Research Article

Development and Validation of Derivative Spectroscopic Method for the Simultaneous Estimation of Rosuvastatin Calcium and Fenofibrate in Tablet

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ABSTRACT

A simple, novel, sensitive and precise validated spectrophotometric method was developed for simultaneous determination of Rosuvastatin calcium and Fenofibrate in synthetic mixture and its dosage form. Methanol was selected as a common solvent for estimation of Rosuvastatin calcium (ROS) and Fenofibrate (FEN) with λ_{max} at 243 nm and 224 nm respectively in methanol. The linearity was obtained in the concentration ranges of 4-12 µg/ml for Rosuvastatin and 16-48 µg/ml for Fenofibrate. The Zero Crossing Point (ZCP) of Rosuvastatin was 224.11 nm and Fenofibrate was 243.29 nm. The correlation coefficient for the ROS was 0.9963and for FEN 0.9996. The % RSD for intraday precision was 0.76-1.05 % for ROS and 0.32-1.16 % for FEN. The interday precision was 0.76-1.82 % and 0.42-1.47 % for ROS and FEN respectively. The detection limit and quantification limit were found to be 1.96 and 5.96 µg/ml for Rosuvastatin and 0.76 and 2.32 µg/ml for Fenofibrate respectively. All the validation parameter was perform as per the International Conference on Harmonization (ICH) guidelines. The recovery study was carried out, result were 100.9-103.2% for ROS and 100.9-101.3% for FEN. No interference form the tablet excipients showed the applicability of method to the routine analysis of the pharmaceutical dosage form.

Keywords: Rosuvastatin calcium, fenofibrate, derivative spectrophotometry, validation.

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INTRODUCTION

Rosuvastatin Calcium (ROS) is an Antihyperlipidemic drug. Rosuvastatin is a competitive inhibitor of HMG-CoA reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, an early rate-limiting step in cholesterol biosynthesis [1]. Rosuvastatin acts primarily in the liver. Decreased hepatic cholesterol concentrations stimulate the upregulation of hepatic low density lipoprotein (LDL) receptors which increases hepatic uptake of LDL [2]. Rosuvastatin also inhibits hepatic synthesis of very low density lipoprotein (VLDL). The overall effect is a decrease in plasma LDL and VLDL. Chemically ROS is bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl-(methyl-sulfonyl) amino] pyrimidin-5-yl] (3R,5S)-3,5 dihydroxyhept-6- enoicacid] calcium salt (**Fig. 1**)[3].

Fenofibrate (FEN) was Antihyperlipidemic drug and exerts its therapeutic effects through activation of peroxisome proliferator activated receptor a (PPARa). This increases lipolysis and elimination of triglyceride-rich particles from plasma by activating lipoprotein lipase and reducing production of apoprotein C-III [4]. The resulting fall in triglycerides produces an alteration in the size and composition of LDL from small, dense particles, to large buoyant particles. These larger particles

have a greater affinity for cholesterol receptors and are catabolized rapidly [5]. Chemically FEN is 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester (**Fig. 2**) [6]**.**

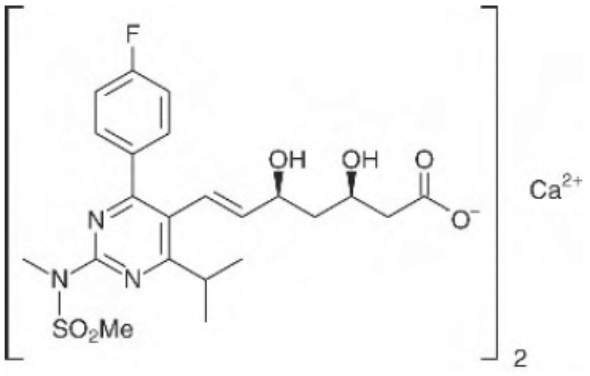

Figure 1: Structure of Rosuvastatin calcium

Figure 2: Structure of Fenofibrate

Few analytical techniques are available for estimation of ROS alone as well as in combine dosage form such as UV, HPLC HPTLC [6- 13]. Similarly few analytical methods are available for estimation of FEN alone and its combination with drugs such as UV and HPLC [14-19].

Keeping this objective in mind an attempt has been made to develop and validate the 1st Derivative UV method for the simultaneous estimation of ROS and FEN which was not developed for the combination earlier. The method would be highly sensitive, having good resolution, reproducible and cost effective. Various validation aspects of the analysis, accuracy, precision, recovery, and the limits of detection and quantification etc. have been measured as per ICH guidelines [20].

MATERIALS AND METHODS

Instrument:

Instrument used was an UV-Visible double beam spectrophotometer, make: Shimadzu Corporation (Japan), Model UV-1800 with a bandwidth of 1.5 nm and a pair of 1 cm matched quartz cells. All weighing was done on analytical balance (Denver instrument, Germany). A sonicator (Electroquip Ultra sonicator, Texas) was used in the study. Calibrated glass wares were used throughout the work.

