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DEVELOPMENT AND VALIDATION OF DERIVATIVE SPECTROSCOPIC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN COMBINED DOSAGE FORM

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ABSTRACT

The Present manuscript describe simple, sensitive, rapid, accurate, precise and economical first derivative spectrophotometric method for the simultaneous determination of Rosuvastatin Calcium and Fenofibrate in combined Pharmaceutical dosage form. The derivative Spectrophotometric method was based on the determination of both the drugs at their respective Zero Crossing Point (ZCP). The First order derivative spectra was obtained in Methanol and the determinations were made at 243nm (ZCP of Rosuvastatin Calcium) for Fenofibrate and 239nm (ZCP of Fenofibrate) for Rosuvastatin Calcium. The linearity was obtained in the concentration range of 2-10 µg/ml for Rosuvastatin Calcium and 3-15 µg/ml for Fenofibrate. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method. The method was found to be Simple, Sensitive, Accurate and Precise as per ICH guideline Q2B (R1). The Proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Pharmaceutical dosage form.

KEY WORDS

Rosuvastatin Calcium (RSC), Fenofibrate (FAN), First order derivative UV Spectrophotometry, Zero Crossing Point, Method validation and ICH guidelines.

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INTRODUCTION[1- 10]

Rosuvastatin Calcium[1] (RSC, Figure No.1) is chemically bis [(E)-7 [4-(4-fluorophenyl)-6 isopropyl-2- [methyl (methylsulphonyl) amino] pyrimidin-5-yl] (3R, 5S) -3, 5-dihydroxyhept-6-enoic acid] Calcium salt. It is a lipid lowering drug. It inhibits the enzyme 3-hydroxry- 3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the rate

limiting enzyme that converts HMG-CoA to mevalonate, a precursor of cholesterol and thereby checks the synthesis of cholesterol. It is used in the treatment of hypercholesterolemia and dyslipidemia. It is used to reduce the amounts of LDL cholesterol, total cholesterol, triglycerides and apolipoprotein B in the blood[2, 3]. Rosuvastatin calcium also modestly increases the level of HDL cholesterol in the blood. These actions are important in reducing the risk of atherosclerosis, which in turn can lead to several cardiovascular complications such as heart attack, stroke and peripheral vascular disease.

Fenofibrate[4] (FAN, Figure No.2) is a drug of the fibrate class Fenofibrate is chemically propan-2-yl 2{4-[(4- chlorophenyl) - carbonyl] phenoxy}-2-methylpropanoate. It is mainly used to reduce cholesterol levels in patients at risk of cardiovascular disease. Like other fibrates, it reduces low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels, as well as reducing triglycerides (TG) level. It also increases high density lipoprotein (HDL) levels. It is used alone or in combination with statins in the treatment of hypercholesterolemia and hypertriglyceridemia. This paper presents simple, rapid, reproducible and economical methods for the simultaneous analysis of Rosuvastatin calcium (RSC) and Fenofibrate (FAN) in tablet dosage forms.

Literature survey [5-9] revealed that a number of analytical methods have been reported for the estimation of Rosuvastatin Calcium and Fenofibrate in individual and combination are spectrophotometry, RP-HPLC method. Apart from this no other spectrophotometric methods like Derivative spectroscopy method were reported for this RSC and FAN in their combined dosage form.

MATERIAL AND METHODS

Instrument

Instrument used was an UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with a pair of 1 cm matched quartz cells. All weighing was done on Shimadzu analytical balance (Model AU-220).

Reagents and chemicals

Pure drug samples of RSC and FAN were provided as a gift sample from Alembic Pharmaceuticals Ltd, Vadodara and Torrent Pharmaceuticals Ltd, Kadi. Methanol LR was used as solvent. Calibrated glass wares were used throughout the work.

Marketed formulation

The commercial formulation Razel-*F10* (Glenmark Pharmaceuticals Ltd, Mumbai) was purchased from Local pharmacy. Each Tablet contains 10mg Rosuvastatin Calcium and 67mg Fenofibrate.

Preparation of standard stock solution

Accurately weighed quantity of RSC (100 mg) and FAN (100 mg) was transferred to two separate 100 ml volumetric flasks, dissolved in Methanol and diluted to the mark with same solvent. (Stock solutions: 1000µg/ml of RSC and 1000µg/ml of FAN).

