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# Development of RP-HPLC Method for Estimation of Rosuvastatin Calcium by Quality by Design Approach

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ABSTRACT:

In this paper Quality by Design (QbD) concepts to the development of a RP-HPLC method for a Antihyperlipidaemic drug (Rosuvastatin Calcium) The present study was undertaken with an objective of developing suitable, sensitive and simple analytical RP-HPLC method for Rosuvastatin calcium. Wavelength 243 nm RP-HPLC method, C18 column was selected The separation was carried on Shimadzu C-18 column (250×4.6 mm, 5-µm particle size) various mobile phase tried and Methanol:water (OPA) pH4 (90:10 %) this was selected. The result of system suitability study the peak was obtained at retention time of 2.6 min with a flow rate of 0.9 ml/min, TP no. 7982 and TF was 1.13 which indicates that the method was suitable to analyze. The method validation was done using optimized chromatographic condition. In accuracy percentage recovery range was found to be 90 to 102% and %RSD was found less than acceptance criteria it indicate that analytical method was accurate. In linearity, it obeys the bear's law calibration curve revealed that absorbance increase linearly as concentration increases from range 10 µg/ml to 50 µg/ml with correlation coefficient of 0.9998. The robustness of proposed analytical method was verified by performed. The result of % RSD was below 2 which gave an indication of its reliability during normal usage. Sensitivity of proposed method was determine by LOD and LOQ which was 0.32 µg/ml and 0.97 µg/ml respectively it indicate the method was sensitive. The methods were found to be accurate, precise, sensitive, robust, rapid and economic also.

**KEYWORDS:** Rosuvastatin Calcium, RP – High Performance Liquid Chromatography, Quality by Design

## 1. INTRODUCTION

Rosuvastatin calcium is official in Indian pharmacopoeia. It is chemically Bis (E) (3R,5S)-7-{4 - (4-fluorophenyl) - 6 -isopropyl - 2 - { methyl (methylsulphonyl amino)] pyrimidin -5-yl}-3,5-dihydroxyhepten-6-enoic acid) calcium. It is used in the treatment of Hyperlipidemia. Rosuvastatin calcium is a selective and competitive inhibitor of HMG CoA reductase, the rate-limiting enzyme that converts 3-hydroxyl-3-methylglutaryl coenzyme A to mevolonate, a precursor of cholesterol Rosuvastatin calcium works to help lower cholesterol Block an enzyme in the liver, causing the liver to make less cholesterol, increases the uptakes and breakdown of cholesterol already in the blood, By the liver[1,9].

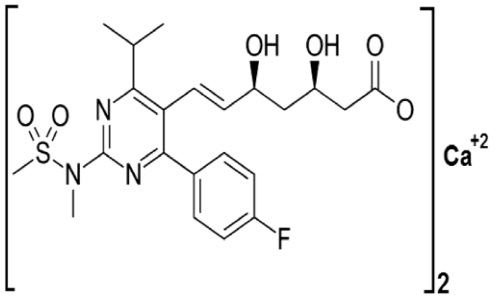



Figure 1: Chemical Structure of Rosuvastatin Calcium

In the present study, a RP-HPLC method for the determination of Rosuvastatin calcium in bulk and in tablet formulation by quality by design approach. This method is simple, rapid, accurate, precise and easy to apply in routine usage and does not need any costly instrumentation.

## 2. MATERIALS AND METHODS

### *2.1 Equipments*

The chromatographic system (Shimadzu, Japan) consisted of a Shimadzu LC- 20AD pump and Shimadzu PDA SPD-M20A detector. The separation was performed on Shimadzu C18 column (250 mm X 4.6 mm, 5 µm particle size) with a mixture of Methanol:water (OPA) pH 4 (90:10 %) as mobile phase. The mobile phase was filtered through a 0.22 µm pore size membrane filter and degassed (Degassing unit- Shimadzu DGU-20A5R) before use. The column was maintained at ambient temperature and the flow rate was 0.9 mL/min in isocratic mode. Injection volume was 20 µL and the PDA detection wavelength was set at 243 nm. The digital pH meter (Equiptronics-EQ-614), electronic balance (Contech CA series) and Mdi 0.2 µm membrane were used.

