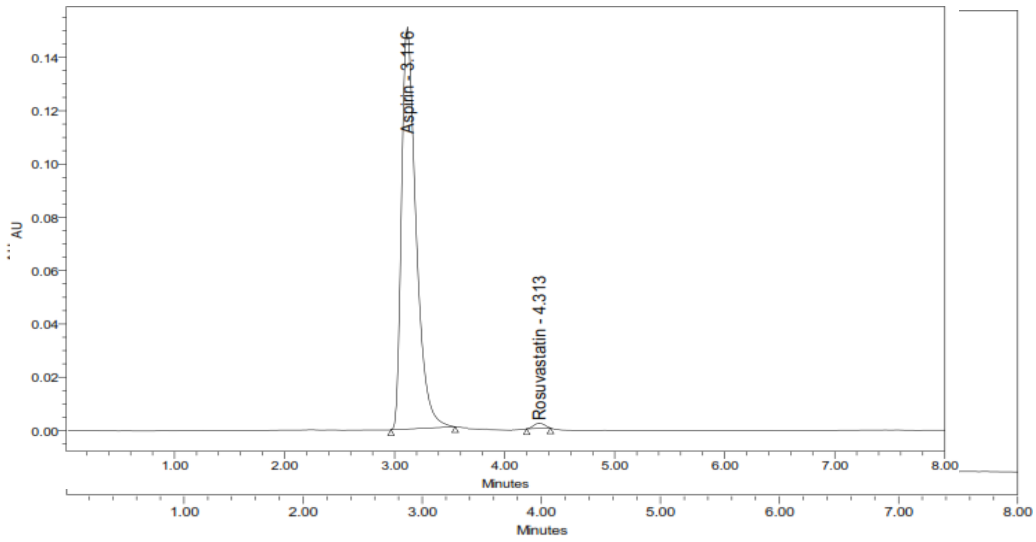



**Fig 6: Chromatogram for Precision**

**Table 7: Results of Precision for Aspirin**

| **Injection** | **Area** |
|---|---|
| Injection-1 | 1023945 |
| Injection-2 | 1027796 |
| Injection-3 | 1026845 |
| Injection-4 | 1036375 |
| Injection-5 | 1020865 |
| **Average** | 1027165.2 |
| **Standard Deviation** | 5817.7 |
| **%RSD** | 0.8 |

**Table 8: Results of Precision for Rosuvastatin**

| **Injection** | **Area** |
|---|---|
| Injection-1 | 168040 |
| Injection-2 | 167914 |
| Injection-3 | 170372 |
| Injection-4 | 175848 |
| Injection-5 | 166068 |
| **Average** | 169648.2 |
| **Standard Deviation** | 3787.4 |
| **%RSD** | 0.5 |

*%RSD for sample should be NMT 2*
*The %RSD for the standard solution is below 1, which is within the limits hencemethod is precise.*

### *Intermediate precision (ruggedness)*

There was no significant change in assay content and system suitability parameters atdifferent conditions of ruggedness like day to day and system to system variation.

