Assay And Dissolution Test Method for The Analysis of Rosuvastatin Drug Dosage Forms-Pellets-15%

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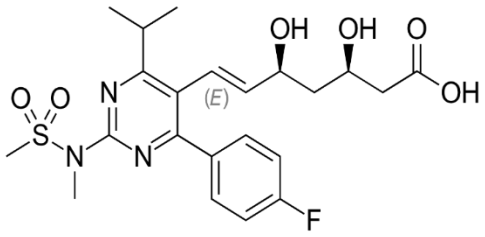
Abstract— Rosuvastatin chemical Name is a di hydroxy mono carboxylic acid that is (6E)-7-{4-(4-fluorophenyl)-2 [methyl (methyl sulfonyl) amino]-6-(propan-2-yl) pyrimidin-5-yl} hept-6-enoic acid.It belongs to a group of medicines called HMG-CoA reductive inhibitors, or statins.Few analytical methods are available for the analysis of Rosuvastatin active pharmaceutical ingredient(API) and formulated dosage forms like tablets and capsules even though no pharmacopoeias method is available for the pellets dosage forms. In-house U.V spectroscopic method was proposed for the analysis of assay and dissolution of ROSUVASTATIN PELLETS-15%. Apparatus USP-II (Paddle), Dissolution medium- pH 6.8 Phosphate buffer, volume1000ml, RPM-100RPM, temperature; 37.0±0.5°C, and time: 60 minutes. The determination was accomplished by U.V Photo Spectrometer at λ (max) 282nm.Proposed method was validated according to ICH guidelines and results are within the limits and satisfactory.

Index Terms- Rosuvastatin Pellets, API, USP-II, pH, RPM, U.V Spectrophoto meter, ICH, Validation.

I. INTRODUCTION

Rosuvastatin chemical Name is a di hydroxy monocarboxylic acid that is (6E)-7-{4-(4-fluorophenyl)-2 [methyl (methyl sulfonyl) amino]-6-(propan-2-yl) pyrimidin-5-yl} hept-6-enoic acid. It is used with a proper diet to lower bad cholesterol (LDL) and triglycerides (fats) in the blood, and to increase good cholesterol (HDL). It is also used to treat adults who cannot control their cholesterol levels by diet and exercise alone. It helps to prevent or slow down medical problems, like atherosclerosis (hardening of the arteries), that are caused by fats clogging the blood vessels. Rosuvastatin may also be used to prevent certain types of heart and blood vessel problems in patients with risk factors for heart problems.

Rosuvastatin belongs to a group of medicines called HMG-CoA reductase inhibitors, or statins. It works by blocking an enzyme that is needed by the body to make cholesterol, so this reduces the amount of cholesterol in the blood [1]. Rosuvastatin and molecular formula is $C_{22}H_{28}FN_3O_6S$, mass is 481.54 g·mol−1 and its chemical structure is shown below [2]:



Chemical structure of Rosuvastatin.

1.1 Objectives:

Objective of the research work is development of analytical method for the analysis of assay and dissolution of Rosuvastatin Pellets dosage forms and validation of proposed method according to ICH(Q2) Guidelines.

II. LITERATURE REVIEW

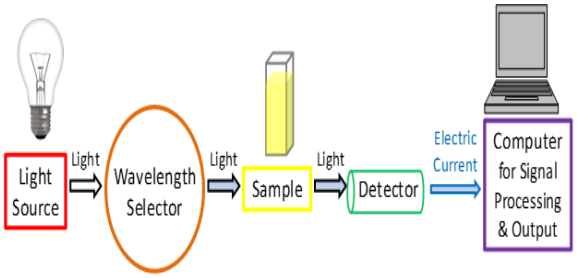
The literature review [3-10] related to determination of assay and dissolution profile by U.V spectrophotometer is taken as a reference for the development of assay and dissolution test method for the analysis of rosuvastatin drug dosage forms-pellets 15%.