### *2.2 Materials*

Working Standards of pharmaceutical grade Rosuvastatin Calcium was received as a gift sample from Swapnaroop agency, Pune, India. The Razel Tablet containing Rosuvastatin calcium (10mg) was purchased from local pharmacy. All the chemicals and reagents used were of HPLC grade and purchased from Merck, India.

### *2.3 Solutions*

**Preparation of Stock Solution**: Standard stock solution of Rosuvastatin calcium (1000 µg/ml) was prepared in Methanol. Working standard solution was prepared by diluting of 1 ml of the Rosuvastatin calcium standard stock Solution up to 10 ml methanol to give the final conc of 100 µg/ml.
**Preparation of Test Solution**: 20 tablets were taken and tablets were crushed to powder weight of powder average weight was calculated equivalent to 10 mg Rosuvastatin calcium was transferred to 100ml volumetric flask and dissolved in methanol. This solution was sonicated and filtered through 0.22 µ membrane filter.

### *2.4 Optimization of Parametres*

Optimization was done by response surface methodology, applying a three level Box Behnken design with three centre points. Three variables selected were flow rate, pH and mobile phase composition. Evaluation of main factor, their interaction and quadric effect on peak USP tailing factor, theoretical plate, retention time and area were done. Injection volume was 20µl. Experiments were conducted by making 17 injections of standard Rosuvastatin calcium solution and the average of USP area, tailing factor, theoretical plate, retention time were analyzed using Design Expert 11 software. Application of multivariate regression analysis resulted in a fitted full quadrate model for the average responses for peak USP tailing given by the equation 1.

$$Y= \beta0+\beta1X1+\beta2X2+ \beta3X3+ \beta11X12+ \beta22X22+ \beta33\ X32 + \beta12\ X1\ X2+ \beta13\ X1X3+ \beta23X2X3.$$

Where, Y is the response, β0 is the arithmetic mean response. β1 β2 and β3 are regression coefficients of the factor X1, X2 and X3 respectively. B11, β22 β33 are squared coefficients β12, β13and β23 are interaction coefficients.

### *2.5 Calibration curve peak area vs. absorbance.*

A stock solution of Rosuvastatin Calcium was prepared in RP-HPLC Methanol of 100 µg/ml aliquots of working standard solution (0.1,0.2,0.3,0.4,and 0.5) were transferred into series of 10 ml volumetric flack and diluted up to mark with methanol these yielded solution of 10,20,30,40 and 50 µg/ml of it. An aliquot of 20 µl of each solution was injected under the operating chromatographic condition.

### *2.6 method validation*

Analytical method validation is a component as Accuracy, Precision, Specificity, Limit of Detection, Limit of Quantitation, Ruggedness, (intermediate precision), Linearity, Range, Robustness, The accuracy of an analytical method is the closeness of test result.

#### *2.6.1 Accuracy (Recovery Studies)*

Accuracy is represented and determined by recovery experiments. In this process, it was tested at three different levels i.e. 50,100 and 150% of the label claim and analyzing chromatograms.

#### *2.6.2. Assay specificity*

The specificity of the method was assessed by injecting the placebo solution (without Rosuvastatin calcium) and Rosuvastatin calcium solution into the HPLC system.

### *2.6.3. Precision*

System and method precision was verified by repeatability and intermediate precision studies with the tablet formulation. Repeatability studies were performed by using analysis of six replicates of standard and sample solution containing 30 µg/mL of the rosuvastatin calcium. The intraday and interday precision studies for rosuvastatin was carried out by estimating the corresponding responses three times on the same day and on three different days for the three level of linearity concentrations (20µg/ml, 30µg/ml and 40µg/ml).

### *2.6.4. Linearity*

It was demonstrated by preparing and analyzing standard stock solutions corresponding to 10-50 µg/mL of Rosuvastatin calcium in five replicates. Calibration curve was obtained by plotting the peak area on the abscissa and the respective drug concentrations of standard Rosuvastatin on the ordinate. The unknown samples concentrations were calculated from the linear regression equation.

### *2.6.5. Limit of detection (LOD) & limit of quantification (LOQ)*

LOD and LOQ for Rsuvastatin calcium were estimated by injecting a series of dilute solutions with known concentration. The parameters LOD and LOQ were determined on the basis of peak response and slope of the regression equation.

### *2.6.6. Robustness of Method*

For the determination of method's robustness, flow rate was varied within a realistic range and the quantitative influence of the variables was determined. Robustness of the method was evaluated at a concentration level 30µg/mL of drug (n=3).

