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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN AND FENOFIBRATE IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A new precise, accurate, reliable validated method for the determination of Rosuvastatin and Fenofibrate has been developed by using reverse phase high performance liquid chromatography (RP-HPLC) in pharmaceutical dosage form. Chromatographic separation was carried out by using mobile phase 0.01M Potassium dihydrogen phosphate: methanol (55:45v/v, PH-2.6 adjusted with Orthophosphoric acid) on Agilent XDB C18 (150 x 4.6 mm, 5µ) at a flow rate 1ml/min with UV detection at 220nm.The retention times for Rosuvastatin and Fenofibrate were 2.36 and 5.80 min respectively and both drugs showed good linearity in the range of 5-20 µg/ml and 80-320 µg/ml. The proposed method has been successfully applied to pharmaceutical formulation and was validated according to ICH guidelines and method showed good precision with percentage relative standard deviation less than 2%. The percentage recovery for Rosuvastatin and Fenofibrate was found between 99.06-100.94% and 99.12-100.95% respectively indicating the proposed method was accurate and precise.

Key words: Rosuvastatin (ROS), Fenofibrate (FEN), RP-HPLC, Simultaneous estimation.

INTRODUCTION

Rosuvastatin calcium (ROS) is 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]

Fenofibrate (FEN) 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). [2]

Literature survey revealed few analytical techniques are available for estimation of ROS alone as well as in combine dosage form such as UV ,HPLC, HPTLC.[3-7] Similarly few analytical methods are available for estimation of FEN alone and its combination with drugs such as UV and HPLC.[8-17] keeping this objective in mind an attempt has been made to develop and validate the RP-HPLC method for the simultaneous estimation of ROS and FEN which would be highly sensitive having good resolution reproducible and cost effective. Various validation aspects of the analysis accuracy, precision, recovery, the limits of detection and quantification etc have been measured as per ICH guidelines. [18]

MATERIALS AND METHOD

Equipment: Chromatographic separation was performed on HPLC system - Water's alliance 2695

with 2996 module Photo Diode Array (PDA) detector equipped with a solvent delivery pump, automatic sample injector and column thermostats. Waters Empower2 software was applied for data collecting and processing.

Chemicals and reagents: Methanol, Acetonitrile (HPLC grade) was used. Buffer used was Potassium dihydrogen ortho phosphate. Reference standards Rosuvastatin and Fenofibrate were obtained from SPECTRUM PHARMA. ROZAVEL-F Tablets of ROS (10mg) and FEN (160mg) manufactured by sun pharmaceuticals Ltd were procured from local market.

Preparation of standard solutions: Accurately weighed 10 mg of Rosuvastatin and 160 mg of Fenofibrate each was transferred into a clean and dry 100ml volumetric flask, dissolved with sufficient volume of diluent and sonicate for 5min. The volume made up to 100ml with diluent to obtain 100µg/ml of Rosuvastatin and 1600µg/ml of Fenofibrate stock solutions. 1ml of standard stock solution of Rosuvastatin (100µg/ml) and 1ml of standard stock solution of Fenofibrate (1600 µg/ml) are transferred in to a 10 ml volumetric flask and the volume made with diluent. The resulting solution was sonicated for 10 min.

Preparation of sample solution: 5 tablets of ROZAVEL-F containing 10mg of rosuvastatin and 160mg of fenofibrate were weighed and crushed into powder. From that powder weight equivalent to 10mg of Rosuvastatin and 160mg of Fenofibrate were transferred into a 500 mL volumetric flask, 300mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipetted out into a 10 ml volumetric flask and made up to 10ml with diluent.

Preparation of buffer: Accurately weighed 1.36gm of Potassium dihydrogen orthophosphate was transferred into a 1000ml of Volumetric flask, about 900ml of milli-Q water was added and sonicate to degassed and finally make up the volume with water. Finally pH is adjusted to 2.6 with dilute orthophosphoric acid solution.

