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SIMULTANEOUS RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF FENOFIBRATE AND ROSUVASTATIN CALCIUM IN BULK AND TABLET DOSAGE FORM

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Abstract:

An isocratic reversed-phase liquid chromatograpic assay method was developed for the quantitative determination of fenofibrate and rosuvastatin calcium in bulk and tablet dosage form. A Lichrosphere Select-B C8 (250x4.6mm & 5.0µm) column with a mobile phase containing Solution A (Milli-Q water has pH3.0 made by orthophosphoric acid): Methanol (20:80). The flow rate was 1.0 mL min^{-1}: 1.5 ml/min and the detection of fenofibrate and rosuvastatin calcium was carried out on absorbance detector at 254nm.The retention times was 12 min (rosuvastatin- 3.40, fenofibrate-7.75). A linear response r^2 > 1.0 for fenofibrate in the range of 40-300µg/ml and r^2 > 0.9997 in the range of 2.8-21µg/ml for rosuvastatin calcium was observed. The proposed method was validated with respect to system suitability, specificity and selectivity, stability of analytical solutions linearity, accuracy, precision, and robustness. The method was successfully applied to the estimation of fenofibrate and rosuvastatin calcium in bulk and tablet dosage form.

Keywords: *RP-HPLC, fenofibrate, rosuvastatin calcium, Validation*

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INTRODUCTION:

Rosuvastatin calcium is chemically Bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl (methylsulfonyl) amino] pyrimidi-5-yl] (3R, 5S) -3, 5-dihydroxyhept-6-enoic acid] calcium. It is used in the treatment of Hyperlipidemia. Rosuvastatin Calcium is a selective and competitive inhibitor of HMG CoA reductase, the rate- limiting enzyme that converts 3-hydroxyl-3-methylglutaryl coenzyme A to mevalonate, a precursor of cholesterol. [1] Fenofibrate is chemically Propane-2-yl-[4-(4-chlorobenzoyl) phenoxy]-2-methyl propanate. It is the lipid regulating drug. It increases lipolysis and elimination of triglyceride-rich particles from plasma by activating lipoprotein lipase and reducing production of apoprotein C-III (an inhibitor of lipoprotein lipase activity). [2] Literature survey revealed few analytical techniques are available for estimation of ROS alone as well as in combine dosage form such as UV ,HPLC, HPTLC.[3-7] Similarly few analytical methods are available for estimation of Fenofibrate alone and its combination with drugs such as UV and HPLC.[8-17] keeping this objective in mind an attempt has been made to develop and validate the RP-HPLC method for the simultaneous estimation of Rosuvastatin and Fenofibrate which would be highly sensitive having good resolution reproducible and cost effective. Various validation aspects of the analysis accuracy, precision, recovery, the limits of detection and quantification etc have been measured as per ICH guidelines. [18]

Chemicals and Reagents

Rosuvastatin calcium (Active Pharmaceutical Ingredient) and working standard were supplied by Cadila Healthcare Limited Ankleshwar, India whereas fenofibrate (Active Pharmaceutical Ingredient) and working standard were supplied by Ami Lifesciences Limited Baroda, India. Ortho-Phosphoric Acid was obtained from Spectrochem Pvt. Ltd., India. Acetonitrile was obtained from Spectrochem Pvt. Ltd, India. Methanol was obtained from Spectrochem Pvt. Ltd., India. Milli-Q Wateras produced by In-house production of company. Triethylamine was obtained from Spectrochem Pvt. Ltd, India.

Chromatographic System

The HPLC system (Shimadzu Corporation, Japan), model Shimadzu VP, consisted of a system controller (CLASS-VP), on-line degasser (LC 2010C, Shimadzu), low pressure gradient valve (LC 2010C, Shimadzu), solvent delivery module (LC 2010C, Shimadzu), auto injector (LC 2010C, Shimadzu), column oven (LC 2010C, Shimadzu), and CLASS – VP software version = SPI, binary pump, auto injector (SIL-10AD VP, Shimadzu), column oven (CTO-10AS VP, Shimadzu) and PDA detector (PDA-SPD-M10A VP, Shimadzu Diode Array Detector) and Chem station (software) were used for analytical purpose.

