Analytical method development and validation for the simultaneous estimation of Rosuvastatin and Finofibate in tablet dosage form by reverse phase high performance liquid chromatography

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ABSTRACT
A new, simple, precise, accurate and reproducible RP-HPLC method for Simultaneous estimation of Rosuvastatin (ROS) and Fenofibrate (FEN) in bulk and pharmaceutical formulations was developed. Separation of ROS and FEN was successfully achieved on a Hypersil C18 (4.6 x 250mm, 6.5 μm, Make: Waters) or equivalent in an isocratic mode utilizing OPA buffer (pH 3.0): Methanol (65:35%v/v) at a flow rate of 1.2 mL/min and eluate was monitored at 238 nm, with a retention time of 1.950 and 3.858 minutes for ROS and FEN. The method was validated and the response was found to be linear in the drug concentration range of 50 μg/mL to 150 μg/mL for ROS and 50 μg/mL to 150 μg/mL for FEN. The values of the slope, intercept and the correlation coefficient were found to be 22507, 7467 and 0.999 for ROS and 21157, 16980 and 0.999 for FEN respectively. The LOD and LOQ for Rosuvastatine were found to be 0.0053, 0.017 respectivly. The LOD and LOQ for Fenofibrate were found to be 0.00019, 0.00063 respectively. This method was found to be good percentage recovery for Rosuvastatine and Fenofibrate were found to be 99.00 and 99.00 respectively indicates that the proposed method is highly accurate. The specificity of the method shows good correlation between retention times of standard with the sample so, the method specifically determines the analyte in the sample without interference from excipients of tablet dosage forms. The method was extensively validated according to ICH guidelines for Linearity, Range, Accuacy, Precesion, Specificity and Robustness.
Key words: Rosuvastatin, Fenofibrate, High performance liquid chromatography.

INTRODUCTION

Rosuvastatin (3R, 5S, 6E) – 7 - [4 - (4 – fluorophenyl) – 2 (*N* - methylmethanesulfonamido) – 6 - (proan-2-yl) pyrimidine-5-yl] - 3, 5-dihydoxyhept-6-enoic acid. Rosuvastatin is an antilipidemic agent that competitively inhibits hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase.HMG-CoA reducuase catalyzes the conversion 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.

Fenofibrate propan - 2 - yl2 - {4 - [(4-chlorophenyl) carbonyl] phenoxy} - 2methylpropanoate is an antilipidemic agent which reduces both cholesterol and triglycerides in the blood. Fenofibrate exerts its therapeutic effects through activation of peroxisome proliferator activated receptor a (PPARa). This increases lipolysis and elimination of triglyceride-rich particles from plasma by activating lipoprotein lipase and reducing production of apoprotein C-III. The resulting fall in triglycerides produces an alteration in the size and composition of LDL from small, dense particles, to large buoyant particles. These larger particles have a greater affinity for cholesterol receptors and are catabolized rapidly.

Literature survey revealed that very few methods have been reported for the analysis of Rosuvastatin and Fenofibrate combinational dosage forms which include UV spectroscopy, Reverse Phase High performance Liquid Chromatography, Densitometric method, HPTLC methods. The present study illustrate development and validation of simple, economical, selective, accurate, precise RP-HPLC method for the determination of Rosuvastatin and Fenofibrate in bulk and Pharmaceutical dosage forms as per ICH guidelines. The goal of this study is to develop rapid, economical HPLC method for the analysis of Rosuvastatin and Fenofibrate in combined dosage form using most commonly employed column (C18) and simple mobile phase preparation. In the present proposed work a successful attempt had been made to develop a method for the simultaneous estimation of Rosuvastatin and Fenofibrate pharmaceutical dosage form and validate it. From the economical point of view and for the purpose of routine analysis, it was decided to develop a more economical RP-HPLC method with simple mobile phase preparation for the estimation of Rosuvastatin and Fenofibrate combinational dosage form. The method would help in estimate of drugs in single run

which reduces the time of analysis and does not require separate method for each drug. Thus, the paper reports an economical, simple and accurate RP-HPLC method for the above said pharmaceutical dosage forms.