Optimized chromatographic conditions:

Flow rate	:	1ml/min
Column	:	Agilent XDB C18, 150 x 4.6 mm, 5µ.
Detector wave length	:	220nm
Column temperature	:	30°C
Injection volume	:	20µL
Run time	:	10 min
Diluent	:	Acetonitrile

METHOD VALIDATION

System suitability test: This parameter was evaluated before each stage of validation. Six replication injections of standard preparation were injected. Asymmetry, number of theoretical plates and relative standard deviation of peak area were determined.

Linearity: Solutions were prepared containing 5µg/ml, 7.5µg/ml, 10µg/ml, 12.5µg/ml, 1.5µg/ml, 2µg/ml concentrations of Rosuvastatin calcium and 80µg/ml, 120µg/ml, 160µg/ml, 200µg/ml, 240µg/ml, 320µg/ml concentrations of Fenofibrate which corresponding to 50, 75, 100, 125, 150 and 200% respectively of the test solution concentration. Each solution was injected, linearity was evaluated by linear- regression analysis.

Accuracy: Accuracy was determined by the recovery studies at three different concentrations (corresponding to 50, 100 and 150% of the test solution concentration) by addition of known amounts of standard to pre-analysed sample preparation. For each concentration, three sets were prepared and injected.

Precision: Intraday and interday variations were determined by using six replicate injections of one concentration and analyzed on the same day and different days. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Robustness: The robustness was evaluated by assaying test solutions after slight but deliberate changes in the analytical conditions. The factors chosen for this study were the flow rate (±0.1ml/min), mobile phase composition (buffer: methanol by 5%), temperature (±5^{o}c).

Limit of detection (LOD) and Limit of quantification (LOQ): LOD and LOQ was calculated from linear curve using formulae

LOD= 3.3 * σ / slope, LOQ= 10 * σ / slope

(Where σ = the standard deviation of the response and S = Slope of calibration curve).

Specificity: Specificity was checked for the interference of impurities in the analysis of blank solution and injecting sample solution under optimized chromatographic conditions to

demonstrate separation of both ROS and FEN from impurities.

RESULTS AND DISCUSSIONS

Several mobile phase compositions were tried to resolve the peak of ROS and FEN. The mobile phase containing buffer: methanol in proportion of 55:45v/v was found ideal to resolve the peak of ROS and FEN satisfactory. Retention time of ROS and FEN were 2.361 and 5.806 min respectively (Figure 1&2). Result of assay is shown in Table-1. The proposed method was found to be linear in concentration range 5-20µg/ml for ROS and 80-320µg/ml for FEN. The data was shown in Table-2 and Figure-3&4 system suitability parameters were evaluated and results shown in (Table-3), which were within acceptance criteria. The mean percentage recovery for ROS and FEN was found to be between 99.06-100.94% and 99.12-100.95% respectively, which are well within the limit and hence the method was found to be accurate (Table-4). LOD and LOQ values were 1.09µg/ml and 3.31µg/ml for Rosuvastatin and 1.82µg/ml and 5.53µg/ml for Fenofibrate (Table-5). Results of intraday and interday precision were shown in the Table (6a&6b). The robustness of the method was investigated by varying experimental conditions such as changes in flow rate, mobile phase composition and temperature. The result obtained implies method is robust for routine qualitative analysis (Table-7).

CONCLUSION

The proposed RP-HPLC method was validated as per International conference on harmonization (ICH) guidelines, and found to be applicable for routine quality control analysis for the simultaneous estimation of ROS and FEN using isocratic mode of elution. The results of linearity, precision, accuracy and specificity, proved to be within the limits. The method provides selective quantification of ROS and FEN without any interference. The proposed method is highly sensitive, reproducible, reliable, rapid and specific.

