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Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Bulk and in Tablet Dosage Form by UV-Spectrophotometry and RP-HPLC

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Original Research Article

ABSTRACT

Two methods are described for the simultaneous estimation of Rosuvastatin Calcium and Fenofibrate in binary mixture. The first method was based on UV Spectrophotometric determination of two drugs, using simultaneous equation method. It involves absorbance measurement at 243nm (λ_{max} of Rosuvastatin Calcium) and 287nm (λ_{max} of Fenofibrate) in methanol; linearity was obtained in the range of 1-6 µg/ml and 4-28 µg/ml for Rosuvastatin Calcium and Fenofibrate, respectively. The second method was based on HPLC separation of two drugs in reverse phase mode using Luna C_{18} column. Linearity was obtained in the concentration of 1-7 µg/ml and 4-28 µg/ml for Rosuvastatin Calcium and Fenofibrate, respectively. Both these methods have been successively applied to pharmaceutical formulation and were validated according to ICH guidelines.

***Key words*:** Rosuvastatin Calcium, Fenofibrate, UV Spectrophotometry, HPLC, Method validation.

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 (Figure 1). It is used in the treatment of Hyperlipidemia (IP 2007). 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 UV Spectrophotometric Determination of Rosuvastatin Calcium in Pure Form and in Pharmaceutical Formulations (Alka Gupta *et al.,* 2009) and Determination of rosuvastatin in rat plasma by HPLC: Validation and its application to pharmacokinetic studies (Thammera Ranjith Kumar *et al.,* 2006) have been reported for estimation of Rosuvastatin Calcium from its formulations and biological fluids. Also Development and Validation of Stability-Indicating HPLC Methods for Quantitative Determination of Pravastatin, Fluvastatin, Atorvastatin, and Rosuvastatin in Pharmaceuticals. (Fabio Pereria Gomes *et al.,* 2009) and Determination of Simvastatin, Pravastatin sodium and Rosuvastatin in Tablet Dosage Forms by HPTLC (Chaudhari *et al.,* 2007) methods were reported for the simultaneous estimation of Rosuvastatin Calcium in combination with other drugs.

Fenofibrate, chemically Propan - 2 - yl 2 - [4 - (4 - chlorobenzoyl) phenoxy] - 2 - methyl propaneate (Figure 2). It is the lipid regulating drug (BP 2009). 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). There are few methods were reported for the estimation of Fenofibrate in pharmaceutical dosage form, which includes a Electronic structure and UV spectrum of fenofibrate in solutions. (Le *et al.,* 2008) and Method development and validation of Fenofibrate by HPLC using human plasma (Zzaman *et al.,* 2009). Also a Stability Indicating UPLC Method for

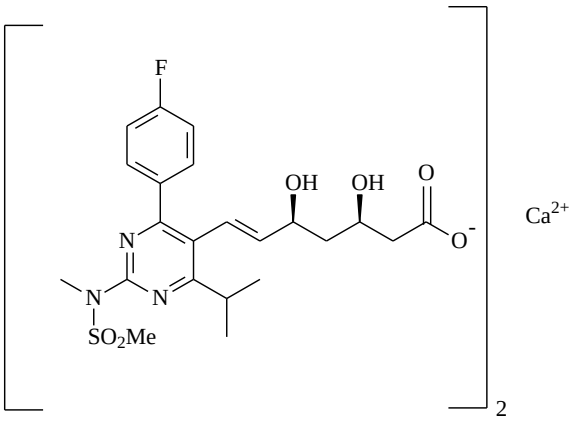

Figure 1. Structure of Rosuvastin Calcium.

simultaneous determination of atorvastatin, fenofibrate and their degradation products in tablets (Kadav *et al.*, 2008) was 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 UV- spectrophotometric and RP-HPLC methods for the simultaneous determination of Rosuvastatin Calcium and Fenofibrate in bulk and in tablet dosage form.