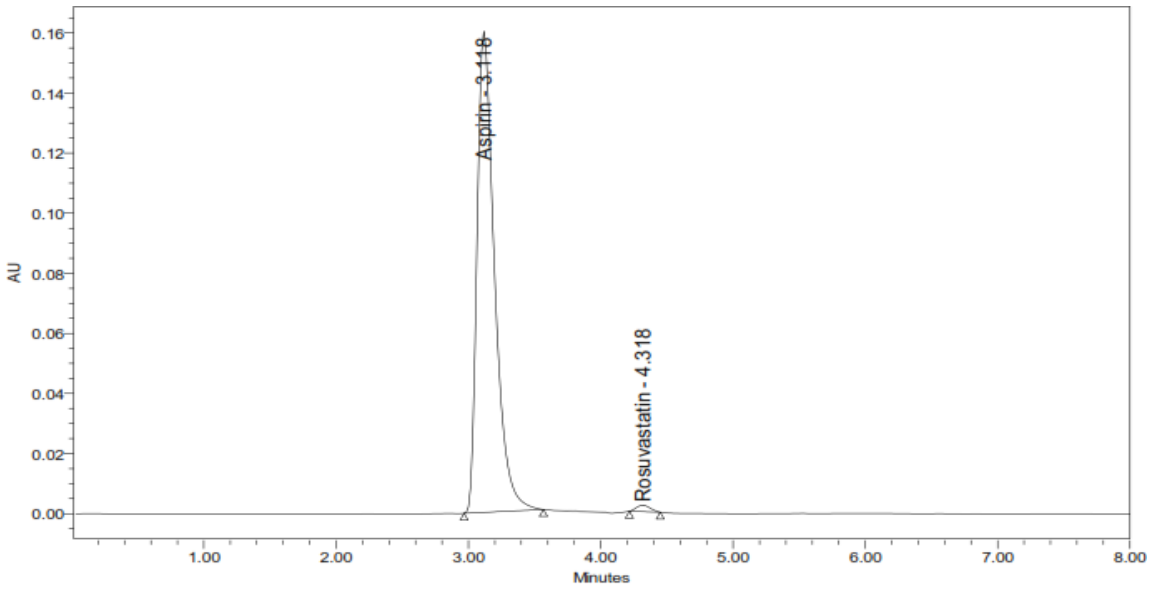



**Fig 7: Chromatogram for ID Precision -1**

**Table 9: Results of Intermediate precision for Aspirin**

| **Injection** | **Area** |
|---|---|
| Injection-1 | 1003903 |
| Injection-2 | 1018214 |
| Injection-3 | 1012117 |
| Injection-4 | 1018518 |
| Injection-5 | 1009168 |
| Injection-6 | 1020368 |
| **Average** | 1013714.4 |
| **Standard Deviation** | 6435.6 |
| **%RSD** | 0.6 |

**Table 10: Results of Intermediate precision for Rosuvastatin**

| **Injection** | **Area** |
|---|---|
| Injection-1 | 164423 |
| Injection-2 | 165485 |
| Injection-3 | 166719 |
| Injection-4 | 165469 |
| Injection-5 | 166045 |
| Injection-6 | 165226 |
| **Average** | 165561.0 |
| **Standard Deviation** | 774.2 |
| **%RSD** | 0.5 |

*%RSD of five different sample solutions should not more than 2*
*The %RSD obtained is within the limit, hence the method is rugged.*

### *Accuracy*

Sample solutions at different concentrations (50%, 100%, and 150%) were prepared andthe % recovery was calculated.

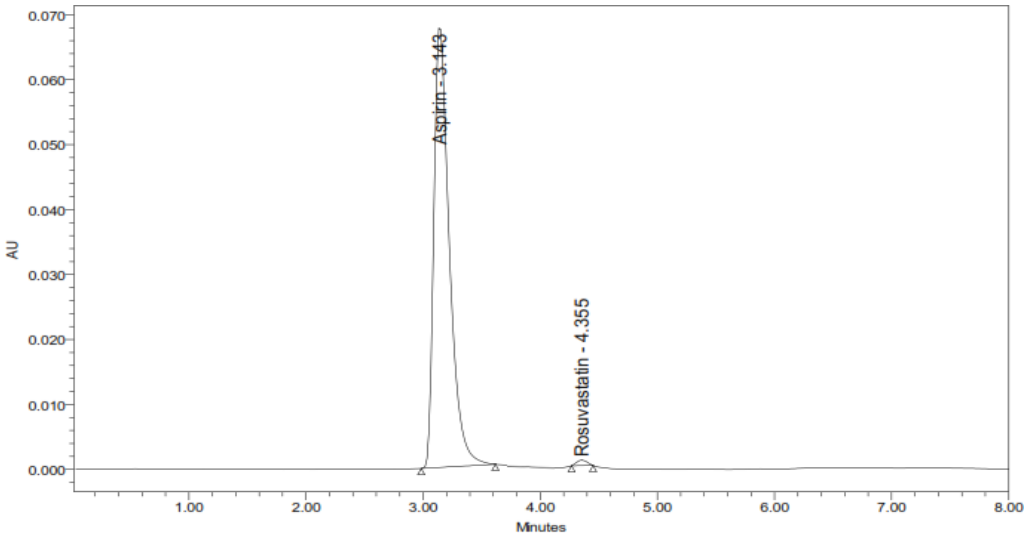



**Fig 8: Chromatogram for Accuracy 50%**

**Table 11: Accuracy (recovery) data for Aspirin**

| %Concentration(at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 526473 | 37.5 | 37.51 | 100.2% | 100.34% |
| 100% | 1011675 | 75 | 75.64 | 100.85 | |
| 150% | 1481480 | 112.5 | 112.64 | 100.15 | |

**Average of three determinations*

**Table 12: Accuracy (recovery) data for Rosuvastatin**

| %Concentration(at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| 50% | 620862 | 5 | 4.98 | 99.62% | 100.22% |
| 100% | 1194715 | 10 | 10.06 | 100.59% | |
| 150% | 1798274 | 15 | 15.07 | 100.47% | |

**Average of three determinations*
*The percentage recovery was found to be within the limit (97-103%).*
*The results obtained for recovery at 50%, 100%, 150% are within the limits. Hencemethod is accurate.*

### *Limit of detection for aspirin and rosuvastatin*

The lowest concentration of the sample was prepared with respect to the base line noiseand measured the signal to noise ratio.

