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DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC ABSORPTION RATIO METHOD FOR ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN TABLET DOSAGE FORM

J.A. Dave*, R. R. Parmar, D. A. Shah, P. P. Nayak

Department of Quality Assurance, APMC College of Pharmaceutical Education and Research, College campus, Motipura, Himatnagar-383001, Gujarat-INDIA.

ABSTRACT

Keywords:

Q-absorption ratio, distilled water, rosuvastatin calcium, fenofibrate, spectrophotometric, validation

For Correspondence:

J.A. Dave

APMC College of
Pharmaceutical Education
and Research, Motipura,
Himatnagar-383001,
Gujarat-INDIA

E-mail:

jaysuz_b.pharmacy@yahoo.co.in

A simple, rapid, accurate, precise and economical UV spectrophotometric method for the determination of Rosuvastatin Calcium and Fenofibrate in combined tablet dosage form using absorption ratio method has been developed. The method is based on the absorption ratio method for analysis of both the drugs using combine methanol and distilled water as solvent. Rosuvastatin calcium has absorbance maxima at 241 nm and Fenofibrate has absorbance maxima at 290 nm, and isosbetic point at 251.5nm. The linearity was obtained in the concentration range of 2-17 µg/ml and 2-22 µg/ml for rosuvastatin calcium and fenofibrate respectively at 241nm and 251.5nm. The concentrations of the drugs were determined by using absorption ratio method. The mean recovery was 100.40 ± 0.43 and 100.25 ± 0.37 for rosuvastatin calcium and fenofibrate respectively. The method was found to be simple, accurate and precise and was applicable for the determination of rosuvastatin calcium and fenofibrate in pharmaceutical tablet dosage form. The results of analysis have been validated statistically and by recovery studies.

INTRODUCTION

Rosuvastatin-fenofibrate combination is widely used in the treatment of hypercholesterolemia and Hypertriglyceridemia. Rosuvastatin Calcium (RSV) is (E)-(3R,5S) - 7-{4-(4-fluorophenyl)-6-isopropyl-2-{methyl(methylsulphonyl amino)}pyrimidin-5-yl}-3,5-dihydroxyhepten-6-oic acid calcium salt^[1]. It is a lipid lowering drug. It inhibits the enzyme 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the rate limiting enzyme that converts HMG-CoA to mevalonate ;a precursor of cholesterol and there by checks the synthesis of cholesterol. It is used in the treatment of hypercholesterolemia and dyslipidemia . A survey of literature showed few UV spectrophotometric, few HPLC , few HPTLC, two chromatography stability indicating method, for the estimation of rosuvastatin in pharmaceutical preparation and in biological fluids.

Fenofibrate is a drug of the fibrate class. Fenofibrate(FENO) is chemically 1-methyl ethyl 2-[4-(chloro benzoyl) phenoxy]-2-methylpropanoate^[2-3]. Like other fibrates, it reduces both low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels. A survey of literature showed few UV spectrophotometric ,few HPLC , are available for estimation of fenofibrate in pharmaceutical preparation and in biological fluids. On the combined dosage form of rosuvastatin calcium and fenofibrate UV spectrophotometric simultaneous equation method and RP-HPLC method are reported. ^[17-18]

No literature survey have been revealed for spectroscopic methods using distilled water and methanol combined as a solvent. The present manuscript describes simple, accurate, precise, rapid and economic spectrophotometric method based on absorption ratio for estimation of RSV and FENO in tablet dosage form using distilled water and methanol as a combined solvent.

MATERIALS AND METHODS

Apparatus:

A shimadzu model 1700 (Japan) double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. A Reptech electronic weighing analytical balance based on EMFC technology and a Toshcon ultrasonic bath (Toshniwal process instrument pvt ltd.) was used in the study.

Reagents and Materials

Rosuvastatin calcium and fenofibrate bulk powder was kindly gifted by Torrent Research Center, Gandhinagar, Gujarat, India. The commercial fixed dose combination Razel-F10 tablet was procured from the local market. All other chemicals used were of analytical grade. Distilled water and calibrated glasswares were employed throughout the work.

Preparation of standard stock solutions:

An accurately weighed quantity of RSV (50 mg) and FENO (50 mg) were transferred to a separate 50 ml volumetric flask. Add 20 ml methanol, sonicate and diluted to the mark with methanol (1000 µg/ml) each. From the above solutions prepare solutions having concentration RSV and FENO (100 µg/ml) each with methanol. From the above solutions prepare solutions having concentration RSV (10 µg/ml) and FENO (10 µg/ml) with distilled water.

Method:

The standard solutions of RSV (10 µg/ml) and FENO (10 µg/ml) were scanned separately in the UV range of 200-400 nm to determine the max of both the drugs. The max of RSV and FENO were found to be 241 nm and 290 nm respectively. Isosbetic point was found to be at 251.5 nm. Six standard solutions having concentration 2, 5, 8, 11, 14 and 17 µg/ml for RSV and 2, 6, 10, 14, 18 and 22 µg/ml for FENO were prepared in distilled water using the solutions having concentration 100 µg/ml. 241 nm (absorption max. of RSV) and 251.5 nm (isosbetic point) were selected for analysis. The absorbance of resulting solutions was measured at 241 nm and 251.5 nm and calibration curves were plotted at these wavelengths. The absorptivity coefficients of these two drugs were determined using calibration curve equations. The concentration of RSV and FENO in sample solution was determined by solving the respective absorption ratio equations generated by using absorptivity coefficients and absorbance values of RSV and FENO at these wavelengths.