Preparation of working standard solution

100µg/ml of RSC solution was prepared by diluting 10.0 ml of stock solution with methanol in 100 ml volumetric flask up to the mark. 100µg/ml of FAN solution was prepared by diluting 10.0 ml of stock solution with Methanol in 100 ml volumetric flask up to the mark.

Selection of wavelength for analysis

1.0 ml of working standard solution of RSC (100µg/ml) and 1.0 ml of working standard solution of FAN (100µg/ml) was pipette out into two separate 10 ml volumetric flask and volume was adjusted to the mark with Methanol to get 10µg/ml of RSC and 10µg/ml of FAN. Each solution was scanned between 200-400 nm against methanol as a reagent blank for zero order spectra (Figure No.3). The first order derivative spectra of each solution were obtained using smoothing ($\Delta\lambda$ = 2, Scaling Factor = 26). The zero crossing points were found to be 243 nm and 239 nm for RSC and FAN respectively (Figure No.4). Wavelengths selected for quantitation were 243 nm for Fenofibrate (zero crossing point for Rosuvastatin Calcium) and 239 nm for Rosuvastatin calcium (zero crossing point for Fenofibrate).

Calibration curves for RSC and FAN

Standard RSC solution from 2-10µg/ml were prepared by pipetting out, 0.2, 0.4, 0.6, 0.8, 0.10 ml of the working standard solution of RSC (100µg/ml) into series of 10 ml volumetric flasks and the volume was adjusted to mark with Methanol. Absorbance of each solution was measured at 239 nm using first order derivative spectrophotometry. A calibration curve was prepared by plotting absorbance against respective concentration (Figure No.7). Standard FAN solution from 3-15µg/ml were prepared by pipetting out 0.3, 0.6, 0.9, 1.2, 1.5 ml of the working standard stock solution of FAN (100µg/ml) into series of 10ml volumetric flasks and the volume was adjusted to mark with Methanol. Absorbance of each solution was measured at 243 nm using first order derivative spectrophotometry. A calibration curve was obtained by plotting absorbance against respective concentration (Figure No.8).

Analysis of marketed formulation

Twenty Tablets were weighed and crushed to obtain a fine powder. An accurately weighed powder equivalent to about 10mg of RSC and 67mg of FAN was transferred to 100 ml volumetric flask and the volume was made up to the mark using Methanol as solvent. The solution was sonicated for 20minutes. The solution was filtered through whatman Filter Paper No.42. First few ml of filtrate were discarded. 2 ml of the solution from above filtrate was diluted to 100 ml with Methanol. The absorbance of the resulting solution was measured using first order derivative spectrophotometry at 239nm for RSC and 243nm for FAN. The concentration of each drug was calculated using equation of straight line (Table No.7).

METHOD VALIDATION[10]

Linearity and range

Aliquots of standard stock solutions of RSC and FAN were taken in volumetric flasks and diluted with Methanol to get final concentrations in range of 2-10µg/ml for RSC and 3-15µg/ml for FAN. This calibration range was prepared five times and absorbances were measured at respective wavelengths for each drug separately (Table No.1) (Figure No.5 and 6).

Precision

Precision of the method was determined by performing interday variation, intraday variation and method repeatability studies. In interday precision, the absorbance of standard solutions of RSC (2, 6 and 10µg/ml) and FAN (3, 9 and 15µg/ml) were measured on Three consecutive days. In intraday variation the absorbances were measured Three times in a day. Repeatability study, one concentration of both the drugs was measured Six times (Table No.2, 3 and 4).

Recovery studies

To study the accuracy of the proposed method, recovery studies were carried out by standard addition method at three different levels. A known amount of drug was added to preanalyzed Tablet powder and percentage recoveries were calculated (Table No.5 and 6).

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were calculated from the data obtained from the linearity studies. The slope of the linearity plot was determined. For each of the six replicate determinations of same conc. (6 µg/ml of RSC and 9 µg/ml of FAN), standard deviation (SD) of the responses was calculated. From these values, the parameters Limit of Detection (LOD) and Limit of Quantitation were determined on the basis of standard deviation and slope of the regression equation.

