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Research Article

A NEW IMPROVED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN TABLETS

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ABSTRACT

A rapid, specific, sensitive and simple high performance liquid chromatography was developed for simultaneous estimation of Rosuvastatin Calcium and Fenofibrate in tablet formulation. The separation was achieved by phenomenex C_{18} column (250× 4.6 mm, particle size 5µm) with a mobile phase consisting of Methanol: 0.02M ammonium di hydrogen phosphate buffer (75:25v/v, PH 5.5 adjusted with ortho phosphoric acid), at a flow rate of 1.0 ml/min. Detection was carried out at 272 nm. Retention time of Rosuvastatin calcium and Fenofibrate were found to be 4.18 and 5.18 min, respectively. The linear dynamic range was 12-32µg/ml and 174-464µg/ml for Rosuvastatin and Fenofibrate, respectively. The method is validated for accuracy, Precision, ruggedness and Robustness. The proposed method is successfully applied for the simultaneous determination of both drugs in commercial tablet preparation. The results of the analysis have been validated statistically and by recovery studies.

Keywords: Rosuvastatin, Fenofibrate, High performance liquid chromatography, Simultaneous estimation.

INTRODUCTION

Rosuvastatin calcium (ROS) is chemically, bis[(E)-7[4-(4-fluorophenyl)-6-isopropyl- 2-[methyl (methyl-sulphonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt . It belongs to a class of drugs called statins, which are employed to lower hypercholesterolemia and related conditions and to prevent cardiovascular diseases. It increases the number of hepatic low-density lipoprotein receptors involved in the catabolism of LDL and also inhibits hepatic synthesis of very low-density lipoprotein [1-3]. Fenofibrate (FEN) is 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester. It is indicated for the treatment of hypercholesterolemia and mixed dyslipidemia [4-5].

New tablet formulation in combination of Rosuvastatin 10mg and Fenofibrate145 mg is commercially available in market (Rosulip®F10) for the treatment of mixed Dyslipidemia, Hypercholesterolemia and hypertension. Literature survey shows that various analytical methods have been reported for estimation of Rosuvastatin and Fenofibrate individually and combination with other drugs [6-18] .Only one HPLC[19] method was reported for its simultaneous estimation but it is less sensitivity and time consuming.

In this work we introduce an simple,fast,economic and isocratic RP-HPLC method for simultaneous determination of this combination in tablet formulation . The total run time was less than 6 min. Most of the HPLC methods developed for these two drugs so far have shown use of acetonitrile as organic component of mobile phase for separation An acute shortage of acetonitrile has lead to increase in demand and thus higher prices. In an effort to practice "green chemistry," this study was planned so as to substitute acetonitrile with a safer and less toxic chemical (methanol).In this method, methanol alone with change in pH of buffer was used to increase retention time of the analytes , making the method easy and safe. The proposed method was developed,optimized,and validated according to International conference on Hormonization(ICH) guidelines [20].

MATERIALS AND METHODS

Equipment

Chromatographic separation was performed on Waters HPLC system consist of model 2695 having PDA detector and Rheodyne injector with 20µl loop volume. Waters Empower software was applied for data collecting and processing.

Reagents and chemicals

Methanol and water of HPLC grade were procured from Rankem lab ltd. ROS and FEN were received as gift samples from Madras pharmaceuticals,Tamilnadu,India, respectively. Ortho phosphoric acid and Ammonium di hydrogen ortho phosphate A.R grade was purchased from E.Merck chemicals Mumbai, India.

HPLC conditions

A Phenomenex C_{18} (25cm×4.6mm, 5µ) column was used as the stationary phase. A mixture of Methanol and 0.02M ammonium di hydrogen phosphate buffer (80:20v/v, PH 5.5 adjusted with ortho phosphoric acid), was used as a mobile phase and P^H 5.5 adjusted with orthophosphoric acid. It was filtered through 0.45µ membrane filter and degassed. The mobile phase was pumped at 1 ml/min. The eluents were monitored at 272nm.The injection volumes of samples and standard were 20µl.

Standard solutions

A stock solution containing 1000µg/ml of ROS and FEN were prepared separately by dissolving ROS and FEN in methanol. A working standard solution containing 12-32µg/ml and 174-464µg/ml of ROS and FEN were prepared from the above stock solution. All the stock solutions were covered with aluminum foil to prevent photolytic degradation until the time of analysis.

Assay in formulations

Twenty tablets, Rosulip-F10,(Cipla),each containing 10mg of ROS, 145mg of FEN were weighed and finely powdered. A quantity of powder equivalent to20mg of ROS,290 mg of FEN were weighed and transferred in to 100ml of standard volumetric flask and added 50 ml of methanol. The sample was kept in an ultrasonic bath for 20 min and furthur diluted to 100ml by using mobile phase.

