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SIMULTANEOUS ESTIMATION AND QUANTIFICATION OF ROSUVASTATIN AND FENOFIBRATE USING HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC) IN PHARMACEUTICAL DOSAGE FORM

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Keywords:

Fenofibrate, Rosuvastatin, HPTLC, Pharmaceutical dosage form, Simultaneous estimation

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ABSTRACT: A sensitive, specific and precise HPTLC method for simultaneous estimation of Fenofibrate and Rosuvastatin has been developed and validated. The method employed aluminium plates pre-coated with silica gel 60 F_{254} as the stationary phase. The solvent system consisted of n-Hexane: Acetone: Ethyl acetate: Glacial acetic acid (4: 5: 0.9: 0.1, v/v/v/v) which was found to give compact and dense spots for Fenofibrate and Rosuvastatin (RF value 0.83 ± 0.05 and 0.35 ± 0.05 respectively). Densitometric analysis of both drugs was carried out at 300 nm. Linear relationship was found with calibration curve for linearity in concentration range of 25-75 mg/spot and 167-501 mg/spot for Rosuvastatin and Fenofibrate with Correlation coefficient value 0.992 and 0.991 respectively. This method was found to be good percentage recovery for Rosuvastatin and Fenofibrate in between 98% to 102%. All parameters of validation of the formulations were found within the limit as per ICH guidelines.

INTRODUCTION: Rosuvastatin Calcium, 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 **Fig. 1**. 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]. Rosuvastatin belongs to a class of medications called Statins and is used to reduce plasma cholesterol levels and prevent cardiovascular disease [2].

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Fenofibrate is chemically Propane-2-yl-[4-(4-chlorobenzoyl) phenoxy]-2-methyl propanate **Fig. 2**. 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) [3]. Rosuvastatin calcium and fenofibrate are official in Indian, United States, and European Pharmacopoeia. But the combination of Rosuvastatin calcium and fenofibrate is not official in any one of the pharmacopoeia.

The tablet combination dosage form contains 10 mg of Rosuvastatin calcium equivalent to Rosuvastatin and 67 mg of Fenofibrate [4]. Rosuvastatin is co-formulated with Fenofibrate in Rosumac-F® tablets indicated [5] to lower the levels of lipids known as cholesterol and triglycerides in the blood when lifestyle changes (eg. low-fat diet) on their own have failed. This medicine helps to reduce the risk of heart disease [6].

Various analytical methods, including UV spectrophotometry, High-Performance Liquid Chromatography (HPLC), and High-Performance Thin Layer Chromatography (HPTLC), have been used to detect Rosuvastatin calcium and Fenofibrate in pharmaceutical formulations, either alone or in combination with other drugs. The current work aims to develop and validate simple, accurate and precise an HPTLC method in accordance with ICH guidelines Q2(R2) for the simultaneous estimation of Rosuvastatin Calcium and Fenofibrate in combined tablet dosage form.[7] Most of the reported methods are based on hyphenated techniques and overall cost of the analysis using these techniques is more as compared to High performance thin layer chromatography. The simple, easy, high throughput and automated prospective of HPTLC makes it a better choice over conventional analytical tool. So the main objective of current work was to develop and validate a simple, accurate method for determination of Rosuvastatin calcium and Fenofibrate in tablet dosage form as per International Conference on Harmonisation Guidelines (ICH) [8].