## 3. RESULTS AND DISCUSSION

### 3.1 Characterization of Rosuvastatin Calcium (RC).

#### 3.1.1 Solubility study

**Table 1: Solubility study.**

| Sr no. | Solvents | Result |
|---|---|---|
| 1 | Methanol | Freely soluble |
| 2 | Acetonitrile | Freely soluble |
| 3 | Water | Sparingly soluble |

#### 3.1.2 Melting point

The melting point of the drug was determined by capillary method by using Thieles tube. The Melting point of drug was found to be 154°C.

#### 3.1.3. Infra Red spectrum of Rosuvastatin Calcium

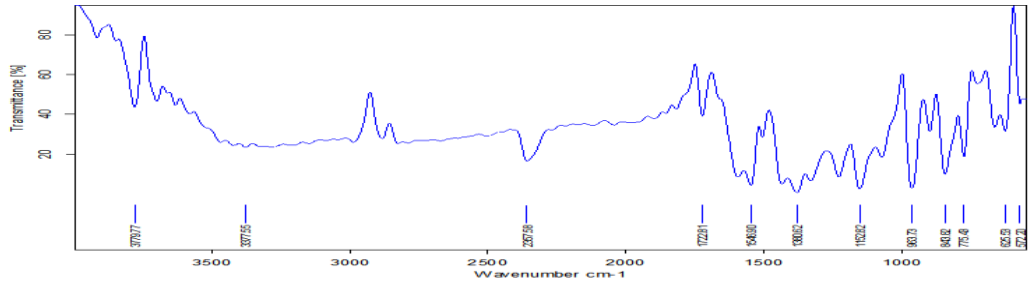



**Figure 2: IR Spectrum of Rosuvastatin**

### 3.1.4 Determination of wavelength maxima of RC

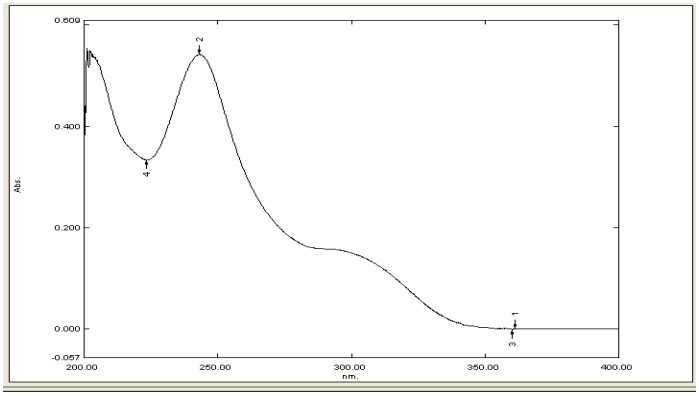
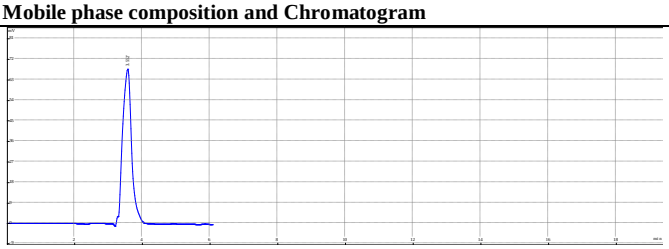
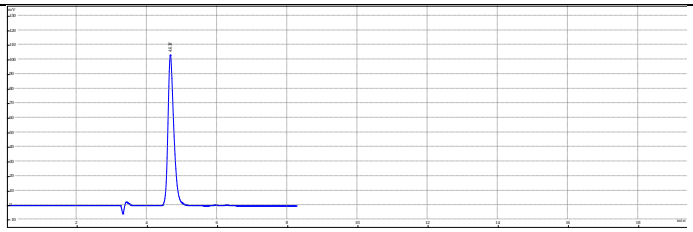
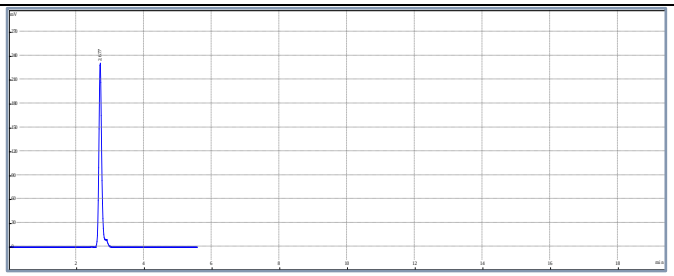
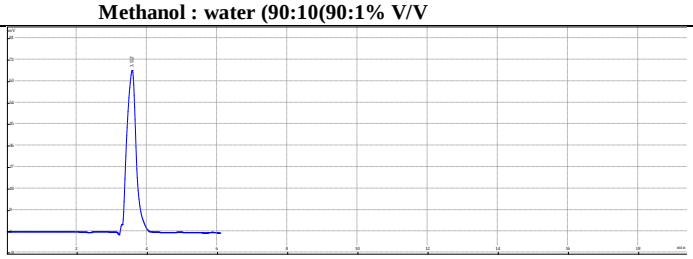
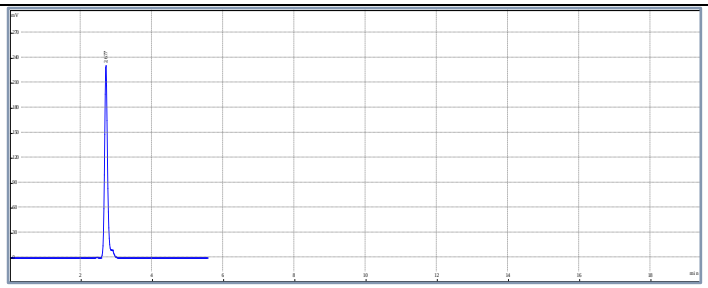