LOD = (3.3 x SD) / Slope

LOQ = (10 x SD) / Slope

RESULTS AND DISCUSSION

The proposed method was validated as per ICH guideline Q2B (R1). The plot of absorbance's versus respective concentrations of RSC and FAN were found to be linear in the concentration range of 2-10µg/ml and 3-15µg/ml respectively with correlation coefficient 0.999 at 243nm and 0.999 at 239nm (as shown in Table No.1 and Figure No.5,6,7 and 8). Precision was calculated as repeatability, intraday and interday variations and % RSD

(Relative Standard Deviation) was found to be in the range (Table No.2, 3, and 4). The accuracy of method was determined at 80, 100 and 120% level. The mean recovery was 100.96 ±1.23 and 100.94 ± 1.09 for Rosuvastatin Calcium and Fenofibrate, respectively (Table No.5 and 6).

The derivative spectrophotometric method can be successfully used for simultaneous estimation of RSC and FAN in their combined Tablet dosage form (Table No.8). Marketed Tablets were analyzed and results obtained were within the range (Table No.7).

Table No.1: Linearity Study

S.No	Concentration (µg/ml)	Absorbance at 239nm	Concentration (µg/ml)	Absorbance at 243nm
1	2	0.115	3	0.101
2	4	0.195	6	0.161
3	6	0.287	9	0.222
4	8	0.377	12	0.291
5	10	0.464	15	0.351

Table No.2: Intra-Day Precision Study

S.No	RSC Concentration (µg/ml)	Absorbance* ± S.D.	%RSD	FAN Concentration (µg/ml)	Absorbance* ± S.D.	%RSD
1	2	0.11533 ± 0.00058	0.5	3	0.101 ± 0.001	0.99
2	6	0.288 ± 0.001	0.3472	9	0.222 ± 0.001	0.45
3	10	0.465 ± 0.001	0.2151	15	0.351 ± 0.001	0.2849

*Average of Three determination, S.D. = Standard Deviation, RSD= Relative Standard Deviation

Table No.3: Inter-Day Precision Study

S.No	RSC Concentration (µg/m)	Absorbance* ± S.D.	%RSD	FAN Concentration (µgml)	Absorbance* ± S.D.	%RSD
1	2	0.11622 ± 0.00137	1.17	3	0.1025 ± 0.00151	1.46
2	6	0.2885 ± 0.00151	0.5224	9	0.224 ± 0.00168	0.75
3	10	0.4654 ± 0.00138	0.2971	15	0.35289 ± 0.0025	0.70

*****Average of Three determination

Table No.4: Repeatability Study

S.No	RSC Concentration (µg/ml)	Absorbance* ± S.D.	%RSD	FAN Concentration (µg/ml)	Absorbance* ± S.D.	%RSD
1	6	0.2885 ± 0.001871	0.6485	9	0.223667 ± 0.00216	0.9657

*****Average of Six determination

Table No.5: Recovery Study of Rosuvastatin Calcium

S.No	Drug	Level of Recovery	Amt. of Drug taken(µg/ml)	Amt. of Std. drug taken (spiked amt.) (µg/ml)	% Recovery* ± S.D.	%RSD
1	Rosuvastatin Calcium	80%	2	4	100.98± 1.15	1.13
		100%	2	5	100.60± 1.94	1.92
		120%	2	6	101.30± 0.60	0.60

*Average of Three determination

Table No.6: Recovery Study of Fenofibrate

S.No	Drug	Level of Recovery	Amt. of Drug taken(µg/ml)	Amt. of Std. drug taken (spiked amt.) (µg/ml)	% Recovery* ± S.D.	%RSD
1	Fenofibrate	80%	3	4	101.23± 1.43	1.41
		100%	3	5	100.47± 0.82	0.81
		120%	3	6	101.12± 1.04	1.02

*Average of Three determination

Table No.7: Results of simultaneous estimation of RSC and FAN in Marketed Formulation

S.No	Brand Name	Drugs	Label Claim (mg)	Amount Found (mg)	% Label Claim*	S.D.	%RSD
1	Razel-F10	RSC	10	9.7	97.04%	0.002	1.83
		FAN	67	67.57	100.85%	0.001	0.31