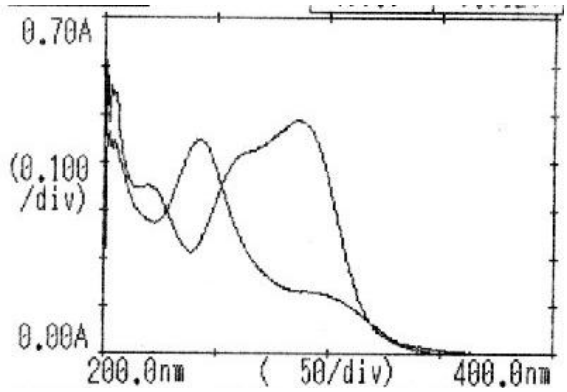

Figure 3. Overlain spectrum of Rosuvastatin Calcium and Fenofibrate in methanol. ROS is Rosuvastatin Calcium, FEN is Fenofibrate (each 10 µg/ ml) taken on UV – VIS Spectrophotometer (SHIMADZU 1700).

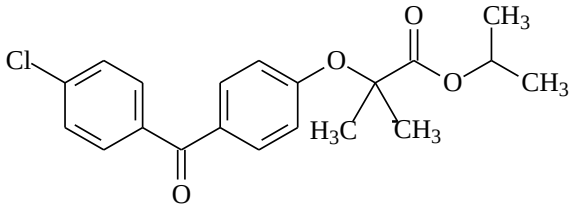

Figure 2. Structure of Fenofibrate.

MATERIALS AND METHODS

Materials

Pharmaceutical grade Rosuvastatin Calcium and Fenofibrate were obtained as gift sample by Glenmark Pharmaceutical Pvt. Ltd., Hyderabad. India, and these samples were used without further purification and certified to contain 99.53% (w/w) and 99.66% (w/w), respectively on dried basis. Rozavel - FLS containing 10 mg of Rosuvastatin Calcium and 80 mg Fenofibrate was obtained from a Hetro Pharmacy, Hyderabad. Methanol (AR grade), Methanol (HPLC grade), Water for HPLC, Acetonitrile (HPLC grade) were purchased from Qualigens India Pvt. Limited and Loba Chemie India Limited.

UV-Spectrophotometry Method

A double beam Shimadzu UV-Visible spectrophotometer-1700 Pharmaspec, with spectral bandwidth of 2nm, wavelength accuracy±0.5nm and a pair of 10 mm matched quartz cells was used.

Standard stock solutions of 100µg/ml were prepared by dissolving 10mg of each in 100ml of methanol. From these stock solutions, working standard solutions containing the concentrations of 10µg/ml of each were prepared by appropriate dilutions. They were scanned in the wavelength range of 400-200nm and the overlain spectrum were obtained (Figure 3). Two wavelengths 243nm (λmax of Rosuvastatin Calcium) and 287nm (λmax of Fenofibrate) were selected for the formation of simultaneous equation. The calibration curves were found to be linear in the concentration range of 1-6µg/ml for Rosuvastatin Calcium and 4-28µg/ml for Fenofibrate. The absorptivity coefficients of each drug at both wavelengths were determined. The

concentration of two drugs in the mixture were calculated using the following equations,

$$c_{ROS} = \frac{A_2 a_{y_1} - A_1 a_{y_2}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \quad \ldots\ldots\ldots (1)$$

$$c_{FEN} = \frac{A_2 a_{x_2} - A_2 a_{x_1}}{a_{x_2} a_{y_1} - a_{x_1} a_{y_2}} \quad \ldots\ldots\ldots (2)$$

Where, A1 and A2 are absorbance of mixture at 243nm and 287nm; ax1 and ax2, absorptivities of Rosuvastatin Calcium at 243nm and 287nm, respectively; ay1 and ay2, absorptivities of Fenofibrate at 243nm and 287nm, respectively. C_{ROS} and C_{FEN} are concentration of Rosuvastatin Calcium and Fenofibrate in mixture. The absorptivities reported are the mean of six independent determinations (Table 1).

HPLC Method

LC system used consisted of pump (model Shimadzu; LC–10 ATvp solvent deliver module) with universal loop injector (Rheodyne 7725 i) of injection capacity 20µL. Detector consists of UV-Visible detector SPD-10 Avp, Shimadzu; the column used was Luna C_{18} (5µm, 25cmX4.6mm i.d) phenomenex, USA, at ambient temparture.

Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously. The optimal composition of mobile phase was determined to be Acetonitrile: Methanol: Water (50:40:10, v/v). The flow rate was set to 0.5ml/min and UV detection was carried out at 252nm.

