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SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN PHARMACEUTICAL DOSAGE FORMS BY USING RP-HPLC METHOD

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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 XTERRA column RP-C18 (150×4.6mm, particle size 3.5µm with a mobile phase consisting of sodium dihydrogen phosphate buffer (PH 3.5 adjusted with orthophosphoric acid): Acetonitrile (35:75), at a flow rate of 0.8 ml/min. Detection was carried out at 256 nm. Retention time of Rosuvastatin calcium and Fenofibrate were found to be 2.006 and 3.856 min, respectively. The linear dynamic range was 10-50µg/ml and 160-800µg/ml for Rosuvastatin and Fenofibrate, respectively. The method is validated for accuracy, Precision, Specificity, ruggedness, Robustness, LOD and LOQ. 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.

Key Words: Rosuvastatin, Fenofibrate, High performance liquid chromatography, Simultaneous estimation.

INTRODUCTION

Rosuvastatin is an anti-hyperlipidemic agent. It 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. Chemically it is 3R,5S,6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propan-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid.

Fenofibrate is an anti-hyperlipidemic agent.

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

Rosuvastatin calcium and Fenofibrate combination are used in the treatment of hyperlipidemics. (Anti-cholesterol). Literature survey reveals a few spectrophotometric and chromatographic methods for the estimation of both drugs as a single component and in combination with other drugs (Uyar B *et al.*, 2007; Lan K *et al.*, 2007; Vittal *et al.*, 2006; Kadav *et al.*, 2008; Nakarani *et al.*, 2007; Sevda RR *et al.*, 2011; Kumar TR *et*

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al., 2006; Suslu *et al.*, 2007; Lossner *et al.*, 2001; Masnatta *et al.*, 1996; Yardmici *et al.*, 2004). The main objective of my present work is to develop a simple, rapid, and precise RP-HPLC method for the estimation of Rosuvastatin calcium and Fenofibrate using Acetonitrile and Buffer in the ratio of 65: 35, v / v at a flow rate of 0.8ml/min.

MATERIALS AND METHODS

Materials

Acetonitrile, methanol, orthophosphoric acid and sodium dihydrogen phosphate were procured from Merck, India. Formulations of Rosuvastatin calcium and Fenofibrate are purchased from local market. Standards of Rosuvastatin and Fenofibrate were procured from AUROBINDO PHARMA LTD (Bachypalley, 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.

Equipment

The instrument used was waters HPLC instrument. The instrument is equipped with a Alliance 2695 with 2487 detector and variable wavelength programmable UV detector and a Inject port. The other equipment used is UV-Visible spectrophotometer. Sonication was done using Meltronicks Brason 2510 bath sonicator. Weighing was done on Mettler Toledo XS205DU weighing balance.

Chromatographic Conditions

XTERRA, RP-C18, 150×4.6mm, 3.5μ was used for separation. The mobile phase containing Acetonitrile and Buffer were mixed in the ratio of 65: 35, v / v was delivered at a flow rate 0.8 ml/min and the elution was monitored at 256 nm. The buffer used was Sodium Phosphate (P^H adjusted to 3.5 with Orthophosphoric acid). Injection volume was 20μl and the analysis was performed at ambient temperature.

Preparation of Standard solution

Accurately weigh and transfer 10mg of Rosuvastatin and 160mg of Fenofibrate working standard into a 100ml clean dry volumetric flask. Add about 30ml of diluent (methanol) 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 above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent (methanol).

Preparation of Sample solution

Accurately weigh and transfer 205.6mg of Rouvastatin and Fenofibrate tablet powder into a 100ml clean dry volumetric flask. Add about 30ml of diluent (methanol) 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 (methanol).

METHOD VALIDATION (The method was validated as per ICH guidelines)

System Suitability

A Standard solution of working standard was prepared and was injected six times into the HPLC system (30ppm for Rosuvastatin calcium and 480ppm for Fenofibrate). 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. The results were shown in Table.2.

Linearity

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 diluents(methanol) and sonicate to dissolve it completely and make volume up to the mark with the same solvent(Stock solution). From this 10-50 μg/ml solutions were prepared.

Inject each level into the chromatographic system and measure the peak area. The results were shown in Table.3. 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

The standard solution (30ppm for Rosuvastatin calcium and 480ppm for Fenofibrate)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. The results were shown in Table.4.

Intermediate Precision

To evaluate the intermediate precision of the method, Precision was performed on different day by using different make column of same dimensions.

The standard solution (30ppm for Rosuvastatin calcium and 480ppm for Fenofibrate) 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 noted. The results were shown in Table.5.