**Figure 3:UV spectrum of RC**

### 3.1.5 Mobile phase optimization

| Trial no | Mobile phase composition and Chromatogram | Remark |
|---|---|---|
| 1. |  **Methanol water 70:30%V/V** | **Tailing was observed in peak** |
| 2. |  **Methanol : water80:20%V/V** | **Tailing was observed in peak** |
| 3. |  **Methanol : water (90:10(90:1% V/V** | **Sharp peak, RT=2.78, No of theoretical plate= 7218** |
| 4. |  **Methanol : water (OPA) pH-2 90:10%V/V** | **Tailing was observed in peak** |

| | | |
|---|---|---|
| 5. |  **Methanol : water (OPA) pH-4 90:10%V/V** | **Sharp peak, RT=2.6, No of theoretical plate= 2982, Tailing factor-1.2** |

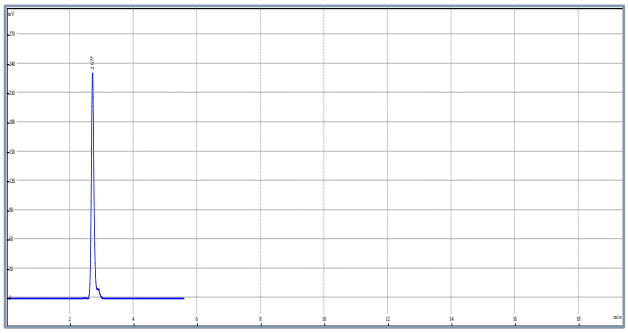


**Fig. 4.** A **representative chromatogram of standard RC obtained with optimized chromatographic conditions**

**Table 2: Optimized chromatographic condition.**

| Sr no. | Parameter | Selected value. |
|---|---|---|
| **1** | Mobile phase | Methanol: water OPA pH4 |
| **2** | Flow rate | 0.9 (ml/min) |
| **3** | Analytical Wavelength | 243 |
| **4** | Column temp. | Ambient |
| **5** | Injection volume | 20 µl |

**3.1.6 System Suitability**

**Table 3. System suitability study**

| Injection No. | RT | Peak Area | Tailing factor | No of Theoretical plate |
|---|---|---|---|---|
| 1 | 2.673 | 1155394 | 1.18 | 7967 |
| 2 | 2.670 | 1156461 | 1.19 | 7935 |
| 3 | 2.673 | 1159919 | 1.19 | 7630 |
| 4 | 2.673 | 1151933 | 1.13 | 8034 |
| 5 | 2.673 | 1160984 | 1.19 | 7891 |
| 6 | 2.673 | 1158796 | 1.19 | 7874 |
| Mean | - | 1157248 | 1.178 | 7872.8 |
| SD | | 3340.48 | 0.02683 | 149.280 |
| % RSD | _ | 0.28865 | 2.27758 | 1.89615 |

**3.2 RP-HPLC method development by QbD approach:**

**3.2.1 Identification of analytical target profile (ATP):**

HPLC parameters targeted here are retention time, Number of theoretical plate, tailing factor.