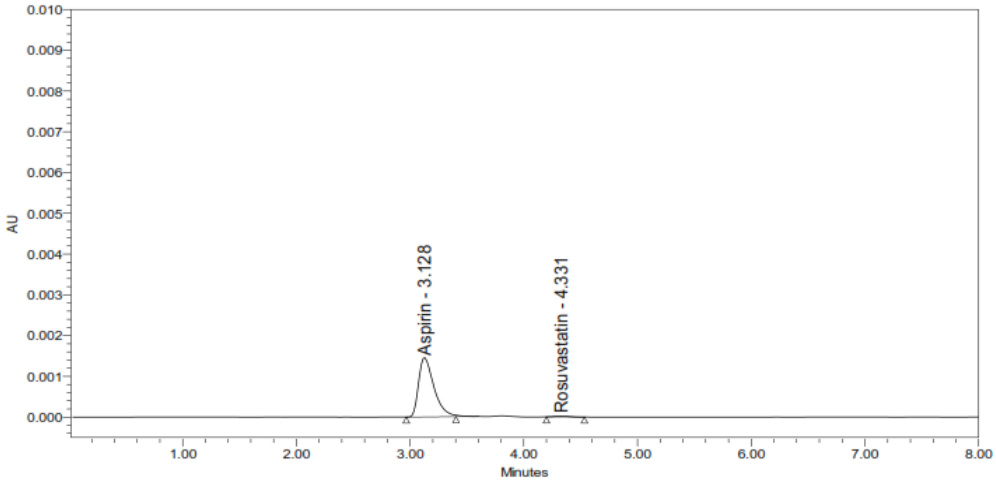



**Fig 9: Chromatogram of Aspirin, Rosuvastatin showing LOD**

**Table 13: Results of LOD**

| Drug name | Baseline noise(µV) | Signal obtained (µV) | S/N ratio |
|---|---|---|---|
| Aspirin | 66 | 198 | 3.00 |
| Rosuvastatin | 66 | 199 | 3.02 |

*Signal to noise ratio shall be 3 for LOD solution*
*The result obtained is within the limit.*

## *LIMIT OF QUANTIFICATION FOR ASPIRIN AND ROSUVASTATIN*

The lowest concentration of the sample was prepared with respect to the base line noiseand measured the signal to noise ratio.

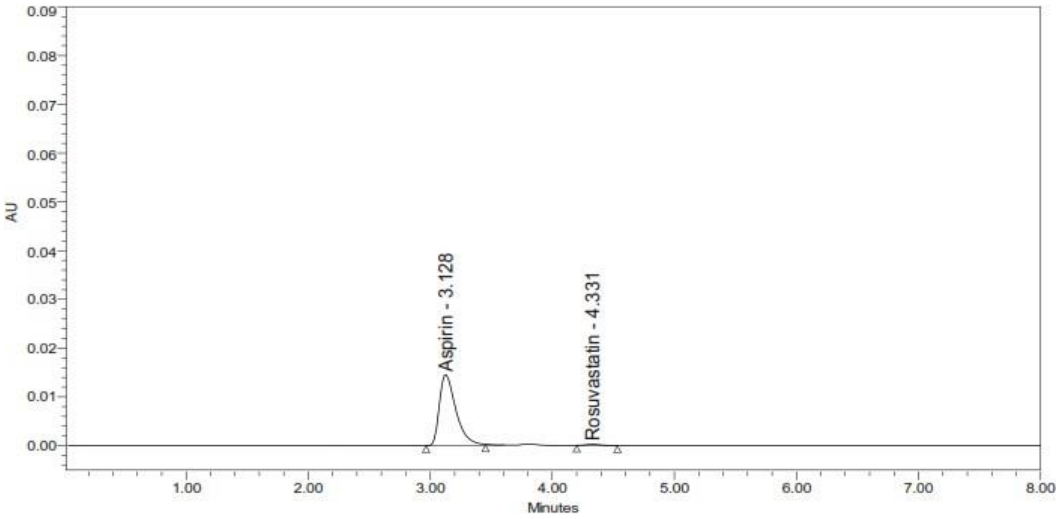



**Fig 10: Chromatogram of Aspirin, Rosuvastatin showing LOQ**

**Table 14: Results of LOQ**

| Drug name | Baseline noise(µV) | Signal obtained (µV) | S/N ratio |
|---|---|---|---|
| Aspirin | 66 | 659 | 9.98 |
| Rosuvastatin | 66 | 660 | 10.00 |

*Signal to noise ratio shall be 10 for LOQ solution*
*The result obtained is within the limit.*

## *Robustness*

The standard and samples of Aspirin and Rosuvastatin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

