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Research Article

A VALIDATED STABILITY INDICATING UV-SPECTROPHOTOMETRIC SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT

Objective: The present work deals with the development and validation of the absorbance ratio method for the estimation of rosuvastatin calcium and fenofibrate in bulk and pharmaceutical formulation. Studied forced degradation characteristics of bulk and pharmaceutical formulation as per stability guidelines.

Methods: The bulk and pharmaceutical formulation studied by the absorbance ratio method. It is the ratio of absorbances at two selected wavelengths. One wavelength is the isoabsorptive point and another wavelength is λ max of one of the components. From the overlay spectra of the two drugs, ROS and FEN showed the isoabsorptive point at 249.5 nm. The second wavelength used was 287 nm, which was the λ max of FEN.

Results: The drugs obeyed Beer's law and showed a good correlation. The correlation coefficient for the ROS was 0.999 and for FEN 0.999. The RSD for intraday precision was 0.57 for ROS and 0.057 for FEN. The interday precision was 0.05 for ROS and 0.03for FEN, respectively. The detection limit and quantification limit were found to be 0.048 and 0.14 µg/ml for ROS and 0.069 and 0.21µg/ml for FEN, respectively. More degradation was found in acid hydrolysis and photostability degradation.

Conclusion: A simple, precise, accurate, validated, stability-indicating method for simultaneous estimation of rosuvastatin calcium and fenofibrate in bulk and pharmaceutical formulation has been developed.

Keywords: Correlation coefficient, Detection limit, Precision, Pharmaceutical, Quantification limit, Spectrophotometric, Ultraviolet, λ max, % RSD

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INTRODUCTION

Chemically rosuvastatin calcium is (3R, 5S, 6E)-7-[4-(4-fluorophenyl)-2-(N-methylmethanesulfonamido)-6-(propane-2-yl)pyrimidin-5-yl]-3,5-dihydroxyhept-6-enoic acid. It belongs to the statin group of drugs. Rosuvastatin reduces the amount of cholesterol made by the liver reducing LDL, triglyceride, and raising HDL in the blood. It decreases the risk of heart disease and helps to prevent heart attack and stroke [1].

Chemically fenofibrate is (Propan-2-yl 2-[4-(4-chlorobenzoyl) phenoxy]-2-methylpropanoate). Fenofibrate is a fabric acid derivative. It is used to improve cholesterol levels in mixed dyslipidemia, severe hypertriglyceridemia, and Primary hypercholesterolemia. It helps lower triglycerides and helps increase levels of HDL cholesterol [2, 3].

In the treatment of mixed dyslipidemia, there is a need to reduce the LDL-C and triglyceride levels. The combined therapy of rosuvastatin calcium and fenofibrate is the most effective option to increase HDL-C [4].

A literature survey showed Anandakumar K *et al.* [5] reported UV-spectrophotometry and RP-HPLC method for simultaneous estimation of this combination in bulk and tablet dosage form. Sevda RR *et al.* [6] reported UV-spectrophotometric simultaneous equation method for the estimation of rosuvastatin calcium and fenofibrate in bulk drug and dosage form. Potawale RS *et al.* [7] revealed the HPTLC method for the simultaneous determination of this combination bulk and pharmaceutical formulation. Sharma S *et al.* [8] reported UV-spectrophotometry and RP-HPLC method. Borole TC *et al.* [9] reported the HPLC method for simultaneous estimation. Patel H *et al.* [10] reported simultaneous quantification by using HPLC-UV of rosuvastatin and fenofibric acid in rat plasma and its application to a pharmacokinetic study. Trivedi RK *et al.* [11] determined rosuvastatin and fenofibric acid in human plasma by LC-MS/MS. Kumar SA *et al.* [12] developed a validated sensitive RP-HPLC method for simultaneous estimation of this combination in tablet dosage form by using a PDA detector in gradient mode. Vyas S *et al.* [13] developed a validated derivative spectroscopic method in a combined dosage form.

The literature review revealed that rosuvastatin-fenofibrate is not studied by the absorbance ratio method (isoabsorptive point method) and their stability is also not determined. So the present work was directed towards try to develop UV-spectrophotometric simultaneous estimation of rosuvastatin calcium and fenofibrate by absorbance ratio method in bulk as well as the tablet dosage form. The method was validated as per ICH guidelines and a forced degradation study performed to check their stability.