Then it is filtered through 0.22µ membrane filter paper. one ml of this solution further diluted to 10ml to get a concentration of 20 µg/ml of ROS, 290µg/ml of FEN.20µl of this solution was injected in to HPLC system and chromatograms were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the chromatograms were recorded. The amount of Rosuvastatin and Fenofibrate present in each tablet was calculated by comparing the peak area of the standard solution and sample. The amount of the drugs were calculated and tabulated in Table 1.

Table 1: Table for Assay

S.No	Tablet sample	Label claim (mg/capsule)	Peak Area	*Amount Present (mg/capsule)	*Percentage (Label claim) (%w/w)
1	Rosuvastatin	10	645792	148.98±0.02	99.32±0.05
2	Fenofibrate	145	49134782	10.11±0.04	101.11±0.08

*Average of six determinations, mean ± Standard Deviation

Optimization of the method

The goal of this study was to develop a single isocratic phase HPLC method for the simultaneous determination of Rosuvastatin calcium and Fenofibrate. During optimizing the method some important parameters like pH of the mobile phase , concentration of the acid or buffer solution ,percentage and type of the organic modifier,etc.,were tested for a good chromatographic separation [21-22]. Trials showed that an basic phase with reverse phase a Purospher BDS C18 column gives symmetric and sharp peaks. For this reason ,0.01M Ammonium di hydrogen phosphate solution was preferred as an basic buffer.Methanol was chosen as organic solvent because it dissolves drugs very well. Mobile phase composition of methanol and 0.01M potassium di hydrogen phosphate buffer (75:25 v/v) at flow rate of 1.0mL/min showed good resolution. When ortho phosphoric acid was used as modifier resolution between ROS and FEN was much better than pH 5.5,with decrease in peak tailing. Retention time of the drugs obtained under these conditions were 4.12and 5.18 min for ROS and FEN respectively. For the quantitative analytical purposes the wave length was set at 272 nm.The typical chromatogram of the standard and sample were shown in Figure 1 and Figure 2.

