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# Quantitative estimation of Rosuvastatin in bulk and tablet dosage form by using area under curve method

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**Abstract**

Simple, precise and economical UV spectrophotometric method has been developed for the estimation of Rosuvastatin in pharmaceutical dosage form and bulk. Method applied was area under curve (AUC) in which area under curve was integrated in the wavelength range of 234.60-251.00 nm. Calibration curves were plotted for method by using instrumental response at selected wavelength and concentrations of analyte in the solution. Linearity for the detector response was observed in the concentration range of 2-12 µg/ml for the method. Tablet formulation was analyzed and % assay determined was 98.35%-102.07%. Accuracy and precision studies were carried out and results were satisfactory. The results of the analysis were validated statistically. Limit of detection and limit of quantitation was determined for methods. The methods were validated by following the analytical performance parameters suggested by the International Conference on Harmonization. All validation parameters were within the acceptable range. The developed methods were successfully applied to estimate the amount of Rosuvastatin in pharmaceutical formulation and bulk.

*Keywords:* Rosuvastatin, UV-spectrophotometry, Area under curve, Validation.

## INTRODUCTION

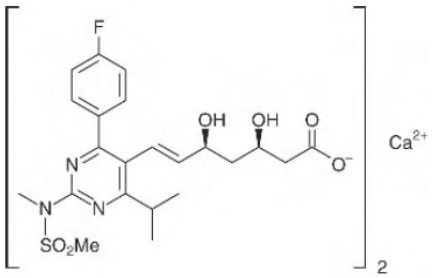


**Figure 1: chemical structure of Rosuvastatin**

Rosuvastatin is chemically known as (E)-(3R,5S)-7-{4-(4-fluorophenyl)-6-isopropyl-2-{methyl(methylsulphonylamino)pyrimidin-5-yl}-3,5-dihydroxyhepten-6-oic acid [figure 1]. The molecular formula is (C22H27FN3O6S)2 which corresponds to molecular weight of 1001.1[1].It is used to prevent cardiovascular disease. The use of Rosuvastatin is for the treatment of dyslipidemia. Rosuvastatin 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 thereby checks the synthesis of cholesterol. Rosuvastatin reduces total cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides (TG) and increases high density lipoprotein (HDL) in patients with hypercholestrolemia and dyslipidemia [2]. A detailed literature survey for Rosuvastatin revealed that several analytical methods such as Spectrophotometric and chromatographic methods were reported for the quantification of Rosuvastatin. There are few UV-spectroscopic [3-7], RP-HPLC [8,9], and HPTLC[10] methods reported for the determination of Rosuvastatin calcium. The objective of study is to develop a simple, precise and accurate spectroscopic method for the estimation of Rosuvastatin in Pharmaceutical formulation and in bulk by zero order derivative area under curve. The method was validated according to ICH guidelines.

## MATERIALS AND METHOD

Rosuvastatin calcium was obtained as a gift sample from Macleods Pharma. It was used without further

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purification. A tablet formulation Roseday-10 of USV limited (HP) containing 10mg of Rosuvastatin was purchased from local market.

**Instrument**

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe with 10mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 200-400 nm; scan speed: medium; sampling interval: 1.0 nm; derivative mode: zero order derivative, dA/dλ); band width (Δλ):10.0 nm; spectral slit width: 1 nm. All weights were taken on electronic balance (Model ShimadzuAUX120).

**Method: Area under curve**

The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration [11].The spectrum obtained was used to calculate AUC. The calibration curve was constructed by plotting concentration (2-12µg/ml) versus AUC

**Preparation of standard stock solution**

The standard stock solution of Rosuvastatin was prepared by dissolving accurately weighed 10mg of the drug in methanol and diluted to 100mLwith same solvent to obtain a final concentration of 100µg/ml.

**Selection of wavelength for analysis of Rosuvastatin**

Appropriate volume 0.2 ml of standard stock solution of Rosuvastatin was transferred into 10 ml volumetric flask, diluted to mark with methanol to give concentration of 2µg/ml. The resulting solution was scanned in UV range (200nm–400nm). In spectrum Rosuvastatin showed absorbance maximum at 244 nm [figure 2].