### 3.2.2 Determination of Critical Quality Attribute (CQA)

Quality attribute which would critically impacted on ATP are mobile phase composition, Flow rate, pH.

### 3.2.3. Risk assessment

Three input variables were selected for method design and three levels were taken as a low, central and high level to assess the risk.

**Table 4. Chromatographic factors for Box Behnken experimental design**

| Chromatographic condition | Level used | | |
|---|---|---|---|
| | Low (-) | Center (0) | High (+) |
| Methanol conc.(X1) | 70 | 80 | 90 |
| Flow rate (X2) | 0.7 | 0.8 | 0.9 |
| pH unit (X3) | 2 | 4 | 6 |

**Table 7: Run Sheet and found value**

| RUN no. | FACTOR. 1 methanol Conc.(%) | FACTOR. FLOW RATE (ml/min) | FACTOR. 3 pH units | Response1Area, units. | Response 2.Retention time Min. | Response 3.Theoretical Plates units. | Response 4.Asymmetryfactor unit. |
|---|---|---|---|---|---|---|---|
| 1 | 90 | 0.8 | 6 | 1.23511E+06 | 3.705 | 2739 | 0.94 |
| **2** | **90** | **0.9** | **4** | **1.95107E+06** | **2.601** | **7483** | **1.12** |
| 3 | 80 | 0.8 | 4 | 1.45075E+06 | 2.784 | 5506 | 1.26 |
| 4 | 80 | 0.8 | 4 | 1.45075E+06 | 2.784 | 5506 | 1.26 |
| 5 | 80 | 0.9 | 2 | 1.03366E+06 | 3.11 | 4388 | 1.28 |
| 6 | 80 | 0.9 | 6 | 1.06974E+06 | 3.663 | 2074 | 2.04 |
| 7 | 80 | 0.7 | 2 | 1.33787E+06 | 4.023 | 5819 | 1.19 |
| 8 | 80 | 0.8 | 4 | 1.45075E+06 | 2.784 | 5506 | 1.26 |
| 9 | 80 | 0.8 | 4 | 1.45075E+06 | 2.784 | 5506 | 1.26 |
| 10 | 70 | 0.8 | 2 | 1.57046E+06 | 4.809 | 2984 | 1.38 |
| 11 | 70 | 0.7 | 4 | 1.70827E+06 | 5.472 | 6467 | 1.19 |
| 12 | 80 | 0.7 | 6 | 1.33186E+06 | 3.919 | 1781 | 1.87 |
| 13 | 70 | 0.9 | 4 | 1.44414E+06 | 2.891 | 4766 | 1.42 |
| 14 | 90 | 0.7 | 4 | 1.62449E+06 | 2.913 | 6270 | 1.15 |
| 15 | 90 | 0.8 | 2 | 1.42064E+06 | 2.948 | 2660 | 1.46 |
| 16 | 80 | 0.8 | 4 | 1.45075E+06 | 2.784 | 5506 | 1.26 |
| 17 | 70 | 0.8 | 6 | 1.12627E+06 | 3.503 | 1649 | 1.29 |

#### 3.2.3.1 Box Behnken method design for Area Analyzed.

Multivariate regression analysis was applied and fitted full quadratic model was obtained for the Area Analyzed factor of peak. Factor considered here are methanol concentration, flow rate and pH unit. Regression coefficient and p-values obtained from software generated.