MATERIALS AND METHODS

Chemicals and reagents

Working standards of fenofibrate and rosuvastatin calcium were procured from yarrowchem products Pvt. Ltd. Thane. Methanol AR grade, NaOH (AR grade), HCl (AR grade), 30 % H_2O_2 (AR grade) were used in the present study. Razat F10 tablet was procured from the market and used for the present study.

Apparatus

A UV spectrophotometer Shimadzu corporation KYOTO, Japan (1700 pharmaspec A-11024302261 LP) was used for the experiment. Afcoset electronic balance (FX 300), Ultrasonicator labline 1-5L-50, Microwave oven york scientific industries, Delhi, Photostability chamber makes newtronic (Model IC DAC version 1.2) was used.

Preparation of standard stock solution

20 mg of standard ROS were weighed and transferred to 100 ml of volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 200 µg/ml of rosuvastatin calcium.160 mg of standard FEN were weighed and transferred to 100 ml of volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 1600 µg/ml of fenofibrate.

Selection of detection wavelength

An aliquot portion of the stock solution of ROS and FEN was diluted to 10 ml of methanol in 10 ml of the volumetric flask to obtain a standard stock solution of 16µg/ml. These solutions were scanned over the range of 200–400 nm and the spectra were obtained. Rosuvastatin calcium showed considerable absorbance at 243 nm and fenofibrate showed considerable absorbance at 287 nm.

Preparation of calibration curve

Prepared the concentrations of 4, 8, 12, 16, 20, and 24 µg/ml respectively by taking aliquot portions of ROS and FEN from stock and diluted individually to 10 ml with methanol in 10 ml of volumetric flasks. The absorbance of diluted solutions was measured at 243 nm and 287 nm respectively, against methanol as blank. The graph is plotted as Absorbance vs. concentration.

Absorbance ratio method

To calculate conc. of drugs in the mixture absorbance ratio method was used. The absorbance ratio method uses the ratio of absorbance at two selected wavelengths, one wavelength is the isoabsorptive point and the other being the λ max of other components. From the overlay spectra of the two drugs, ROS and FEN showed the isoabsorptive point at 249.5 nm. The second wavelength used was 287 nm, which was the λ max of FEN.

The concentration of two drugs in a mixture can be calculated using the following equations.

$$Cx = \frac{QM - QY}{QX - QY} \times \frac{A1}{aX1}$$

$$Cy = \frac{QM - QX}{QY - QX} \times \frac{A1}{aY1}$$

Where A1 and A2 are absorbances of the mixture at 249.5 nm and 287 nm.

aX1 and aY1 are absorptivities of ROS and FEN, respectively [14].

Preparation of mixed standard solution

Accurately weighed 10 mg of rosuvastatin calcium and 160 mg fenofibrate transferred into 100 ml dry and clean volumetric flask and 50 ml of methanol was added. This mixed standard solution was sonicated for 10 min and then volume made up to mark with methanol. The prepared solution was subjected to UV analysis. Take appropriate aliquot in a 10 ml volumetric flask and diluted up to mark with methanol to get the resulting solution containing the 16 µg/ml FEN and 1 µg/ml ROS. The absorbance of the resulting solutions was measured at 249.5 nm and 287 nm. The concentration of both drugs in the mixture was calculated by using the equation of the absorbance ratio method. The Amount of drug estimated in the sample in (mg) and percent estimation was calculated.

Analysis of marketed formulation

Razat F10 (Rosuvastatin calcium 10 mg, Fenofibrate 160 mg)

Accurately weighed 10 mg of ROS and 160 mg of FEN of tablet powder was transferred into 100 ml of a volumetric flask, 20 ml of methanol was added, sonicated, and diluted up to 100 ml. The resultant solution was filtered through Whatman filter paper and transferred to a 100 ml volumetric flask and diluted with methanol to get a solution containing 100 µg/ml ROS and 1600 µg/ml of FEN. 0.1 ml of solution was transferred to a 10 ml volumetric flask and diluted up to mark with methanol to get a solution containing the 1 µg/ml ROS and 16 µg/ml FEN. The absorbances of resulting solutions were measured at 249.5 nm and 287 nm. The concentration of ROS and FEN present in standard mixture and amount of drug estimated in mg/tab and percent label claim was calculated by the equation of absorbance ratio method.