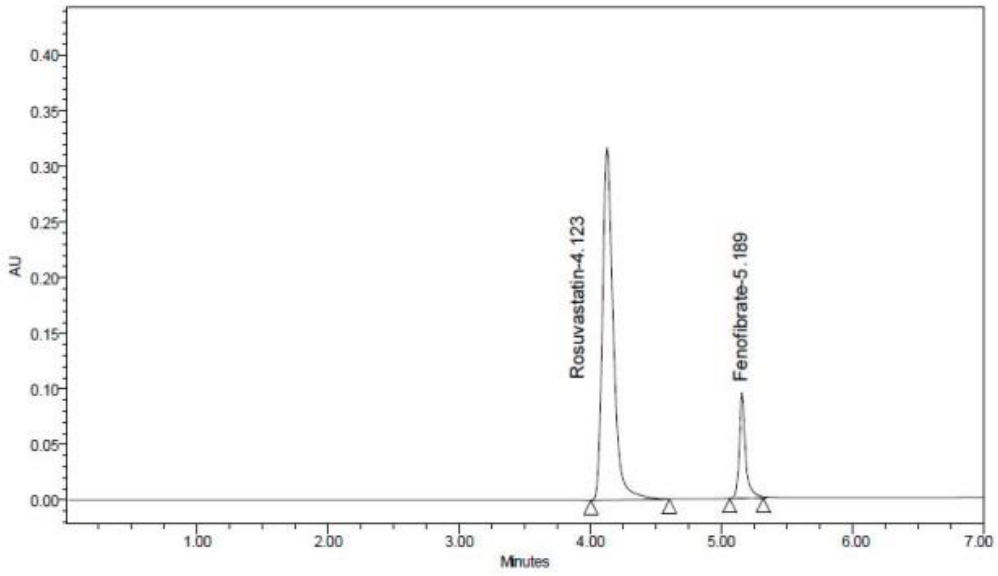

Fig. 1: Chromatogram of standard

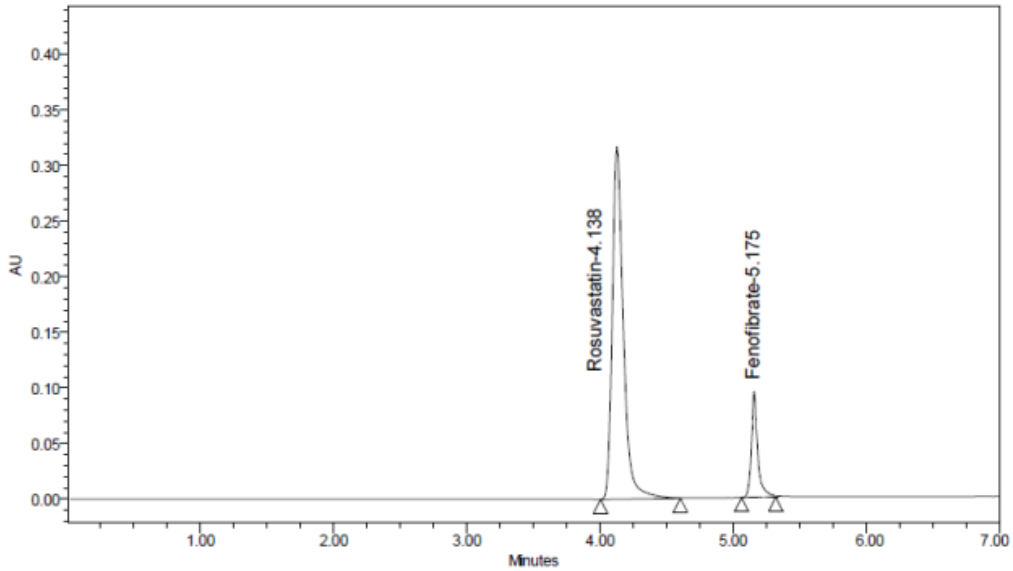

Fig. 2: Chromatogram of sample

Method Validation

The chromatographic conditions were validated by evaluating linearity, recovery, method and system precision ,accuracy, system suitability, solution stability, limit of detection(LOD), Limit of Quantification (LOQ), robustness, ruggedness studies in accordance with ICH guideline Q2(R1).

System suitability

The column efficiency, resolution and peak symmetry were calculated for the standard solutions. (Table.2). The values obtained demonstrated the suitability of the system for the analysis of this drug combination and the system suitability parameters fall within ± 2% standard deviation range during performance of the method. Here asymmetric factor for peaks of ROS and FEN was less than 2% and resolution was satisfactory. The peaks obtained for ROS and FEN were sharp and have clear base line separation.

Solution stability

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 hr at room temperature. The results indicated that both the solutions, retention time and peak area of ROS and FEN did not show much variation (%RSD less than 2.0). There was no significant degradation with in the indicated period. Hence, it was concluded that both the solutions were stable for 24 hr at room temperature.

Specificity of the method

The specificity of the method was checked for the interference of impurities in the analysis of a blank solution (without any sample) and then a drug solution of 20 µg/ml was injected into the column,(Figure.3) under optimized chromatographic conditions, to demonstrate the separation of both ROS and FEN from any of the impurities, if present. As there was no interference of impurities and also no change in the retention time, the method was found to be specific and also confirmed with the results of analysis of formulation.

Linearity study

The peak areas of ROS and FEN were linear with respect to the concentrations over the range of 12-32µg/ml and 174-464µg/ml respectively. The slope and intercept value for calibration curve Y =32255X+925.6 (R^2= 0.999) for FEN and 16988X—23448(R^2= 0.999) for FEN

The results showed that excellent correlation exists between peak area and concentration of the drugs within the concentration range indicated previously. The data was analyzed by "linear regression least squares fit", and the parameters are listed in Table.3.

Table 2: system suitability

S.No	Parameters	Rosuvastatin calcium	Fenofibrate	Acceptance criteria
1	Retention time	4.12	5.18	
2	RSD of replicate injections	0.165	0.368	Not more than 2%
3	Asymmetric factor	0.56	0.62	Not more than 2
4	Theoretical plates	5476	4940	Not more than 3000
5	Resolution factor		3.41	More than 2