Validation of the proposed method:

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines^[6].

Linearity (Calibration curve):

The calibration curves were plotted over a concentration range of 2-17 µg/ml and 2-22 µg/ml for RSV and FENO respectively. Accurately measured standard solutions of RSV (100 µg/ml) (2, 5, 8, 11, 14, and 17 ml) and FENO (100 µg/ml) (2, 6, 10, 14, 18 and 22 ml) were transferred to a series of 100 ml of volumetric flasks and diluted to the mark with distilled water. The absorbances of the solutions were measured at 241 nm and 251.5 nm against distilled water as blank. The calibration curves were constructed by plotting absorbances versus concentrations and the regression equations were calculated.

Method precision (repeatability):

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions ($n = 6$) for RSV and FENO (10 µg/ml for both RSV and FENO) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility):

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days 3 different concentrations of standard solutions of RSV and FENO.

Accuracy (recovery study):

The accuracy of the method was determined by calculating recovery of RSV and FENO by the standard addition method. Known amounts of standard solutions of RSV and FENO were added at 80, 100 and 120 % level to prequantified sample solutions of RSV and FENO. The amounts of RSV and FENO were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

Limit of detection and Limit of quantification:

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \sigma/S$$

$$\text{LOQ} = 10 \times \sigma/S$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of RSV and FENO in combined tablet dosage form:

Twenty tablets were weighed and powdered. The powder equivalent to 10 mg of RSV and 67 mg of FENO was transferred to a 100 ml volumetric flask. Methanol (30 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the mark with methanol. From the above stock solution 10 ml of solution is taken and diluted to the mark in 100ml volumetric flask. The above solution was suitably diluted with distilled water to get a final concentration of 2 µg/ml of RSV and 13.4 µg/ml of FENO. The absorbances of the tablet sample solution i.e. A1 and A2 were recorded at 251.5 nm (isosbetic point) and 241 nm and ratios of absorbance were calculated, i.e. A2/A1. Relative concentration of two drugs in the sample solution was calculated using respective absorption ratio equations generated by using absorptivity coefficients and absorbance values of RSV and FENO at these wavelengths.

The concentration of RSV and FENO in solutions obtained by solving equation (1) and (2).

$$CX = QM - QY / QX - QY \times A_1 / a_{X1} \text{ ----- (1)}$$

$$CY = QM - QX / QY - QX \times A_1 / a_{Y1} \text{ ----- (2)}$$

Where, $QX = a_{X2} / a_{X1}$ $QY = a_{Y2} / a_{Y1}$ $QM = A_2 / A_1$

where, A_1 and A_2 are absorbance of sample solution at Isosbetic point and λ_{\max} of drug RSV respectively; a_{x1} and a_{x2} are the absorptivities of drug RSV, a_{y1} and a_{y2} are the absorptivities of drug FENO at the two wavelengths respectively

RESULTS AND DISCUSSION

In this method, two wavelengths were used for the analysis of the drugs. 251.5 nm (isosbetic point) and 241 nm (λ_{\max} of RSV) are the wavelengths at which calibration curves were prepared for both the drugs. This method is applicable to the drugs that obey Beer's law at all wavelengths and the ratio of absorbances at any two wavelengths are a constant value, independent of concentration or pathlength. The criteria for obtaining maximum precision^[18] by this method were calculated and ratio of the absorbance of different dilution of same substances at two wavelengths are constant. Once the absorptivity values are determined very little time is required for analysis, as would require determination of absorbances of the sample solution at two selected wavelengths and few

simple calculations. Linear correlation was obtained between absorbances and concentrations of RSV and FENO in the concentration ranges of 2-17 $\mu\text{g/ml}$ and 2-22 $\mu\text{g/ml}$ for both drugs respectively at 251.5nm and 241nm. The linearity of the calibration curve was validated by the high values of correlation coefficient of regression. LOD and LOQ values for RSV were found to be 0.33 and 1.01 $\mu\text{g/ml}$ and 0.27 and 0.81 $\mu\text{g/ml}$ at 251.5nm and 241 nm respectively. LOD and LOQ values for FENO were found to be 0.39 and 1.19 $\mu\text{g/ml}$ and 0.42 and 1.29 $\mu\text{g/ml}$ at 251.5nm and 241 nm respectively. These data show that method is sensitive for the determination of RSV and FENO. All the regression analysis data and summary of validation parameters for the proposed method is reported in Table 1

The recovery experiment was performed by the standard addition method. The mean recoveries were 100.40 ± 0.43 and 100.25 ± 0.37 for RSV and FENO respectively indicates accuracy of the proposed method (Table 3). The proposed validated method was successfully applied to determine RSV and FENO in their combined dosage form. The results obtained for RSV and FENO were comparable with the corresponding labeled amounts (Table 2). The RSD values for assay of RSV and FENO were found to be 0.84 and 2.0 respectively. Relative standard deviation was less, which indicates that proposed method is repeatable (Table 4). No interference of the excipients with the absorbance of interest appeared; hence the proposed method is applicable for the routine simultaneous estimation of RSV and FENO in pharmaceutical tablet dosage forms.