Validation of the proposed method

Linearity

The linearity of the established method is achieved by running a series of a standard mixture of ROS and FEN. Standard calibration data was calculated.

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 (interday precision). Precision studies were performed by preparing nine determinations of the specified range for the procedure (3 x 3 replicates for each concentration). Low % RSD shows that the method has good precision [12].

Accuracy

The standard addition method is used to determine accuracy. Known amounts of standard solutions of ROS and FEN were added at 80 %, 100 %, and 120 % level to quantified sample solutions of ROS and FEN (1µg/ml for ROS and 16µg/ml for FEN). The amount of drug recovered (mg) and percent recovery was calculated [13].

Robustness

The robustness of this proposed method was checked by changing the analyst and other conditions remained the same as UV spectrophotometer, solvent, dilutions [13].

Limit of detection and limit of quantification

The LOD and LOQ were determined based on the standard calibration curve. The residual standard deviation of the y-intercept of regression lines may be used to calculate LOD and LOQ using the following equations [12].

$$LOD = 3.3 *D/S$$

$$LOQ = 10*D/S$$

Stability studies of standard mixture and formulation

Acid hydrolysis

0.1 ml working standard solution and formulation of ROS and FEN was mixed with 1 ml of 0.1 N HCL and volume makeup to 10 ml with methanol (i. e 1:16 µg/ml). The solution was kept overnight. The absorbance of the resulting solution was measured at 249.5 nm and 287 nm [15].

Alkali hydrolysis

0.1 ml working standard solution of ROS and FEN was mixed with 1 ml of 0.1N NaOH and volume makeup to 10 ml with methanol in 10 ml of the volumetric flask to give (i. e1:16 µg/ml). The solution was kept for 6 h. The absorbance of the resulting solution was measured at 243 nm and 287 nm [16].

Oxidative hydrolytic degradation

0.1 ml working standard solution and formulation of ROS and FEN was exposed with 1 ml of 3% H_2O_2 and volume makeup to 10 ml with methanol in 10 ml of the volumetric flask to give (i. e 1:16 µg/ml). The solution was kept for 6 h. The absorbance of the resulting solution was measured at 249.5 nm and 287 nm [17].

Degradation under dry heat

Dry heat studies were performed by keeping the drug sample in an oven at 80 °c for 24 h. 10 mg of ROS and 160 mg of FEN was weighed and dissolved in 100 ml methanol. 0.1 ml working standard solution of was mixed with 9.9 ml methanol in 10 ml of the volumetric flask (i. e 1:16 µg/ml). The solution was kept for 6 h. The absorbance of the resulting solution was measured at 249.5 nm and 287 nm [17].

Photodegradation study

The photostability study of the drug was determined by exposing the drug under UV light, which provides illumination of NLT 200 watt hr/m²followed by cool white sunlight. After each exposure, accurately 1:16µg/ml photo exposed solution made for absorbance. The absorbance of the resulting solution was measured at 249.5 nm and 287 nm [18].