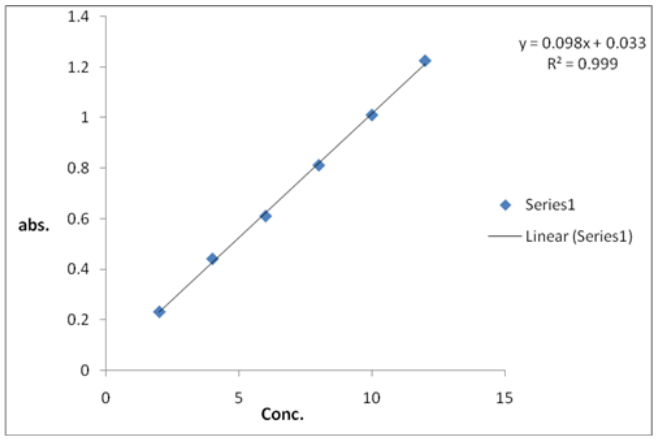


**Figure 2: linearity of area under curve**

**Validation of the method**

The method was validated in terms of linearity, accuracy, precision, and ruggedness as per ICH guidelines [12].

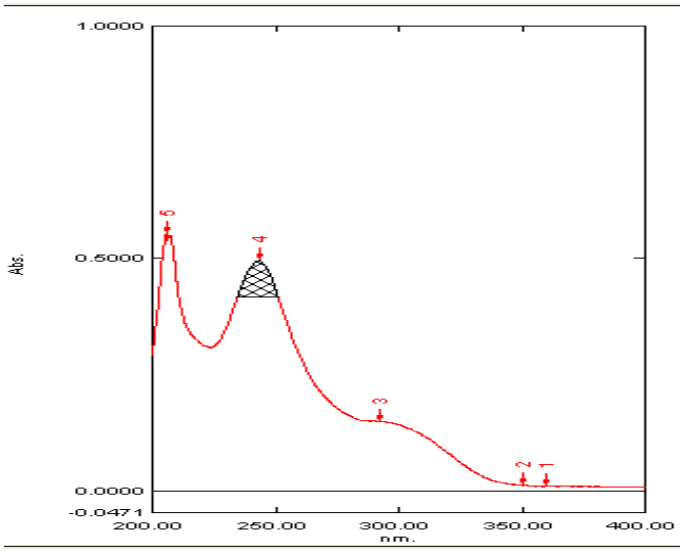


**Figure 3: area under curve spectrum of Rosuvastatin**

**Linearity study**

Different aliquots of Rosuvastatin in range 0.2-1.2 ml were transferred into series of 10 ml volumetric flasks and the volume was made up to the mark with methanol to get concentrations 0.4, 0.8, 1.2, 1.6 and 2 µg/ml, respectively. The solutions were scanned on spectrophotometer in the UV range 200 - 400 nm. The

spectrum was recorded at 244 nm. The calibration plot was constructed as Absorbance vs concentration [figure 3].

**Accuracy**

To the pre-analysed sample solutions, a known amount of standard stock solution was added at different levels i.e. 80%, 100% and 120 %. The solutions were reanalyzed by proposed method.

**Precision**

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing the 0.4, 0.6 and 0.8µg/ml of Rosuvastatin solutions for three times in the same day. Inter-day precision was determined by analyzing the 0.4, 0.6 and 0.8µg/ml of Rosuvastatin solution daily for three days over the period of week.

**Sensitivity**

The sensitivity of measurements of Rosuvastatin by the use of the proposed method was estimated in terms of the Limit of Quantification (LOQ) and Limit of Detection (LOD). The LOQ and LOD were calculated using equation LOD = 3.3 x N/B and LOQ = 10 x N/B, where, 'N' is standard deviation of the peak areas of the drugs (n = 3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve.

**Repeatability**

Repeatability was determined by analyzing 0.6µg/ml concentration of Rosuvastatin solution for six times.