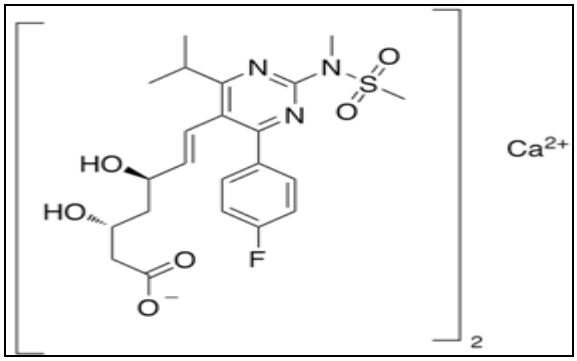

FIG. 1: ROSUVASTATIN CALCIUM

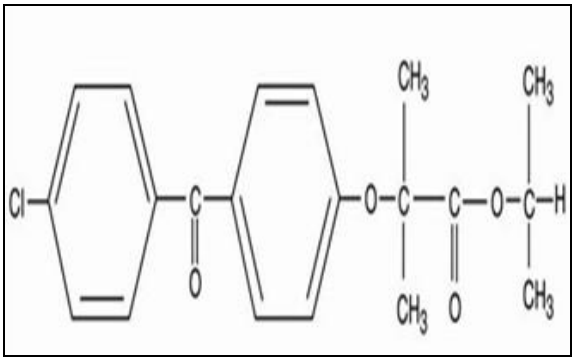

FIG. 2: FENOFIBRATE

MATERIALS AND METHODS: Fixed dose combination tablet Rosumac-F® was purchased from local market, Goregaon (West), Maharashtra, India. N-Hexane, Acetone, Ethyl acetate and Glacial acetic acid used were AR grade, purchased from Rankem Chemicals. All dilutions were performed in standard volumetric flasks.

Instrumentation and Chromatographic Conditions: Chromatography was performed on 10 cm × 10 cm and 20 cm × 10 cm Aluminium-backed TLC plates coated with silica gel 60 F_{254} from Merck. The solvent system of n-Hexane: Acetone: Ethyl acetate: Glacial acetic acid in the ratio 4:5:0.9:0.1 was the most appropriate solvent system for the HPTLC analysis of Rosuvastatin calcium and Fenofibrate in methanol. Separate HPTLC pre-coated plates of silica gel G 60 F254 (20x10) were employed for the spotting of standard solutions [9]. Reference standard and sample solutions (10 µl) were applied to the plates as bands 6 mm long, 10 mm from the bottom, and 11.9 mm apart by CAMAG Linomat 5 sample applicator. Densitometric scanning with a TLC Scanner 4 equipped with vision CATS software was performed at 300 nm.

Rosuvastatin Calcium Standard Stock Solution: Accurately weighed 16.72 mg Rosuvastatin calcium Standard to a 25mL volumetric flask, dissolve in the Methanol, and dilute to volume with the Methanol.

Fenofibrate Standard Stock Solution: Accurately weighed 10.02 mg Fenofibrate Standard to a 100mL volumetric flask, dissolve in the Methanol, and dilute to volume with the Methanol.

Standard Solution Preparation: From the above prepared Stock solution, pipette out 5.0 ml from each stock solution, transfer to 10 ml volumetric flask, mix well.

Sample Solution Preparation: Weigh 10 tablets determine the average weight of the tablet .Weigh accurately and transfer powder equivalent to 10 mg Rosuvastatin Calcium and 67 mg of Fenofibrate into 100 ml volumetric flask add 10 ml methanol, sonicated for 20 minutes with intermittent shaking cool to room temperature. Make up volume with Methanol. Filter the solution through 0.45µ filter.

Method Validation: ICH guidelines for method validation were followed for the developed HPTLC

method [10]. After the development of HPTLC method for the simultaneous determination of Rosuvastatin calcium and Fenofibrate was established. he objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose [11].

Validation of the method was carried out with respect to Specificity, precision, linearity, accuracy, Limit of Detection and quantification and Robustness.

Specificity: The specificity of the method was determined by analyzing standard drug and test samples. The peak for, Rosuvastatin Calcium and Fenofibrate test sample were confirmed by comparing the Rf value and the spectrum of the peak with that of the standard.

