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A NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF FOR THE SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN TABLET DOSAGE FORM

Madala Anuradha*

Academic Consultant, Division of Pharmacy, SV University, Tirupati, Andhra Pradesh.

Abstract:

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost HPLC method. It is successfully applied for the determination of Rosuvastatin and Fenofibrate in pharmaceutical preparations without the interferences of other constituents in the formulations. The optimum wavelength for detection was 256 nm at which better detector response for drug was obtained. The average retention time for Rosuvastatin and Fenofibrate was found to be 2.006 and 3.856 min respectively. System suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The calibration was linear in concentration range of 10 – 50 µg/ml and160-800 µg/ml with regression 0.999 and 0.999, for Rosuvastatin and Fenofibrate respectively. The low values of % R.S.D. indicate that method is precise and accurate. Sample to sample precision and accuracy were evaluated using six samples of same concentration and three samples each of three different concentrations respectively, which were prepared and analyzed on same day. The mean recovery values obtained were between 99 and 100.5 % confirming accuracy of the proposed method. There is no interference due to placebo at the retention time of analyte. Hence the method is specific.

Keywords: *Rosuvastatin , Fenofibrate, chromatographic, LOD, LOQ.*

Corresponding author:

Madala Anuradha*

Academic Consultant,
Division of Pharmacy,
SV University, Tirupati,
Andhra Pradesh.

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INTRODUCTION:

HPLC is a modern technique, it is a much more reliable and reproducible method for the standardization of both single and compound formulations. HPLC is a separation technique based on a stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption or ion exchange process, depending upon the size of stationary phase used. HPLC is one of the most versatile instruments used in the field of pharmaceutical analysis. It provides the following features:

- High resolving power
- Speedy separation
- Continuous monitoring of the column effluent
- Accurate quantitative measurement
- Repetitive and reproducible analysis using the same column
- Automation of the analytical procedure and data handling

Reversed phase HPLC (RP-HPLC) consists of a non-polar stationary phase and a moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe2SiCl, where R is a straight chain alkyl group such as C18H37 or C8H17. The retention time is therefore longer for molecules which are more non-polar in nature, allowing polar molecules to elute more readily. Retention time is increased by the addition of polar solvent to the mobile phase and decreased by the addition of more hydrophobic solvent.

RPLC operates on the principle of hydrophobic interactions which result from repulsive forces between a relatively polar solvent, the relatively non-polar analyte, and the non-polar stationary phase. The driving force in the binding of the analyte to the stationary phase is the decrease in the area of the non-polar segment of the analyte molecule exposed to the solvent. This hydrophobic effect is dominated by the decrease in free energy from entropy associated with the minimization of the ordered molecule-polar solvent interface. The hydrophobic effect is decreased by adding more non-polar solvent into the mobile phase.

The sample or solute is analyzed quantitatively by either peak height or peak area measurements. Peak areas are proportional to the amount of constant rate. Peak heights are proportional to the amount of material only when peak width are constant and are strongly affected by the sample injection techniques. Once the peak height or the peak areas are measured, there are five principle evaluation methods for quantifying the solute.

Basic Components of an HPLC System:

Pump System. Mobile phase pressures up to 6000 psi are necessary to achieve reasonable column elution times (~ minutes). Typical flow rates are 0.1 to 10 mL/ minute. Injection System. Used to introduce small samples (0.1 to 500 µL) into the carrier stream under high pressure. Reservoirs (Solvents). Multiple solvents are necessary for performing gradient elution's (i.e. changing the polarity of the mobile phase during a run). Chromatographic Column. Typically 10-30 cm in length containing a packing of 5-10 µm diameter. Many types of columns are available, depending on the type of liquid chromatography desired. Detector. Many types are available including UV, IR, refractive index, fluorescence, conductivity, mass spectrometry, and electrochemical. Diode array detectors are used when wavelength scans are desired.

MATERIAL AND METHODS:

Rosuvastatin and Fenofibrate were procured from AUROBINDO PHARMA LTD (Bachupalley, hyd A.P, India). Commercial Pharmaceutical preparations from AUROBINDO PHARMA, which were claimed to contain 10 mg of Rosuvastatin and 160 mg of Fenofibrate was used in analysis.