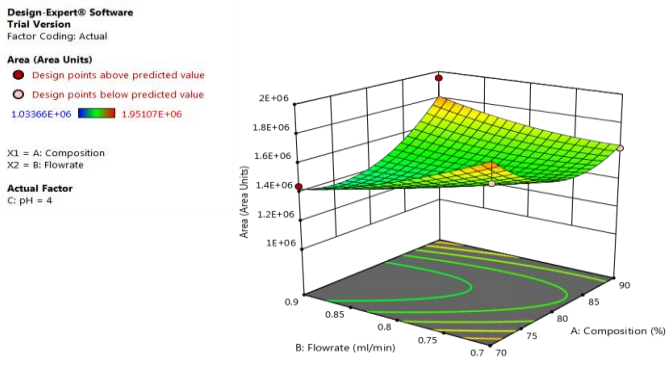



**Figure 4:Effect of Flow rate & Mobile phase composition on peak area**

### 3.2.3.2 Box Behnken method design for Retention Time

Multivariate regression analysis was applied and fitted full quadratic model was obtained for the Retention Time factor of peak. Factor considered here are methanol concentration, flow rate and pH unit. Regression coefficient and values obtained from software generated. Regression coefficients and associated probability values (p-values) for Retention Time of Rosuvastatin calcium.

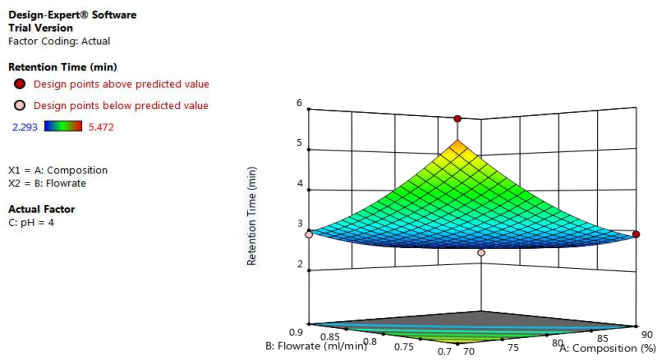



Figure 5: Effect of Flow rate & Mobile phase composition on retention time

### 3.2.3.3 Box Behnken method design for Theoretical plate

Multivariate regression analysis was applied and fitted full quadratic model was obtained for the Theoretical plate factor of peak. Factor considered here are methanol concentration, flow rate and pH. Regression coefficient and p- values obtained from software generated. Regression coefficients and associated probability values (p-values) for Theoretical plate of Rosuvastatin calcium.

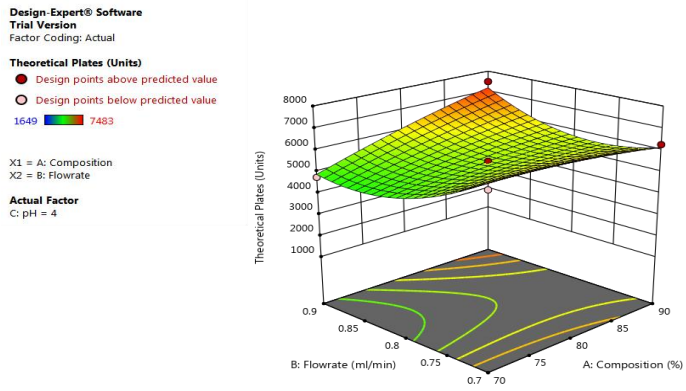



**Figure 6: Effect of Flow rate & Mobile phase composition on number of Theoretical plate**

### 3.2.3.4 Box Behnken method design for Tailing factor

Multivariate regression analysis was applied and fitted full quadratic model was obtained for the Tailing factor of peak. Factor considered here are

methanol conc., flow rate and wavelength. Regression coefficient and p-values obtained from software generated. : Regression coefficients and associated probability values (p-values) for Tailing factor of Rosuvastatin calcium.

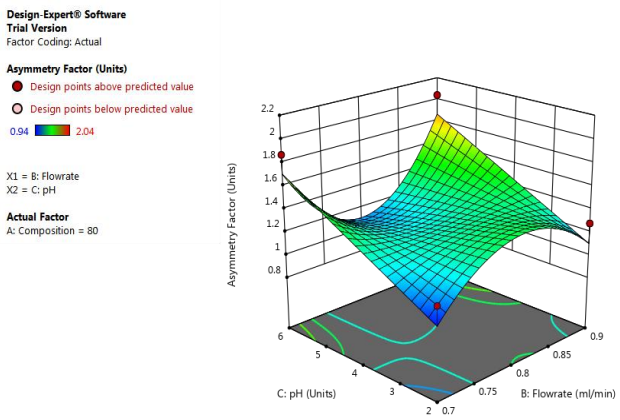



**Figure 7: Effect of pH & Flow rate on tailing factor**

### 3.3 Validation of chromatographic method

Validation of the method for the standard drug and marketed formulation was carried out with respect to different parameters as mentioned in ICH guidelines for method validation. RC showed good correlation coefficient in concentration range of 10-50µg/mL (R2= 0.9998).

