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

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In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and reproducible stability indicating RP- HPLC method.. 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 and 160-800 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. The drugs were subjected to stress conditions such as acid hydrolysis, alkali hydrolysis, oxidative and thermal degradation as per ICH guidelines. The present method can be successfully used for routine quality control and stability studies.

Keywords: Rosuvastatin , Fenofibrate, chromatographic, ICH, Forced degradation.

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INTRODUCTION:

Stress studies were carried out under the conditions mentioned in ICH Q1A (R2) viz dry heat, hydrolysis, oxidation and photolysis. Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21 CFR section 211 all requires the development and validation of stability indicating assays [1-4].

Rosuvastatin calcium, 3R, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3, 5-dihydroxyhept-6-enoic acid, is a well known Antihyperlipidemic. Rosuvastatin is a selective and competitive inhibitor of HMG-CoA reductase, the rate limiting enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme-A to mevalonate, a precursor of cholesterol [5].

Fenofibrate, 3R, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl) pyrimidin-5-yl]-3, 5-dihydroxyhept-6-enoic acid, is a known Antihyperlipidemic. Fenofibric acid, the active metabolite of Fenofibrate, produces reductions in total cholesterol, LDL-C, apolipoprotein B, total TG and TG-rich lipoprotein (VLDL)[6].

The literature shows that some analytical methods were developed for estimation of these drugs by individually or in combination with other drugs. Suslu et al [7] developed and validated a capillary zone electrophoretic method with diode array detection for the determination of Rosuvastatin calcium in pharmaceutical formulations. Uyar et al[8] developed a simple, rapid and reliable spectrophotometric method for the determination of Rosuvastatin calcium in pharmaceutical preparations. Kadav et al[9] developed and validated a stability indicating UPLC method for the simultaneous determination of atorvastatin, Fenofibrate and their impurities in tablets. Some other analytical methods that have been reported for estimation of Rosuvastatin and Fenofibrate in individual or in combined dosage forms are HPLC[10-17] and LC-MS/MS[18].

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 C₁₈ and C₈ 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 (isoabsorptive 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 Orthophosphoric 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 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&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 Rosuvastatin 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 sonicated 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.

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.

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.

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. 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.

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 & Fenofibrate of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. 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.

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.

Forced degradation study:

To conform the stability indicating nature of the analytical method, forced degradation of Rosuvastatin calcium and Fenofibrate were carried out acid/alkali hydrolysis, oxidative, photolytic and thermal degradation as per ICH recommended test conditions¹⁹.The drugs were subjected to acid hydrolysis using 0.1N Hydrochloric acid for 6 hrs, Alkali hydrolysis using 0.1N Sodium hydroxide for 6 hrs, oxidation by using 3% v/v hydrogen peroxide for 6 hrs, thermal stress in a controlled temperature oven at 60°C for 48 hrs and photolytic stress using UV lamp for 48 hrs.

RESULTS AND DISCUSSION:

Method Development:

Several mobile phase composition were tried to resolve the peaks of Rosuvastatin calcium,

Fenofibrate and degraded components. The optimum mobile phase containing Acetonitrile and Buffer (70: 30, v / v) was selected.

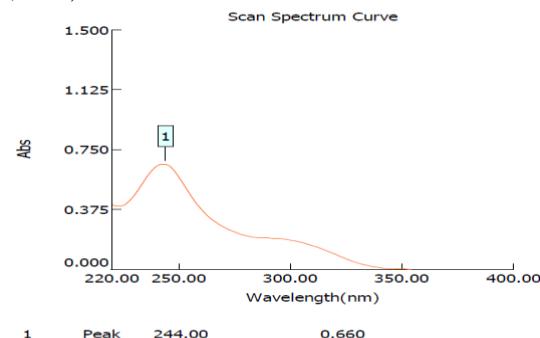


Fig 1: λ_{max} for Rosuvastatin calcium

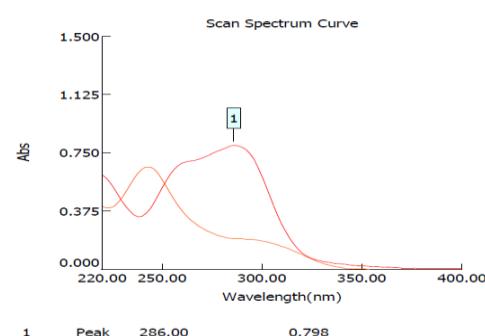
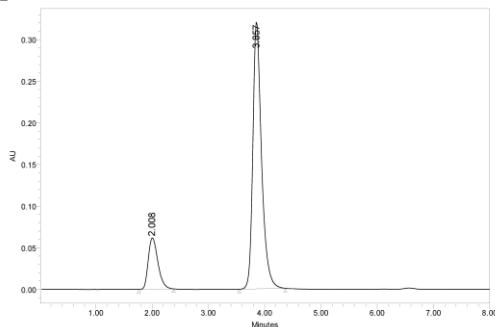