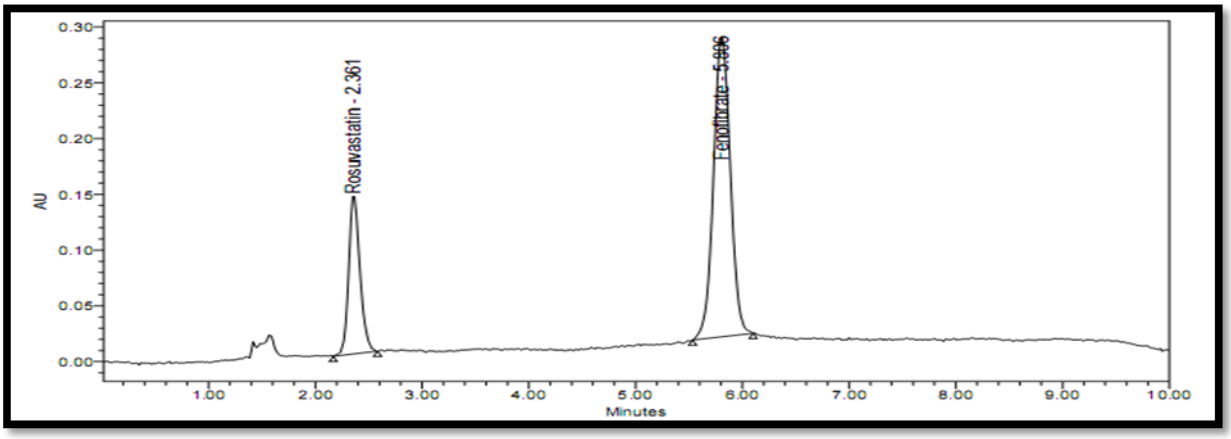

Figure-1: Chromatogram of ROS (10µg/ml) and FEN (160µg/ml) standard

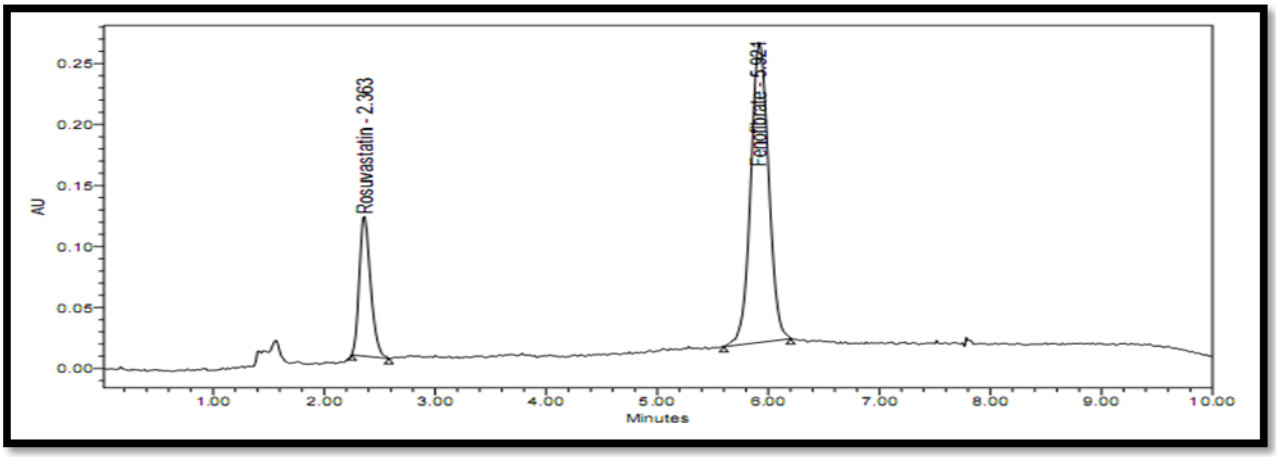

Figure-2: Chromatogram of ROS (10µg/ml) and FEN (160µg/ml) sample

Table -1 Analysis data of tablet formulation (ROZAVEL-F)

TABLET	Label claim(mg)	Assay ± SD (% label claim)	%RSD
ROS	10	99.25±0.26	0.26
FEN	160	99.24±0.15	0.15

RSD – relative standard deviation; SD – standard deviation

Table – 2: Result of Linearity

S. no	Rosuvastatin		Fenofibrate	
	Conc. (µg/ml)	**Peak area**	**Conc. (µg/ml)**	**Peak area**
1	5	572902	80	1645895
2	7.5	825608	120	2585271
3	10	1095022	160	3446232
4	12.5	1346646	200	4268134
5	15	1595989	240	5257245
6	20	2102324	320	6924643