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of RSV and FENO in tablet dosage form. The method utilizes easily available and low cost solvent like distilled water for analysis of RSV and FENO. Hence the method was also found to be economic for estimation of RSV and FENO from tablet.

ILLUSTRATIONS: TABLES

ABSORPTION RATIO METHOD				
Parameters	RSV		FENO	
Wavelength (nm)	251.5	241	251.5	241
Beer's law limit ($\mu\text{g/ml}$)	2-17	2-17	2-22	2-22
Regression equation ($y = mx + c$)	$y = 0.032x + 0.004$	$y = 0.040x + 0.014$	$y = 0.033x + 0.031$	$y = 0.026x - 0.051$
Slope (m)	0.032	0.040	0.031	0.026
Intercept (c)	0.004	0.014		0.051
Absorptivity values (l / mole cm)	0.034×10^4	0.040×10^4	0.033×10^4	0.026×10^4
Correlation coefficient(r^2)	0.999	0.999	0.999	0.998
LOD ($\mu\text{g/ml}$)	0.33	0.27	0.39	0.42
LOQ ($\mu\text{g /ml}$)	1.01	0.81	1.19	1.29

II. RESULTS OF ANALYSIS OF TABLET FORMULATION

Drugs	Absorption ratio method
	% \pm RSD(n=6)
RSV	100.19 \pm 0.8
FENO	101.68 \pm 2.0

n = number of replicates

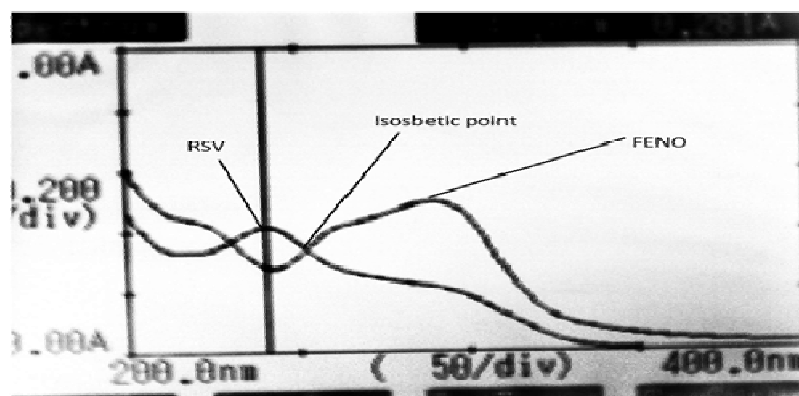
III. RESULTS OF THE RECOVERY STUDIES

Level of recovery %	Amount of pure drug added ($\mu\text{g/ml}$)		Absorption ratio method method % recovery	
	RSV	FENO	RSV	FENO
80	7.9	53.0	100.86	100.34
100	9.9	66.6	99.98	99.84
120	11.9	79.0	100.38	100.58
Mean % recovery RSD*			100.40 0.4389	100.25 0.3765

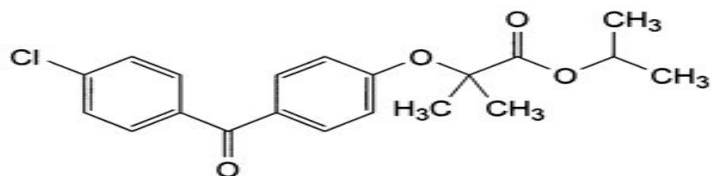
IV. RESULTS OF INTERMEDIATE PRECISIONS

PRECISION	RSV		FENO	
	241	251.5	241	251.5
INTERDAY(n=3)%RSD	0.43	0.64	0.54	0.58
INTRADAY(n=3) %RSD	0.56	0.27	0.31	0.31

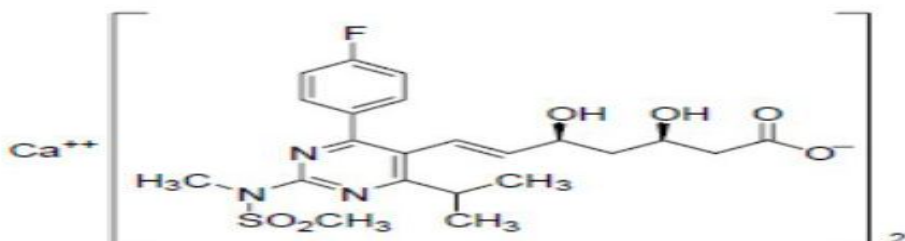
FIGURES:



OVERLAIN SPECTRA OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN METHANOL AND WATER COMBINED SOLVENT.



FENOFIBRATE



ROSUVASTATIN CALCIUM

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