MATERIALS AND METHODS

Quantitative HPLC was performed on a high performance liquid chromatograph -Waters e2695Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC and Hypersil ODS C18 column of dimension 250 × 4.6, 5µm particle size. In addition an analytical balance (DENVER 0.1mg sensitivity), digital pH meter (Eutech pH 510), a sonicator (Unichrome associates UCA 701) were used in this study.

Standards and chemicals used: The reference samples of Rosuvastatin and Fenofibrate standards were kindly supplied as gift samples by Dr.Reddy's., Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade.ortho phosphoric acid from Dr.Reddy's., Hyderabad,Andhra Pradesh, India, while Methanol (HPLC grade) and Water (HPLC grade) from Merck Pharmaceuticals Private Ltd., Mumbai, India. Rosuvastatin and Fenofibrate Tablets available in the market as RosvasF (J.B. Chemicals & Pharmaceuticals Ltd.) in composition of Rosuvastatin (190mg), Fenofibrate (6mg).

Preparation of mobile phase: Transfer 1000ml water into 1000ml of beaker.Then adjust its pH-3.0 with Ortho phosphoric acid (OPA) and filtered through 0.45µ membrane filter and degassed by sonication.

Preparation of calibration standards: Rosuvastatin 6mg and 190mg Fenofibrate was taken into a 10, 25ml of volumetric flask and add 10ml of Diluent and sonicated for 10 minutes and made up with Diluent. It was further diluted to get stock solution of Rosuvastatin and Fenofibrate.This is taken as a 100% concentration. Working standard solutions of Rosuvastatin and Fenofibrate was prepared with mobile phase. To a series of 10 ml volumetric flasks, standard solutions of Rosuvastatin and Fenofibrate in the concentration range of 50- 150µg/ml were transferred respectively.

System suitability: System suitability is an integral part of chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solutions at 100% concentration level for

Rosuvastatin and Fenofibrate to check the reproducibility of the system. At first the HPLC system was stabilized for 40 min. One blank followed by six replicate analysis of solution containing 100% target concentration of Rosuvastatin and Fenofibrate were injected to check the system suitability. To ascertain the system suitability for the proposed method, a number of parameters such as theoretical plates, peak asymmetry, and retention time were taken and results were presented in Table 1.

Calibration curves for Rosuvastatin and Fenofibrate: Replicate analysis of solution containing 50-150µg/mL of Rosuvastatin and Fenofibrate sample solutions respectively were injected into HPLC according to the procedure in a sequence and chromatograms were recorded. Calibration curves were constructed by plotting by taking concentrations on X-axis and ratio of peak areas of standards on Y-axis and regression equation were computed for both drugs and represented in Table .6

Analysis of marketed formulation: The content of ten tablets was weighed accurately. Their average weights were determined. Powder of tablets equivalent to one tablet weight (488mg) were weighed and taken in a 50 ml volumetric flask, dissolved in diluents, shaken and sonicated for about 20 minutes then filtered through 0.45µ membrane filter. The filtered solution was further diluted (5 to 50ml) in the diluents to make the final concentration of working sample equivalent to 100% of target concentration. The prepared sample and standard solutions were injected into HPLC system according to the procedure. From the peak areas of Rosuvastatin and Fenofibrate the amount of the drugs in the sample were computed. The contents were calculated as an average of six determinations and experimental results were presented in Table 4. The representive standard and sample chromatograms were shown in fig.2 and fig.3.

Validation study of Rosuvastatin and Fenofibrate: An integral part of analytical method development is validation. Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. The newly developed RP-HPLC method was validated as per International Conference on Harmonization (ICH) guidelines for parameters like specificity, system suitability, accuracy, linearity, precision (repeatability), limit of detection (LOD), limit of Quantification (LOQ) and robustness.

Specificity: The effect of wide range of excipients and other additives usually present in the formulation

of Rosuvastatin and Fenofibrate in the determination under optimum conditions were investigated. The specificity of the RP-HPLC method was established by injecting the mobile phase and placebo solution in triplicate and recording the chromatograms.