**Determination of Rosuvastatin in bulk**

Accurately weighed 10 mg of Rosuvastatin was transferred into 100 ml volumetric flask containing 20 ml methanol and volume was made up to the mark using same. Appropriate volume 0.6ml of this solution was transferred to 10 ml volumetric flask and volume was adjusted to mark using methanol. The resulting solution was scanned on spectrophotometer in the UV range 200-400 nm. The concentrations of the drug were calculated from linear regression equations.

**Application of proposed method for pharmaceutical formulation**

For analysis of commercial formulation 10 tablets of Rosuvastatin were weighed and average weight equivalent to 10 mg taken in 100 ml volumetric flask and the volume was made up to the mark with methanol to give 100µg/ml concentration. From this 1 ml was taken and transferred to 10 ml volumetric flask and volume was made up to the mark with methanol to give 10 µg/ml concentration. It was scanned on spectrophotometer in the UV range 200-400 nm. The spectrum was recorded at 244 nm. The concentrations of the drug were calculated from linear regression equation.

## RESULTS AND DISCUSSION

**Method Validation**

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adopted procedure given in the experiment.

**Linearity studies**

The linear regression data for the calibration curves showed good linear relationship over the concentration range 0.2-1.2µg/ml for Rosuvastatin. Linear regression equation was found to be Y = 0.098 X + 0.033 (r2 = 0.999) (Table 1).

**Table 1: Linearity of Rosuvastatin**

| Conc.(µg/ml) | Area |
|---|---|
| 2 | 0.23145 |
| 4 | 0.4411 |
| 6 | 0.61025 |
| 8 | 0.8115 |
| 10 | 1.0104 |
| 12 | 1.22515 |

**Accuracy**

The solutions were reanalyzed by proposed method. The results of recovery studies showed that the % amount found was between ho jayenge
98.35% to 102.07% with %R.S.D. less than 2 (Table 2).

**Table 2: Accuracy (Recovery study)**

| Nominal value in % | Initial amount (n=3) | Amount added | % Recovery | % RSD |
|---|---|---|---|---|
| 80 | 4 | 3.2 | 98.43 | |
| 100 | 4 | 4 | 98.0 | 1.15345 |
| 120 | 4 | 3.8 | 100 | |

**Precision**

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These result shows reproducibility of the assay. The % R.S.D. values found to be less than 2, indicating that the method is precise for the determination of the drugs in formulation (Table 3).

**Table 3: Precision studies**

| Conc (µg/ml) | SESSION | | | SD | %RSD |
|---|---|---|---|---|---|
| | 1 (area) | 2 (area) | mean | | |
| | | | Intra-day | | |
| 4 | 0.2577 | 0.2524 | 0.2533 | 0.003983 | 1.5721 |
| 6 | 0.4087 | 0.4013 | 0.4081 | 0.006616 | 1.6209 |
| 8 | 0.5255 | 0.5259 | 0.5229 | 0.004796 | 0.9171 |
| | | | Inter-day | | |
| 4 | 0.2487 | 0.2418 | 0.2452 | 0.004879 | 1.989 |
| 6 | 0.4322 | 0.4225 | 0.4273 | 0.006859 | 1.6049 |
| 8 | 0.542 | 0.5353 | 0.5386 | 0.004738 | 0.8795 |

**Sensitivity**

The linearity equation was Y = 0.098 X + 0.033. The LOD and LOQ for Rosuvastatin is reported to be 0.1581 µg and 0.4792µg, respectively. (Table 4)

**Repeatability**

Repeatability was determined by analyzing 0.6 µg/ml concentration of Rosuvastatin solution for six times and the % amount found was between 98% to 102% with % R.S.D. less than 2. (Table 5)

**Ruggedness**

Ruggedness of the proposed method is determined for 6 µg/ml concentration of Rosuvastatin by analysis of aliquots from homogenous slot by two analysts using same operational and environmental conditions (Table 6).