Accuracy

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy -150% solutions. Calculate the amount found and amount added for Rosuvastatin & Fenofibrate and calculate the individual recovery and mean

recovery values. The results were shown in Table.6.

Robustness

As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. (30ppm for Rosuvastatin calcium and 480ppm for Fenofibrate).

The flow rate was varied at 0.8 ml/min to 1.2ml/min.

The Organic composition in the mobile phase was varied from 65% to 75%.

The results were shown in Table.7.

Limit of Detection (for Rosuvastatin)

Inject the 0.15% solution into HPLC system and record the chromatogram.

Limit of Quantification (for Rosuvastatin)

Inject 0.48% solution into HPLC system and record the chromatogram.

Limit of Detection (for Fenofibrate)

Inject the 0.02% solution into HPLC system and record the chromatogram.

Limit of Quantification (for Fenofibrate)

Inject the 0.09% solution into HPLC system and record the chromatogram. The results were shown in Table.8.

Forced Degradation Studies

Acid hydrolysis degradation

Weighed and transferred 205.6 tablet powder into a 100 ml volumetric flask. 25 ml of 0.1 N Hydrochloric acid was added and reflux the sample at 60°C for about 12 hour in water bath and then neutralized with 25 ml of 0.1N sodium hydroxide. 25 ml diluent (methanol) was added and sonicated for 30 minutes and diluted to volume with diluent (methanol) and mixed well and filtered the solutions through 0.45µ Millex-HV PVDF filter.

Transfer 1ml of above solution into 10ml of volumetric flask and make upto the mark with diluent (methanol) and filter the solution through 0.45µm nylon filter.

Inject the above solution into HPLC system and record the chromatogram.

Alkali hydrolysis degradation

Weighed and transferred 205.6 tablet powder into a 100 ml volumetric flask. 25 ml of 0.1N sodium hydroxide was added and reflux the sample at 60°C for about 12 hour in water bath and then neutralized with 25 ml of 0.1N Hydrochloric acid. 25 ml diluent (methanol) was added and sonicated for 30 minutes and diluted to

volume with diluent and mixed well and filtered the solution through 0.45µ Millex-HV PVDF filter.

Transfer 1ml of above solution into 10ml of volumetric flask and make upto the mark with diluent (methanol) and filter the solution through 0.45µm nylon filter.

Inject the above solution into HPLC system and record the chromatogram.

Peroxide Oxidation degradation

Weighed and transferred 205.6 tablet powder into a 100 ml volumetric flask. 50 ml of 3% hydrogen peroxide was added and reflux the sample at 25°C for about 1 hour in water bath. 25 ml diluent (methanol) was added and sonicated for 30 minutes and diluted to volume with diluent and mixed well and filtered the solution through 0.45µ Millex-HV PVDF filter.

Transfer 1ml of above solution into 10ml of volumetric flask and make upto the mark with diluent (methanol) and filter the solution through 0.45µm nylon filter.

Inject the above solution into HPLC system and record the chromatogram.

Thermal degradation

Heat about 205.6 mg of tablet powder at 105°C for 12 hrs and transfer into a 100ml volumetric flask. Add 50ml of diluents and sonicate for 30mins with intermittent shaking and make up to the mark with diluent (methanol).

Transfer 1ml of above solution into 10ml of volumetric flask and make upto the mark with diluent (methanol) and filter the solution through 0.45µm nylon filter. Inject the above solution into HPLC system and record the chromatogram. The results were shown in Table.9.

RESULTS

Optimized Method

Buffer: Sodium Phosphate (P^H adjusted to 3.5 with Orthophosphoric acid).

Mobile phase: Acetonitrile and Buffer were mixed in the ratio of 65: 35, v / v and sonicated to degas.

Column: XTERRA, RP-C18, 150×4.6mm, 3.5µ

Pump mode: Isocratic

Flow rate: 0.8 ml/min

Detection wavelength: UV, 256 nm

Temperature: Ambient

Injection volume: 2

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.