Parameters for method development with Specifications are given in table 1

Table 1: Parameters for method development

Parameters	Specifications
Stationary Phase	Lichrosphere Select B C8 (250mm x4.6mm) 5 µ.
Mobile Phase	Buffer: Methanol (20:80)
Diluent	Buffer : methanol (20:80)
ssFlow rate	1.0 ml/min
Injection volume	10µl
Detection	254 nm
Temperature	30˚C
Run time	12 min (Rosuvastatin- 3.40, Fenofibrate-7.75)
Buffer	Buffer is milliQ water whose pH 3.0made by H_3PO_4

Selection Criteria

Working Standard and sample from reliable source in pure form was collected. Solubility was determined of fenofibrate and rosuvastatin calcium in appropriate solvent or their mixture of solvents. On the basis of solubility studies and literature survey, the mobile phase composition for further development work was decided. The λmax for fenofibrate and rosuvastatin calcium was obtained with the help of UV Spectroscopy. Concentration or µg/ml solution was prepared for standard by help of their label claim mentioned. Selection of column carried out on the basis of previous work on individual drugs or combination with other drugs, mainly C-8 & C-18 column. The column was selected on the basis of their retention time, area, peak shape and asymmetry. Isocratic mode for the analysis was decided by primary run on HPLC system. Injection volume was determined on the basis of their symmetry and resolution in chromatogram by several run on HPLC method. Run time was determined on the basis of the retention time of both mentioned components. Optimization was performed by changing the proportion of mobile phase or adjusts the pH of mobile phase, as well as trials made on different grade column. The mobile phase was selected on the basis of resolution, asymmetry, peak shape and area.

Method Development

Fenofibrate and rosuvastatin calcium showed λ_{max} at 254 nm. Proper selection of the HPLC method depends upon the nature of the sample (ionic or ionizable or neutral molecule), its molecular weight and solubility. RP-HPLC was selected for the initial separation because of its simplicity and suitability. To optimize the chromatographic conditions the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied and the chromatographic parameters such as capacity factor, asymmetric factor, and resolution and column efficiency were calculated. The condition was chosen that gave the best resolution and symmetry was selected for estimation. The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be In the present study, standard solution of fenofibrate and rosuvastatin calcium were scanned over the range of 200–400 nm wavelengths. The both drugs have shown absorbance maxima nearer 254 nm. So the 254 nm wavelength was selected for simultaneous estimation of fenofibrate and rosuvastatin calcium in solid dosage forms. For RP-HPLC method, various columns are available but our main aim to resolve the drugs in the presence of degradation products and other impurities. So the C-8 column was selected over the other columns. For fenofibrate and rosuvastatin calcium, Lichrosphere Select B C8 (250mm x4.6mm) 5 µ column was chosen to give good peak shape and high resolution as compared to other C- 8 columns. This column has an embedded polar group and which are more stable at lower pH and carbon loads, which provide high peak purity and more retention to polar drugs and facilitates the separation of impurity peaks within a very short run time.

Method Validation

Validation was done as per ICH guideline Q2 (R1). The developed RP-HPLC methods were validated with respect to parameters such as linearity, precision, accuracy, specificity, ruggedness, robustness and solution stability. [18]

System Suitability

System suitability is the checking of a system to ensure system performance before or during the analysis of unknowns. Parameters such as plate count, tailing factors, resolution and reproducibility (% RSD, retention time and area for six repetitions) are determined and compared against the specifications set for the method. These parameters are measured during the analysis. The Assymetry for analyte peak should be not more than (NMT) 1.2 and % RSD of five replicate standared injections should be NMT 2.0.

System Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. This is usually expressed as the standard deviation or the relative standard deviation (coefficient of variation). Precision is a measure of the degree of reproducibility or of the repeatability of the analytical method under normal operating circumstances. Repeatability involves analysis of replicates by the analyst using the same equipment, method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, and different batch of reagent, different analysts and different equipments. The Standard Solution is prepared at working Concentration and analyzed in replicate. The % RSD of five replicate standard injection is NMT 2.0.

Linearity and Range

The linearity of an analytical method is its ability to elicit test results that are directly (or by a well defined mathematical transformation) proportional to the analyte concentration in samples within a given range. Linearity usually expressed in terms of the variance around the slope of regression line calculated according to an established

mathematical relationship from test results obtained by the analysis of samples with varying concentrations of analyte. The linear range of detect ability that obeys Beer's law is dependent on the compound analyzed and the detector used. Linearity was determined at five levels over the range of 20% to 150% of test concentration. Standard linearity solutions were prepared to different concentration of 20%, 50%, 80%, 100%, 120%, and 150% of the test concentration. Each linearity solution was injected in duplicate. The correlation coefficient is should be not less than (NLT) 0.995.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Limit of Detection

The limit of detection is the parameter of limit tests. It is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, using a specific method under the required experimental conditions. The limit test thus merely substantiates that the analyte concentration is above or below a certain level. A signal-to-noise ratio of 2:1 or 3:1 is generally accepted. The signal-to-noise ratio is determined by dividing the base peak by the standard deviation of all data points below a set threshold. Limit of detection is calculated by taking the concentration of the peak of interest divided by three times the signal-to-noise ratio. The standard deviation of the intercept (Sa) which may be related to LOD and the slope of the calibration curve, b, by: LOD = 3.3 Sa / b.

