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Development and Validation of Spectrophotometric Method for the Determination of Rosuvastatin Calcium in Solid Dosage Form and Pharmaceutical Formulation

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Abstract: **A new, simple, precise, accurate and economical and sensitive spectrophotometric method in ultraviolet region has been developed for the determination of rosuvastatin calcium in bulk and in pharmaceutical formulation. The drug exhibits an absorption maximum at a wavelength of 242 nm with Acetonitrile: Methanol (60:40v/v) as diluents. Linearity of rosuvastatin calcium was observed in the concentration range of 4-24µg/ml. Percentage purity and accuracy were in the limit of 98%-102%.The method was found precise (% RSD< 2%). The Correlation coefficient for drug methods was greater than 0.99. Percentage purity is found to be 100.21%.LOD and LOQ values are found to be 0.75 and 2.27µg/ml. The assay was found to be in range of 99.77%-101.81% .The developed method is validated statistically as per ICH guidelines and the results obtained are within acceptance criteria related to linearity, accuracy and precision.**

Keywords: **Rosuvastatin calcium, Methanol, Acetonitrile, UV spectrophotometric method, Validation.**

I. INTRODUCTION

Rosuvastatin calcium is used as an antihyperlipidemic, Rosuvastatin calcium, (E)-(3R,5S)-7-[4-(4-fluorophenyl)-6-isopropyl-2{methyl(methylsulphonylamino)}pyrimidin-5-yl]-3,5-dihydroxyhepten-6-oic acid calcium, is a HMG Co-A Reductase inhibitor which is used in Hyperlipidemia1,2.It is used in the treatment of dyslipidemia, which is effective at low doses and its half-life is more compared to other statins. The proposed method was optimized and validated in accordance with International Conference on Harmonization (ICH) guidelines .Rosuvastatin is a, synthetic lipid-lowering agent, is a selective and competitive inhibitor of 3-hydroxy -3-methylglutaryl-coenzyme A(HMG-CoA reductase), the key –limiting enzyme of cholesterol biosynthesis in liver.

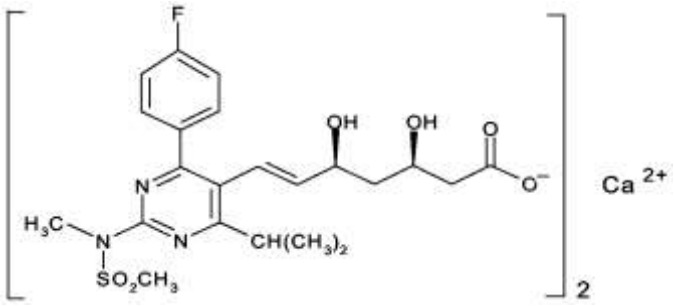

Fig.1.Structure of Rosuvastatin calcium

Extensive literature survey reveals that a few spectrometric [3-10] were available for the estimation of Rosuvastatin calcium in bulk and formulations. Several methods have been reported for the determination of Rosuvastatin calcium.

Literature survey revealed that various analytical methods such as high performance liquid chromatography (HPLC),spectrophotometry, gas chromatography have been reported for the estimation of Rosuvastatin calcium. The spectrophotometric methods, which are reported, require the need of chromophoric agent for the absorbance[1]. Thus, there is a need to develop a simple, accurate, precise, specific method for Rosuvastatin calcium estimation by spectrophotometry. The aim is to develop a simple, accurate, precise, specific spectrophotometric method for the direct quantitative estimation of Rosuvastatin calcium in bulk and formulation. The developed method was validated as per the guidelines of International Conference on Harmonization (ICH) and demonstrated excellent specificity, linearity, precision and accuracy for Rosuvastatin calcium.

II. MATERIALS AND METHODS

Instrumentation

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matched quartz cells with 1 cm light path length and loaded with UV probe software was used for the recording of spectra and measuring absorbance for method development and validation study.