*Average of Six determination

Table No.8: Results of Validation Parameters

S.No	Parameter	Rosuvastatin Calcium	Fenofibrate
1	Zero Crossing Point	243nm	239nm
2	Concentration Range	2-10 µg/ml	3-15 µg/ml
3	Correlation Coefficient	0.999	0.999
4	Precision (%RSD[b]) Intraday (n=3) Interday (n=3)	 0.21-0.50 0.29-1.17	 0.28-0.99 0.70-1.46
5	Repeatability (%RSD, n=6)	0.6485	0.9657
6	Accuracy ± [a]SD (% Recovery)	100.60 ± 1.94 - 100.98 ± 1.15	100.47 ± 0.82 - 101.23 ± 1.43
7	[c]LOD (µg/ml)	0.14	0.33
8	[d]LOQ (µg/ml)	0.42	1.02
9	Assay (%RSD, n=6)	97.04 ± 1.83 %	100.85 ± 0.31 %

[a]SD= Standard Deviation, [b]RSD=Relative Standard Deviation, [c]LOD= Limit of detection, [d]LOQ= Limit of Quantification.

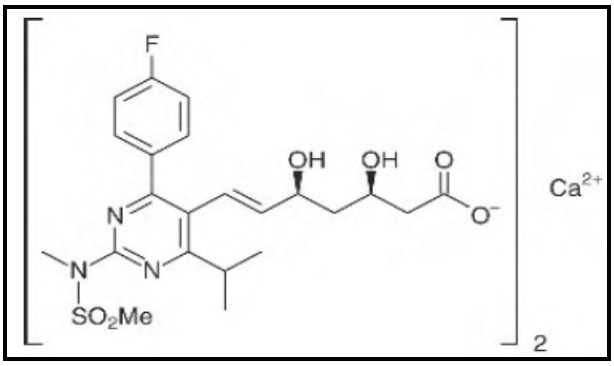

Figure No.1: Chemical Structure of Rosuvastatin calcium

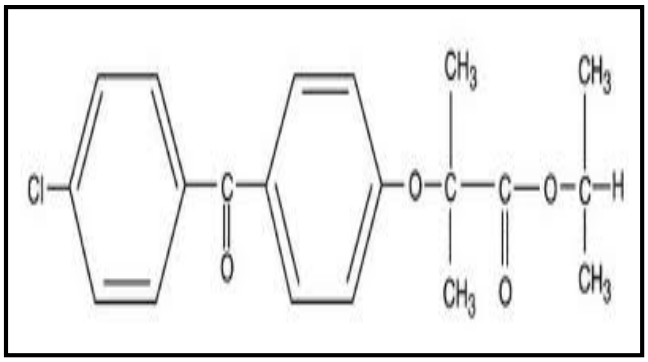