The specificity of the method was ascertained by overlaying UV spectra of spots for standard drug and sample [12]. The Confirmation of sample with standard by matching Rf values shown in **Fig. 3.**

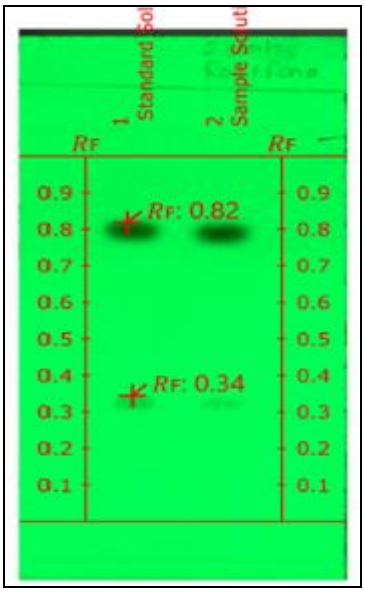

FIG. 3: COMPARISON OF STANDARD AND TEST SOLUTION

Linearity: According to the ICH-Q2 (R2) guidelines, for the establishment of linearity, a minimum of five concentrations is recommended [13]. Representative calibration curves of Fenofibrate and Rosuvastatin calcium were obtained by plotting the mean peak area of Fenofibrate and Rosuvastatin calcium against concentration over the range of concentration 25-75 µg/spot for Rosuvastatin and 167-500 µg/spot for Fenofibrate (n=5). They were found to be linear in the above mentioned range with correlation coefficient of 0.991 for Fenofibrate and 0.992 for Rosuvastatin calcium [14]. the graphical representation of calibration curves of both drugs shown in **Fig. 4** and **Fig. 5.** The statistical data represent in tabular form in **Table 1.**

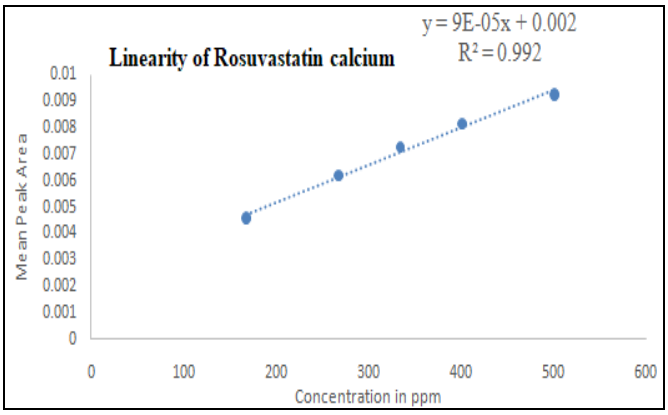

FIG. 4: LINEARITY OF ROSUVASTATIN CALCIUM

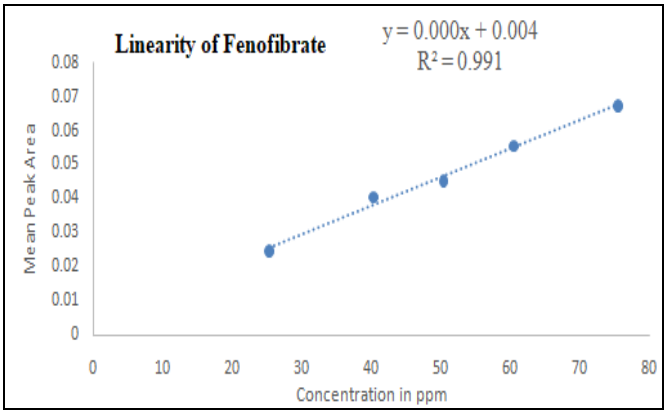

FIG. 5: LINEARITY OF FENOFIBRATE

TABLE 1: ANALYSIS PERFORMANCE DATA OF LINEARITY

	Rosuvastatin	Fenofibrate
Linear working range	25-75 µg/spot	167-500 µg/spot
Slope	0.00009	0.00013
Intercept	0.00234	0.00453
Correlation coefficient	0.992	0.991