III. METHODS (EXPERIMENTAL)

Chemicals and reagents: Rosuvastatin working standard procured from Adyah pharmaceuticals (p) Lt.d Hyderabad. Methanol AR grade supplier maruthi chemisol, Hyderabad, Potassium hydrogen orthophosphate AR grade suppliers Dradis chemicals Hyderabad. Sodium hydroxide AR grade, hydrochloric acid LR grade supplier Sannidhhi chemical Industries, Hyderabad. Double distilled water prepared in the lab,Glass ware ‘’A” grade

Instruments:U.V Photo Spectrometer,(Shimadzu) L.C solutions software version 2010 ,semi micro balance make Sartorius, Dissolution tester- USPII((Paddle) make electro labs model EDT08Lx,

Assay method by U.V Spectro photometer.



Standard Solution Preparation:

Accurately weigh, and transfer 30 mg of Rosuvastatin working standard into a 100 ml volumetric flask dissolve and make up to the mark with methanol. Transfer 2 mL of this solution in to a 50 mL volumetric flask, make the volume with methanol.

Sample Solution Preparation:
Weigh and transfer the pellets equivalent* to 30 mg of Rosuvastatin in to a 100 mL volumetric flask, add about 5 mL of methanol and Sonicate for 20 minutes to dissolve and make up the volume with methanol. Filter the solution through Filter through 0.45 micron nylon filter. Transfer 2 mL of the filtrate solution in to a 50 mL volumetric flask, dilute to volume with methanol. Prepare the samples in duplicate.

Procedure: Measure the absorbance’s of standard and sample at 282 nm using methanol as a blank. Calculate and report the quantity, in % of Rosuvastatin present in the pellets.

Calculation:

$$\text{Assay (\%)} = \frac{\text{Sample Abs}}{\text{Std Abs}} \times \frac{\text{Std wt}}{\text{Spl. wt}} \times \text{Std purity}$$

Determination of Release rates:

Dissolution Tester:

Dissolution conditions:

Apparatus	:	USP Apparatus 2 (Paddle)
Medium	:	pH 6.8 Phosphate buffer, 1000ml
Time Interval	:	1 hour.
RPM	:	100
Temperature	:	37°C ± 0.5°C

Preparation of pH 6.8 phosphate buffer:

Dissolve 6.8 g of potassium hydrogen orthophosphate in 1000ml of purified water. Adjust with 2N sodium hydroxide or 2N hydrochloric Acid to a pH 6.8 ±0.05.

Standard solution preparation:
Accurately weigh, and transfer 30 mg of Rosuvastatin working standard into a 100 ml volumetric flask dissolve and make up to the mark with methanol. Transfer 2 mL of this solution in to a 50 mL volumetric flask, make the volume with pH 6.8 phosphate buffer and mix well.

Sample Preparation:
Weigh and transfer the pellets equivalent* to 30 mg of Rosuvastatin individually in each of the 6 dissolution jars, containing 1000 mL of pH 6.8 phosphate buffer which has been equilibrated to the temperature of 37± 0.5°C. Immediately operate the apparatus at the rate specified in the individual monograph. After completion of specified interval with draw a specimen from a zone midway between the surface of

dissolution medium and the top of rotation blade, not less than 1 cm from the top of the rotation blade, not less than 1 cm from the vessel wall. Replace the aliquots withdrawn for analysis with equal volumes of fresh dissolution medium at 37 ^{0} C and filter the solution through a 0.45 micron nylon filter. Transfer 5 mL from the filtrate in to a 10 mL volumetric flask, dilute to volume with dissolution media.

Procedure:
Measure the absorbance of standard and sample at 282 nm using pH 6.8 phosphate buffer as a blank. Calculate the amount of Rosuvastatin dissolved in pH 6.8 phosphate buffer.
Calculation:
Drug release (%) =

$$\frac{\text{Spl Abs}}{\text{Std Abs}} \times \frac{\text{Std wt}}{100} \times \frac{2}{50} \times \frac{1000}{\text{Spl wt}} \times \frac{10}{5} \times \frac{100}{\text{Assay}} \times \text{Std purity}$$

Method Validation: The proposed method was validated according to ICH(Q2) guideline.