Figure No.2: Chemical Structure of Fenofibrate

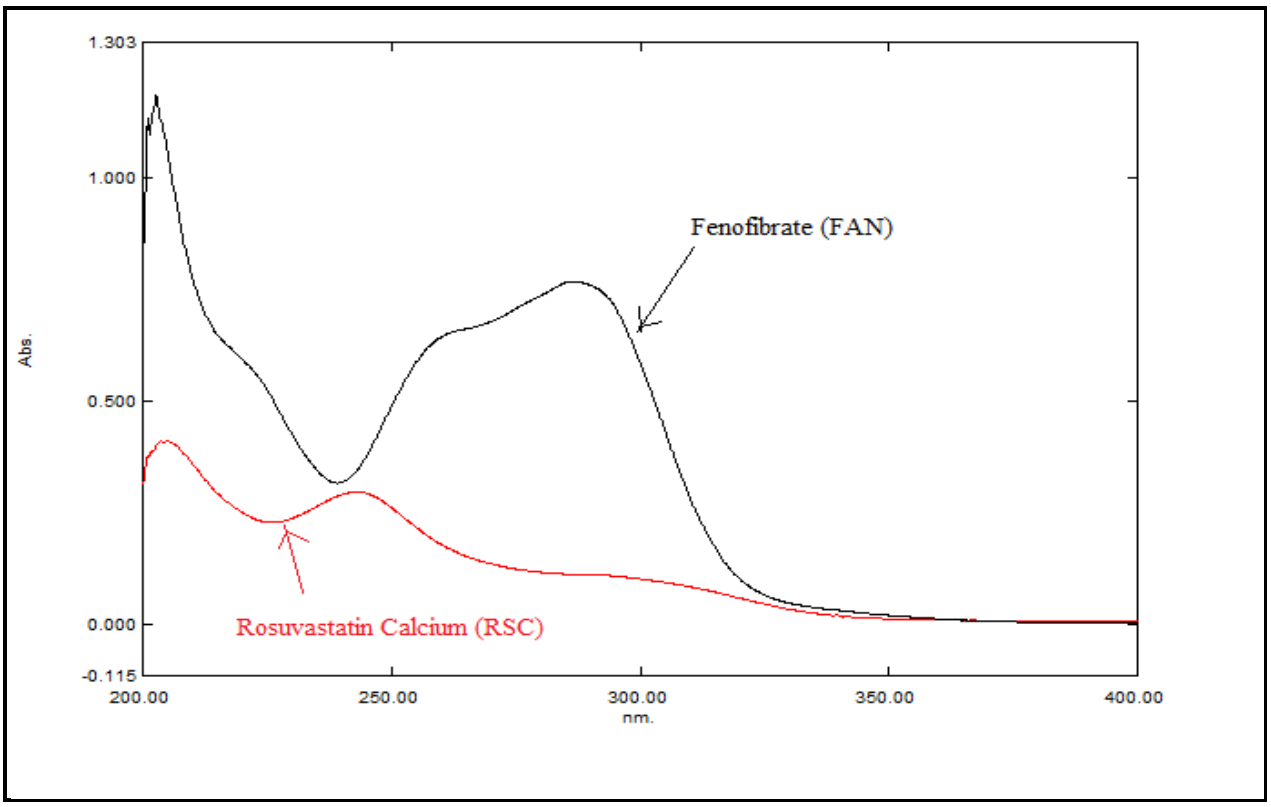

Figure No.3: Overlain Zero order spectra of RSC and FAN in methanol

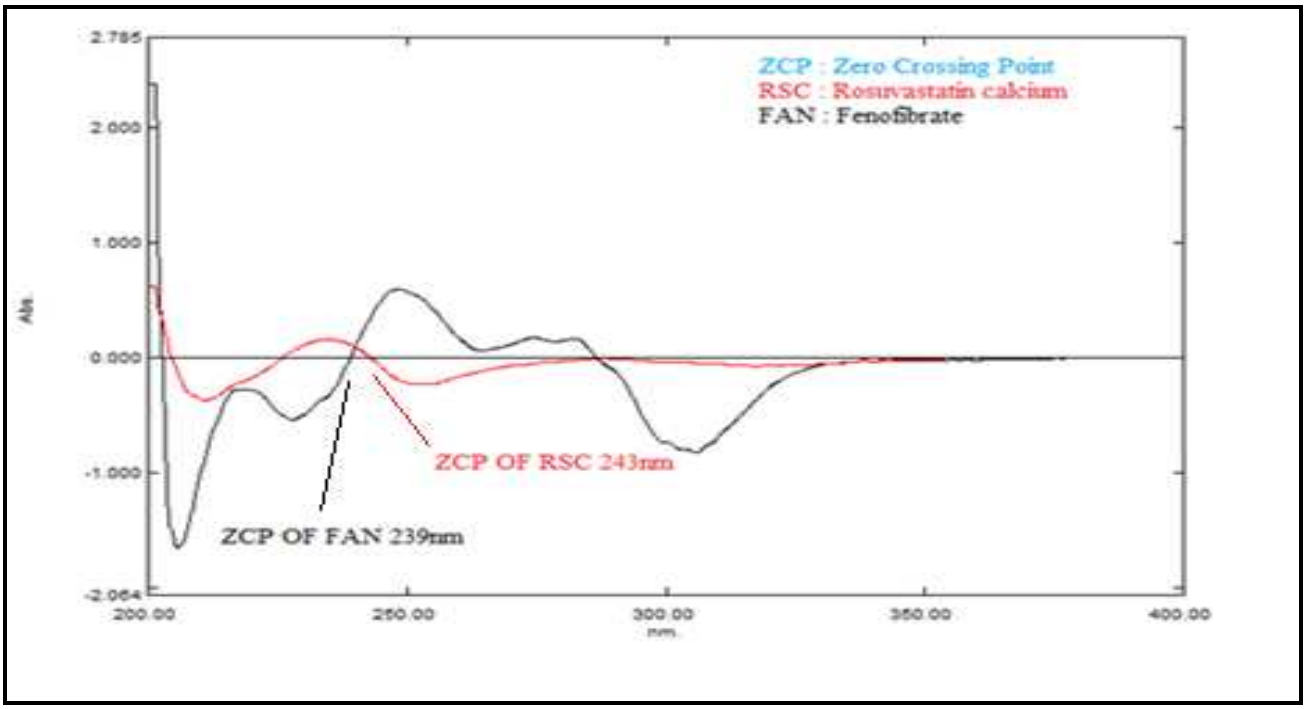

Figure No.4: Overlain First order Derivative spectra of RSC (2µg/ml) and FAN (15µg /ml) in Methanol

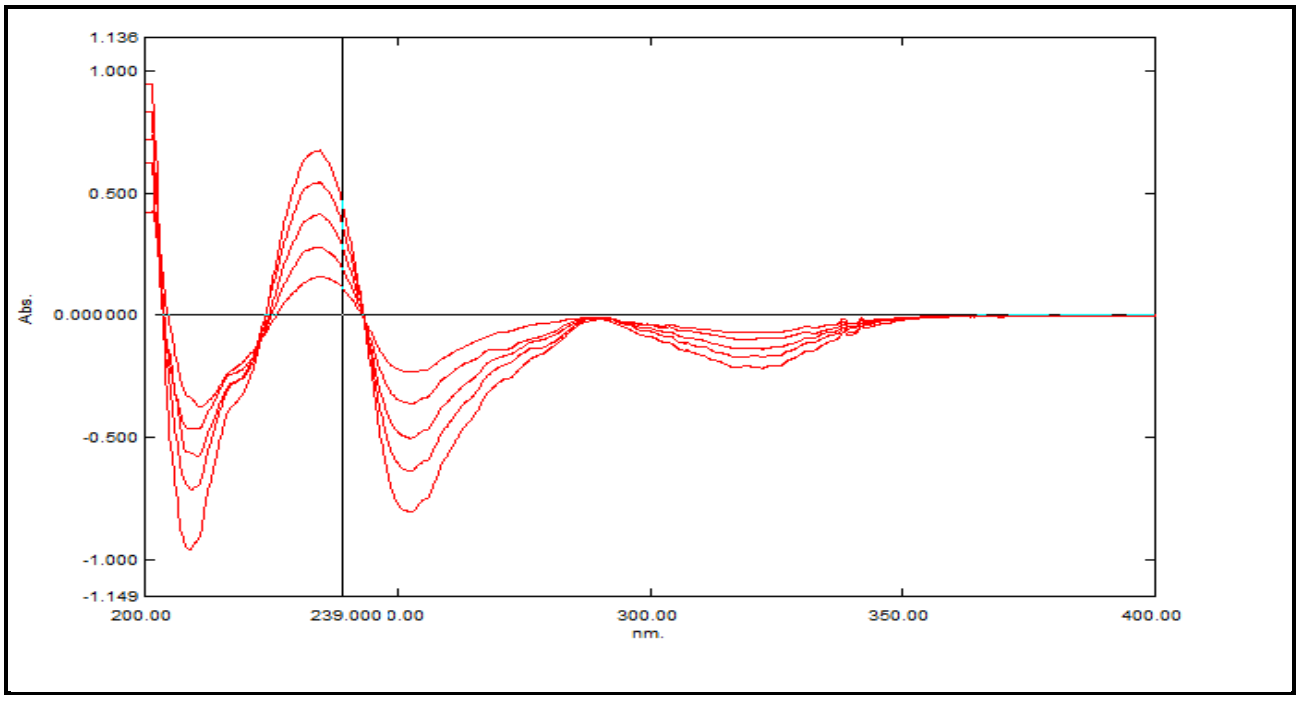

Figure No.5: Overlain First order Derivative spectra of RSC (2-10µg/ml) at 239nm (ZCP of FAN) in Methanol