Table 1. Assay of Rosuvastatin calcium & Fenofibrate

S.No	Drug	Sample Area	% of Assay
1	Rosuvastatin calcium	728921	99.3
2	Fenofibrate	3459281	100.3

Table 2. Results for system suitability (30ppm for Rosuvastatin and 480 ppm for Fenofibrate)

S.No	Injection		Retention time		Peak area		Tailing	
		Rosuvastatin	Fenofibrate	Rosuvastatin	Fenofibrate	Rosuvastatin	Fenofibrate	
1	1	2.009	3.859	733208	3437120	1.3	1.4	
2	2	2.010	3.863	730197	3507129	1.3	1.3	
3	3	2.008	3.861	732167	3506221	1.3	1.4	
4	4	2.008	3.859	728675	3509917	1.3	1.4	
5	5	2.010	3.859	734206	3513133	1.2	1.4	
6	6	2.009	3.860	730983	3512730	1.3	1.3	
7	MEAN	2.009	3.859	731572	3497708	1.28	1.36	
8	SD	0.008	0.006	2029.7	29815.3	0.02	0.024	
9	%RSD	0.39	0.15	0.27	0.8	1.43	1.5	

Table 3. Linearity Results

S.No	Rosuvastatin		Fenofibrate	
	Concentration	Peak area	Concentration	Peak area
1	10	377579	160	1830393
2	20	560627	320	2556258
3	30	729627	480	3447394
4	40	883969	640	4310362
5	50	1090217	800	5101860
Correlation coefficient		0.999	0.999	

Table 4. Repeatability results (30ppm for Rosuvastatin and 480 ppm for Fenofibrate)

S.No	Injection	Peak area	
		Rosuvastatin	Fenofibrate
1	1	729669	3507129
2	2	730197	3506221
3	3	732167	3509917
4	4	728675	3513133
5	5	734206	3512730
6	6	733208	3437120
7	Average	731353	3497708
8	SD	2205.7	3148.4
9	%RSD	0.30	0.09

Table 5. Intermediate precision results (30ppm for Rosuvastatin and 480 ppm for Fenofibrate)

S.No	Condition	Peak area	
		Rosuvastatin	Fenofibrate
1	Day 1	734876	3515429
2	Day 2	733658	3519104
3	Day 3	734026	3526904
4	Day 4	730810	3530000
5	Day 5	735165	3533438
6	Average	733707	3524975
7	SD	1731.1	7519.9
8	%RSD	0.24	0.21z

Table 6. Accuracy results

S.No	Concentration (%)	Peak area		%Recovery (%)		Mean recovery (%)	
		Rosuvastatin	Fenofibrate	Rosuvastatin	Fenofibrate	Rosuvastatin	Fenofibrate
1	50	379560	1820017	101.3	101.3	100.5	99.9
2	100	731695	3444806	100.5	100.0		
3	150	1087515	5087789	99.6	98.4		

Table 7. Results for robustness (30ppm for Rosuvastatin and 480 ppm for Fenofibrate)

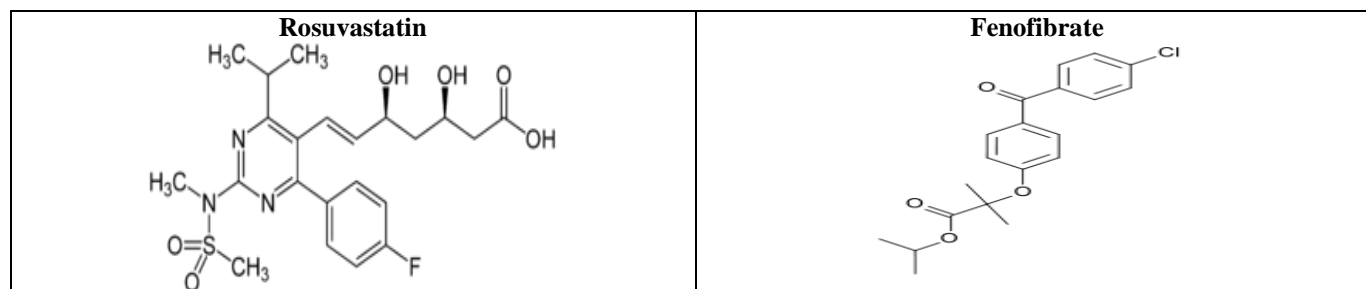
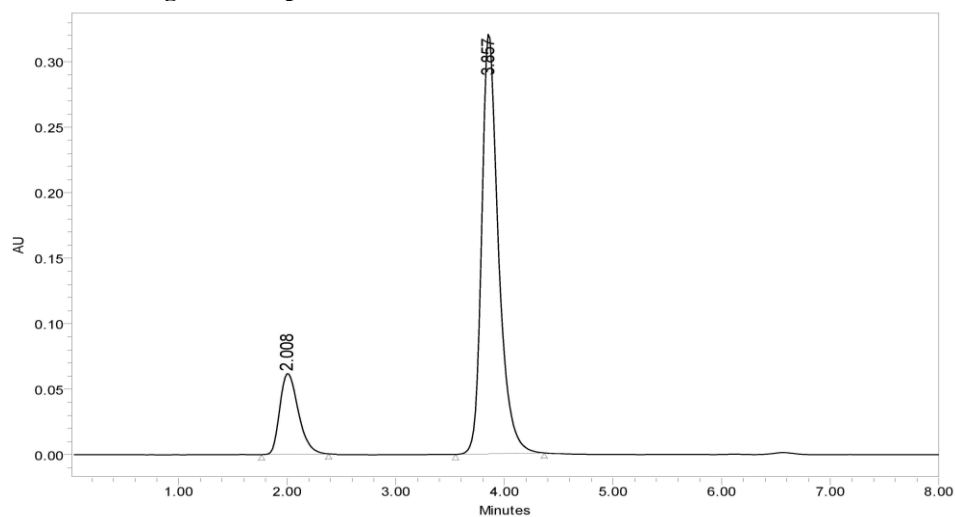
S.No	Flow rate	USP plate count		USP Tailing	
		Rosuvastatin	Fenofibrate	Rosuvastatin	Fenofibrate
1	0.8	2530.0	3296.1	1.4	1.4
2	1.0	2491.3	3185.8	1.3	1.4
3	1.2	2420.0	3119.8	1.3	1.3