Method development

Selection of column

Initially different C_{18} and C_{8} columns were tried for selected composition of mobile phase and quality of peaks were observed for the drug. Finally the column was fixed upon the satisfactory results of various system suitability parameters such as retention time, column efficiency, tailing factor, peak asymmetry of the peaks.

Selection of detection wavelength

The absorption maximum of Rosuvastatin calcium and Fenofibrate were taken by using primarily UV-Visible spectrophotometer. They were scanned in the range of 200- 400 nm against methanol as a blank.

- Rosuvastatin calcium showed maximum absorbance at 244nm.
- Fenofibrate showed maximum absorbance at 286nm.

The overlain spectra showed λ_{max} of both drugs was recorded (is absorptive point) at 256nm.Hence 256 was selected as detection wavelength.

Selection of mobile phase

The pure drug of Rosuvastatin Calcium and Fenofibrate were injected into the HPLC system and run in different solvent systems. Different mobile

phases like acetonitrile and water; methanol and water; acetonitrile, methanol and water, Buffer and acetonitrile were tried in order to find the best conditions for the separation of Rosuvastatin Calcium and Fenofibrate. It was found that Buffer and acetonitrile gives satisfactory results as compared to other mobile phases. This mobile phase system was tried with different proportions.

Selection of mode of separation

The selection of method depends upon the nature of the sample, its molecular weight and solubility. The drug selected in the present study was polar in nature and hence RP-HPLC method was preferred because of its suitability.

Preparation of sodium phosphate buffer

Weighed 0.6 grams of Sodium dihydrogen phosphate into a 250ml beaker add 30 ml of HPLC water and sonicate to dissolve it completely and diluted to 250ml with HPLC water and pH adjusted to 4 with Orthophosporic acid.

Diluent

Methanol was used as diluent.

Preparation of Standard solution

Accurately weigh and transfer 10 mg of Rosuvastatin and 160mg of Fenofibrate working standard into a 100mL clean dry volumetric f
Further pipette 1mL of Rosuvastatin&Fenofibratethe above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Sample solution

Accurately weigh and transfer 205.6 mg of Rouvastatin and Fenofibrate Tablet powder into a 100mL clean dry volumetric flask add about 30mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 1ml of Rosuvastatin & Fenofibrate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation of Placebo

The amount of powdered inactive ingredient supposed to be present in 10 tablets was accurately weighed and transferred in to 100 ml volumetric flask, 70 ml of diluent was added and shaken by mechanical stirrer and sonicate for about 30 minutes by shaking at intervals of five minutes and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for dilution. 1 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 µm filter before injecting into HPLC system.

Optimized Method

Buffer: Sodium Phosphate (P^H adjusted to 4 with Orthophosporic acid).

Mobile phase: Acetonitrile and Buffer were mixed in the ratio of 70: 30, v / v and sonicate to degas.
Column: XETERRA, RP-8, 150×4.6mm, 3.5µ
Pump mode: Isocratic
Flow rate: 1 ml/min
Detection wavelength: UV, 256 nm
Temperature: Ambient
Injection volume: 20µL
Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for the Rosuvastatin and Fenofibrate peaks and calculate the %Assay by using the formulae.

Calculation:

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{Avg.\ Wt}{Label\ Claim} \times X100$$

Where:
AT = average area counts of sample preparation.
As= average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
Lc = Label Claim of Drug mg/ml.

Method Validation

System Suitability

A Standard solution of working standard was prepared as per procedure and was injected six times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention times, tailing factor, theoretical plates and peak areas from six replicate injections.

Procedure

Inject sample and standard solution into the chromatographic system and measure the peak area, USP plate count, tailing factor.

Acceptance criteria

- The % RSD for the retention times of principal peak from 6 replicate injections of each Standard solution should be not more than 2.0 %
- The number of theoretical plates (N) should be not less than 2000.

Linearity:

Preparation of stock solution:

Accurately weigh and transfer 10 mg of Rosuvastatin and 160mg of Fenofibrate working standard into a 100mL clean dry volumetric flask add about 30mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock solution).