25mg of Rosuvastatin Calcium was weighed accurately and transferred into 50ml volumetric flask and dissolved in methanol, after dissolution, the volume was made up to the mark with methanol (500µg/ml). Further dilution was made by pipetting 1ml of mother liquor into 50ml with mobile phase to acquire 10µg/ml solution. 10mg of Fenofibrate was weighed accurately and transferred into 10ml volumetric flask and dissolved in methanol and made up to the volume with methanol (1000 µg/ml). 2ml of the solution was diluited to 50ml with mobile phase to acquire 40µg/ml solution.

From the above stock solutions, dilutions were made in the concentrations range of 1-7µg/ml for Rosuvastatin Calcium and 4-28µg/ml for Fenofibrate. A volume of 20µl of each sample was injected and the chromatograms were recorded at 252nm. The above concentration range was found to be linear and obeys Beer's law. The procedure was repeated for six times. The peak areas were plotted against concentration and the calibration curve was constructed.

Analysis of Pharmaceutical Dosage Form

To determine the content of Rosuvastatin Calcium and Fenofibrate simultaneously in tablets (label claim: 10mg of Rosuvastatin Calcium and 80mg of Fenofibrate). Ten tablets were weighed, their average weight was determined and were finely powdered. Weighed accurately a quantity of the tablet powder equivalent to 10mg of Fenofibrate was dissolved in methanol and the solution was sonicated for 15 minutes and centrifuged for 10 minutes at 2000rpm. The supernatant liquid was separated and the excipients were removed by filtration. Appropriate aliquots were subjected to above methods and the amount of Rosuvastatin Calcium and Fenofibrate were determined (Table 2).

Table 1. Absorptivity values at 243 nm (λmax of Rosuvastatin Calcium) and 287 nm (λmax of Fenofibrate).

	Absorptivity at 243 nm		Absorptivity at 287 nm	
	ROS	FEN	ROS	FEN
Mean[a]	ax1= 601.14	ay1= 216.61	ax2= 193.11	ay2= 491.1
± S.D.	1.71	1.35	1.31	1.68

[a]Absorptivity values are the mean of six determinations. S.D. is standard deviation. ax1 and ax2 absorptivites of Rosuvastatin Calcium at 243 nm and 287 nm, respectively; ay1 and ay2 absorptivites of Fenofibrate at 243 nm 287 nm, respectively

Table 2. Analysis data of tablet formulations.

Parameters	UV – spectrophotometry		HPLC	
	ROS	FEN	ROS	FEN
Label Claim	10	80	10	80
Drug content[a]	100.52	100.40	100.33	100.67
± S.D[b]	1.5453	0.4238	1.1226	0.6907
% R.S.D[c]	1.5373	0.4221	1.1188	0.6861

[a] Value for drug content (%) are the mean of six estimations; [b] ± S.D is standard deviation and [c]R.S.D. is relative standard deviation.

Recovery Studies

To check the accuracy of the developed methods and to study the interferernce of formulation additives, analytical recovery experiments were carried out by standard addition method at 80, 100, 120 % levels. From the total amount of drug found percentage recovery was calculated (Table 3).

RESULT AND DISCUSSION

Both, UV spectrophotometric and RP - HPLC methods were found to be simple, accurate, economic and rapid for routine simultaneous estimation of Rosuvastatin Calcium and Fenofibrate in bulk and in tablet dosage forms. For UV spectrophotometric method, linearity was obtained in the concentration range of 1-6µg/ml for Rosuvastatin Calcium and 4-28 µg/ml for Fenofibrate. The Correlation coefficient for Rosuvastatin Calcium was found to be 0.99998 and 0.99993 at 243nm and 287nm, respectively. The slope and intercept was found to be -0.05977, 0.01938 and 0.00073, -0.00011 at 243nm and 287nm, respectively. The correlation coefficient for Fenofibrate was found to be 0.99978 and 0.99994 at 243nm and 287nm, respectively. The slope and intercept was found to be 0.02190, 0.04891 and - 0.00197, 0.00108 at 243nm and 287nm, respectively. The percentage recovery was found to be in the range of 98.41 – 99.17 % and 100.02-100.18% for Rosuvastain Calcium and Fenofibrate, respectively. The standard deviation and % RSD values were found to be less than 2% shows the high precision and accuracy of the method.