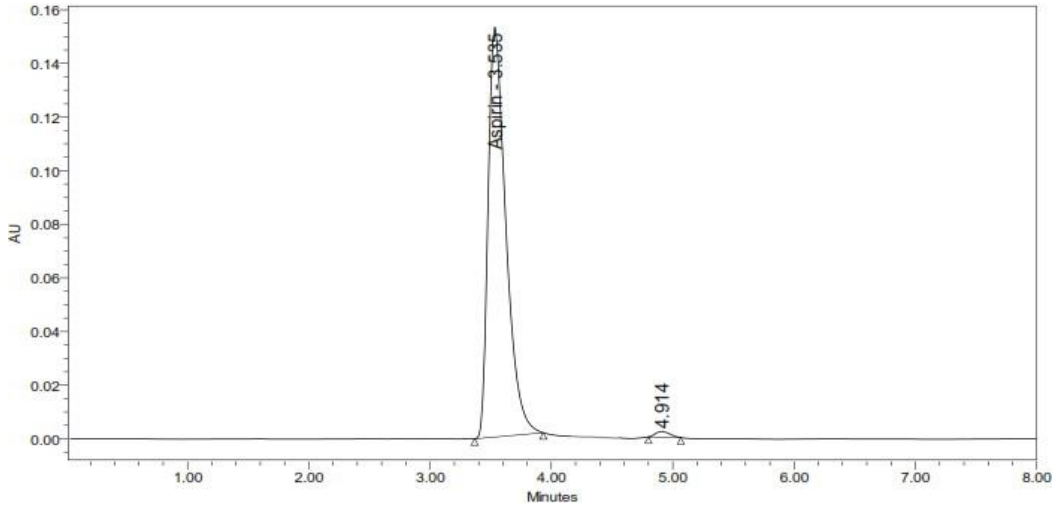



**Fig 11: Chromatogram showing less flow**

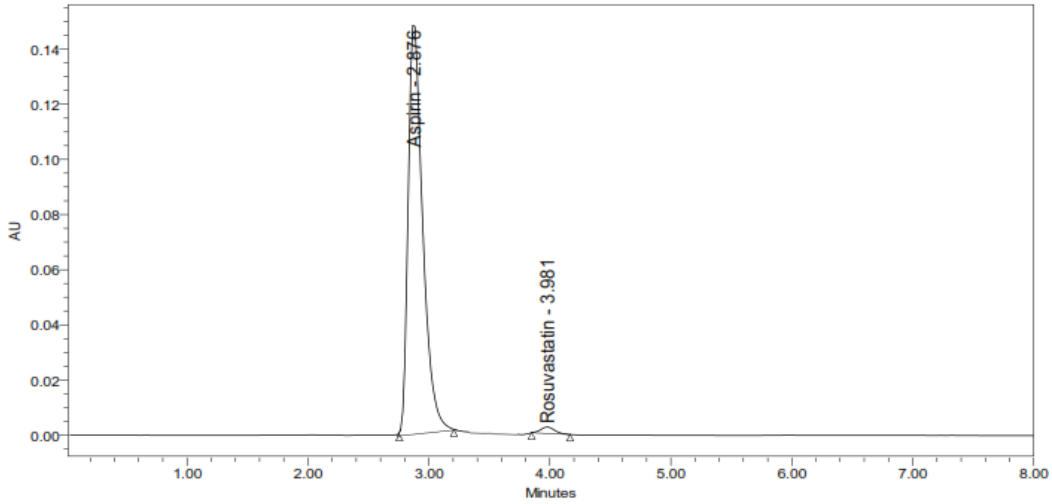



**Fig 12: Chromatogram showing more flow Variation of mobile phase organic composition**

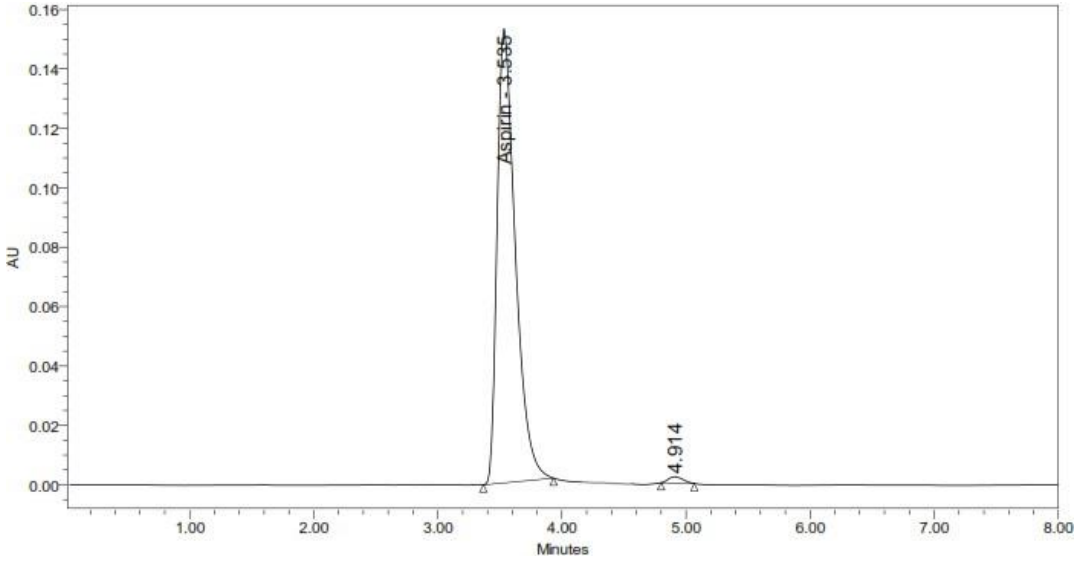



**Fig 13: Chromatogram showing less organic composition**

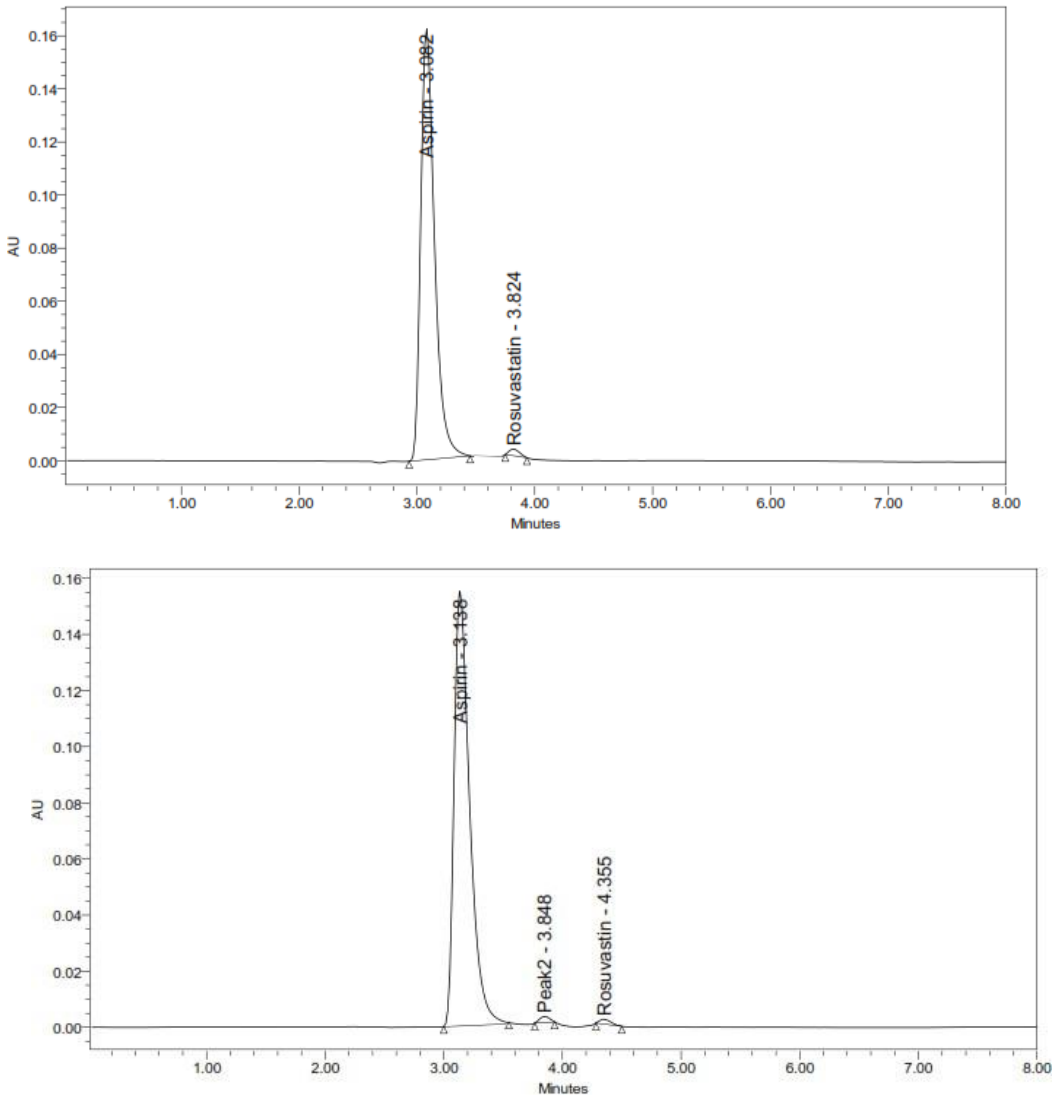



**Fig 14: Chromatogram showing more organic composition**

**Table 15: Results for variation in flow for Aspirin**

| S. No | Flow Rate (ml/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Plate Count |
| 1 | 0.9 | 2680.7 | 1.3 |
| 2 | 1 | 2524.84 | 1.3 |
| 3 | 1.1 | 2124.4 | 1.3 |

**Table 16: Results for variation in flow for Rosuvastatin**

| S. No | Flow Rate (ml/min) | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Tailing |
| 1 | 0.9 | 3200.8 | 1.3 |
| 2 | 1 | 3177.99 | 1.4 |
| 3 | 1.1 | 2973.7 | 1.4 |

** Results for actual flow (1.0ml/min) have been considered from Assay standard.*

**Table 17: Results for variation in mobile phase composition for Aspirin**

| S. No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Taling |
| 1 | 10% less | 2573.8 | 1.3 |
| 2 | *Actual | 2524.84 | 1.3 |
| 3 | 10% more | 2124.4 | 1.3 |

**Table 18: Results for variation in mobile phase composition for Rosuvastatin**

| S. No | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|
| | | USP Plate Count | USP Taling |
| 1 | 10% less | 3579.6 | 1.3 |
| 2 | *Actual | 3177.99 | 1.4 |
| 3 | 10% more | 2973.7 | 1.4 |

***** *Results for actual Mobile phase composition have been considered from Accuracystandard.*

The Retention time, USP plate count, USP tailing factor obtained for change of flow rate, variation in mobile phase was found to be within the acceptance criteria. Hence the method is robust.

