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Development and Validation of Reversed-Phase HPLC Method for Simultaneous Estimation of Rosuvastatin and Fenofibrate in Tablet Dosage Form

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Abstract: A simple, precise and accurate reversed-phase liquid chromatographic method has been developed for the simultaneous estimation of Rosuvastatin and Fenofibrate in tablet formulations. The chromatographic separation was achieved on (Inertsil ODS, 250 x 4.6mm, 5µ column) analytical column. And mobile phase as mixture of Water (pH adjusted to 2.5 with ortho phosphoric acid) and acetonitrile in ratio (30:70 v/v) at flow rate of 1.0ml/min and dual detector wavelength 248 nm for Rosuvastatin and 286 nm for Fenofibrate. The retention time of Rosuvastatin and Fenofibrate was found to be 3.6 and 20.5 minutes respectively. The validation of the proposed method was carried out for its specificity, linearity, accuracy, precision, limit of detection and quantification for both atorvastatin calcium and telmisartan. The developed method can be used for routine quality analysis of titled drugs in combination in tablet formulation.
Key words: Rosuvastatin, Fenofibrate, RP-HPLC, validation, simultaneous estimation.

1. Introduction

Rosuvastatin, new member of a class of cholesterol-lowering drugs commonly referred to as "statins", was approved for the treatment of dyslipidemia [1–3]. Rosuvastatin (RST) is chemically bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl-(methyl-sulfonyl)amino] pyrimidin-5-yl](3R,5S)-3,5-dihydroxy hept- 6-enoicacid] calcium salt. RST, a synthetic lipid-lowering agent, is a selective and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyne A (HMG-CoA) reductase, the key rate-limiting enzyme of cholesterol biosynthesis in liver. RST is used to reduce the amounts of LDL cholesterol, total cholesterol, triglycerides and apolipoprotein B in the blood. RST also modestly increases the level of HDL cholesterol in the blood. These actions are important in reducing the risk of atherosclerosis, which in turn can lead to several cardiovascular complications such as heart attack, stroke and peripheral vascular disease. RST peak plasma concentrations were reached by 3–5 h following oral administration in humans [4].

Fenofibrate has been widely used drug in the treatment of dyslipidaemia. The current formulations of FBT shown an improved bioavailability due to the incorporation of a micronized process in product development [5,6]. Chemically, FBT is 2-[4-(4-chlorobenzoyl) phenoxy]-2- methyl-propanoic acid, 1-methylethyl ester. Fenofibric acid (FFA), the active metabolite of FBT, contributes for the reductions in total cholesterol, LDL cholesterol, apolipoprotien B, total triglycerides and triglyceride rich lipoprotein [7,8].

A detailed survey of analytical literature for estimation of Rosuvastatin revealed several methods based on varied techniques viz, HPLC [9-11], Capillary Zone Electrophoresis [12], Spectrophotometry [10] and High Performance Thin Layer Chromatography (HPTLC) [13-14]. Estimation of Fenofibrate are reported in bulk and formulations using nuclear magnetic resonance (NMR) spectrometry and LC (15), and in human plasma by LC/tandem mass spectrometry (LC/MS/MS) with electrospray ionization (16).

However no references have been found for simultaneous determination of Rosuvastatin and Fenofibrate in pharmaceutical preparations. The present manuscript describes a simple, rapid, precise and accurate isocratic reversed-phase HPLC method for simultaneous determination of Rosuvastatin and Fenofibrate in the same tablet dosage form.

2. Experimental

2.1. Chemicals

Rosuvastatin (94.51%) and Fenofibrate (94.41%) were obtained from Biocon Limited, Bangalore, India and Troikaa Pharmaceuticals Ltd. respectively as gift samples. Acetonitrile (HPLC Grade) and Methanol (HPLC Grade) were purchased from E. Merck (India) Ltd. Worli, Mumbai, India. The 0.45-µm nylon filters were purchased from Advanced Micro Devices Pvt. Ltd. Chandigarh, India. Mili-Q water was used throughout the experiment.