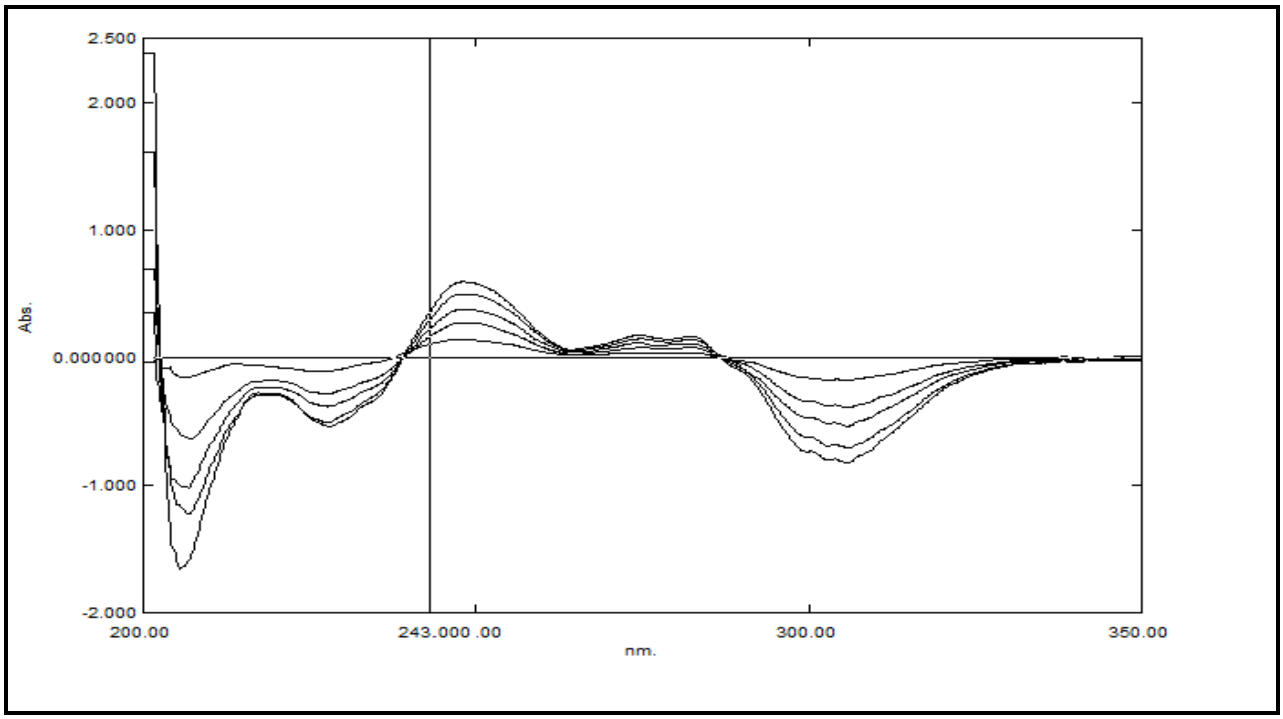

Figure No.6: Overlain First order Derivative spectra of FAN (3-15µg/ml) at 243nm (ZCP of RSC) in Methanol