Precision: precision study of sample (Rosuvastatin and Fenofibrate) was carried out by estimating corresponding responses 6 times on the same day for the 100% target concentration. The percent relative standard deviation (%RSD) is calculated which is within the acceptable criteria of not more than 2.0.

Linearity: The linearity graphs for the proposed assay methods were obtained over the concentration range of 50-150µg/ml Rosuvastatin and Fenofibrate respectively. Method of least square analysis is carried out for getting the slope, intercept and correlation coefficient, regression data values. The representative chromatograms indicating the sample were shown in fig.2&3. A calibration curve was plotted between concentration and area response.

Accuracy (Recovery studies): The accuracy of the method is determined by calculating recovery of Rosuvastatin and Fenofibrate by the method of addition. Known amount of Rosuvastatin and Fenofibrate at 50%, 100%, 150% is added to a pre quantified sample solution. The recovery studies were carried out in the tablet in triplicate each in the presence of placebo. The mean percentage recovery of Rosuvastatin and Fenofibrate at each level is not less than 98% and not more than 102%.

Robustness: The robustness is evaluated by the analysis of Rosuvastatin and Fenofibrate under different experimental conditions such as making small changes in flow rate (±0.2 ml/min), λmax (±5), column temperature (±5), mobile phase composition (±5%), and pH of the buffer solution.

LOD and LOQ: Limit of detection is the lowest concentration in a sample that can be detected but not necessarily quantified. Under the stated experimental conditions, the limit of quantification is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of detection and limit of quantification were calculated using following formula LOD=3.3(SD)/S and LOQ=10(SD)/S, where SD= standard deviation of response (peak area) and S= average of the slope of the calibration curve.

RESULTS AND DISCUSSION

Reverse phase HPLC method was preferred for the determination of Rosuvastatin and Fenofibrate.

Preliminary experiments were carried out to achieve the best chromatographic conditions for the simultaneous determination of the drug substances. Several column types and lengths were tried considering other chromatographic parameters. C18 column with a 4.6 mm inner diameter and 5µm particle size was chosen. The detection wave length was selected as 238nm with PDA detector. Chromatographic conditions were optimized by changing the mobile phase composition and buffers used in mobile phase. Different experiments were performed to optimize the mobile phase but adequate separation of the drugs could not be achieved. By altering the pH of buffer results a good separation. Different proportions of solvents were tested. Eventually the best separation was obtained by the isocratic elution system using a mixture of OPA buffer, Methanol in the ratio of (65:35) adjusted to pH- 3.0 at a flow rate of 1.2 ml/min. A typical chromatogram for simultaneous estimation of the two drugs obtained by using a above mentioned mobile phase. Under these conditions Rosuvastatin and Fenofibrate were eluted at 1.950min and 2.858 minutes respectively with a run time of 6 minutes.

The OPA buffer, MeOH in the ratio of (65:35) adjusted to pH- 3.0 was chosen as the mobile phase. The run time of the HPLC procedure was 6 minutes at flow rate of 1.2ml/min was optimized which gave sharp peak, minimum tailing factor. The system suitability parameters were shown in Table 1 were in within limit, hence it was concluded that the system was suitable to perform the assay. The method shows linearity between the concentration range of 50-150µg/ml for Rosuvastatin and Fenofibrate. The experimental results were shown in table 6 and fig.6&7.

The % recovery of Rosuvastatin and Fenofibrate was found to be in the range of 98.00 to 101.00 % and 98.00to 101.00% respectively. As there was no interference due to excipients and mobile phase, the method was found to be specific. As both compounds pass the peak purity, the method was found to be specific. The method was robust and rugged as observed from insignificant variation in the results of analysis by changes in Flow rate, column oven temperature, mobile phase composition and wave length separately and analysis being performed by different analysts. The results were shown in Table 5. The LOD and LOQ values were calculated based on the standard deviation of the response and the slope of the calibration curve at levels approximately the LOD and LOQ. The limit of detection was obtained as 0.0053µg/mL for Rosuvastatin and 0.00019µg/mL for

Fenofibrate. The limit of quantitation was obtained as 0.017µg/mL for Rosuvastatin and 0.00063µg/mL for Fenofibrate which shows that the method is very sensitive.