2.2. Equipments

Analysis was performed on a chromatographic system Agilent 1200 series separation module (Japan) equipped with an auto injector (G1329A), Diode array detector (DAD) SL (G1315C), Quaternary pump (G1311A) and column thermostat (G1316A). A chromatographic separation was achieved by Symmetry C-18, 250 x 4.6mm, 5µ analytical column. Data acquisition was made with Chemstation software. The peak purity was checked with the DAD detector.

2.3 Liquid chromatographic conditions

Chromatographic conditions were obtained using a stainless steel column (Inertsil ODS, 250 x 4.6mm, 5µ column), which was maintained at 25° C. The dual analytical was set, 248 nm for Rosuvastatin and 286 nm for Fenofibrate and samples of 5µl were injected to HPLC system. The mobile phase was a mixture of water (pH 2.5 adjusted with ortho-phosphoric acid) and acetonitrile in ratio of 30:70 (v/v) at a flow rate of 1.0ml/min. The mobile phase was filtered through 0.45µm filter (Sartorius, Germany) and degassed for 10 minutes by sonication.

2.4. Standard solutions and calibration graphs

Standard stock solution of Rosuvastatin (0.1 mg/ml) and Fenofibrate (2.0 mg/ml) was prepared in methanol as diluent. To study the linearity range of each component, serial dilutions were made to obtain working standards in the concentration range of Rosuvastatin (50-150 µg/ml) and Fenofibrate (1000 - 3000 µg/ml). A graph was plotted as concentration of drugs versus peak area response and results were found linear for both analytes. From the standard stock solution, a mixed standard solution was prepared containing Rosuvastatin (100 µg/ml) and Fenofibrate (2000 µg/ml). The system suitability test was performed from five replicate injections of mixed standard solution.

2.5. Sample preparation

Twenty tablets were weighed and finely powdered. The average weight of tablets was determined with weight of 20 tablets. A portion of powder equivalent to the weight of one tablet was accurately weighed into 100 ml A-grade volumetric flask and 70 ml diluent was added. The volumetric flasks were sonicated for about 20min to effect complete dissolution of the telmisartan and atorvastatin calcium, the solutions were then made up to volume with diluent. The solution was filtered through 0.45 µ m nylon filter. The aliquot portion of the filtrate was further diluted to get final concentration of 100 µg/ml of Rosuvastatin and 2000 µg/ml of Fenofibrate. Five microlitres of the test solution was injected and chromatogram was recorded for the same, and the amounts of the drugs were calculated.

2.6. Method validation

The HPLC method was validated in terms of precision, accuracy and linearity according to ICH guidelines [17]. Assay method precision was determined by using nine-independent test solutions. The intermediate precision of the assay method was also evaluated using different analyst on three different days. The accuracy of the assay method was evaluated with the recovery of the standards from excipients. Three different quantities (low, medium and high) of the authentic standards were added to the placebo. The mixtures were extracted as described in Section 2.5 and analyzed using the developed HPLC method. Linearity test solutions were prepared as described in Section 2.4. The LOD and LOQ for analytes were estimated by injecting a series of dilute solutions with known concentration. To determine the robustness of the method, the final experimental conditions were purposely altered and the results were examined. The flow rate was varied by (±) 0.1 ml/min. The percentage of organic modifier was varied by (±) 5% and pH of mobile phase was varied by (±) 0.1.

Table 1: Results of the recovery analysis of Rosuvastatin and Finofibrate

Compound	Wt spiked (mg)	Wt recovered (mg)	Recovery (%)	RSD (%) N=3
Rosuvastatin	5.08	4.96	97.64	0.82
	10.13	10.02	98.91	0.53
	15.19	15.09	99.34	0.94
Finofibrate	100.24	100.14	99.90	0.51
	200.19	200.07	99.94	0.72
	300.21	300.18	99.99	0.84

R.S.D.: relative standard deviation Wt: weight.