IV. RESULTS AND DISCUSSION

Analytical Method validation parameters are as follows
Accuracy, Precision, Linearity, LOD (limit of detection), LOQ (limit of quantification), Specificity, Range, Robustness.

Accuracy
Accuracy is defined as the closeness of the test results to the true value. The accuracy of the method was determined by spiking the drug-release samples with known quantity of the active substance and subjecting them to quantitative determinations.

The results are given in the following tables and demonstrate the accuracy of the method

4.1 Numerical Results

Sample	Concentration		
	Spiking Concentration	Recovered Concentration	Recovery (%)
1	0.02016	0.02015	99.50%
2	0.02016	0.02016	100.00%
3	0.02016	0.02014	99.90%
4	0.02016	0.02014	99.90%
5	0.02016	0.02015	99.50%
6	0.02016	0.02016	100.00%
		Mean	99.80%

Accuracy for Rosuvastatin at 20% of Specified
Table-1

Sample	Concentration		
	Spiking Concentration	Recovered Concentration	Recovery (%)
1	0.04536	0.04537	100.02%
2	0.04536	0.04534	99.95%
3	0.04536	0.04536	100.00%
4	0.04536	0.04533	99.93%
5	0.04536	0.04534	99.95%
6	0.04536	0.04536	100.00%
		Mean	99.98%

Accuracy for Rosuvastatin at 45% of Specified Limit:
Table-2

Sample	Concentration		
	Spiking Concentration	Recovered Concentration	Recovery (%)
1	0.07056	0.07054	99.97%
2	0.07056	0.07057	100.01%
3	0.07056	0.07051	99.92%
4	0.07056	0.07053	99.95%
5	0.07056	0.07056	100.00%
6	0.07056	0.07050	99.91%
		Mean	99.96%

Accuracy for Rosuvastatin at 70% of Specified Limit:
Table-3

Sample	Concentration		
	Spiking Concentration	Recovered Concentration	Recovery (%)
1	0.09576	0.09573	99.96%
2	0.09576	0.09575	99.98%
3	0.09576	0.09576	100.00%
4	0.09576	0.09577	100.01%

5	0.09576	0.09571	99.94%
6	0.09576	0.09569	99.92%
		Mean	99.97%

Accuracy Rosuvastatin at95% of Specified LimitTable-4

Sample	Concentration		
	Spiking Concentration	Recovered Concentration	Recovery (%)
1	0.12096	0.12090	99.95%
2	0.12096	0.12088	99.93%
3	0.12096	0.12094	99.98%
4	0.12096	0.12095	99.99%
5	0.12096	0.12092	99.96%
6	0.12096	0.12078	99.85%
		Mean	99.94%

Accuracy for Rosuvastatin at 120% of Specified LimitTable-5

Precision:

Precision is a measure of Reproducibility or of Repeatability of the analytical method and is usually expressed as the relative standard deviation. Suitable tests have been carried out to ensure the Precession of the method.

Precession of method: This is determined by using the analytical method to a sample, sufficient number of times (Six) to get analytically valid results .Sample to be Weighed Six different times at required Concentration and to be analytical for method.

Sl. No	Std weight in mg	Std Absorbance	Sample weight in mg	Sample Absorbance	%of Drug release	RSD
1			200.6	0.569	98.57	
2			200.8	0.57	98.54	
3			200.8	0.569	98.49	
4			200.7	0.569	98.55	
5		0.465	200.5	0.568	98.41	0.054 %
6	30.3		200.5	0.568	98.41	

Observation: Relative standard deviation of method results to be calculated from the observation using the formula.