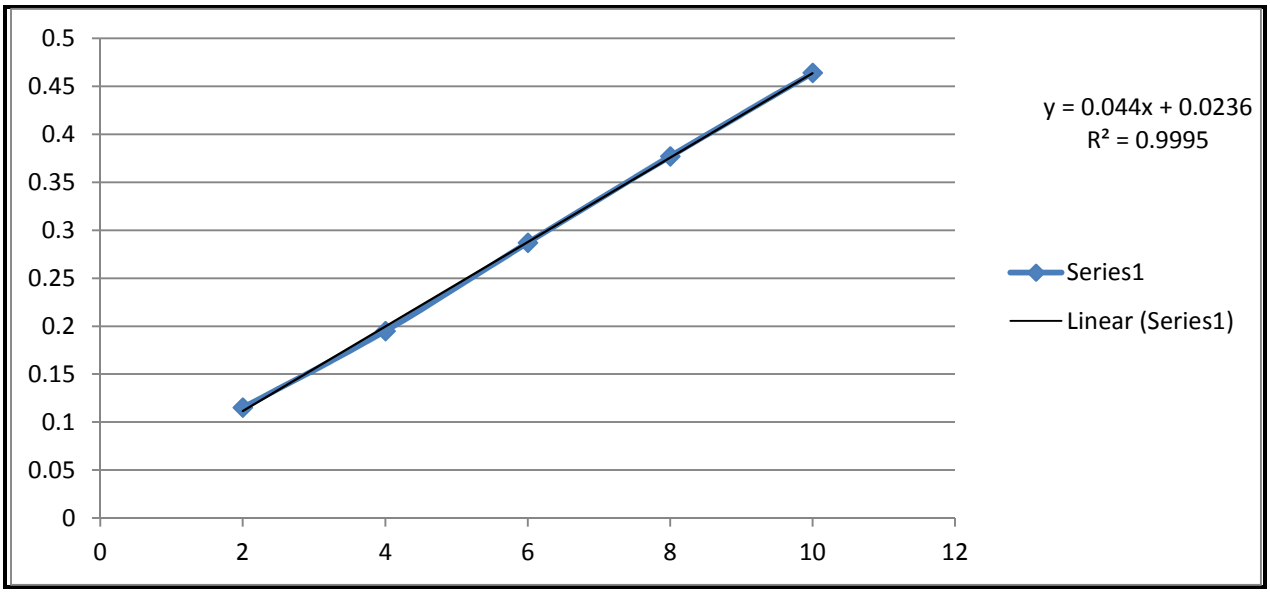

Figure No.7: Calibration curve of standard RSC at 239nm by first order derivative Spectrophotometry

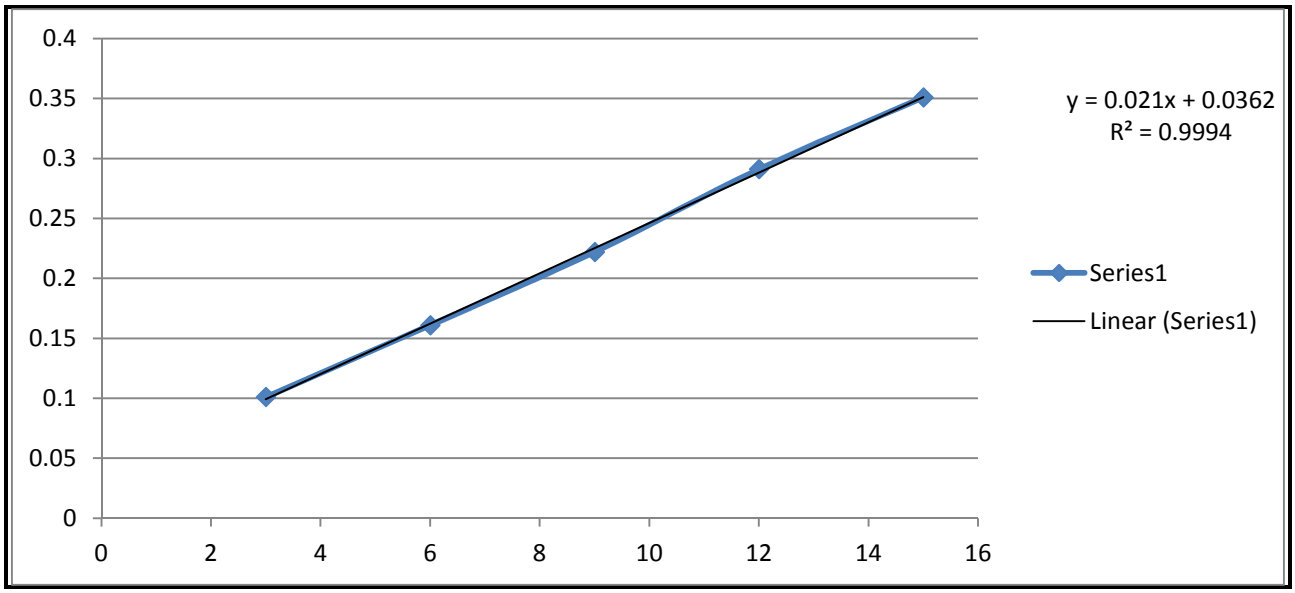

Figure No.8: Calibration curve of standard FAN at 243nm by first order derivative Spectrophotometry

CONCLUSION

The proposed method gives accurate and precise results for determination of RSC and FAN in marketed Tablet formulation and is easily applied for routine analysis. The method is simple, accurate, precise and rapid. The proposed method was successfully applied for the estimation of these drugs in commercial dosage form.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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