RESULTS

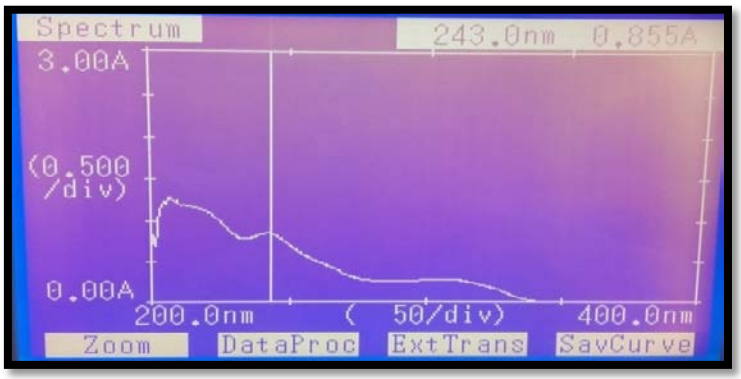


Fig. 1: UV absorption spectrum of ROS

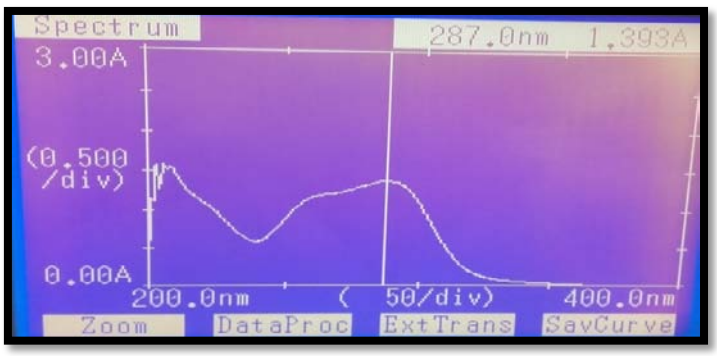


Fig. 2: UV absorption spectrum of FEN

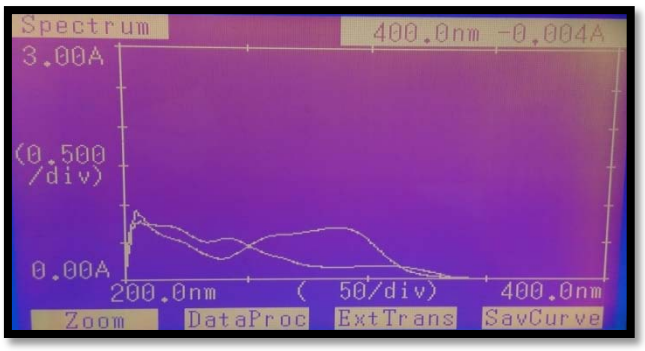


Fig. 3: Overlay spectra of ROS and FEN

The UV scanning spectrum of rosuvastatin calcium and fenofibrate showed the λ max at 243 nm as shown in fig. 1 and 287 nm as shown in fig. 2, respectively. Overlay spectra of both drugs showed is absorptive point at 249.5 nm as shown in fig. 3.

Table 1: Standard calibration data for rosuvastatin calcium and fenofibrate

	Rosuvastatin calcium	Fenofibrate
Concentration range	4–24	4–24
Correlation coefficient	0.999	0.999
Intercept	0.042x	0.053x
Slope	0.0477	0.054

Table 1 shows the linearity of this method was studied in the range 4-24 µg/ml for ROS and FEN, respectively. Calibration curved showed a linear relationship between absorbance and concentrations. The linearity equation for ROS was found to be y = 0.042x+0.001 with a correlation coefficient 0.999 as shown in table 1 and fig. 4. The linearity equation for FEN was found to be y = 0.052x+0.008 with a correlation coefficient 0.999 as shown in table 1 and fig. 5.

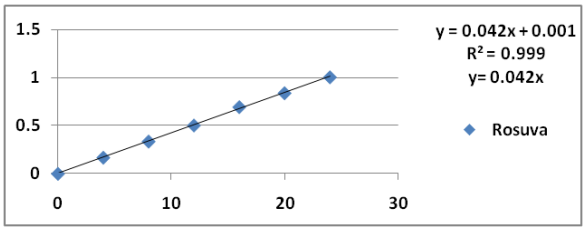


Fig. 4: Calibration curve of rosuvastatin calcium

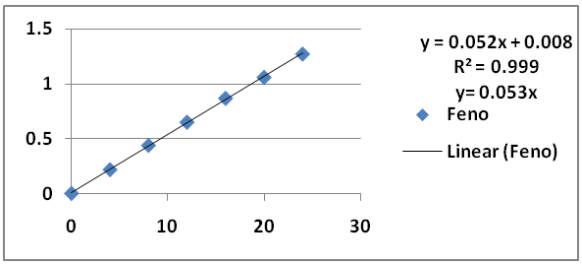


Fig. 5: Calibration curve of fenofibrate

Table 2: Results analysis of standard laboratory mixture (n=6)

S. No.	Amt. of a drug taken (µg/ml)		Absorbance (nm)		Amt. of drug estimated (mg)		% Estimation	
	ROS	FEN	249.5	287	ROS	FEN	ROS	FEN
1	1	16	0.580	0.920	10	160	100	100
2	1	16	0.580	0.920	10	160	100	100
3	1	16	0.582	0.923	10.02	161	100.2	100.61
4	1	16	0.580	0.920	10	160	100	100
5	1	16	0.581	0.921	10.02	161	100.2	100.61
6	1	16	0.580	0.920	10	160	100	100
						mean±SD	100.06±0.103	100.20±0.315
						% RSD	0.102	0.314

Mean Average of six determinations, SD: Standard Deviation, % RSD: Percentage relative standard deviation, The amount of drug estimated and percentage estimation are shown in table 2.