Table 2: System suitability parameters.

Parameters	Rosuvastatin	Finofibrate
Theoretical plates[a]	8836	15389
USP resolution[a]	41.37	--
peak symmetry[a]	1.20	0.98
% RSD	0.82	0.97

[a] USP-NF 29 section 621, pp.2135

Table 3: Intra and Inter-day assay precision data (n=9)

Actual Concentration	Measured concentration (µg/ml), RSD. (%)	
	Intra -day	Inter-day
Rosuvastatin (µg/ml)		
5.14	5.02 (0.54)	5.09 (0.30)
10.35	10.36(0.34)	10.21 (0.42)
15.19	15.04 (0.91)	15.03 (0.78)
Finofibrate (µg/ml)		
100.43	100.07 (0.23)	100.21 (0.61)
200.01	199.38 (0.49)	200.03 (0.67)
300.33	300.01 (0.51)	300.35 (0.42)

Data expressed as mean for "measured concentration" values.

Table 4:Results of robustness study

Factor	Level	Rosuvastatin	Finofibrate
		Mean % assay (n=3) (% R.S.D.)	Mean % assay (n=3) (% R.S.D.)
pH of mobile phase	2.4	100.1 (0.31)	99.7(0.53)
	2.6	99.6(0.76)	100.2(0.91)
Flow rate (ml/min)	0.9	99.7(0.54)	100.7(0.47)
	1.1	100.4(0.62)	99.1(0.44)
% of acetonitrile	25	99.2(0.21)	99.3(0.71)
	35	99.5(0.84)	99.0(0.39)

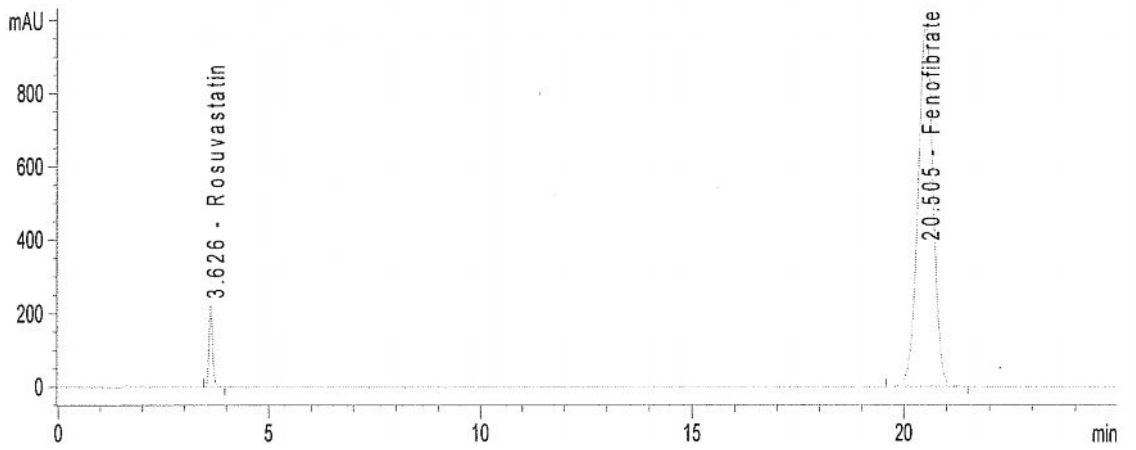

Fig 1. A typical chromatogram of sample solution containing 100 µg/ml of rosuvastatin and 200 µg/ml of fenofibrate

3. Results and discussion

3.1. Optimization of the chromatographic conditions

In order to develop RP-HPLC method for combination of cardiovascular drugs Rosuvastatin and Fenofibrate in single formulation. The chromatographic conditions were optimized for better resolution by using water and various buffers like phosphate, acetate and citrate for mobile phase preparation. After a series of screening experiments, it was concluded that water (pH at 2.5 with ortho phosphoric acid) gave better peak shapes than different buffer at different pH. With methanol as solvent both the peaks shows less theoretical plates and bad peak shapes, on changing to acetonitrile the peak shape improved along with theoretical plates. Further optimization experiments were carried out with varying percentage of acetonitrile in mobile phase. The best peak shape and maximum separation was achieved with mobile phase composition comprising mixture of water-acetonitrile (30:70 v/v).