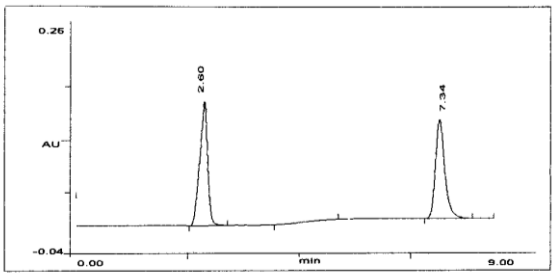

Figure 4. Chromatogram of Standard Rosuvastatin Calcium (10 µg/ ml); (Rt 2.60), and Fenofibrate (10 µg/ ml); (Rt 7.34) measured at 252 nm, mobile phase Acetonitrile: Methanol: Water (50:40:10).

In HPLC method, HPLC conditions were optimized to obtain an adequate separation of

Table 3. Recovery studies.

Drug	UV Spectrophotometry			HPLC		
	% of raw material added	Recovery[a]	% R.S.D	% of raw material added	Recovery[a]	% R.S.D
ROS[b]	80	99.17	0.8428	80	100.07	0.1984
	100	98.87	0.3604	100	101.08	1.4641
	120	98.41	0.1376	120	99.78	0.5760
FEN[c]	80	100.02	0.6376	80	100.14	0.6920
	100	100.18	0.1480	100	99.20	0.6531
	120	100.12	0.2347	120	101.32	0.0782

[a]Recovery is mean of three estimations, [b]Ros - Rosvastatin Calcium, [c]Fen - Fenofibrate

Table 4. System suitability parameters.

Parameters	Rosuvastatin Calcium	Fenofibrate
Tailing factor	0.84	1.31
Theoretical Plates	2397	7952
Asymmetrcal factor	0.61	1.44
Capacity factor	1.68	3.74
Resolution	Between ROS and FEN 8.61	

eluted compounds. Initially, various mobile phase composition were tried to separate drugs. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with Acetonitrile: Methanol: Water (50:40:10 v/v) with 0.5 ml/min flow rate is quite robust. The optimum wavelength for detection was 252nm at which better detector response for drugs was obtained. The average retention times for Rosuvastatin Calcium and Fenofibrate was found to be 2.60±0.03 and 7.34±0.03min, respectively (Figure 4). According to USP XXIV (621), system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions (Table 4). The calibration curve was linear in concentration range of 1–7µg/ml for Rosuvas-tatin Calcium and 4–28µg/ml for Fenofibrate. The correlation coeffiecient was found to be 0.99926 and 0.99935 for Rosuvastatin Calcium and Fenofibrate, respectively. The intercept value was found to be 86608.5556 for Rosuvastatin Calcium and 48148.9207 for Fenofibrate. The slope was found to be 916262.4603 and 848948.0069 for Rosuvastatin Calcium and Fenofibrate, respectively.

Sample to sample precision and accuracy were evaluated using three samples of three different concentrations, which were prepared and analyzed on same day. Day to day variability was assessed using three concentrations analyzed on three different days, over a period of one week. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there was no significant difference on the assay, which was tested on intra-day and inter day basis. The % R.S.D. values shows that proposed methods provides acceptable intra-day and inter day variation of Rosuvastatin Calcium and Fenofibrate (Table 5).

Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot in different laboratories, by different analysts, using similar operational and environmental conditions. The % R.S.D. values were found to be less than 2% (Table 5).

CONCLUSION

The proposed methods are accurate, simple, rapid and selective for the simultaneous estimation of Rosuvastatin Calcium and Fenofibrate in bulk and in tablet dosage form by external standard calibration method. Because of the low run time (less than 10 minutes), it can be conveniently adopted for the routine quality control analysis of these two drugs in bulk and in combined dosage forms. As the drug combination is available in market, hence, work is toward development of analysis.

Table 5. Summary of % R.S.D values of repeatability, precision and ruggedness.

Parameter	UV – Spectrophotometry		HPLC	
	ROS	FEN	ROS	FEN
Repeatability[a]	1.5373	0.4221	1.1188	0.6861
Precision				
Intra day[b]	0.0510	0.6245	0.8314	1.1421
Inter day[b]	0.8245	0.6468	0.9532	0.6534
Ruggedness				
Analyst 1[c]	0.5388	0.2786	1.2134	0.8714
Analyst 2[c]	1.3528	0.3650	0.5433	0.7632

[a] is the number of 6 repetitions for repeatability, [b] is the number of 6 repetitions for intra-day and inter day, [c] is the number of 6 repetitions for different analyst.

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