Limit of Quantification

Limit of Quantification is a parameter of quantitative assays for low levels of compounds in sample matrices such as impurities in bulk drugs and degradation products in finished pharmaceuticals. The limit of quantification is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied. It is measured by analyzing samples containing known quantities of the analyte and determining the lowest level at which acceptable degrees of accuracy and precision are attainable. The standard deviation multiplied by a factor (usually 10) provides an estimate of the limit of quantification. In many cases, the LOQ is approximately twice the limit of detection. Sa is the standard deviation of the intercept which may be related to LOQ and the slope of the calibration curve, b, by: LOQ = 10 Sa / b.

Stability of Analytical Solution

Stability of the sample, standard and reagents is required for a reasonable time to generate reproducible and reliable results. For example, 24 hour stability is desired for solutions and reagents that need to be prepared for each analysis. System suitability test provide the added assurance that on a specific occasion the method is giving, accurate and precise results. System suitability test are run every time a method is used either before or during analysis. Solution stability period for standard and sample preparation was determined by keeping the solution for 12 hour at room temperature. At interval 2, 4, 6, 8, 10, and 12 hour the solutions were analysed. The insignificant changes (<2%) were observed for the chromatographic responses for the solution analysed, relative to freshly prepared standard. The peak areas of analyte in standard and sample solution not differ by more than 2% from initial peak area for the accepted storage time.

Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added. Accuracy may be determined by applying the method to samples or mixtures of excipients to which known amount of analyte have been added, both above and below the normal levels expected in the samples. Accuracy is then calculated from the test results as the percentage of the analyte recovered by the assay. The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was carried out at three levels in the range of 50-150% of the working concentration of sample. Calculated amount of fenofibrate and rosuvastatin calcium working standards were added in placebo containing volumetric flasks to prepare 50%, 100% and 150% level of the working concentration. Each level was prepared in triplicate manner and each preparation was injected in duplicate. The recovery at each level should be 98%-102% and the % RSD NMT 2.0.

Specificity and Selectivity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix. If an analytical procedure is able to separate and resolve the various components of a mixture and detect the analyte qualitatively the method is called selective. On the other hand, if the method determines or measures quantitatively the component of interest in the sample matrix without separation, it is said to be specific Specificity is a procedure to detect quantitatively the analyte in the presence of components that may be expected to be present in the sample matrix. While selectivity is the procedure to detect qualitatively the analyte in

presence of components that may expected to be present in the sample matrix. Specificity of developed method was established by determining peak purity of active component in standard preparation, test preparation and spiked sample preparation using PDA detector.

Interference from Blank and Placebo

A blank preparation, standard preparation, placebo preparation, sample preparation of fenofibrate and rosuvastatin calcium and placebo spiked with targeted concentration of both API were prepared and injected. There is no interference from placebo with analyte and peak purity of analyte in sample solution is NLT 0.995.

Robustness and Ruggedness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage. The determination of robustness requires that methods characteristic are assessed when one or more operating parameter varied. The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The testing of ruggedness is normally suggested when the method is to be used in more than one laboratory. Ruggedness is normally expressed as the lack of the influence on the test results of operational and environmental variables of the analytical method. % RSD of five replicate standard injections should be NMT 2.0.

RESULT AND DISCUSSION:

System Suitability

Table 2: System Suitability

Sr. No.	Parameters (n= 5)	Fenofibrate	Rosuvastatin
1	Retention Time (min)	7.45	3.40
2	Theoritical Plates	9474.35	5148.93
3	Asymmetry	1.11	1.14
4	% RSD	0.1825	0.1837

According to above table the all parameters like theoretical plates, assymetry and %RSD was within the limit so system is suitable for method.

System Precision

Table 3: System Precision

System precision Injection	Area	
	Fenofibrate (mV*sec)	**Rosuvastatin (mV*sec)**
Injection 1	4053570	245539
Injection 2	4103144	247064
Injection 3	4110290	246958
Injection 4	4081802	245988
Injection 5	4095113	246368
% RSD	**0.5**	**0.3**

Five injections were given and % RSD for both fenofibrate and rosuvastatin calcium was calculated which is within the range.

