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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, specific and accurate reverse phase liquid chromatographic method was developed for the estimation of Rosuvastatin Calcium (ROS) and Fenofibrate (FEN) in combination. The separation of two drugs in reverse phase mode using C18 column (Agilent ODS UG 5 COLUMN 250X4.5mm Dimensions) with mobile phase containing acetonitrile: methanol: water (40:40:20) was used at isocratic mode and eluents were monitored at 252nm. The retention times of ROS and FEN were 2.3 and 5.0 min respectively and both the drugs showed good linearity in the concentration of 1-5µg/ml and 8-40µg/ml with a correlation coefficient (R) of 0.99968 and 0.99969 respectively. The proposed methods have been successfully applied to pharmaceutical formulation and were validated according to ICH guidelines and method showed good precision with percent relative standard deviation less than 2%. The percentage assay values of ROS and FEN were found to be 100.06 and 99.59 respectively and recovery values are within the limits of 98-102% indicating the proposed method was accurate and precise for the simultaneous estimation of ROS and FEN in bulk and pharmaceutical dosage forms.

Keywords: Simultaneous estimation, Reverse phase liquid chromatography, Validation.

INTRODUCTION

Rosuvastatin Calcium is official in Indian Pharmacopoeia. It 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. 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 (Rang et al., 2003). Literature survey revealed that various analytical methods such as Spectrophotometric Estimation Of Rosuvastatin Calcium And Glimepiride In Tablet Dosage Form (S.M. Ashraful Islam et.al.,2011),Simultaneous Estimation Of Rosuvastatin Calcium And Ezetimibe In Bulk And Tablet Dosage Form By Simultaneous Equation Method (B.Pandya et al.,2010), Simultaneous Estimation Of Rosuvastatin Calcium And Aspirin In Pharmaceutical Dosage Form By UV Spectrophotometric Method (S. Patel et. al.,2012), methods have been reported for the simultaneous estimation of Rosuvastatin Calcium in combination with other drugs.

Fenofibrate is official in Indian Pharmacopoeia. It is chemically Propane-2-yl-[4-(4-chlorobenzoyl) phenoxy]-2-methyl propanate It is the lipid regulating drug. Fenofibrate 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) (Rang et al., 2003). Literature survey revealed the various analytical methods such as Validated Spectrophotometric Determination of Fenofibrate in Formulation (R. Gupta et al., 2010); Three Simple Spectrophotometric

Methods for Fenofibrate in Tablet Formulation (G. Preetivinodet.al., 2011) has been reported for estimation of Fenofibrate from its formulation. Also Simultaneous Spectrophotometric Estimation Of Atorvastatin And Fenofibrate In Bulk Drug And Dosage Form By Using Simultaneous Equation Method (D.S.Gharge et al., 2010), A New Analytical Method Development And Validation Of Metformin Hydrochloride And Fenofibrate By Absorbance Ratio UV Spectrophotometric Method,(P.C. Bhamare et al., 2011), Development And Validation Of HPLC-UV Method For The Estimation Of Fenofibrate In Human Plasma(T. Manish Kumar et al., 2011), UV Spectrophotometric Estimation Of Ezetimibe And Fenofibrate In Bulk Drug And Dosage Form Using Simultaneous Equation Method (K.H.Maharshi et al., 2011) methods were reported for the simultaneous estimation of Fenofibrate in combination with other drugs.
At present no HPLC and UV Spectrophotometric methods are reported for the simultaneous estimation of Rosuvastatin Calcium and Fenofibrate in bulk and in tablet dosage form. Therefore, an attempt was made to develop simple, precise, accurate RP-HPLC methods for the simultaneous determination of Rosuvastatin Calcium and Fenofibrate in bulk and in dosage form.

MATERIALS AND METHODS

Materials

Pharmaceutical grade Rosuvastatin Calcium and Fenofibrate were obtained as a gift samples by M/S Aurobindo Pharma Ltd, Unit-4, Pydibhimavaram, A.P, India and Matrix Laboratories Limited R&D Centre, Bollaram, Medak Dist. A.P. India. These samples were used without further purification and certified to contain 99.65% w/w and 99.89 % w/w, respectively on dried basis. Rozavel-FLS containing 10 mg of Rosuvastatin Calcium and 80 mg Fenofibrate was obtained from a Hetero Pharmacy, Hyderabad.
Methanol (HPLC grade), Acetonitrile (HPLC grade), water for HPLC, were purchased from RANKEM Chemicals Limited, MERK Chemicals Limited.