Materials

All chemicals and reagents were of analytical grade. Rosuvastatin calcium .was gifted from Optimus Pharmaceutical, Hyderabad, India. The commercially available tablets Rosustat 10 mg Torrent Pharmaceuticals were obtained from the market. HPLC grade Acetonotrile and Methanol Analytical Grade Research Labs was used as a solvent.

Method Development

Preparation of stock solution

Standard stock solution of Rosuvastatin Calcium was prepared by dissolving 100mg of drug in 100ml of ACN :Methanol(60:40v/v) to get a concentration of 1mg/ml or 1000 µg/ml (Stock I). From the above solutions, 1ml was taken in 10ml volumetric flask and volume was made up with 10 ml of ACN: Methanol(60:40v/v) to get a concentration of 100µg/ml (Stock II). This solution was taken as stock solution.

Determination of wavelength of maximum absorption

From the above stock solution, 1 ml of standard stock II solution was transferred into 10 ml volumetric flask and diluted to 10 ml with ACN: Methanol(60:40v/v) to give concentration of 10µg/ml, it was used for spectral scan in the UV range of 400-200 nm, and the wavelength corresponding to maximum absorbance was noted at 242nm.

Preparation of Calibration Curve

For the preparation of standard calibration curve, concentration of 4-24µg/ml were prepared by pipetting out 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 ml from the 100µg/ml solution in to a 10ml volumetric flask and made up the volume with ACN :Methanol (60:40v/v). The absorbance of each solution was measured at 242 nm against ACN: Methanol (60:40v/v) as blank. Calibration curve of the Rosuvastatin Calcium was plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis. The calibration curve is shown in fig. N

Preparation of calibration standards

Accurately 4-24ml of working standard solution of Rosuvastatin Calcium was transferred to a series of 10ml volumetric flasks and the volume was made up to the mark with ACN :Methanol(60:40v/v) to produce 4-24µg/ml solutions and the absorbance of the resulted solutions was measured at 242nm. The calibration curve was constructed by plotting absorbance against concentration.

Preparation of sample solution

Ten tablets of Rosustat each containing 10 mg of Rosuvastatin calcium were weighed accurately and made into a fine powder. The tablet powder equivalent to 10mg of rosuvastatin calcium was weighed accurately and transferred into a 10ml volumetric flask, 4ml of ACN :Methanol(60:40v/v) was added, mixed well and fornicated for10mins using ultra sonicator. The volume was made up to the mark with the same solvent to get 1000µg/ml. Rosuvastatin 100µg/ml sample solution was prepared by diluting 1ml of 1000µg/ml of the stock solution with ACN :Methanol(60:40v/v). Accurately 1ml of 100µg/ml solution was transferred to 10ml volumetric flask and made up to the mark with ACN :Methanol(60:40v/v) to get 10µg/ml of Rosuvastatin and the absorbance of the prepared solution was measured at 242nm.