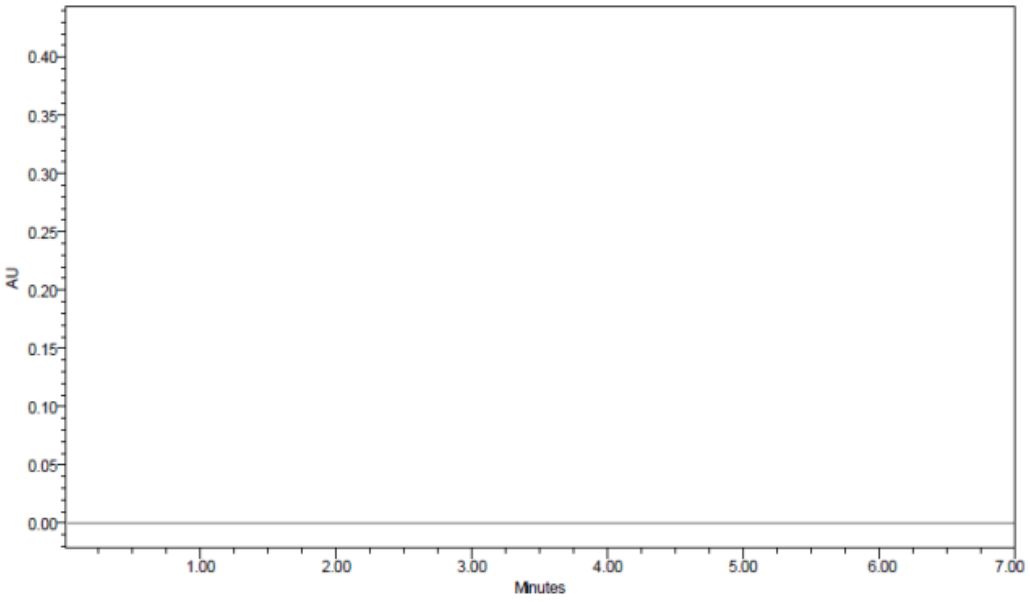

Fig. 3: Chromatogram of placebo

Table 3: Linearity study

Drug	Range µg/mL	Slope	Intercept	R^2	LOD µg/mL	LOQ µg/mL
Rosuvastatin calcium	12-32	32255	+925.6	0.999	0.5	1
Fenofibrate	174-464	16988	—23448	0.999	5	12

Limit of detection and Limit of quantification

The linearity for ROS was performed from12-32µg/ml and that for HCT from 174-464µg/ml. Lineaity graph was plotted and the correlation coefficient (R^2) determined. The limit if detection (LOD) was calculated from the linearity curve using the formula

LOD= 3.3X {Residual Standard deviation/Slope}.

The LOD for ROS was confirmed to be 0.5 µg/ml and for FEN it was confirmed to be 5 µg/ml.

The Limit of quantification (LOQ) was calculated from the linearity curve using the formula.

LOQ= 10X {Residual Standard deviation/Slope}

The LOQ for ROS was confirmed to be1 µg/ml and for FEN it was confirmed to be12 µg/ml

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. It was confirmed by studying the recovery at three different concentrations, 75%, 100%, and 125 % of those expected by spiking a previously analyzed test solution with additional drug standard solutions, the analysis being done in replicate. The %RSD and % relative error in all cases were within the acceptable limit (≤ 2%).

Table 4: Accuracy of the Method

Amount(%) ofdrug added	Theoretical content(µg/ml)	Conc.found (µg/ml)±SD*	Recovery (%)	SEM	RE (%)	RSD (%)
Rosuvastatin Calcium						
0	20	20.12±0.382	100.60	0.214	0.91	1.44
80	36	35.96±0.482	99.88	0.241	0.46	1.13
100	40	40.21±0.261	100.53	0.201	0.78	0.87
120	44	43.59±0.167	99.07	0.323	1.41	0.16
Fenofibrate						
0	290	290.39±0.216	100.15	0.148	0.55	0.89
80	522	522.42±0.314	100.08	0.117	0.69	0.21
100	580	581.51±0.169	100.27	0.418	1.44	0.41
120	638	639.08±0.145	100.11	0.152	1.42	0.36

*SD standard deviation(n=3),SEM= Standard Error of Mean,*RSD=SD/Mean×100,

RE (%)=%Relative Error =(Mean assayed concentration-Added Concentration/ Added Conentration×100)

It is evident from the results of accuracy study, reported in Table 4 that the proposed method enables very accurate quantitative simultaneous estimation of ROS and FEN.

Precision of this method was determined by injecting the standard solution of the three analytes six times. The R.S.D of the peak area of six replicates was found to be less than 1.0 %. Intermediate precision of the method was also evaluated by analyzing five samples of the three analytes at different days(6 days).results which are represented in Table.5.shows good intermediate precision of the method (average percentage of ROSfor the 6 days is 102.0% with R.S.D of 0.7%,while it is 99.7%for FEN with a R.S.D of 0.62%. From the data obtained, the developed RP-HPLC method was found to be precise.

Table 5: Intermediate precision of the method (% of the three active ingredients during 6 days)

Day	Rosuvastatin calcium	Fenofibrate
1	102.1±0.4	99.4±0.8
2	101.1±1.3	99.9±1.8
3	102.2±0.9	99.4±0.6
4	100.5±0.6	99.7±1.2
5	102.4±1.5	99.8±1.0
6	102.1±1.1	99.1±1.5

Ruggedness and Robustness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC 10 AT), Water Alliance 2695 by different operators using different columns. Robustness of the method was determined by subjecting the method to slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP- HPLC method developed is rugged and robust.

CONCLUSION

The method represents a fast analytical procedure for the simultaneous quantitation of Rosuvastatin calcium and Fenofibrate . The sample preparation is simple, the analysis time is short and the elution is isocratic. The method is amenable to the large number of samples with excellent precision and accuracy.

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