Limit of Detection and Quantification: The detection limit of an individual analytical procedure is the lowest amount of analytes in a sample which can be detected but not necessarily quantities as an exact value [15]. The sensitivity of measurement of Rosuvastatin Calcium and Fenofibrate was estimated in terms of the limit of detection (LOD) and in terms of the limit of quantitation (LOQ). LOD and LOQ for Rosuvastatin Calcium were 16.10 and 40.24 µg/mL, respectively. For Fenofibrate they were 8.35 and 25.05 µg/mL, respectively [16]. By injecting triplicate of LOD and six times of LOQ, % RSD was calculated which for Rosuvastatin Calcium were 0.9 and 1.5 respectively, for Fenofibrate were 0.4 and 1.5 respectively.

Precision: The precision of the method was assessed through repeatability and intermediate precision. Repeatability was tested by analyzing the formulation six times at the same concentration. The amount of each drug in the tablet formulation was calculated, and the % relative standard deviation (%RSD) was determined.

The amount of each drug and the %RSD for both analyses were calculated to ensure consistency and reliability of the method [14]. The statistical data of repeatability and intermediate precision shown in **Table 2** and **Table 3**. The comparative data between repeatability and intermediate precision represented by **Table 4.**

TABLE 2: STATISTICAL DATA OF ASSAY (%) OF REPEATABILITY

Sample	Rosuvastatin Calcium	Fenofibrate
Sample solution-1	98.19	99.97
Sample solution-2	102.00	99.72
Sample solution-3	100.01	98.34
Sample solution-4	99.03	101.48
Sample solution-5	98.55	97.40
Sample solution-6	99.43	101.62
Mean	99.54	99.76
STD DEV	1.4	1.68
% RSD	1.4	1.7

TABLE 3: STATISTICAL DATA OF ASSAY (%) OF INTERMEDIATE PRECISION

Sample	Rosuvastatin Calcium	Fenofibrate
Sample solution-1	100.82	100.28
Sample solution-2	103.77	97.35
Sample solution-3	98.60	98.63
Sample solution-4	100.61	99.01
Sample solution-5	98.77	99.92
Sample solution-6	99.59	99.79
Mean	100.36	99.16
STD DEV	1.90	1.08
% RSD	1.90	1.09

TABLE 4: COMPARATIVE STATISTICAL DATA OF REPEATABILITY AND INTERMEDIATE PRECISION

Parameter	Sample	Rosuvastatin Calcium	Fenofibrate
Repeatability	sample solution-1	98.19	99.97
	sample solution-2	102.00	99.72
	sample solution-3	100.01	98.34
	sample solution-4	99.03	101.48
	sample solution-5	98.55	97.40
	sample solution-6	99.43	101.62
Intermediate precision	sample solution-1	100.82	100.28

sample solution-2	103.77	97.35
sample solution-3	98.60	98.63
sample solution-4	100.61	99.01
sample solution-5	98.77	99.92
sample solution-6	99.59	99.79
Mean	99.95	99.46
STD DEV	1.64	1.36
% RSD	1.6	1.4

Accuracy: The accuracy study of the developed method was done by standard addition. The sample solution was spiked with the standard solution and then analyzed to calculate its average percent recovery and percent relative standard deviation (%RSD). This spiking was done at three concentration levels (120%, 130% and 150%) and was conducted in triplicate [17]. The summary of accuracy for both drugs shown in **Table 5.**

TABLE 5: SUMMARY OF RECOVERY

Accuracy level%	% Recovery		Mean recovery		% RSD	
	Rosuvastatin Calcium	Fenofibrate	Rosuvastatin Calcium	Fenofibrate	Rosuvastatin Calcium	Fenofibrate
Accuracy 120%_1	99.56	99.16	100.33	99.99	0.7	1.0
Accuracy 120%_2	100.74	101.08				
Accuracy 120%_3	100.69	99.72				
Accuracy 130%_1	99.36	100.27	100.03	100.46	1.1	1.4
Accuracy 130%_2	99.39	99.14				
Accuracy 130%_3	101.36	101.96				
Accuracy 150%_1	101.56	101.28	100.60	99.37	0.8	1.7
Accuracy 150%_2	99.95	98.40				
Accuracy 150%_3	100.28	98.44				