Table 8. Results for LOD and LOQ

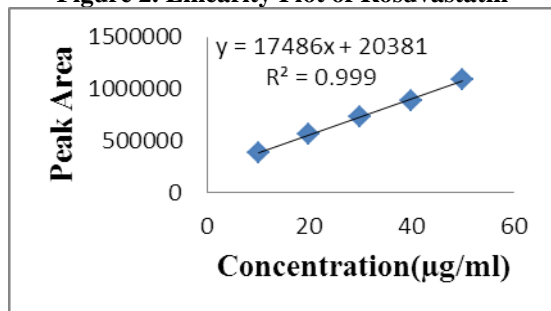
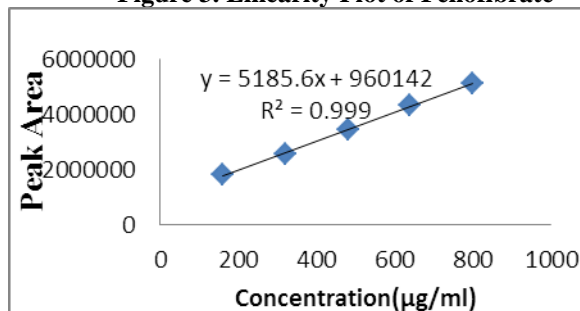
Drug	LOD	LOQ
Rosuvastatin	2.98	9.98
Fenofibrate	2.96	10

Table 9. 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 ₂ O ₂)	84.6%	83.8%
Thermal degradation	88.6%	87.8%

**Figure 1. Standard Chromatogram for optimized method**

	Name	Retention Time (min)	Area (μV*sec)	Height (μV)
1	Rosuvastatin	2.008	731472	61817
2	Fenofibrate	3.857	3435946	321577

Figure 2. Linearity Plot of Rosuvastatin**Figure 3. Linearity Plot of Fenofibrate**

DISCUSSION AND CONCLUSION

In the present work, an attempt was made to provide a newer, sensitive, simple, accurate and low cost RP-HPLC method. It is successfully applied for the determination of Rosuvastatin and Fenofibrate in pharmaceutical preparations without the interferences of other constituent in the formulations.

In this method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to get good optimum results. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with acetonitrile and buffer (65:35) with 0.8 ml/min flow rate is quite robust. The optimum wavelength for detection was 256 nm for which better detector response for the drug were obtained. The average retention time for Rosuvastatin and Fenofibrate were found to be 2.008 and 3.859.

The calibration was linear in concentration range of 10-50 µg/ml for Rosuvastatin and 160-800 Fenofibrate respectively. The sensitivity for the drug has been calculated and the LOD and LOQ of the Rosuvastatin was found to be 2.98 µg/ml and 9.98 µg/ml and Fenofibrate was

found to be 2.96 and 10. The low values of % RSD, indicate the method is precise and accurate. The mean recoveries were found in the range of 100.5% for Rosuvastatin and 99.9% for Fenofibrate respectively.

Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot by different analysts, using similar operational and environmental conditions the % RSD, reported was found to be less than 2%. The proposed method was validated in accordance with ICH parameters and the results of all methods were very close to each other as well as to the label value of commercial pharmaceutical formulation. Therefore, there is no significant difference in the results achieved by the proposed method.

Hence it is suggested that the proposed isocratic RP-HPLC method can be effectively applied for the routine analysis of Rosuvastatin and Fenofibrate in bulk and in tablet formulation.

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