Linearity

The linearity of developed method was achieved in the range of 40-300µg/ml (r^2 = 0.9999) for Fenofibrate and 2.8-21µg/ml (r^2 = 0.9999) for Rosuvastatin, The results show that all validation parameters of method lie within its specific acceptance crieteria.

Table 4: Linearity Data of Fenofibrate

Linearity Range	Stock solution to be taken in ml	Dilute to volume (ml)with diluent	Final concentration in µg/ml Fenofibrate	Area
20%	1.0	25	40	756662
50%	2.5	25	100	1964299
80%	4.0	25	160	3185516
100%	5.0	25	200	3979271
120%	6.0	25	240	4774907
150%	7.5	25	300	5970318

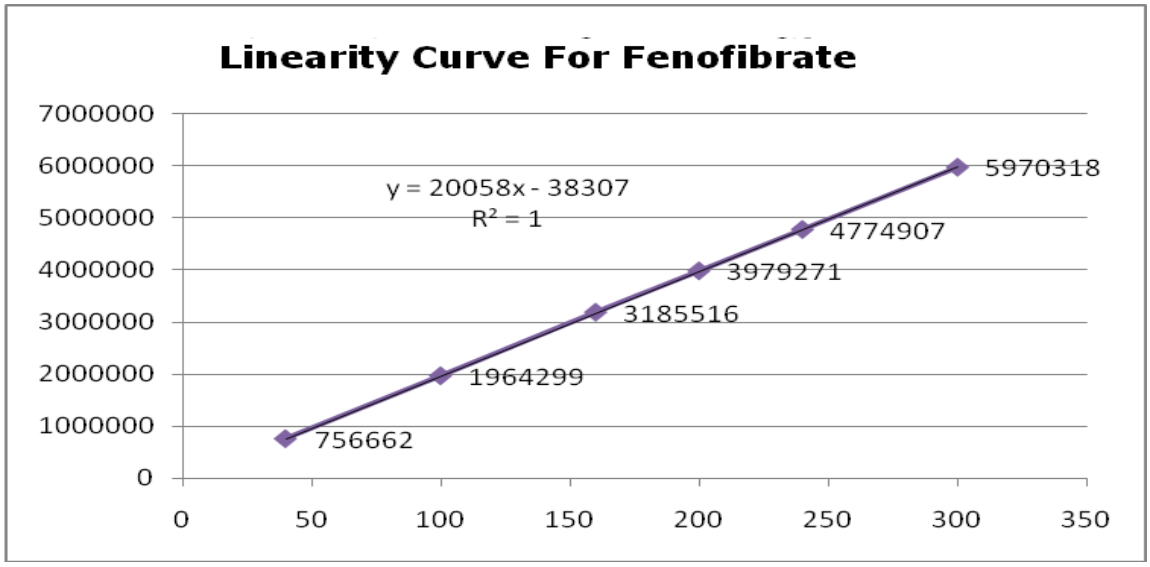

Fig. 1: Linearity Curve for Fenofibrate

Table 5: Linearity Data of Rosuvastatin Calcium

Linearity Range	Stock solution to be taken in ml	Dilute to volume (ml)with diluent	Final concentration in µg/ml Rosuvastatin	Area
20%	2.0	25	2.8	53779
50%	5.0	25	7.0	137045
80%	8.0	25	11.2	219267
100%	10.0	25	14.0	275756
120%	12.0	25	16.8	328650
150%	15.0	25	21.0	415303