Equipment

Agilent 1120 compact LC Chromatographic system with Variable Wavelength Programmable UV detector and Rheodyne Injector with 20l fixed loop were used for the chromatographic separation. EZChrome software was used for data analysis. Chromatographic separation was carried out on a C_{18} column [Agilent ODS UG5 column 250mmX4.5mm]. For spectroscopic detection ELico double beam SL218 UV-VIS Spectrophotometer. AXIS AGN 204-PO electronic balance was used for weighing purpose. Ultrasonic bath sonicator, degasser is used

Chromatographic Conditions

Column : C18 Column
Detector :Variable
Wavelength Detector : 252nm)
Injection Volume : 40µl
Flow Rate : 1 ml/ min
Temperature : Ambient.
Runtime : 10min
Mobile Phase : Acetonitrile: Methanol: Water (40:40:20)

Preparation of Standard Stock Solution

Accurately weighed quantities of 5mg and 10mg of ROS and FEN were dissolved in sufficient quantity of mobile phase in a 10ml volumetric flask. The volume was adjusted up to the mark with mobile phase to obtain the stock solution of 500µg/ml and 1000µg/ml concentrations each of ROS and FEN.

Assay

Twenty tablets containing ROS (10mg) and FEN (80mg) were taken and crushed to fine powder. Then powder equivalent to 10mg to FEN was taken in 10ml volumetric flask and dissolved in mobile phase. It was sonicated for 5-10min. solution was filtered through whatmann filter paper. From 1000µL of filtrate was further diluted with the mobile phase to get a solution containing 100µg/ml. From the above solution each 4.8ml was taken which contains 6µg/ml of ROS and 48 µg/ml of FEN. The solution was injected three times into the column. The amount present in the each tablet was calculated by comparing the areas of standards with the test samples.

VALIDATION OF THE METHOD

Linearity

The linearity responses in the concentration range of 2-10µg/ml and 16-80µg/ml for ROS and FEN were determined and the data was given in Table 1.

Precision

Precision was measured in terms of repeatability of application and measurement. Study was carried out by injecting six replicates of the standard concentrations of 10µg/ml and 80µg/ml for ROS and FEN. The precision values were given in Table 3.

Accuracy

Accuracy of the method was ascertained by performing recovery studies. Recovery studies

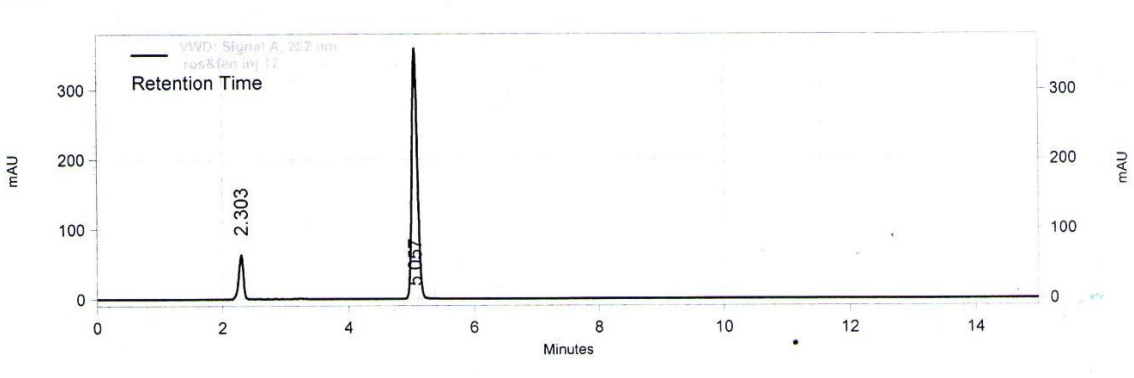
were carried out by addition of standard drug solution to pre-analysed tablet sample solution at three different concentrations levels (80%, 100%, and 120%) within the range of linearity. Results of recovery studies were shown in Table 4.

System suitability

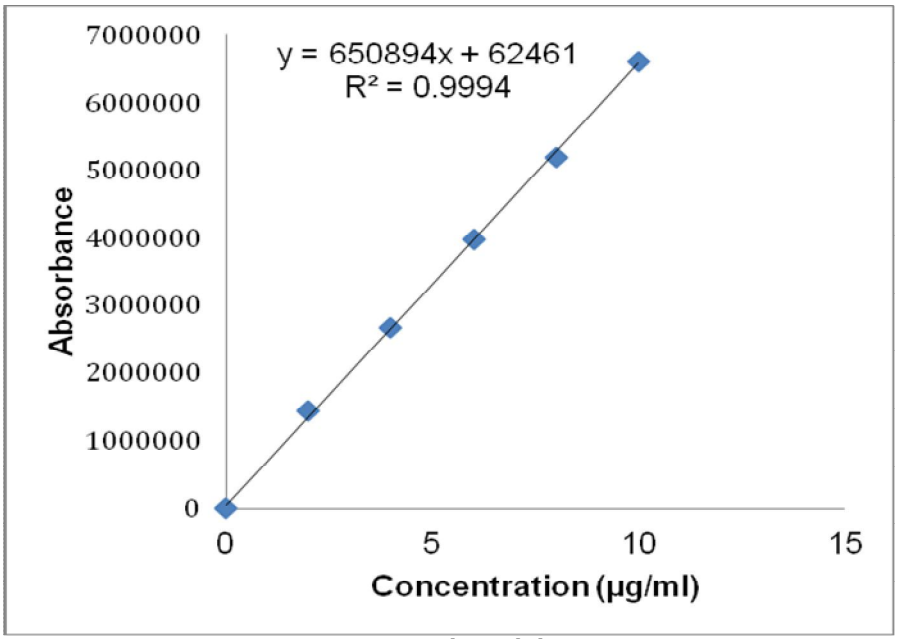
System suitability was carried out by injecting 10µg/ml and 80µg/ml of ROS and FEN at different injection volumes 10-50µg/ml with increment of injection volumes, the %RSD for tailing factor and theoretical plate number was less than 1% and is satisfactory.