Table 3: Results analysis of marketed formulation (n=6)

S. No.	Weight of tablet powder (gm)	Absorbance		Amt. of drug estimated (mg)		% Estimation	
		249.5 nm	287 nm	ROS	FEN	ROS	FEN
1	0.420	0.596	0.937	10	160	100	100
2	0.422	0.598	0.939	10.02	160.02	100.2	100.2
3	0.421	0.597	0.938	10.01	160.01	100.1	100.1
4	0.420	0.596	0.937	10	160	100	100
5	0.422	0.598	0.939	10.02	160.02	100.2	100.2
6	0.420	0.596	0.937	10	160	100	100
					mean±SD	100.08±0.0983	100.08±0.0983
					% RSD	0.098	0.098

Mean: Average of six determinations, SD: Standard Deviation, % RSD: Percentage relative standard deviation, the amount of drug estimated and percentage estimation are shown in table 3.

Validation of the analytical method

Table 4: Results of accuracy study (n=3)

Amount added	Amount found±SD		% Recovery±% RSD	
	ROS	FEN	ROS	FEN
80	0.41±1.44	0.081±0.721	100.83±1.39	100.41±0.71
100	0.51±1.15	0.11±1.15	100.66±1.14	100.66±1.14
120	0.61±0.958	0.121±0.479	100.55±0.95	100.55±0.47

n: no. of determinations taken at each recovery level, SD: Standard Deviation, % RSD: Percentage relative standard deviation, The % recovery was calculated and it was found to be within the prescribed limit as shown in table 4.

Table 5: Results of precision data of ROS and FEN (n=3)

	Interday precision		Intraday precision	
	ROS	FEN	ROS	FEN
mean±SD	0.566±0.00404	0.906±0.004	0.569±0.00057	0.909±0.000577
% RSD	0.57	0.05	0.57	0.03

Mean: Average of 3 determinations SD: Standard Deviation, % RSD: Percentage relative standard deviation, the above table represents the results of interday and intraday precision data of ROS and FEN calculated by taking a mean of 3 determinations. The % RSD of the absorbance is less than 2 % so the method was found to be precise.

Table 6: Results of robustness study (n=3)

ROS		FEN	
mean±SD	%RSD	mean±SD	%RSD
0.336±0.001	0.29	0.436±0.0015	0.34
0.504±0.0015	0.30	0.648±0.0015	0.23
0.695±0.0015	0.21	0.867±0.001	0.11

SD: Standard Deviation, % RSD: Percentage relative standard deviation, the robustness of the method was calculated by changing the analyst and it was found to be within a limit as shown in table 6.

Table 7: Result of LOD and LOQ

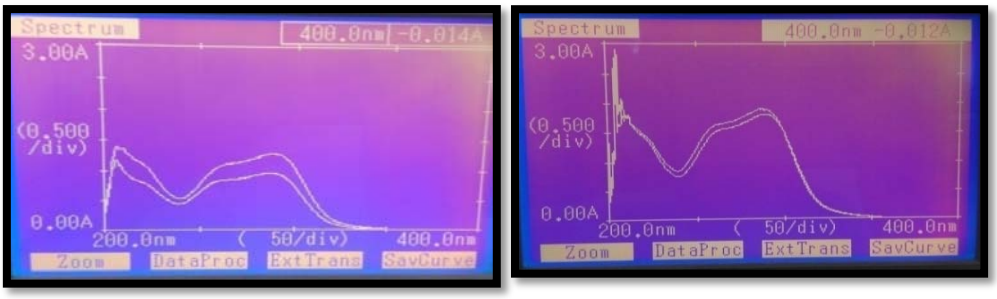
Parameter	ROS	FEN
LOD (µg/ml)	0.0480	0.069
LOQ (µg/ml)	0.145	0.210

LOD: Limit of detection LOQ: Limit of quantification, the detection limit and quantification limit were found to be 0.048 and 0.14 µg/ml for rosuvastatin and 0.069 and 0.210 µg/ml for fenofibrate, respectively, as shown in table 7.