## *Degradation studies*

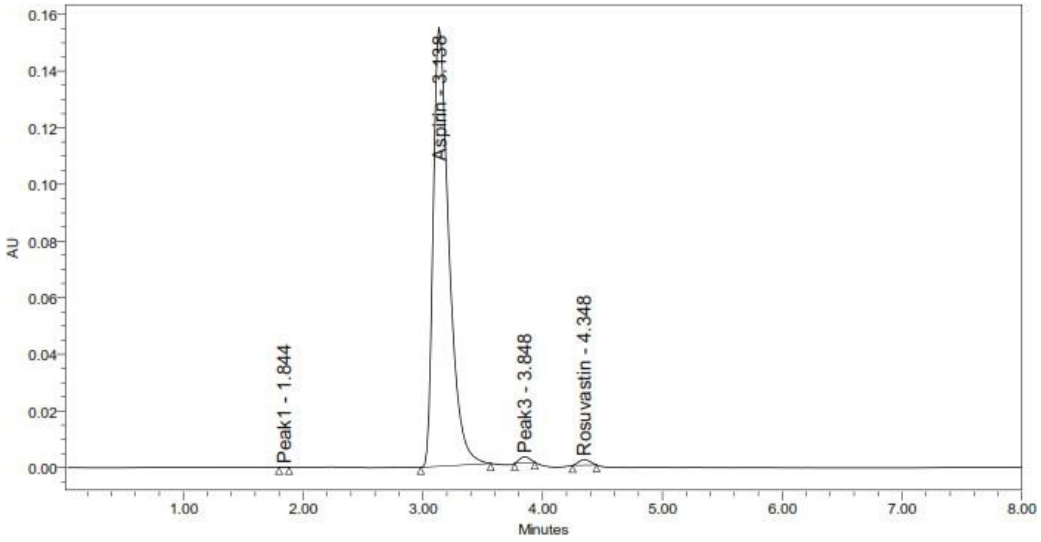



**Fig 16: Chromatogram showing Base**

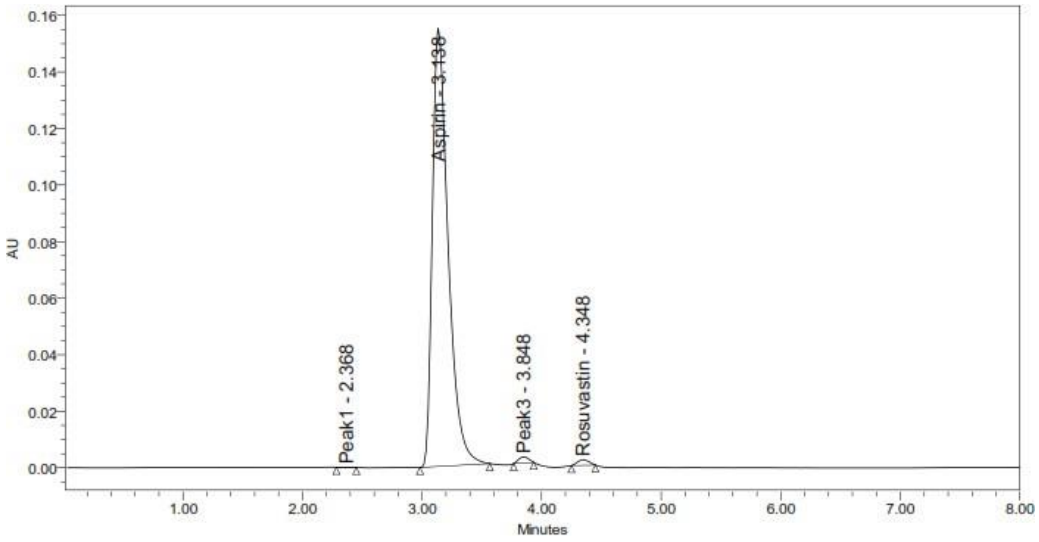



**Fig 17: Chromatogram showing Peroxide**

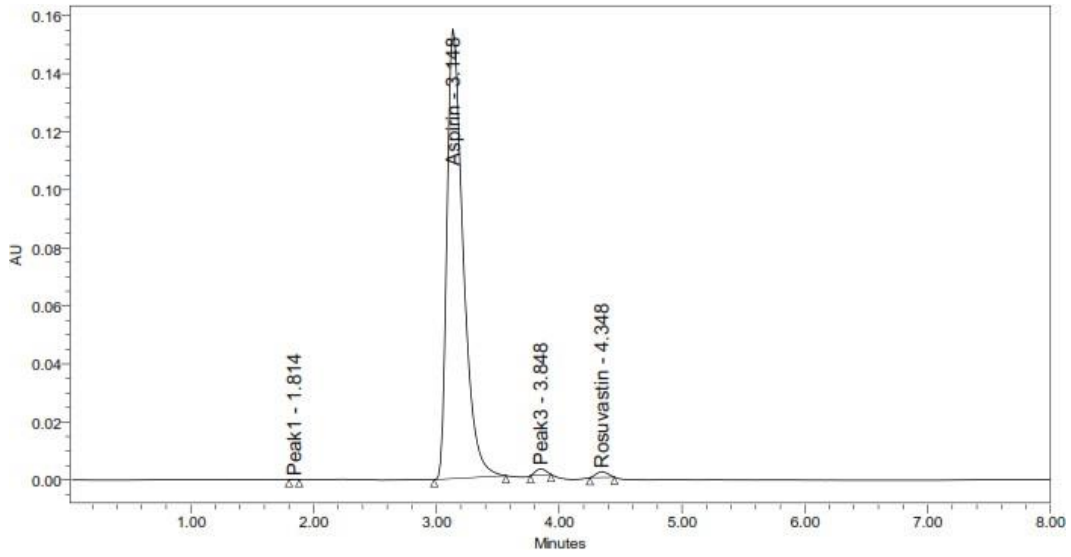



**Fig 18: Chromatogram showing Thermal**

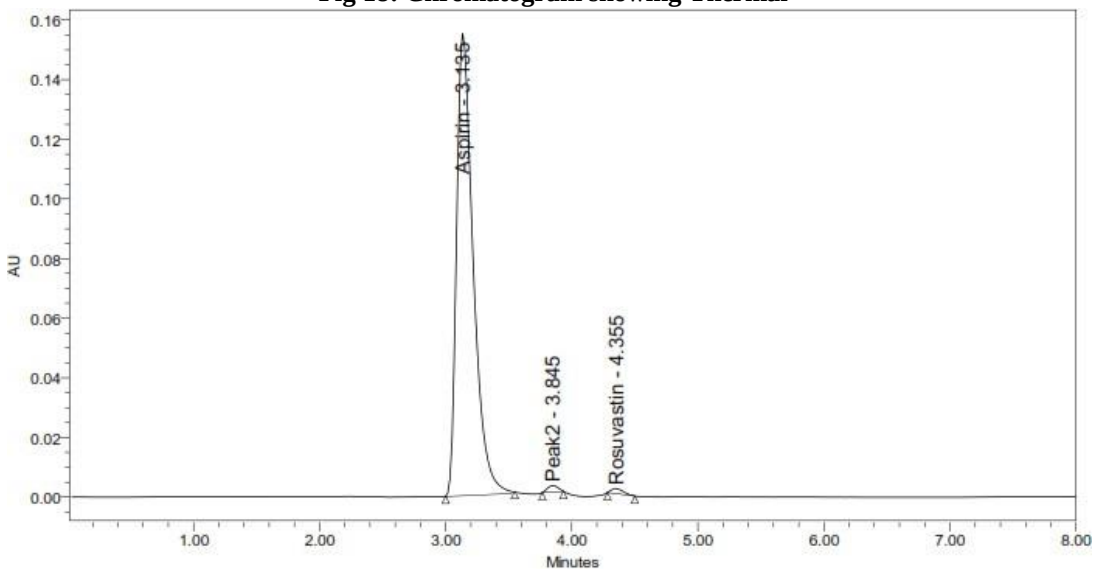



**Fig 19: Chromatogram showing Photo**

**Table 19: Results for variation in mobile phase composition for Rosuvastatin**

| Parameters | Aspirin | | Rosuvastatin | |
|---|---|---|---|---|
| | Area | %Degradation | Area | %Degradation |
| Standard | 1037570 | | 171037 | |
| Acid | 937570 | 9.64 | 157031 | 8.19 |
| Base | 920335 | 11.30 | 152390 | 10.90 |
| Peroxide | 966845 | 6.82 | 152390 | 10.90 |
| Thermal | 1019720 | 1.72 | 158147 | 7.54 |
| Photo | 988518 | 4.73 | 156045 | 8.77 |

## SUMMARY AND CONCLUSION

The estimation of Aspirin and Rosuvastatin was done by RP-HPLC. The assay of Aspirin and Rosuvastatin was performed with tablets and the % assay was found to be 99.11 and 100.76 which shows that the method is useful for routine analysis. The linearity of Aspirin and Rosuvastatin was found to be linear with a correlation coefficient of 0.999 and 0.999, which shows that the method is capable of producing good sensitivity.

The acceptance criteria of precision is RSD should be not more than 2.0% and the method show precision 0.8 and 0.5 for Aspirin and Rosuvastatin which shows that the method is precise.

The acceptance criteria of intermediate precision is RSD should be not more than 2.0% and the method show precision 0.6 and 0.5 for Aspirin and Rosuvastatin which shows that the method is repeatable when performed in different days also.

The accuracy limit is the percentage recovery should be in the range of 97.0% - 103.0%. The total recovery was found to be 100.34% and 100.22% for Aspirin and Rosuvastatin. The validation of developed method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy and reproducibility. The acceptance criteria for LOD and LOQ is 3 and 10.The LOD and LOQ for Aspirinwas found to be 3.00 and 9.98 and LOD and LOQ for Rosuvastatin was found to be 3.02 and 10.00.