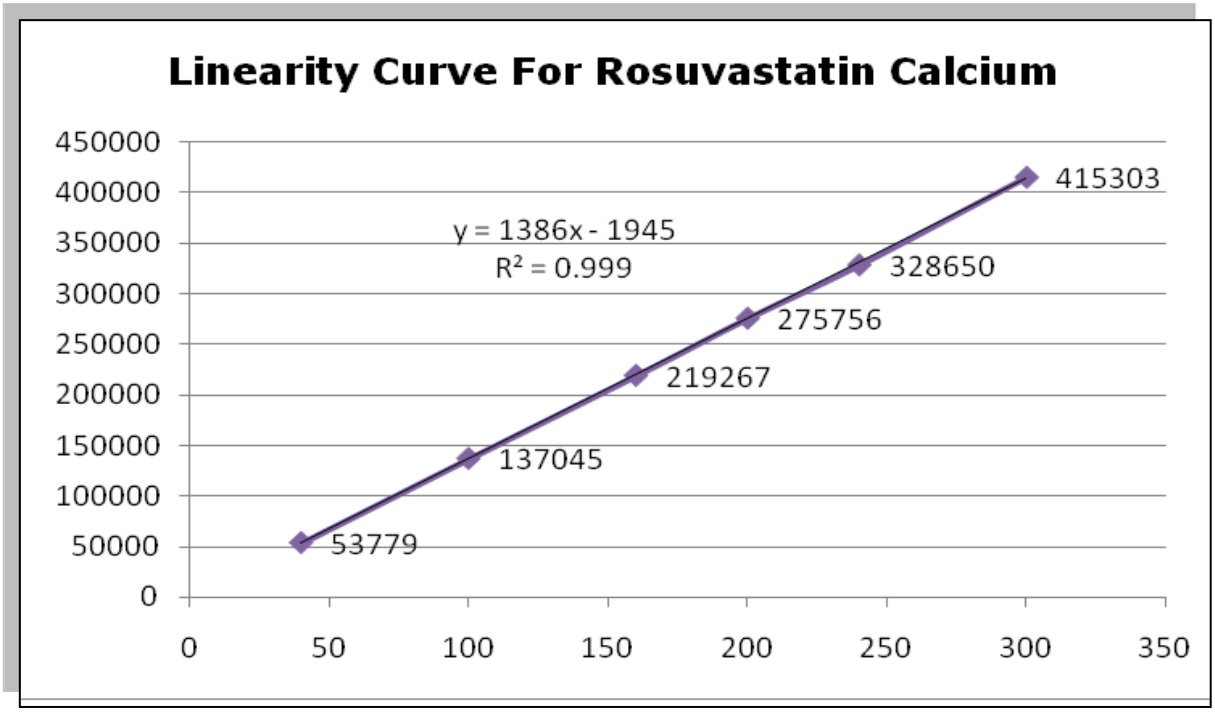

Fig.2: Linearity for Rosuvastatin Calcium

The mean area at each level was calculated and a graph of mean area versus concentration was plotted. The correlation co-efficient, Y intercept and slope of regression line were calculated.

LOD and LOQ

Table 6: LOD and LOQ

Parameters	Fenofibrate	Rosuvastatin calcium
Linearity equation	Y=20058x-38307	Y=1386x-1945
Correlation coefficient	1.0	0.9999
LOD	0.02µg/ml	0.02µg/ml
LOQ	0.05µg/ml	0.05µg/ml

The above data shows that a micro gram quantity of both drugs can be accurately and precisely determined.

Stability of Analytical Solution

Table 7: Results of Standard Solution Stability

Time (hour)	Area		% Difference	
	Fenofibrtae	Rosuvastatin	Fenofibrtae	Rosuvastatin
0 (Initial)	4009869	141792	-----	------
2	4015763	141710	0.1	-0.1
4	4017140	141875	0.2	0.1
6	4016521	141787	0.2	0.0
8	4018345	141758	0.2	0.0
10	4021270	141729	0.3	0.0
12	4019958	141583	0.3	-0.1
14	4024119	141669	0.4	-0.1
16	4023793	141499	0.3	-0.2
% Mean RSD			**0.1825**	**0.1837**

Solution stability lie within its specific acceptance criteria for 12 hrs.

Table 8: Results of Sample Solution Stability

Time (hour)	Area		% Difference	
	Fenofibrtae	Rosuvastatin	Fenofibrtae	Rosuvastatin
0 (Initial)	3960524	151977	------	------
2	3950322	151328	-0.3	0.4
4	3952114	151582	-0.2	-0.3
6	3961673	151907	0.0	0.0
8	3956805	151754	-0.1	-0.1
10	3965695	152010	0.1	0.0
12	3966435	151922	0.1	0.0
14	3962589	151696	0.1	-0.2
16	3965523	151764	0.1	-0.1

The solution stability of standard and sample was performed and the percentage difference was not more than 2%.

Precision

Method Precision (Repeatability)

Table 9: Method Precision Data of Fenofibrate (Feno) and Rosuvastatin calcium (Rosu)

Set No.	% Assay		% Assay Mean		%RSD	
	Feno	Rosu	Feno	Rosu	Feno	Rosu
1	98.00	101.92				
2	98.67	101.80	98.53		0.30	1.20
3	98.77	102.21		101.00		
4	98.42	100.34				
5	98.50	100.73				
6	98.82	99.01				

Individual % assay, mean % assay and % RSD were calculated. The % RSD is 0.30 for Fenofibrate & 1.20 for Rosuvastatin calcium which indicates that the method is precise.