The linear regression data is presented in Table 5. The linearity of calibration graph and adherence of the system to Beer's law was validated by determination of correlation coefficient. These values were found to be well within the accepted limit. The regression plot is shown in Fig. 8.

**Table 5. Linear regression data for calibration curve (n=3) Parameters**

| Parameters | Result |
|---|---|
| Linearity range | 10-50µg/mL |
| R2 | 0.999 |
| Slope | 60610x |
| Intercept | 38536 |
| Y=mX+C | 60610x + 38536 |

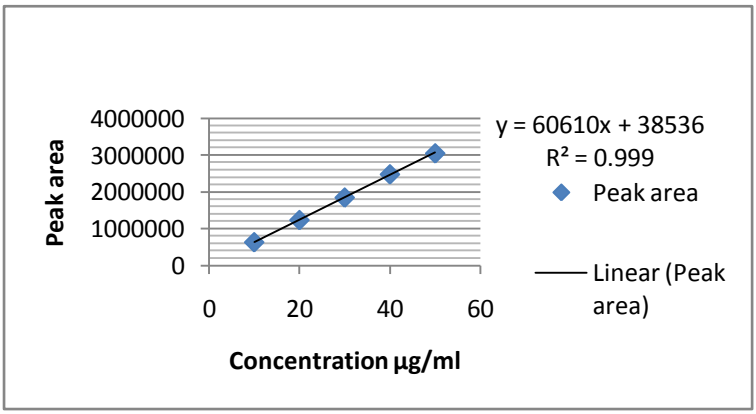



**Fig 8**. Regression plot of RC

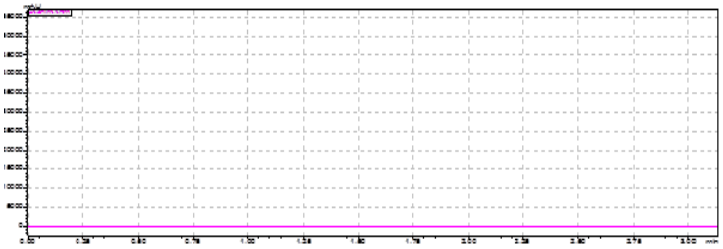


**Fig. 9.** Specificity chromatogram of Placebo

**Table 6: Recovery determination of drug by HPLC (n=3)**

| Sr no | Level of recover % | Amt of std added (µg/ml) | Total amt of recovered (µg/ml) | % Recovery | Mean % Recovery |
|---|---|---|---|---|---|
| 1 | 50 | 15 | 14.8 | 98.66 | 99.10 |
| 2 | 50 | 15 | 15.1 | 100.66 | |
| 3 | 50 | 15 | 14.7 | 98.00 | |
| 4 | 100 | 30 | 29.99 | 99.96 | 99.98 |
| 5 | 100 | 30 | 30 | 100 | |
| 6 | 100 | 30 | 30 | 100 | |
| 7 | 150 | 45 | 44.8 | 99.55 | 100.07 |
| 8 | 150 | 45 | 44.6 | 99.11 | |
| 9 | 150 | 45 | 101. | 101.55 | |

**Table 7:** Statistical Evaluation of Recovery studies.

| Level of Recovery (%) | % mean Recovery* | %SD(±) | %RSD(±) | S.E |
|---|---|---|---|---|
| 50 | 99.10 | 0.1 | 0.1001 | 0.005 |
| 100 | 99.98 | 0.000577 | 0.0005773 | 0.004 |
| 150 | 100.07 | 0.023094 | 0.023097 | 0.003 |

**Table 8: Precision Study (Repeatability)**

| Injection no | Concentration (µg/ml) |
|---|---|
| 1 | 29.84 |
| 2 | 29.86 |
| 3 | 29.95 |
| 4 | 29.75 |
| 5 | 29.97 |
| 6 | 29.92 |
| Mean | 29.8816 |
| SD(±) | 0.08183 |