The best separation, peak symmetry and reproducibility were obtained with Inertsil ODS, 250 x 4.6mm, 5µ column compared to Zorbax C18, 250mm x 4.6mm, 5µm and Waters symmetry C18, 250mm x 4.6mm, 5µm column. The optimum wavelength for detecting both the analytes was ascertained and found to be dual detector wavelength 248 nm for Rosuvastatin and 286 nm for Fenofibrate. Peak tailing was observed for rosuvastatin when the flow rate was 0.8ml/min using optimized mobile phase conditions. However, a flow rate of 1.0ml/min yielded optimum separation and peak asymmetry.

3.2. Validation of method

3.2.1. Specificity

The specificity of the HPLC method is illustrated in Figures-1 which depicts complete separation of Rosuvastatin and Fenofibrate in presence of tablet excipients. And no interfering peaks of endogenous compounds observed at the retention time of the analytes. In peak purity analysis with DAD detector, purity angle was less than purity threshold for both the analytes, which implies that both analytes are pure and excipients in the formulation doesn't interfere the analytes.

3.2.2. Accuracy

Accuracy of the method was calculated by recovery studies at three levels by standard addition method (Table 1). The mean percentage of recoveries obtained for Rosuvastatin and Fenofibrate was found to be 98.63 and 99.94% respectively.

3.2.3. Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The system precision is a measure of method variability that can be expected for a given analyst performing the analysis and was determined by performing five replicate analysis of the same working solution. The relative standard deviation (R.S.D.) obtained for Rosuvastatin and Fenofibrate are 0.82 and 0.97% respectively (Table 2).

The intra- and inter-day variability or precision data are summarized in Table 3. The intra-day precision of the developed LC method was determined by preparing the tablet samples of the same batch in nine

determinations with three concentrations and three replicate each. The R.S.D. of the assay results, expressed as percentage of label claim, was used to evaluate the method precision. The inter-day precision was also determined by assaying the tablets in triplicate per day for consecutive 3 days. The results indicated the good precision of the developed method (Table 3).

3.2.4. Linearity

Linearity was determined for telmisartan in the range of Rosuvastatin 50–150µg/ml and for Fenofibrate 1000–3000µg/ml. The correlation coefficient ('*r*') values for both the drugs were >0.999.

3.2.5. Limit of detection (LOD) and limit of quantitation (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels [17]. The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. The LOD and LOQ were calculated as

$$LOD = \frac{3.3 \times Syx}{b}$$

$$LOQ = \frac{10.0 \times Syx}{b}$$

Where Syx is residual variance due to regression; *b* is slope.

LOD and LOQ for Rosuvastatin were 0.24 and 0.81 µg/ml respectively and for Fenofibrate were 0.14 and 0.45 µg/ml, respectively.

3.2.6. Robustness

The robustness of an analytical procedure is measure of its ability to remain unaffected by small, but deliberate variations in method parameters. Robustness of the method was investigated by varying experimental conditions such as changes in wavelength, flow rate, pH and composition of mobile phase. The mixed standard solution is injected in five replicates and sample solution of 100% concentration is prepared and injected in triplicate for every condition and % R.S.D. of assay was calculated for each condition. The degree of reproducibility of the results obtained implies method is robust for routine quality analysis (Table 4).

4. Conclusion

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of Rosuvastatin and Fenofibrate in new tablet formulation. The method is very simple and specific as both peaks are well separated from its excipient peaks, which makes the developed method suitable for routine quality control analysis.

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