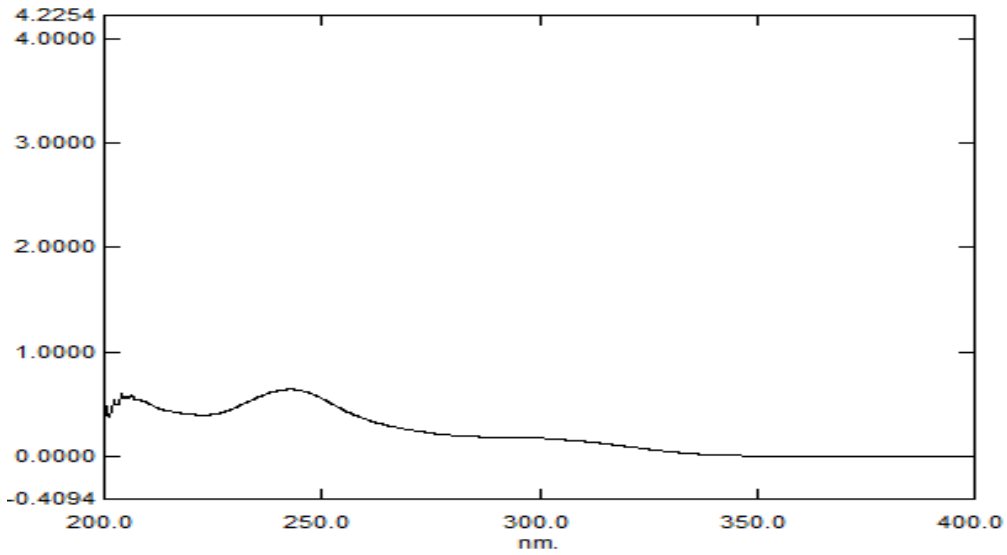

Fig 2- UV Spectrum of Rosuvastatin Calcium

Validation of the Developed Method

The developed method was validated for accuracy, precision, linearity, limit of detection, limit of quantitation and robustness as per ICH guidelines [8].

Linearity

For linearity study, from the stock solution II (100µg/ml) six solutions at different concentrations (4, 8, 12, 16, 20 and 24 mg/mL) were prepared using six point calibration method. The samples were scanned in UV-Vis Spectrophotometer against methanol as blank. The selected drug shows linearity between the ranges of 4-24µg/ml.

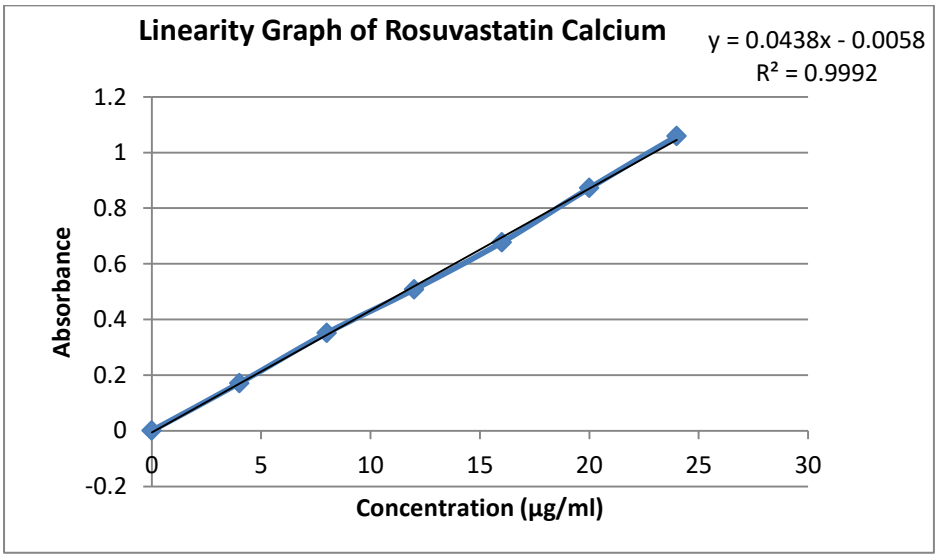

Fig N: Calibration curve of Rosuvastatin Calcium at 242 nm.

Precision

The precision of proposed method was determined by Intra-day and Inter-day precision. Three different solutions of three different concentrations (8, 12,16µg/ml) was analyzed, and it was expressed in terms of percent relative standard deviation (%RSD). For Inter-day and Intra-day %RSD were found in the range of 0.5992 and 0.477respectively.

Accuracy

Accuracy of the present method was carried out by using the drug substance 10ppm as standard solution and spiked solution at three different concentration levels of 80%, 100% and 120% in triplicates. Absorbance was measured at 242 nm and results were expressed in terms of % recoveries. Standard deviation and % RSD was calculated.