Calculation:

Standard Deviation SD = $[\sum(X-X_1)^2/(n-1)]^{1/2}$

Relative standard deviation RSD=[SD/Mean]*100

Characteristics Acceptance Criteria: RSD of absorbance Response Less than 2.00%

pH 6.8Buffer stage-1hr Table-6

Linearity and Range:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

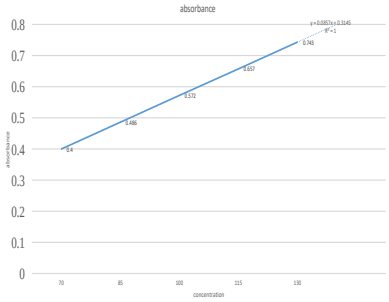
A linear relationship should be evaluated across the range of the analytical procedure. It may be demonstrated directly on the active substance (by dilution of a standard stock solution) and/or on separate weighing of synthetic mixtures of the product components, using the proposed procedure. Table.7

Linearity and Range Table-7

% Of Standard Concentrations	Concentration (mg/ml)	Absorbance
70	0.07056	0.400
85	0.08568	0.486
100	0.10080	0.572
115	0.11592	0.657
130	0.13104	0.743
	Corrlelation coefficent	1
	Slope	0.08571

4.2 Graphical Results

Linearity and Range Table-8



Range

The specified range is normally derived from linearity studies and depend on the intended application of the procedure .The standard calibration curve of Rosuvastatin was prepared in phosphate buffer pH 6.8 media.The equation of line was found to be the equation obtained from these curve was used to calculate cumulative % release of drug from pellets dosage form in dissolution study.

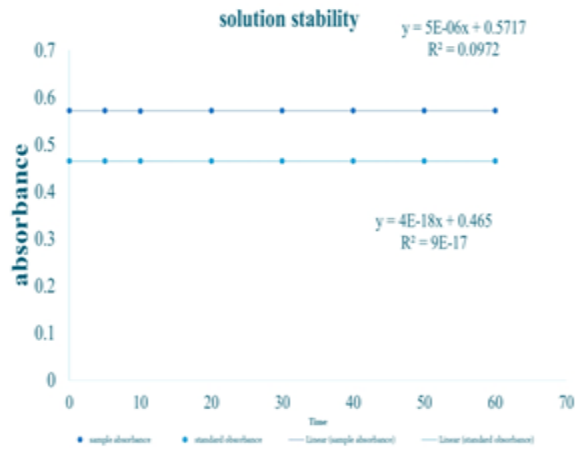
Solution Stability:

The stability of Rosuvastatin in pH 6.8 buffer solution was studied by the UV method. Sample solutions were prepared in duplicate and stored at 37.5ºC for 10, 20, 30, 40, 50, and60.mints. The stability of these solutions was studied by performing the experiment and looking for the change in the spectrophotometric pattern compared with freshly prepared solutions.

SOLUTION STABILITY AT 37.5°C : Table-9
pH 6.8 Buffer STABILITY TEST SOLUTION

SL.NO	Hours	Sample Absorbance	Standard Absorbance
1	0	0.572	0.465
2	5	0.572	0.465
3	10	0.571	0.465
4	20	0.572	0.465
5	30	0.571	0.465
6	40	0.572	0.465
7	50	0.572	0.465
8	60	0.572	0.465

Solution stability Table-10



CONCLUSION

The development of a simple, rapid, sensitive, and accurate analytical method for the routine quantitative determination of samples will reduce unnecessary sample preparations and the cost of materials.

Rosuvastatin is a UV-absorbing molecule with specific chromophores in the structure that absorb at a particular wavelength and this fact was successfully employed for their quantitative determinations using the UV spectrophotometric method. The absorption spectrum of Rosuvastatin in Phosphate buffer pH6.8, maximum absorbance at 282 nm.

Calibration curve data was constructed in the range of the expected concentration of (7.056-13.104)×10-2 Beer's law was obeyed over this concentration range. The regression equation was found to be Y=0.0857X+0.3145,The correlation coefficient R of the standard curve was found to be R_2=1.The stock solution and working standards were made in 6.8 phosphate buffer .The λmax of the drug for analysis was determined by taking scans of the drug sample solution in the entire U.V region.

The characteristic of the calibration plot is presented in Table-8 and the analytical characteristics and necessary validation parameters for the UV techniques for Rasuvastatin is presented. Performing replicate analyses of the standard solutions was used to assess the accuracy, precision, of the proposed methods.

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