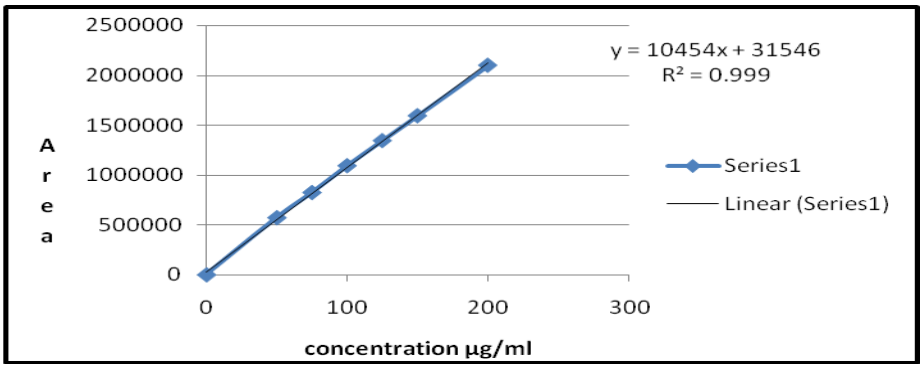

Figure-3: Calibration curve for rosuvastatin

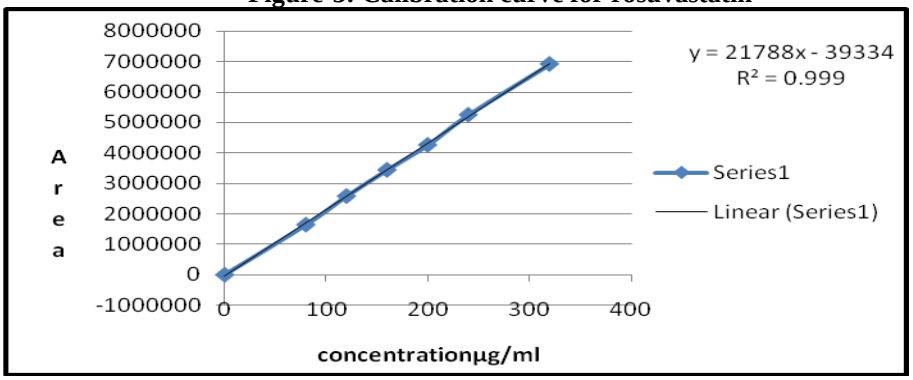

Figure -4: Calibration curve for Fenofibrate

Table-3: System suitability studies

Parameters	Rosuvastatin	Fenofibrate	Acceptance criteria
Theoretical plates	2392	6490	More than 2000
Tailing factor	1.14	1.01	Less than 2
Retention time	2.361	5.806	More than 2

Table-4: Recovery studies for Rosuvastatin and Fenofibrate

DRUG	Spiked level%	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery n=3	% RSD
ROS	50	25.10	25.56	101.86	0.23
	100	50.20	49.99	99.59	0.24
	150	75.30	75.89	100.79	1.04
FEN	50	401.60	404.2	100.65	0.23
	100	803.21	798.5	99.42	0.15
	150	1204.81	1207.7	100.04	0.18

n- Number of replicate injections

Table-5: LOD and LOQ for Rosuvastatin and Fenofibrate

DRUG	LOD (µg/ml)	LOQ (µg/ml)
Rosuvastatin	1.82	5.53
Fenofibrate	1.09	3.31

Table-6a: Results of intraday Precision

DRUG	Conc. (µg/ml)	Peak area (n=6)	% RSD
ROS	10	1089792	1.88
FEN	160	3091457	0.88

Table-6b: Results of interday Precision

DRUG	Conc. (µg/ml)	Peak area (n=6)	% RSD
ROS	10	1084728	0.05
FEN	160	3080896	0.26

Table-7: Results of Robustness study

S. no	Parameter	Condition	Mean Peak area (n=2)		% change	
			ROS	FEN	ROS	FEN
1.	Flow rate	1.1 ml/min	1136211	3137386	1.75	1.65
		0.9 ml/min	1124524	3189236	1.81	1.65
2.	Mobile phase	60:40 v/v	1203320	3183229	1.41	0.96
		50:50 v/v	1167730	3217219	0.20	1.43
3.	Temperature	35ºc	1147718	3224831	0.89	1.72
		25ºc	1152081	3211246	0.62	0.55

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