Intermediate Precision (Ruggedness)

Table 10: Intermediate Precision Data of Fenofibrate and Rosuvastatin calcium

Set No.	% Assay		% Assay Mean		%RSD	
	Feno	Rosu	Feno	Rosu	Feno	Rosu
1	98.00	101.87				
2	98.35	101.31				
3	98.53	101.87	98.29		0.26	1.35
4	98.10	100.01		100.57		
5	98.16	100.02				
6	98.66	98.38				

Individual % assay, mean % assay and % RSD were calculated and recorded in Table10. The % RSD is 0.26 for Fenofibrate & 1.35 for Rosuvastatin calcium which indicate that the method is rugged.

Specificity and Selectivity

Table 11: Results of Peak Purity in Specificity Study of Fenofibrate and Rosuvastatin calcium

Sample	% Assay		Peak purity	
	Fenofibrate	Rosuvastatin	fenofibrate	Rosuvastati
Standard Solution	100.50	98.21	0.9989	0.9998
Test Solution	98.42	101.87	0.9992	0.9998
Spiked Sample	101.65	98.03	0.9998	0.9998

The peak purity index for the main peak in all the standard preparation, sample and placebo preparation was determined there is no interference in main peak.

Robustness

Table 12: Results of Robustness Study

Compound	% RSD (n= 5)		
	Normal Condition	Changed Condition	
Temperature	**Normal**	**(-5°C)**	**(+5°C)**
Fenofibrate	0.10	0.02	0.10
Rosuvastatin	0.10	0.00	0.00
pH	**Normal**	**(-0.2 unit)**	**(+0.2 unit)**
Fenofibrate	0.10	0.20	0.10
Rosuvastatin		0.20	0.10
Flow Rate	**Normal**	**(-10%)**	**(+10%)**
Fenofibrate	0.10	0.02	0.01
Rosuvastatin	0.10	0.10	0.00
Mobile phase ratio	**Normal**	**(-2%)**	**(+2%)**
Fenofibrate	0.10	0.05	0.10
Rosuvastatin	0.10	0.00	0.10
Wavelength	**Normal**	**-5nm**	**+5nm**
Fenofibrate	0.10	0.1	0.05
Rosuvastatin	0.10	0.00	0.01

The low % RSD values (< 2%) reveal that the proposed method is robust for this variation. The Summary of validation parameters is given in table 13.

Summary of Validation Results

Table 13: Summary of Validation Parameters of Fenofibrate and Rosuvastatin calcium by RP-HPLC

Parameter	Acceptance Crieteria	Fenofibrate	Rosuvastatin Cal.
Linearity Range Correlation Coefficient	Correlation coefficient $r^2 > 0.999$ or 0.995	40-300µg/ml $r^2 = 0.9999$	2.8-21µg/ml $r^2 = 0.9999$
LOD	S/N > 2 or 3	0.02µg/ml	0.02µg/ml
LOQ	S/N > 10	0.05µg/ml	0.05µg/ml
Precision Intermediate Precision	RSD < 2% RSD < 2%	%RSD = 1.2 %RSD = 0.8	%RSD = 0.4 % RSD = 1.3
Specificity	1) No intereference from blank, placebo with the main peak. 2) The peak purity index > 0.999	No intereference. Peak purity 1)Test sample = 0.9992 2)Spiked sample = 0.9998	No intereference. Peak purity 1)Test sample = 0.9998 2) Spiked sample = 0.9998
Accuracy	Recovery 98- 102%	% recovery=101.7	% recovery = 98.2- 101.7
Solution Stability	> 12 hour	Stable up to 16 hour	Stable up to 16 hour
Robustness	RSD NMT 2% in modified condition	%RSD = Complies	%RSD = Complies

CONCLUSION:

This developed and validated method for simultaneous analysis fenofibrate and rosuvastatin calcium in pharmaceutical preparations is very simple, rapid, accurate and precise. The method was successfully applied for determination of fenofibrate and rosuvastatin calcium in its pharmaceutical formulations. Moreover, it has advantages of short run time and the possibility of analysis of a large number of samples, both of which significantly reduce the analysis time per sample. Hence, this method can be conveniently used for routine quality control analysis of fenofibrate and rosuvastatin calcium in their pharmaceutical formulations.

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