Chemicals:

Chemicals used were methanol (A.R.Grade, Sisco Chem Pvt Ltd, Andheri, Mumbai) and distilled water (Filtrate obtained through Distillation set). Marketed formulation containing Fenofibrate (67 mg) and Rosuvastatin Ca (10 mg) (Razel-F10) was procured from the local pharmacy.

METHOD

Preparation of standard stock solution:

10 mg of standard ROS and FEN were weighed separately and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml each of ROS and FEN.

Methodology:

The working standard solutions of ROS and FEN were prepared separately in methanol having concentration of 10 µg/ml. They were scanned in the wavelength range of 200-400 nm against

solvent methanol as blank and the absorption spectra thus obtained were derivatised from first to fourth order. First order derivative spectrum was selected for the analysis of both the drugs. From the overlain spectra of both the drugs [Figure 3] wavelengths selected for quantitation were 243.29 nm (zero cross point for FEN) for ROS and 224.11 nm (zero cross point for ROS) for FEN. Overlain derivative spectra of both the drugs individually also shown in Figure 4 and 5 for ROS and FEN respectively.

Validation of the proposed method:

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines [21].

Linearity (calibration curve):

Appropriate aliquots from the standard stock solutions of ROS and FEN were used to prepare two different sets of dilutions: Series A, and B as follows. Series A consisted of different concentration of ROS (4-12 µg/ml). Aliquot from the stock solution of ROS (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with methanol to get final concentration in range of 4-12 µg/ml. Series B consisted of varying concentrations of FEN (16-48 µg/ml). Appropriate volume of the stock solution of FEN (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with methanol to get final concentration in range of 16-48 µg/ml.

Precision:

The reproducibility of the proposed method was determined by performing the assay for the same day (intraday assay precision) and on three different days (inter day precision). Precision studies were performed by preparing nine determinations covering the specified range for the procedure (3 x 3 replicates for each concentration). Low % RSD shows that the method has good precision.

Accuracy:

The accuracy of the method was determined by calculating the recoveries of ROS and FEN by the standard addition method. Known amounts of standard solutions of ROS and FEN were added at 50 %, 100 % and 150 % level to prequantified sample solutions of ROS and FEN (4 µg/ml for ROS and 16 µg/ml for FEN). The amounts of ROS and FEN were estimated by applying obtained values to the respective regression line.

Limit of Detection and Limit of Quantification:

The LOD and LOQ was separately determined based on the standard calibration curve. The residual standard deviation of y-intercept of regression lines may be used to calculate LOD and LOQ using following equations.

LOD = 3.3 * D/S

LOQ = 10 * D/S

Where, D = Standard deviation of the intercepts of regression line.

S = Slope of the calibration curve

Application of the proposed method for the determination of Rosuvastatin Calcium and Fenofibrate

Accurately weighed equivalent to 10 mg of ROS and 67 mg of FEN (166.55 mg) of tablet powder was transferred into 100 ml of volumetric flask, 20 ml of methanol was added to it and sonicated for 30 minutes and diluted up to the mark with methanol. The resulting solution was filtered and the filtered solution (1 ml) was transferred to 10 ml volumetric flask and diluted with methanol to get a solution containing 100 µg/ml ROS and 670 µg/ml of FEN.

Take appropriate aliquot to get resulting solution containing the 32 µg/ml FEN. 1 ml of resulting solution was transferred to 10 ml volumetric flask and diluted up to mark with methanol to get a solution containing the 4 µg/ml ROS.

The absorbance's of resulting solutions were measured at 224.11 nm and 243.29 nm. The concentration of ROS and FEN present in the sample solution was calculated by using the equation generated from calibration curve of respective drugs.

RESULTS AND DISCUSSION

The standard solutions of ROS and FEN were scanned separately in the UV range and First-order spectra for ROS and FEN were recorded. The first order derivative

absorption at 243.29 nm (zero cross point for FEN) was used for Rosuvastatin and 224.11 nm (zero cross point for ROS) was used for Fenofibrate shown in (**Figure 3**).

These two wavelengths can be employed for the determination of ROS and FEN without any interference from the other drug in their combined dosage form.

Standard calibration curves for ROS and FEN were linear with Correlation coefficients (r^2) values in the range of 0.9963 and 0.9996 respectively at all the selected wavelengths and the values were average of five readings and statistical data was shown in (**Table 1**).

Precision study showed co-efficient of variance (% CV) values less than 2 % for both ROS and FEN respectively in all selected concentrations shown in (**Table 2 and 3**) respectively for ROS and FEN.