The robustness limit for mobile phase variation and flow rate variation are well within the limit, which shows that the method is having good system suitability and precision under given set of conditions.

## REFERENCES


1. Beckett AH, Stenlake JB;text book of pharmaceutical chemistry. 4th ed,-part 2. CBS publishers and Distriburots, New Delhi,1998:278,307.
2. Douglas Skoog A, James Hollar F, Nieman T. A principles of instrumental analysis. Vol.110, 300. Singapore: Thomson Learning Inc; 1998. p. 5thed.
3. Sethi PD. Quantitative Analysis of Drugs in Pharmaceutical Formulation, 3rd ed,CBS Publishers and Distributors, 1997; 1-29, 50-64.
4. Mendham RC, Denny JD, Barnis M, Thomas JK, Vogel's Text Book of Quantitative. Chem Anal. 2003; 1;676:6thed.
5. Sharma BK. Instrumental method of Chemical Analysis. 24th ed. Vol. 46. Meerut: GOEL Publishing House; 2005. p. 68.
6. Chatwal GR, Anand KS. instrumental methods of chemical analysis. 5th ed Himalaya publishing House,M umbai; 2002. p. 2-149.
7. Kemp W. Organic. Spectroscopy. 1991;243:3rded.
8. Munson JW. Modern Methods of Pharmaceutical Analysis,Medical book distributors. Mumbai; 2001. p. 17-54.
9. Willard HH, Merritt LL. Dean J.A. and settle F.A:Instrumental Methods of analysis. 7th ed. CBS Publishers and New Delhi: Distributors; 1988. p. 436-9.
10. Synder KL, Krikland JJ, Glajch JL. Practical HPLC method development. 2nd ed. Wiley Interscience Publication; 1983. p. 1-10.
11. O'Brein. Bently and Drivers:textbook of pharmaceutical chemistry. 8th ed. oxford university press; 1985. p. 1-3.
12. Ewing GW. Instrumental Methods of chemical analysis. 2nd ed. MCGraw-Hill book company; 1960. p. 1.
13. International conference on harmonization "Validation of analytical procedures Methodology," 14. Fed Regist(v); 1996. p. 1-8.
14. Tripathi KD. Essentials of Medical pharmacology. 5th ed,Medical Publishes(P)Ltd,New Delhi; 2003. p. 169-77.
15. Divya. A, Raveendra Reddy. J, Ramalingam.P, Simultaneous Estimation of Aspirin and Rosuvastatin Calcium in Dosage forms by RP-HPLC and its Application to Forced Degradation Studies. J Pharm Res. 2012;5(11):5081-3.
16. Dumasiya MinaxiR, Bhatt KK, Patel B, Joshi NK. Development and validation of stability indicating Rphplc method for estimation of rosuvastatin calcium and Aspirin in combined dosage form an international journal of pharmaceutical sciences, pharma science monitor an international. J Pharm Sci, Vol-3. 2012;1(v)(4) [Suppl]:2421-39. http://www.pharmasm.com/pdf_files/20130119084954_minaxi_260.pdf
17. Solanki C, Patel N. Development and validation of Rp-HPLC method for simultaneous estimation of rosuvastatin calcium and Aspirin in capsule dosage form Chiragsinh Solanki and Nishitkumar Patel. Int J Pharm Bio Sci. 2012;3(3) 577-

585.https://www.researchgate.net/publication/288282582_Development_and_validation_of_RP-HPLC_method_for_simultaneous_estimation_of_rosuvastatin_calcium_and_aspirin_in_capsule_dosage_form

18. Kumaraswamy. Gandla1, R. Lalitha1. Konam Kishore2 and Rakam Gopikrishna, A validated RP-HPLC method for simultaneous estimation of Aspirin and rosuvastatin in tablet dosage form International Journal of Pharma And Chemical Research, Jul – Sep I 2015; 1(3):128-133. Available from: http://www.ijpacr.com/files/11-01-16/32610012016.pdf
19. Rashmi DS, Yadav H, Hinge M, patel A. Development and validation of analytical methods for simultaneous estimation of rosuvastatin, clopidogrel and Aspirin in pharmaceutical dosage FormDevelopment and validation of analytical methods for simultaneous estimation of rosuvastatin, clopidogrel and Aspirin in pharmaceutical dosage form. J Pharm sci bioscientific res. 2016;6(2):197-206. ISSN. 2271-3681.
20. Purkar AJ, Balap AR, Sathiyanarayanan L, Mahadik KR. Development and validation of Hptlc method for simultaneous determination of rosuvastatin calcium and Aspirin in its pure and pharmaceutical dosage form. Int J Pharm Pharm Sci. 2014;6(5):704-706. Available from: https://www.researchgate.net/publication/286743120_Development_and_validation_of_HPTLC_method_for_simultaneous_determination_of_rosuvastatin_calcium_and_aspirin_in_its_pure_and_pharmaceutical_dosage_form