Preparation of Level – I (10ppm of Rosuvastatin&160ppm of Fenofibrate):

1ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (20ppm of Rosuvastatin& 320ppm of Fenofibrate):
2ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.
Preparation of Level – III (30ppm of Rosuvastatin&480ppm of Fenofibrate):
3ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.
Preparation of Level – IV (40ppm of Rosuvastatin& 640ppm of Fenofibrate):
4ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.
Preparation of Level – V (50ppm of Rosuvastatin& 800ppm of Fenofibrate):
5ml of stock solution has taken in 10ml of volumetric flask dilute up to the mark with diluent.

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.
Acceptance Criteria
Correlation coefficient should be not less than 0.999

Precision
Repeatability
Preparation of stock solution
Accurately weigh and transfer 10 mg of Rosuvastatin and 160mg of Fenofibrate working standard into a 100mL clean dry volumetric flask add about 30mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 3ml of Rosuvastatin & Fenofibrate 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 five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.
Acceptance Criteria
The % RSD for the area of five standard injections results should not be more than 2%.
Intermediate Precision
To evaluate the intermediate precision of the method, Precision was performed on different day by using different make column of same dimensions.
Preparation of stock solution
Accurately weigh and transfer 10 mg of Rosuvastatin and 160mg of Fenofibrate working standard into a 100mL clean dry volumetric flask add about 30mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock solution)
Further pipette 3ml of Rosuvastatin&Fenofibrateof 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 injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.
Acceptance Criteria
The % RSD for the area of five standard injections results should not be more than 2%.
Accuracy:
Preparation of Standard Stock Solution
Accurately weigh and transfer 10 mg of Rosuvastatin and 160mg of Fenofibrate working standard into a 100mL clean dry volumetric flask add about 30mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock solution).
Further pipette 3ml of Rosuvastatin & Fenofibrate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

RESULTS:

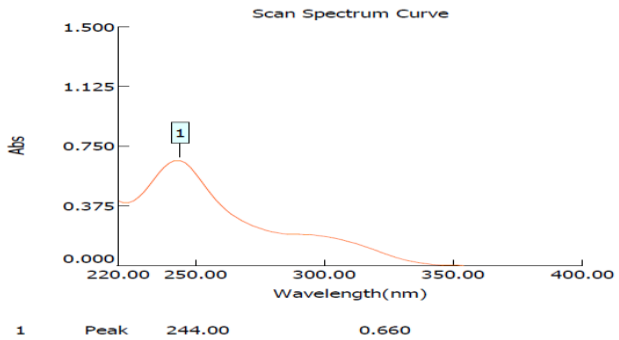

Fig 1: λ_{max} for Rosuvastatin calcium

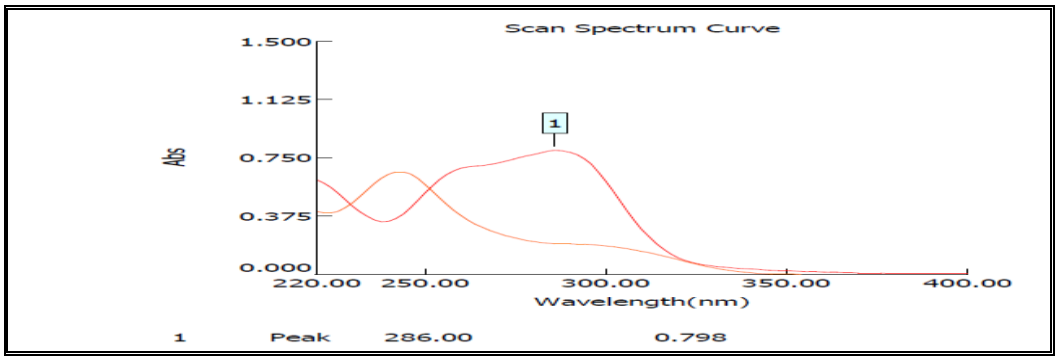

Fig 2: UV overlain spectra for Fenofibrate and Rosuvastatin

Method Validation

System suitability

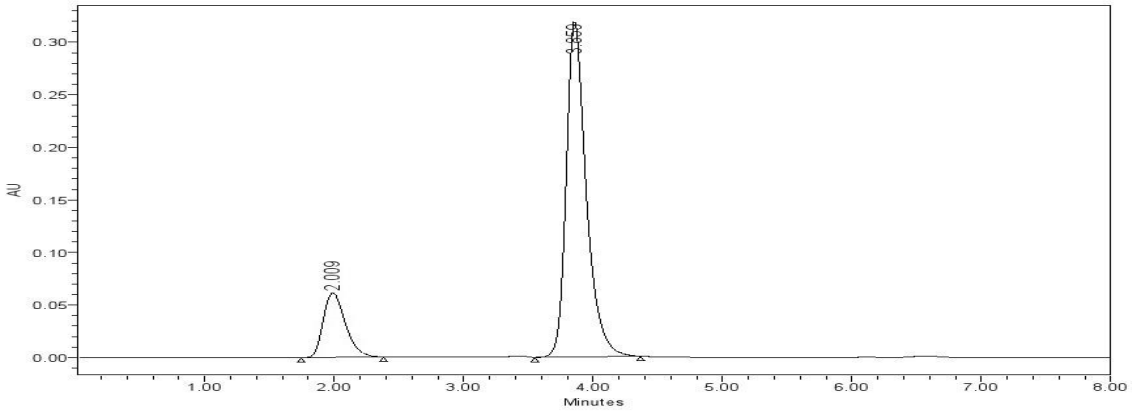

Fig 3: Chromatogram for System suitability

Linearity

Table 1: Linearity of Rosuvastatin

S.No.	Lnearity Level	Concentration(µg/ml)	Area
1	I	10	377579
2	II	20	560627
3	III	30	729627
4	IV	40	883969
5	V	50	1090217
Correlation Coefficient			0.999

Table 2: Linearity of Fenofibrate

S.No.	Linearity Level	Concentration (µg/ml)	Area
1	**I**	**160**	**377579**
2	**II**	**320**	**560627**
3	**III**	**480**	**729627**
4	**IV**	**640**	**883969**
5	**V**	**800**	**1090217**
Correlation coefficient			**0.999**

Precision
Repeatability

Table 3: Repeatability results for Rosuvastatin

Injection	Area
Injection-1	729669
Injection-2	730197
Injection-3	732167
Injection-4	728675
Injection-5	734206
Injection-6	733208
Average	731353
Standard Deviation	2205.7
%RSD	0.30

Table 4: Repeatability results for Fenofibrate

Injection	Area
Injection-1	3507129
Injection-2	3506221
Injection-3	3509917
Injection-4	3513133
Injection-5	3512730
Injection-6	3437120
Average	3497708
Standard Deviation	3148.4
%RSD	0.09

Table 5: Intermediate precision results for Rosuvastatin

Condition	Area
day-1,analyst-1	734876
day-2, analyst-2	733658
day-3,analyst-3	734026
day-4, analyst-4	730810
day-5, analyst-5	735165
Average	733707
Standard Deviation	1731.1
%RSD	0.24

Table 6: Intermediate precision results for Fenofibrate

Injection	Area
day-1,analyst-1	3515429
day-2, analyst-2	3519104
day-3,analyst-3	3526904
day-4, analyst-4	3530000
day-5, analyst-5	3533438
Average	3524975
Standard Deviation	7519.9

Table 7: The accuracy results for Rosuvastatin

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	379560	5.15	5.21	101.3%	100.5%
100%	731695	10.0	10.0	100.5%	
150%	1087515	15.0	14.9	99.6%	

Table 8: The accuracy results for Fenofibrate

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1820017	83.5	84.5	101.3%	99.9%
100%	3444806	160.0	160.1	100.0%	
150%	5082887	240.0	236.2	98.4%	

Table 9: Results for robustness Rosuvastatin

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	2530.0	1.4
2	1.0	2491.3	1.3
3	1.2	2420.0	1.3

Table 10: Results for robustness Fenofibrate

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	3296.1	1.4
2	1.0	3185.8	1.4
3	1.2	3119.8	1.3

Table 11: LOD and LOQ

DRUG	LOD	LOQ
Rosuvastatin	2.98	9.98
Fenofibrate	2.96	10

CONCLUSION:

A simple specific and reliable RP- HPLC method was developed for the determination of Rosuvastatin calcium and Fenofibrate in tablet dosage form. It is successfully applied for the determination of Rosuvastatin and Fenofibrate in pharmaceutical preparations without the interferences of other constituents in the formulations .It can, therefore, be easily and conveniently used for routine quality control analysis, particularly when large numbers of samples are encountered. The developed method was found to be specific as there was no interference of the excipients, which is confirmed by the absence of extra peaks.

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