Assay

Twenty tablets of Rosuvastatin calcium were weighed accurately and powdered. Powder equivalent to 50 mg of was weighed and transferred to a 50 ml volumetric flask and make up the volume up to 50ml with ACN :Methanol(60:40v/v), which gives 1000µg/ml solution and sonicated for 15 minutes to get homogeneous solution. Then it was filtered through a whatman filter paper. A final concentration of 100 mg/ml of Rosuvastatin calcium was prepared. From this 1 ml was taken and diluted to 10 ml with ACN: Methanol (60:40v/v) which gives 10µg/ml solution and the absorbance of the solution was measured at 242 nm.

Percent Recovery Study

Recovery study was carried out by spiking standard working solution to sample solution (formulation) at three different levels 80%, 100% and 120%. The final concentration of Rosuvastatin calcium determined. The percentage recovery was calculated as mean± standard deviation and %RSD.

Limit of detection and Limit of Quantification

Limit of detection (LOD) and Limit of quantification (LOQ) of Rosuvastatin calcium was calculated by using equation given in the ICH guidelines[2].

Limit of Detection

Limit of Detection was determined on the basis of slope and standard deviation of the calibration curve.

LOD = 3.3 σ/S

Where, σ = standard deviation of Y intercept of regression lines

S = slope of the calibration curve

Limit of Quantitation

Limit of Quantitation was determined on the basis of slope and standard deviation of the calibration curve.

LOQ = 10 σ/S

Where, σ = standard deviation of Y intercept of regression lines

S = slope of the calibration curve**.**

III. METHOD VALIDATION

The proposed method was validated for various parameters such as linearity and range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), Assay, Percent recovery and specificity according to ICH Q2 (R1) guideline and USP guidelines [8].

Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 0.4–24 mg/mL was linear with a correlation coefficient (R^2) greater than 0.998.

Precision

The precision (measurement of intraday, interday) results showed good reproducibility with percent relative standard deviation (%RSD) was below 2.0%. This indicated that method is highly precise.

Accuracy

The accuracy of analytical method results found within the range of 100–100.90%, which indicate that the method is accurate.

Assay (Content of Rosuvastatin Calcium in marketed formulation)

Rosuvastatin Calcium content of marketed products determined by the proposed method was in good agreement with the label claim and was found at 100.79 %recovery with the mean RSD value 0.82 respectively.

Limit of detection

The limits of detection (LOD) which represent the sensitivity of the proposed method were determined. The LOD value obtained was 0.75µg/ml. It indicates the high sensitivity of the proposed method.

Limit of quantitation

The limits of Quantitation (LOQ), which represent the sensitivity of the proposed method, were determined. The LOQ value obtained was 2.27µg/ml. It indicates the high sensitivity of the proposed method.

IV. RESULT AND DISSCUSSION

This study was focused on development of a new spectrometric method for the analysis of Rosuvastatin in bulk drug and tablet dosage form. Spectrophotometric analysis was performed using double beam UV-Visible spectrophotometer (LABINDIA 3000) with 1cm path length supported by UV-WIN Software.

Linearity

TABLE 1: LINEARITY DATA OF ROSUVASTATIN CALCIUM

S.NO.	Concentration (µg / ml)	Absorbance at 242nm
1	4	0.171
2	8	0.351
3	12	0.508
4	16	0.677
5	20	0.872
6	24	1.059

Intraday Precision

TABLE 2: INTRADAY PRECISION OF ROSUVASTATIN CALCIUM

Concentration (µg/ml)	Absorbance (nm)			Mean	Standard Deviation	% Relative standard Deviation	Average of % RSD
	1	2	3				
8	0.351	0.360	0.360	0.357	0.005196	1.455505	0.295168
12	0.508	0.498	0.497	0.501	0.006083	1.214124	
16	0.677	0.672	0.668	0.672	0.004509	0.670687	