**Table 4: LOD & LOQ**

| Conc. | Dilutions 1 | 2 | Mean | SD | LOD | LOQ |
|---|---|---|---|---|---|---|
| **1** | 0.0766 | 0.09 19 | 0.08425 | 0.0108 19 | | |
| **1.5** | 0.1 226 | 0.12 71 | 0.12485 | 0.0031 8 | | |
| **2** | 0.1 606 | 0.15 45 | 0.1575 | 0.0043 13 | | |
| **2.5** | 0.2 065 | 0.19 35 | 0.2 | 0.0091 92 | 0.15 816 | 0.47 928 |
| **3** | 0.2 23 | 0.22 33 | 0.22315 | 0.0002 12 | | |
| **3.5** | 0.2 65 | 0.26 58 | 0.2654 | 0.0005 66 | | |
| **4** | 0.3 025 | 0.30 9 | 0.30575 | 0.0045 96 | | |
| **Mean SD** | | | | 0.0046 97 | | |

**Table 5: Repeatability study of Rosuvastatin**

| Conc. (µg/ml) | Area | Amount found | % Amount found |
|---|---|---|---|
| 6 | 0.6348 | 6.1408 | 102.34 |
| 6 | 0.6321 | 6.1132 | 101.88 |
| 6 | 0.6332 | 6.1244 | 102.07 |
| 6 | 0.6287 | 6.078 | 101.3 |
| 6 | 0.6311 | 6.1030 | 101.71 |
| 6 | 0.6255 | 6.0459 | 100.7 |
| Mean | 0.6309 | 6.1008 | 101.66 |
| SD | 0.00334 | 0.0342 | 0.5886 |
| %RSD | 0.53036 | 0.5606 | 0.5789 |

**Table 6: Ruggedness**

| Analyst | Amount found in % | % RSD |
|---|---|---|
| 1 | 100.13 | 0.3411 |
| 2 | 101.54 | 0.6396 |

**Analysis of Rosuvastatin in bulk**

The concentrations of the drug were calculated from linear regression equation. The amount found was between 100.5% to 101.36% (Table 7).

**Table 7: Analysis of Rosuvastatin in bulk**

| Conc. (µg/ml) | Area | Amount found | % Amount found |
|---|---|---|---|
| 6 | 0.6243 | 6.0336 | 100.5 |
| 6 | 0.6325 | 6.1173 | 101.95 |
| 6 | 0.6328 | 6.1204 | 102.0 |
| 6 | 0.6326 | 6.1183 | 101.97 |
| 6 | 0.6212 | 6.002 | 100.03 |
| 6 | 0.6311 | 6.1030 | 101.71 |
| mean | | 6.1312 | 101.36 |
| SD | | 0.08638 | |
| %RSD | | 1.4089 | |

**Determination of Rosuvastatin in formulation**

The concentrations of the drug were calculated from linear regression equations. The % amount found was between 98.35% to 102.07% (Table 8).

**Table 8: Analysis of Rosuvastatin in formulation**

| **Conc. (µg/ml)** | **Area** | **Amount found** | **%Amount found** |
|---|---|---|---|
| 6 | 0.6113 | 5.9010 | 98.35 |
| 6 | 0.6146 | 5.9346 | 98.91 |
| 6 | 0.6238 | 6.0285 | 100.47 |
| 6 | 0.6189 | 5.3255 | 99.6 |
| 6 | 0.6330 | 6.122 | 102.04 |
| 6 | 0.6252 | 6.0428 | 100.71 |
| mean | | 6.00115 | 100.01 |
| SD | | 0.08010 | 1.3383 |
| RSD | | 1.3347 | 1.33818 |

**CONCLUSION**

Area under curve method was developed for the determination of Rosuvastatin based on analytical technique. The method was validated and found to be simple, sensitive, accurate, and precise. Hence, this method can be used successfully for routine analysis of pharmaceutical dosage forms of Rosuvastatin. The proposed Spectrophotometric methods will not replace the presently known methods available for the analysis of Rosuvastatin. However, it can serve as an alternative where advanced instruments (e.g. HPLC) are not available for routine analysis.

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