Table.1.Optimized chromatographic conditions and system suitability parameters for proposed method

Parameter	Chromatographic conditions
Instrument	Waters e2695 Alliance HPLC with Empower2 software
Column	C18 Hypersill ODS (250×4.6mm.5µ)
Detector	PDA Detector 2998
Diluents	Methanol
Mobile phase	Methanol :OPA buffer pH 3.0(35:65)
Flow rate	1.2ml/min
Detection wavelength	238nm
Temperature	25°c
Injection volume	10µl
Retention time	Rosuvastatin:1.950; Fenofibrate:30858
Theoretical plate count	Rosuvastatin:4726; Fenofibrate:3170
Tailing factor	Rosuvastatin:1.138; Fenofibrate:1.400
USP Resolution	10.310

Table.2.Specificity study

Name of the solution	Retention time in min
Blank	No peaks
Rosuvastatin	1.950
Fenofibrate	3.858

Table.3.Recovery data
Table.3a.Recovery data of the proposed Rosuvastatin

Spiked level	Sample weight	Sample area	µg/ ml added	µg/ ml found	% Recovery	% Mean
50%	732.00	1132260	29.400	29.71	101	
100%	1464.00	2203343	57.800	57.81	98	100
150%	2196.00	3363910	88.200	88.25	100	

Table.3b.Recovery data of the proposed Fenofibrate

Spiked level	Sample weight	Sample area	µg/ ml added	µg/ ml found	% Recovery	% Mean
50%	732.00	10666213	950.400	951.38	100	
100%	1464.00	21488849	1900.800	1916.72	100	100
150%	2196.00	31569646	2851.00	2815.89	99	

Table.4.Results of Precision study

Sample	Injection number	Precision	
		RT	Peak area
Rosuvastatin	1	1.941	2255177
	2	1.944	2252365
	3	1.941	2223745
	4	1.938	2223773
	5	1.938	2252119
	6	1.938	2252119
	Mean		2243216
	%RSD(NMT 2.0)		0.72
Fenofibrate	1	3.838	21070608
	2	3.838	21107354
	3	3.836	21246011
	4	3.831	21284382
	5	3.830	21532673
	6	3.830	21532673
	Mean		21295617
	%RSD(NMT 2.0)		0.94

Table.5.Robustness studies
Table.5a.Robustness data for Rosuvastatin

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate	2.205	2670	1.474
Actual flow ate	1.950	3233	1.403
Increased flow rate	1.752	2473	1.502
Decreased temperature	2.358	2567	1.461
Actual temperature	1.950	3233	1.403
Incrased temperature	2.659	3045	1.486

Table.5b.Robustness data for Fenofibrate

Parameter	RT	Theoretical plates	Asymmetry
Decreased flow rate	4.366	4011	1.218
Actual flow ate	3.858	4734	1.128
Increased flow rate	3.426	3658	1.244
Decreased temperature	4.874	3729	1.266
Actual temperature	3.858	4734	1.128
Incrased temperature	5.317	4770	1.313

Table.6.Linearity data
Table.6a.Linearity data of the Rosuvastatin

S.No	Conc(µg/ml)	RT	Area
1	50	1.930	1136335
2	75	1.922	1691065
3	100	1.917	2259388
4	125	1.916	2817812
5	150	1.916	3386369
Std.dev			889685
Slope			22507
Intercept			7467
Correlation coefficient (r^2)			0.999

Table.6b.Linearity data of the Fenofibrate

S.No	Conc(µg/ml)	RT	Area
1.	50	3.801	10624304
2.	75	3.786	15847690
3.	100	3.784	21180479
4.	125	3.789	26450357
5.	150	3.798	31769938
Std.dev			8363306
Slope			21157
Intercept			16980
Correlation coefficient (r^2)			0.999