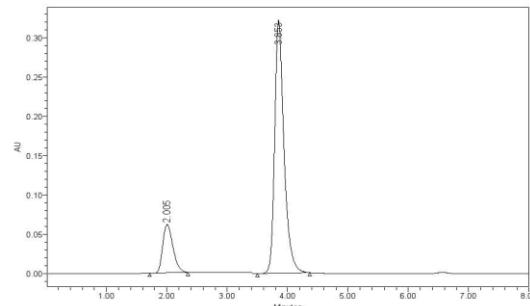
Fig 2: UV overlain spectra for Fenofibrate and Rosuvastatin

Optimized method:



	Name	Retention Time (min)	Area ($\mu\text{V}^*\text{sec}$)	Height (μV)
1	Rosuvastatin	2.008	731472	61817
2	Fenofibrate	3.857	3435946	321577

Fig 3: Standard Chromatogram for optimized method



	Name	Retention Time (min)	Area ($\mu\text{V}^*\text{sec}$)	Height (μV)
1	Rosuvastatin	2.005	728775	61557
2	Fenofibrate	3.853	3461420	321794

Fig 4: Sample Chromatogram for optimized method

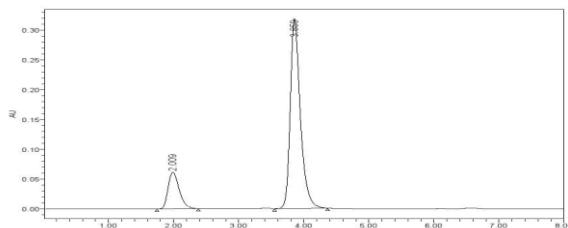
Assay Results: (Rosuvastatin)

% Assay = $\frac{728921}{732340} \times 100 = 99.3\%$

Assay Results :(Fenofibrate)

3459281 160 1 100 10 99.7 205.6
 % Assay = $\frac{3436533}{3459281} \times 100 = 99.7\%$
 ----- x -----x----- x -----x----- x -----x----- x100 =100.3%

Method Validation System suitability



	Name	Retention Time (min)	Area ($\mu\text{V}^*\text{sec}$)	Height (μV)	USP Plate Count	USP Tailing
1	Rosuvastatin	2.009	733208	61345	2480.0	1.3
2	Fenofibrate	3.859	3437120	320842	3167.1	1.4

Fig 5: Chromatogram for System suitability

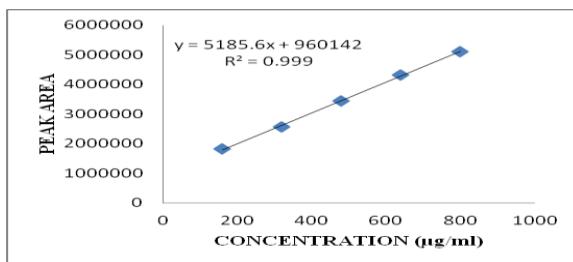


Fig 6: Linearity plot of Rosuvastatin

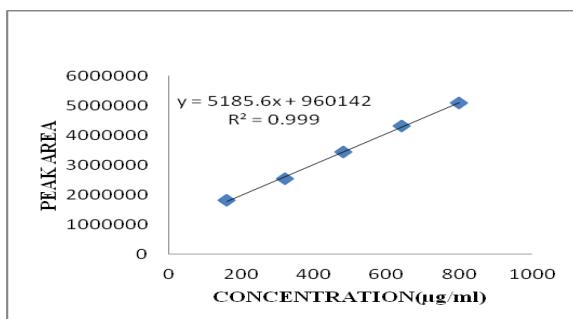
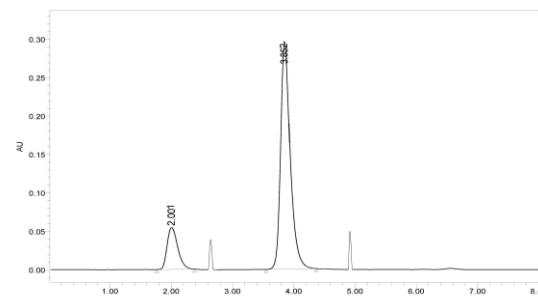


Fig 7: Linearity plot of Fenofibrate

Forced Degradation Studies:

Forced Degradation studies: Forced degradation studies were carried out for the simultaneous estimation of Rosuvastatin calcium and Fenofibrate in acid/alkali hydrolysis, oxidative, thermal and photolytic stress. The peaks of the degradation components were well resolved from peaks of main components.