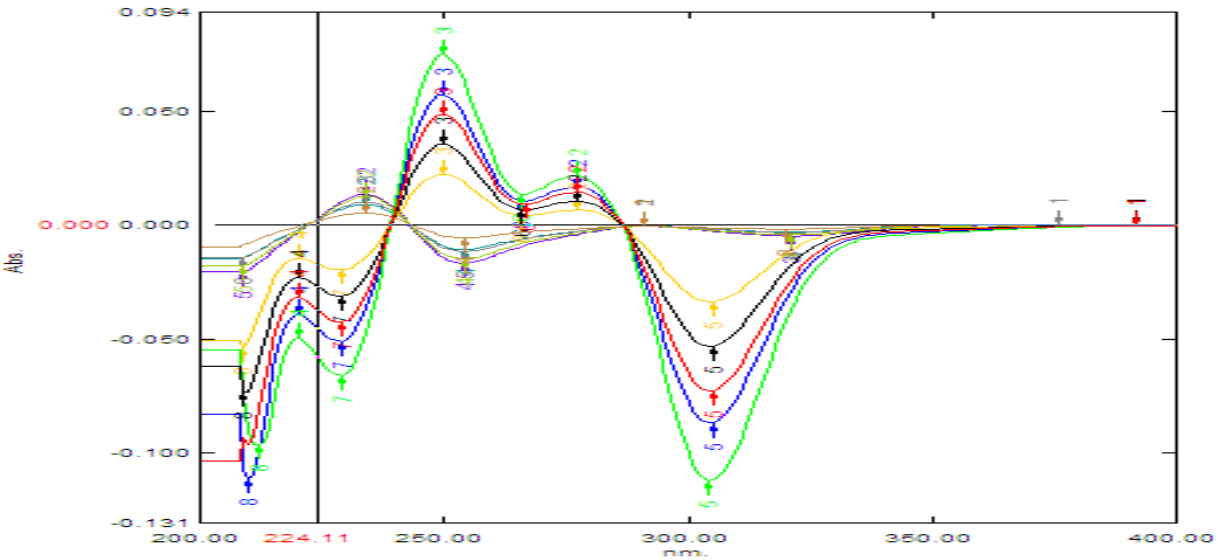

Figure 3: Overlain of Derivative Spectrum (1st Order) of FEN and ROS

Table 1: Statistical Parameter from the Calibration Curve

Statistical Parameter	ROS	FEN
λ_{max}	243nm	224nm
Linearity range	4-12µg/ml	16-48µg/ml
Linearity eqution	y = 0.0013X+0.0017	y = 0.0009X+0.0022
Slope	0.000136	0.00096
Intercept	0.0016	0.00176
Standard deviation of slope	0.0000894	0.0000548
Standard deviation of intercept	0.000316	0.000576
Correlation co-efficient	0.9963	0.9996

Table 2: Interday Precision for ROS

Concentration	1	2	3	Mean	SD	RSD
6	0.0097	0.0095	0.0096	0.0096	0.0001	1.04
8	0.0128	0.0127	0.0127	0.0127	0.0001	0.79
10	0.0146	0.0143	0.0146	0.0145	0.0002	1.19

Table 3: Interday Precision for FEN

Concentration	1	2	3	Mean	SD	RSD
24	0.0243	0.0249	0.025	0.0247	0.0003	1.53
32	0.0312	0.0321	0.0317	0.0317	0.0004	1.42
40	0.0395	0.0404	0.0398	0.0400	0.0005	1.15

The accuracy of the method was confirmed by recovery studies from tablet at three different levels of 50 %, 100 %, 150 % recovery in the range of 100.92 – 103.26 % justifies the accuracy of method. The results obtained from the recoveries of both drugs showed excellent accuracy which was shown in (**Table 4**).

Table 4: Accuracy Data

Amt. of sample (Formulation) µg/ml (n=3)		Amt. of std drug added µg/ml		Amt. recovered		% Recovery	
ROS	**FEN**	**ROS**	**FEN**	**ROS**	**FEN**	**ROS**	**FEN**
4	16	0	0	4.03	16.22	100.96	101.39
4	16	4	16	8.071	32.44	100.90	101.39
4	16	8	32	12.39	48.44	103.27	100.92

The influence of excipients was studied by mixing two drugs with excipients as per the ratio. LOD and LOQ were found to be 0.76 µg/ml and 2.32 µg/ml for ROS and 1.96 µg/ml and 5.96 µg/ml for FEN. (**Table 5**) shows the summary of all validation parameter.

Table 5: Statistical Data of Validation Parameter for ROS AND FEN

Parameter	ROS	FEN
Linearity Range (µg/ml)	4-12	16-48
Regression equation	y = 0.0013X+0.0017	y = 0.0009X+0.0022
correlation co-efficient	0.9963	0.9996
Precision (% RSD)		
Intraday (n=3)	0.76-1.05	0.32-1.16
Interday (n=3)	0.76-1.82	0.42-1.47
Accuracy or Recovery (%)	100.9-103.2	100.9-101.3
LOD (µg/ml)	0.76	1.96
LOQ (µg/ml)	2.32	5.96

There was no interference was observed from the presence of excipients in the amounts, which are commonly present in tablet dosage forms. The results of pharmaceutical dosage forms analysis of the combinations are shown in Table 6 which showed good agreement with the labelled claim.

Table 6: Assay Result of Marketed Formulation

	Label claim (mg/tablet)		Assay ± SD (% of label claim)	
Tablet	**ROS**	**FEN**	**ROS**	**FEN**
Razel-F10	10	67	99.038 ± 0.2903	101.2346 ± 0.2672

CONCLUSION

From all the present work we can conclude that the proposed UV spectrometric method for quantitative determination of FEN and ROS in combined dosage form is found to be simple, rapid, precise, accurate and sensitive.

The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these formulation.

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