LOD and LOQ

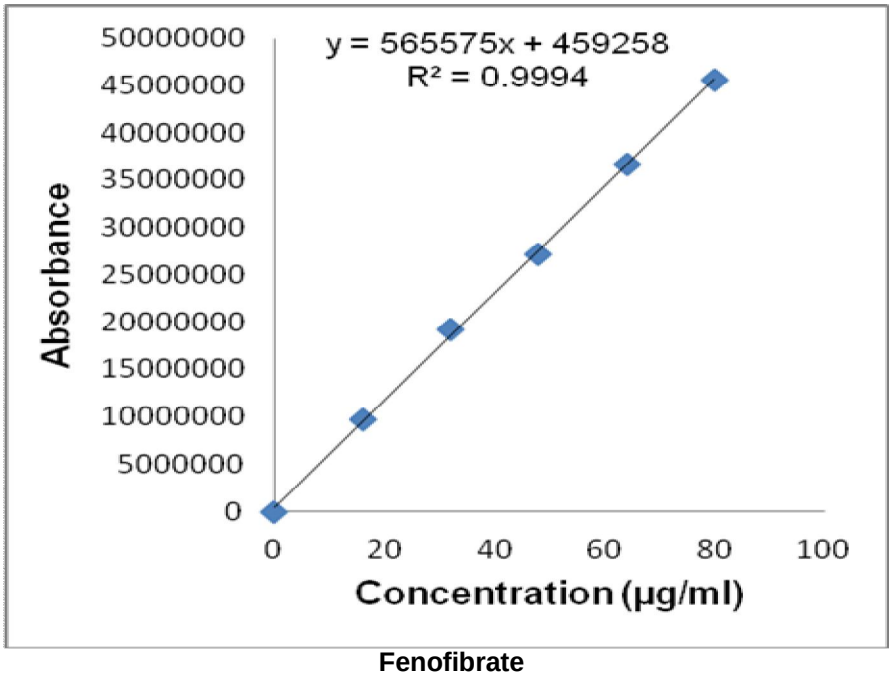
The LOD and LOQ values were determined by formulae LOD = 3.3 σ/m and LOQ = 10 σ/m (where, σ is the standard deviation of the responses and m is the mean of the slope of the calibration curve).



Chromatogram of Rosuvastatin Calcium and Fenofibrate



Rosuvastatin Calcium



Fenofibrate

Table 1: Linearity

S.no	Rosuvastatin Calcium			Fenofibrate		
	Conc. (µg/ml)	Retention time (min)	Peak area	Conc. (µg/ml)	Retention time (min)	Peak area
1	2	2.3	1449813	16	5.0	9763487
2	4	2.263	2671592	32	5.0	19220557
3	6	2.267	3982885	48	5.0	27226901
4	8	2.3	5192882	64	5.0	36703491
5	10	2.3	6604420	80	5.0	45579141
Correl (R)	0.99968			0.99969		

Table 2: System suitability studies

Parameters	Rosuvastatin calcium	Fenofibrate	Limit
Retention time (min)	2.30	5.06	
Theoretical plates (N)	7650	15638	N > 2000
Tailing factor (T)	1.34	1.5	T of $\leq$ 2
Resolution (R_s)	3.0		R_s of > 2

Table 3: Precision

Drug	Rosuvastatin Calcium	Fenofibrate
Mean	7068468	48830711
Standard Deviation	57666.82	366752.7
%RSD	0.815	0.751

Table 4: Accuracy

Drug	Spiked level (%)	Amount taken (µg/ml)	Amount found (µg/ml)	Percent recovery (% w/w)
ROS	80	6.4	6.37	99.6
	100	8	8.06	100.77
	120	9.6	9.59	99.9
FEN	80	51.2	51.19	99.99
	100	64	63.33	98.9
	120	76.8	76.79	99.9

RESULTS AND DISCUSSION

The proposed method was found to be linear in the concentration range of 2-10µg/ml and 16-80µg/ml for Rosuvastatin Calcium and Fenofibrate. The method was specific since excipients in the formulation did not interfere in the estimation of ROS and FEN. Accuracy of the method was indicated by the recovery values 98.9-100.7% for ROS and FEN. Precision is reflected by %RSD as 0.815 for ROS and 0.751 for FEN which was less than 2. The LOD and LOQ values were 0.19µg/ml and 0.58µg/ml for ROS and 0.28µg/ml and 0.87µg/ml for FEN.

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.

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