Interday Precision

TABLE 3: INTERDAY PRECISION OF ROSUVASTATIN CALCIUM

Concentration (µg/ml)	Absorbance (nm)			Mean	Standard Deviation	% Relative standard Deviation	Average of % RSD
	1	2	3				
8	0.360	0.360	0.360	0.360	6.79	1.8885	0.754566
12	0.494	0.495	0.494	0.495	0.001	0.2020	
16	0.668	0.666	0.666	0.666	0.173205	0.173205	

Accuracy

TABLE 4: ACCURACY OF ROSUVASTATIN CALCIUM

No of Preparation	Concentration (µg/ml)		Percent Recovery	Mean %Recovery ±SD	% RSD	Mean RSD
	Formulation	Pure Drug				
S1: 80%	10	8	100.00	100.09±0.362	0.15	0.14
S1: 80%	10	8	100.00			
S1: 80%	10	8	100.28			
S2: 100%	10	10	100.90	100.64± 0.454	0.12	
S2: 100%	10	10	100.90			
S2: 100%	10	10	100.13			
S3: 120%	10	12	100.18	100.18± 0.539	0.	
S3: 120%	10	12	100.18			
S3: 120%	10	12	100.18			

Assay

TABLE 5: ASSAY OF ROSUVASTATIN CALCIUM AVERAGE (N=6)

Tablet formulation	Label claim	Amount taken	Assay (Amount found)	%rsd
Rosustat	10 mg	10 mg	100.79±0.0037	0.82

Percent Recovery

TABLE 6: PERCENT RECOVERY OF ROSUVASTATIN CALCIUM

Test(µg/ml)	Accuracy level	Initial Concentration (µg/mL)	Amount of drug added	% Recovery (n=3)	Standard deviation	%RSD
10(µg/ml)	80%	10	8	98.68	0.001155	0.32
	100%	10	10	100.00	0.000577	0.12
	120%	10	12	102.0833	0.001732	0.31

LOD & LOQ

TABLE 7: LOD & LOQ OF UV-VIS SPECTROPHOTOMETRIC METHOD FOR ROSUVASTATIN CALCIUM

S. no	Parameters	S.D*	b**	Formula	Calculation (µg/ml)
1	LOD	0.010	0.044	$3.3(S.D/b)$	0.75
2	LOQ	0.010	0.044	$10(S.D/b)$	2.27

V. SUMMERY OF VALIDATION PARAMETERS

TABLE 8: SUMMERY OF VALIDATION PARAMETERS

SR.NO	PARAMETERS	RESULTS
1	Absorption maxima(nm)	242
2	Correlation coefficient (R^2)	0.9992
3	Linear regression Equation	y = 0.0438x - 0.0058
4	Linearity Range (µg/ml)	4-24
5	Interday Precision	0.754566
6	Intraday Precision	0.295168
7	Assay in percentage(n=6)	100.79±0.0037
8	Accuracy (Mean RSD) (n=3)	0.14
9	LOD (µg/ml)	0.75
10	LOQ (µg/ml)	2.27

VI. CONCLUSION

The present study was focused on development of a new spectrometric method for the analysis of Rosuvastatin in bulk drug and tablet dosage form. Spectrophotometric analysis was performed using double beam UV-Visible spectrophotometer (LABINDIA 3000) with 1cm path length supported by UV-WIN Software. For the method development suitable solvent, concentrations of the drug and detection wavelength were studied. The solvent selected for the study was with ACN: Methanol (60:40 v/v) and the drug showed absorption maxima at 242nm. The concentration range of the drug selected for linearity was 4-24µg/ml. The calibration curve of Rosuvastatin calcium was found to be linear from 4-24µg/ml with correlation coefficient value of 0.9992 which was within the specified limit. The % RSD was found within the specified limits. Thus the results and the statistical parameters demonstrate that the proposed spectrophotometric method is simple, precise, rapid, and accurate. Therefore, this method can be used for routine analysis of Rosuvastatin Calcium in bulk and pharmaceutical dosage formulation.

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