**Table 9: Interday Precision**

| Day | Injection no | Average* Concentration (µg/ml) | Mean | (±)SD | %RSD(±) |
|---|---|---|---|---|---|
| Day1 | 1 | 29.84 | 29.905 | 0.06363 | 0.23649 |
| | 2 | 29.86 | | | |
| | 3 | 29.95 | | | |
| Day2 | 1 | 29.89 | 29.084 | 0.07 | 0.26080 |
| | 2 | 29.76 | | | |
| | 3 | 29.87 | | | |
| Day3 | 1 | 29.75 | 29..87 | 0.070711 | 0.26316 |
| | 2 | 29.92 | | | |
| | 3 | 29.82 | | | |

**Table 10: Intraday Precision**

| Time in hrs. | Injection No | Average* Concentration (µg/ml) | Mean | SD(±) | %RSD(±) |
|---|---|---|---|---|---|
| 0 | 1 | 29.84 | 29.883 | 0.0585946 | 0.2179615 |
| | 2 | 29.86 | | | |
| | 3 | 29.95 | | | |
| 3 | 1 | 29.75 | 29.945 | 0.0353553 | 0.1312128 |
| | 2 | 29.97 | | | |
| | 3 | 29.92 | | | |
| 6 | 1 | 29.97 | 29.895 | 0.0353553 | 0.1314567 |
| | 2 | 29.92 | | | |
| | 3 | 29.87 | | | |

*Mean of three reading

**Table 11: System suitability study**

| Injection No. | RT | Peak Area | Tailing factor | No of Theoretical plate |
|---|---|---|---|---|
| 1 | 2.673 | 1155394 | 1.18 | 7967 |
| 2 | 2.670 | 1156461 | 1.19 | 7935 |
| 3 | 2.673 | 1159919 | 1.19 | 7630 |
| 4 | 2.673 | 1151933 | 1.13 | 8034 |
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| 6 | 2.673 | 1158796 | 1.19 | 7874 |
| Mean | - | 1157248 | 1.178 | 7872.8 |
| SD | | 3340.48 | 0.02683 | 149.280 |
| % RSD | _ | 0.28865 | 2.27758 | 1.89615 |

**Table 12: Robustness Study (Change in flow rate)**

| Sr No | RT | Theoretical plate | TF |
|---|---|---|---|
| 1 | 3.768 | 7899 | 1.17 |
| 2 | 3.731 | 7893 | 1.17 |
| 3 | 3.780 | 7891 | 1.13 |
| Mean | 3.759667 | 7894.333 | 1.156667 |
| SD(±) | 0.025540 | 4.16333 | 0.023094 |
| %RSD(±) | 0.679315 | 0.052738 | 1.996598 |

**Table 13: Robustness Study (Change in Wavelength)**

| Sr.no | RT | Concentration (µg/ml) | Theoretical plate | TF |
|---|---|---|---|---|
| 1 | 2.674 | 29.81 | 7476 | 1.17 |
| 2 | 2.673 | 29.80 | 7428 | 1.16 |
| 3 | 2.674 | 29.82 | 7481 | 1.16 |
| Mean | 2.673667 | 29.81 | 7461.667 | 1.163333 |
| SD(±) | 0.0057735 | 0.123567 | 29.263173 | 0.00577350 |
| % RSD(±) | 0.021593 | 0.059488 | 0.392180 | 0.496291 |

## 4. CONCLUSION

The Quality by Design approach has been successfully used to develop RP-HPLC method for Rosuvastatin calcium. All key aspect of QbD was implemented in said study. Systematic approach was utilized to develop an efficient and robust method which includes beginning with determination of target profile characteristics, risk assessment, design of experiment and validation. Three factors that were determined to significantly affect the peaks were then analyzed to determine their interactions and quadratic effects with the least possible runs by

using Box-Behnken model in conjunction with response surface methodology. Response surface diagrams and contour plots were studied for coming to conclusion which factor are affecting response and their limits were recorded. A desirability function was applied to determine the optimum conditions. Optimum conditions were obtained; the one with higher desirability was selected. Replicates of run having optimized condition were taken to confirm the predicted response with actual response. From result it was conclude that, after multivariate regression analysis was applied on Area, TP, RT, TF. This Box-Behnken model was fitted statistically significant for this CQA. Found significant factor were shown inversely relationship to the CQA and insignificant factor which did not shown any physical effect on response. This method reduced the risk errors. The developed RP-HPLC method for estimation of rosuvastatin calcium by using QbD approach was simple, accurate, precise, linear, robust and highly sensitive. Hence this method can be routinely used for analysis.

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