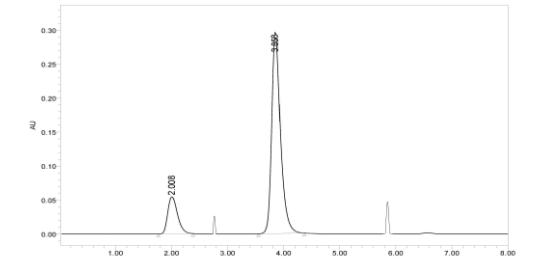
Acid degradation



	Name	Retention Time (min)	Area ($\mu\text{V}\cdot\text{sec}$)	Height (μV)	USP Plate Count	USP Tailing
1	Rosuvastatin	2.001	679506	57311	2282.6	1.3
2	Fenofibrate	3.852	3163417	298828	3228.8	1.3

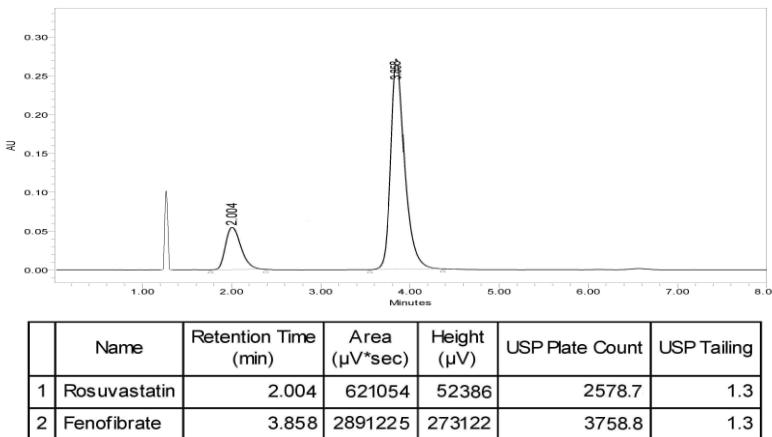
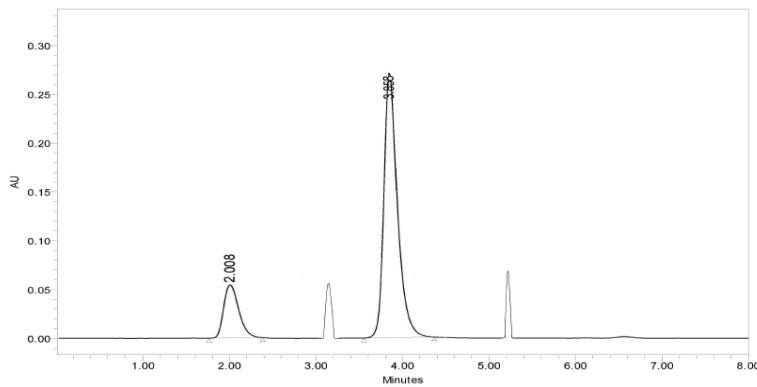
Fig 8: Chromatogram for Acid degradation

Alkali degradation



	Name	Retention Time (min)	Area (μ V·sec)	Height (μ V)	USP Plate Count	USP Tailing
1	Rosuvastatin	2.008	672199	56695	2582.6	1.3
2	Fenofibrate	3.858	3129408	295615	3258.5	1.3

Fig 9: Chromatogram for Alkali degradation

**Fig 10: Chromatogram for oxidative degradation****Fig 11: chromatogram for thermal degradation****Humidity:****Table 1: Results for forced degradation studies**

Stress conditions	% Assay of active substance	
	ROS	FEN
Acid hydrolysis(0.1 M HCl)	92.5%	91.7%
Base hydrolysis(0.1 N NaOH)	91.6%	90.7%
Oxidation(3% H_2O_2)	84.6%	83.8%
Thermal degradation	88.6%	87.8%

CONCLUSION:

A simple specific and reliable RP- HPLC method was developed for the determination of Rosuvastatin calcium and Fenofibrate in tablet dosage form. the two compounds were subjected to forced degradation

applying several stress conditions. The results showed that it was highly sensitive to oxidative, thermal conditions followed by liable to photolytic, alkali, acidic and neutral stress conditions. The degraded products were well resolved from the

analyte peak with significant difference in their RT values. The method was validated as per ICH guidelines.

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