Robustness: The analytical procedure robustness is small, but deliberate variations in method parameters measurement. The capacity of the method to remain unaffected and provides an indication of its reliability when compared to normal usage [18]. The standard deviation of peak areas of samples was calculated for each parameter and the % RSD was found to be less than 2 %. The data obtained by changing mobile phase ratio i.e., n-Hexane: acetone: Ethyl acetate: Acetic acid (4:5.1:0.8:0.1, 4:4.9:1.0:0.1), and by changing the detection wavelength ±2 nm (302nm & 298 nm). The cumulative percentage RSD obtained for Method precision and Robustness shown in **Table 6.**

TABLE 6: CUMULATIVE PERCENTAGE RSD BETWEEN METHOD PRECISION AND ROBUSTNESS

Sr. no.	Parameter	% RSD of Samples (Rosuvastatin Calcium)	% RSD of Samples (Fenofibrate)
1	Robustness of Detection wavelength (302nm)	1.4	1.5
2	Robustness of Detection wavelength (298 nm)	1.9	1.5
3	Robustness of change in Mobile phase ratio (4: 4.9: 1.0: 0.1)	1.3	1.5
4	Robustness of change in Mobile phase ratio (4: 5.1: 0.8: 0.1)	1.2	1.3

RESULTS AND DISCUSSION: The developed HPTLC method for Rosuvastatin Calcium and Fenofibrate provided results with a mobile phase of hexane: acetone: Ethyl acetate: Acetic acid (v/v/v/v) using HPTLC silica gel F254 plates. A linear relationship was observed by plotting a calibration curve which included a range of concentrations theory, establishing the linear dynamic range for the method. Rosuvastatin Calcium and Fenofibrate depicted a constant linear response between the ranges of 25-75 µg/spot for Rosuvastatin and 167-500 µg/spot for Fenofibrate. The corresponding linear regression coefficients were found to be 0.992 and 0.991 for Rosuvastatin Calcium and Fenofibrate respectively. Results for parameters such as repeatability and intermediate precision data are shown in **Tables 2** and **3**. The developed method was found to be precise as the %RSD values were found to be within range as per the (ICH Q2 (R2) Guidelines. The sensitivity of the

method was derived from the trend line equation and regression coefficient obtained. Limit of Detection (LOD) was found to be for Rosuvastatin Calcium was 16.10 µg/mL and For Fenofibrate was 8.35 µg/mL whereas the Limit of Quantitation (LOQ) obtained for Rosuvastatin Calcium was 40.24 µg/mL and for Fenofibrate was 25.05 µg/mL which indicated the sensitivity of the method developed. The proposed method showed adequate percentage of recovery i.e. between 98-102%. All 3 concentrations were spiked with the required known amount of standard as per the concentration of each and hence the percent recovery was estimated. The results depicting recovery/accuracy of the method are given in **Table 5**. Robustness of the method was determined by changing mobile phase composition and wavelength. The cumulative percentage recovery between Method precision and Robustness as shown in **Table 6**, which ensured robustness of the method.

CONCLUSION: In present study the HPTLC analytical methods were developed for widely used drug combination Rosuvastatin Calcium and Fenofibrate for the treatment of high cholesterol and heart attack. The developed method was found be accurate, precise, robust and sensitive for the estimation of Rosuvastatin Calcium and Fenofibrate in pharmaceutical dosage forms. Proposed method is useful for quality control labs and routine analysis.

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CONFLICT OF INTEREST: The authors confirm that there are no conflicts of interest to declare.

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