Table 8: Forced degradation study

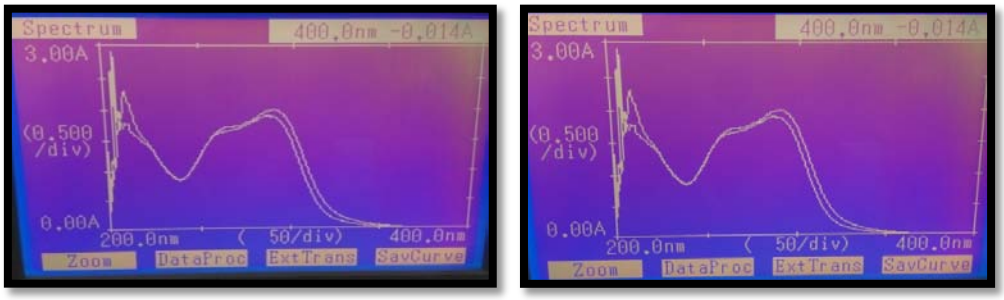
S. No.	Stress condition control	Time	Standard Mix. % obtained	Standard mix. % degraded	Formulation % obtained	Formulation % degraded
1	Acid hydrolysis(0.1 N HCl)	6h	84.2	15.8	87.21	12.7
2	Alkali hydrolysis (0.1 N NaOH)	6h	95.77	4.3	99.68	0.32
3	Oxidation (3% H2O2)	6h	99.32	0.68	96.85	3.14
4	Thermal (80 °Cfor 6 h)	6h	94.9	5.10	61.86	1.14
5	Photo Stability Sun Light 6 h	5h	61.86	38.14	48.44	51.56

The above table represents the results of the forced degradation study of standard mixture and formulation. % degradation and % obtained was calculated for each stress condition. Photostability degradation and acid hydrolysis showed prominent degradation.