Table.7.Limit of Detection and Limit of Quantification

Name	LOD	LOQ
Rosuvastatin	0.0053	0.017
Fenofibrate	0.00019	0.00063
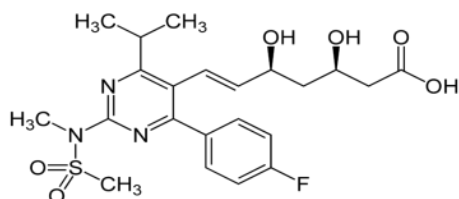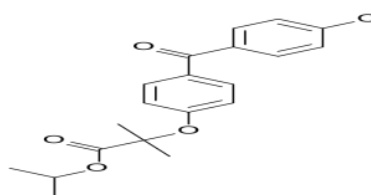
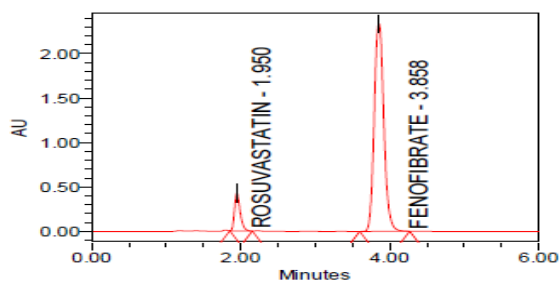**Rosuvastatin**   **Fenofibrate**

Figure.1.Typical Chromatogram of standard Rosuvastatin and Fenofibrate
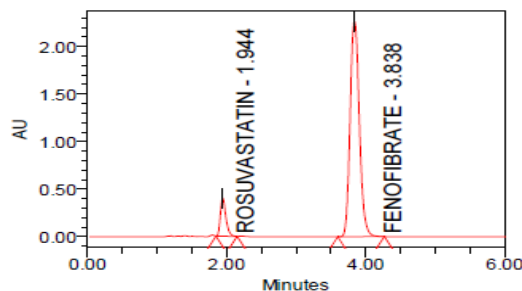

Figure.2.Typical chromatogram of Rosuvastatin and Fenofibrate tablets in marketed formulation
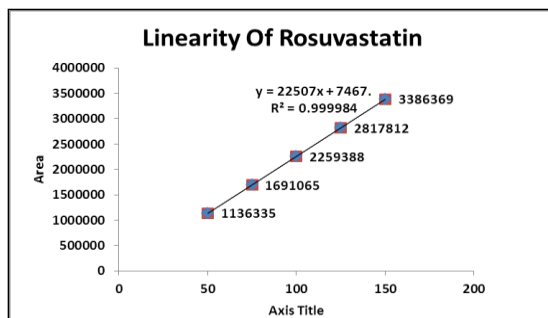

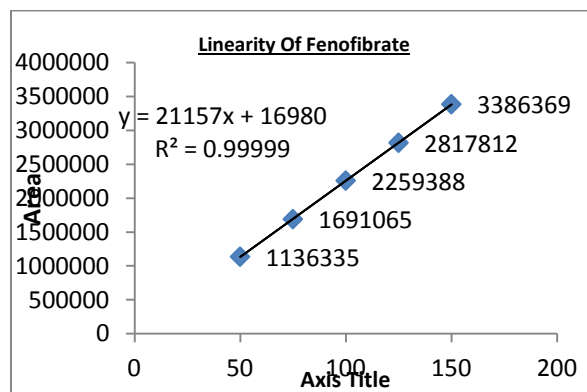**Figure.3.Linearity of Rosuvastatin**

Figure.4.Linearity of Fenofibrate

CONCLUSION

A new validated RP-HPLC method has been developed for the quantitative and Qualitative determination of Rosuvastatin and Fenofibrate in tablet dosage forms in bulk and pharmaceutical dosage forms was established. The method was completely validated shows satisfactory results for all the method validation parameters tested and method was free from interferences of the other active ingredients and additives used in the formulation. Infact results of the study indicate that the developed method was found to be simple, reliable, accurate, linear, sensitive, economical and reproducible and have short run time which makes the method rapid. Hence it can be concluded that the proposed method was a good approach for obtaining reliable results and

found to be suitable for the routine analysis of Rosuvastatin and Fenofibrate in Bulk drug and Pharmaceutical formulations.

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