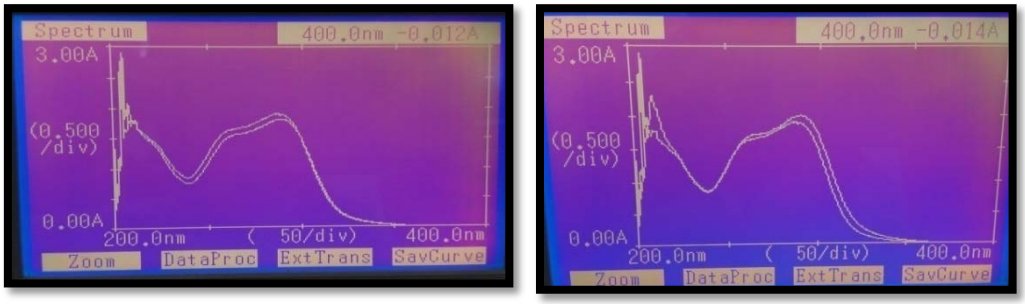
Std. mixture **Formulation**

Fig. 6: Acid degradation of standard mixture and formulation



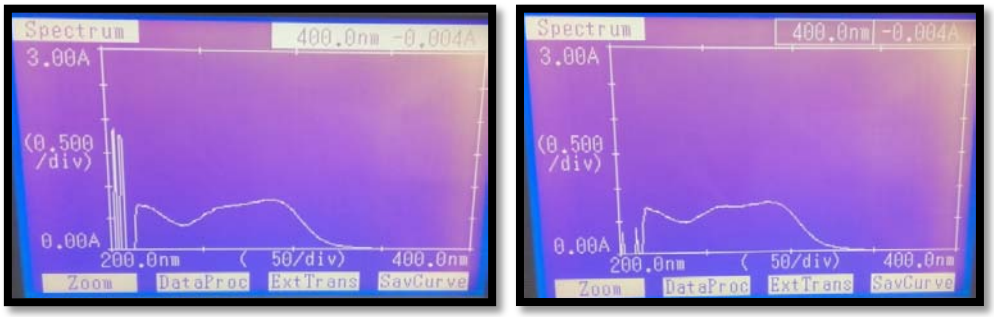
Std. mixture **Formulation**

Fig. 7: Base degradation of standard mixture and formulation



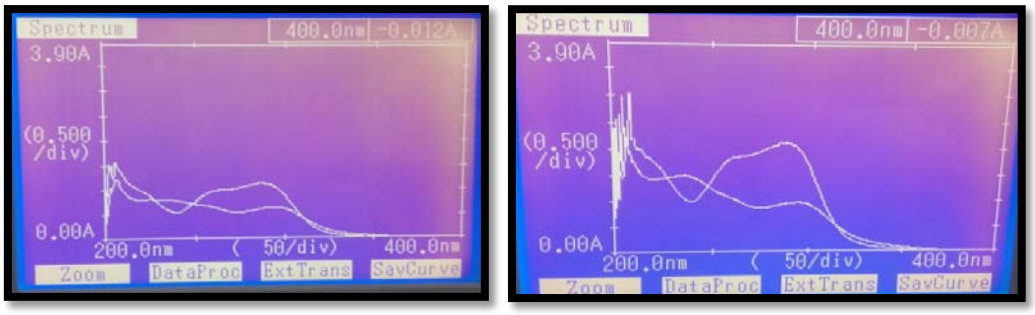
Std. mixture **Formulation**

Fig. 8: H_2O_2 degradation of standard mixture and formulation



Std. mixture **Formulation**

Fig. 9: Thermal degradation of standard mixture and formulation



Std. mixture **Formulation**

Fig. 10: Photodegradation of standard mixture and formulation

The spectrum of all degradation study of standard mixture and formulation as shown in fig. 6-10. From that spectrum % degradation was calculated and observed that more degradation was found in acid hydrolysis and photostability degradation.

DISCUSSION

For determination of ROS and FEN absorbance ratio method used. From the overlay spectra of both drugs, 249.5 nm selected as is absorptive point for estimation and another wavelength used was λ max of fenofibrate. For this method, linearity was obtained in the range of 4-24 µg/ml for ROS and FEN, respectively. For ROS the correlation coefficient, intercept, the slope was found to be 0.999, 0.042, 0.047, respectively. For FEN the correlation coefficient, intercept, the slope was found to be 0.999, 0.053, 0.054, respectively. The standard mixture and formulation studied by absorbance ratio method and amount of drug estimated, % estimation was calculated by using the equation of absorbance ratio method. It was found within the prescribed limit. The validation of the proposed method was performed as per ICH guidelines with linearity, accuracy, precision, LOD, LOQ. In the accuracy study 100.83, 100.66, 100.55 percentage ROS and 100.41, 100.66, 100.55 percentage FEN recovered at 3 different recovery levels. The standard deviation and % RSD value were found to be less than 2% shows the high precision and accuracy of the method. The robustness of the proposed method was calculated by changing the analyst and it was found within a limit. The detection limit and quantification limit were found to be 0.048 and 0.14 µg/ml for rosuvastatin and 0.069 and 0.210 µg/ml for fenofibrate, respectively. The forced degradation studies were performed by subjecting to the different stress conditions like acidic, alkaline, hydrolytic, oxidative, thermal, photostability. Major degradation was observed in acidic and photolytic conditions. In acidic condition, degradation of standard mixture and formulation was found to be 15.8 % and 12.7 %. In photolytic conditions, degradation of standard mixture and formulation was found to be 38. 14% and 51.50%, respectively. This spectroscopic method provides versatile techniques for the analysis of drugs in the multi-component formulation. The method was found to be simple, precise, accurate and reproducible during an analysis of standard mixture and formulation containing both drugs.

CONCLUSION

The present work describes a simple, accurate, reproducible, stable, economical, and non-interfering spectrophotometric method for the simultaneous estimation of rosuvastatin calcium and fenofibrate in bulk as well as pharmaceutical formulation using the isoabsorptive point method.

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Nil

AUTHORS CONTRIBUTIONS

Apeksha Funde developed a method and performed validation. Jayshree Kokat performed stability testing. Both authors discussed the results and contributed to writing the manuscript.

CONFLICT OF INTERESTS

Declared none

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