RESEARCH ARTICLE

Implementation of Quality by Design Approach to the Analytical Method Development and Validation for the Estimation of Rosuvastatin Calcium

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ABSTRACT

Quality by Design (QbD) refers to the achievement of certain predictable quality with a predetermined and desired specification. The current studies details QbD enable the development of a simple, rapid, sensitive, and cost-effective high-performance liquid chromatographic method for the estimation of rosuvastatin calcium. The factor screening studies were performed using a 3-factor 12-trials 2-level factorial design. System thematic optimization was performed employing split-plot design by selecting the mobile phase ratio, buffer pH, and column type as the critical method parameter (CMPs) identified from screening studies, thus, evaluating a critical quality attribute (CQA), *viz.*, retention time, peak tailing, and theoretical plate as per the parameter of the method robustness. The optimal chromatographic separation was achieved using acetonitrile and water 75:25 v/v as the mobile phase with a flow rate of 1 mL/min by using a PDA detector at 246 nm. The method was validated as per the ICH recommended conditions, which ensure a high degree of linearity, accuracy, precision, sensitivity, and robustness over the exiting liquid chromatography methods of the drug. Moreover, the lower solvent consumption along with the short analytical run time of 10 minutes leads to a cost-effective and environment-friendly chromatographic procedure. Thus, the proposed method reviled that rapid and represented a good procedure for rosuvastatin calcium.

Keywords: Critical analytical attributes (CAA), Critical method parameter (CMP), Critical quality attributes (CQA), Quality by design (QbD), Rosuvastatin calcium.

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INTRODUCTION

Rosuvastatin calcium (ROSU) is chemically bis [(E)-7[4-(4-fluorophenyl)-6 isopropyl- 2-[methyl (methyl-sulphonyl) amino] pyrimidin-5-yl] (3R, 5S) -3,5-dihydroxyhept-6-enoic acid] calcium salt (Figure 1). It 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. It is used in the treatment of hypercholesterolemia and dyslipidemia.

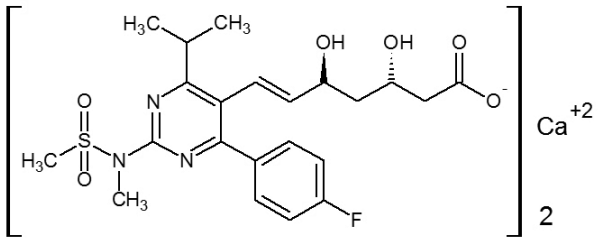

Figure 1: Structure of rosuvastatin calcium

QbD approach suggests looking into the quality of the analytical process during the development stage itself. It says that quality should be built into the process design rather than testing into the final results of the analytical process. QbD is defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on sound science and quality risk management." In alignment with the approach proposed in the draft food and drug administration (FDA) guidance for process validation, a three-stage approach can be applied to method validation.

The conception of "QbD" was outlined as an approach which covers a better scientific understanding of critical process and product qualities, designing controls and tests based on the scientific limits of understanding during the development phase and using the knowledge obtained during the life-cycle of the product to work on a constant improvement environment. QbD does not essentially mean less analytical testing; rather it means that proper analysis at the right time and is based on science and risk assessment. Implementation of QbD helps to develop a rugged and robust (strong) method that

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helps to go with ICH, therefore, for that reason pharmaceutical industries are adopting the concept of QbD. Factors that affect the robustness are considered for development of the analytical method in QbD environment.[6-7]

According to the information extracted from literature to data, there is not even a single method reported for the reverse phase high performance liquid chromatography (RP-HPLC) of ROSU using the QbD approach in the pharmaceutical formulation. The method was validated for linearity, accuracy, precision, limits of detection (LOD), limits of quantitation (LOQ), system suitability, and selectivity as per ICH guidelines.[8] The primary objective of this study was to implement the QbD approach to develop and validate the RP-HPLC method and to established and in-depth understanding of the method and build in the quality during the method development to ensure optimum method performance over the lifetime of the product.[9-13]

EXPERIMENTAL

Standards and Reagents

ROSU provided by Alkem Pharmaceutical Ltd., Ankaleshvar, India. The commercially available tablet formulation of ROSU Rosuvas (M/s Ranbaxy Ltd.) was used for the assay. HPLC grade acetonitrile obtained from Merck Specialities Pvt. Ltd., Worli, Mumbai was used for the study, while all other chemicals and reagents were used as obtained.

Instrumentation and Chromatographic Conditions

A Shimadzu (Model no. LC20AD) high-performance liquid chromatographic system fitted with the quaternary solvent manager, sample manager, PDA detector controlled by Design Expert 9 software, the analytical column used for the method development. Chromatographic separations were performed on a reversed-phase C18 column of dimension 4.6 × 250 mm, particle size 5.0 µm, (kromasil 100-5C8 part no. M05CMA25). The isocratic solution was employed with acetonitrile and water in the ratio of 75:25 v/v as the mobile phase with PDA detector at 245 nm. The column was equilibrated with mobile phase for saturation of the stationary phase to chromatographic analysis.

Preparation of Solvent and Solution

10 mg of working standard of ROSU was accurately weighed and transferred to 10 mL of volumetric flask, added about 4 mL of distilled water (diluent), and sonicated to dissolve. The solution was then cooled to room temperature and the volume was made with diluent to give stock solution of 1,000 µg/mL (solution A). 1 mL of solution A was transferred into a 10 mL volumetric flask and diluted to volume with diluent to give 100 µg/mL solution (solution B). Then, 1 mL of solution B was diluted to 10 mL with diluent to give 10 µg/mL solution which was used as the standard solution.

Initial Method Development Choice of Column

In order to choose the appropriate column, initial experimental runs were carried out as shown in Tables 1 and 2. According to the observations of the above initial trials and its chromatograms, the C18 column was selected for further trials.

Factor Screening Studies

A new reverse phase-HPLC method was developed for ROSU using Design Expert 9 software. In this software, Box-Behnken statistical screening design was used to optimize the critical process parameters (CPP) or CMPs, and to evaluate the interaction effects of these parameters on the CQAs. This Box-Behnken statistical screening design is a 3-factor 2-level design that was specifically selected since it requires fewer experimental runs than other screening designs. This screening phase includes the following steps:

Selection of CMPs

CMPs are selected number of factors that impact the analytical technique under development. So, the CMPs selected for the study are buffer pH, organic phase (% acetonitrile), and organic modifier (methanol) design summary for CMP tabulated in

Table 1: Experimental trial for choice of column

Column	*Observation*	*Inference*
C8	Poor retention of the analyte	Broad and poor peak shape
C18	Improved retention of analyte	Better peak shape

Table 2: Experimental trials for choice of mobile phase

Mobile phase composition	*Observation*	*Inference*
Water:acetonitrile	No precision in retention time. Broad peak with tailing	Use of buffer required and use of methanol to improve peak shape.
Water:methanol	No precision in retention time. Better peak shape	Use of buffer and methanol required.
Water:acetonitrile:methanol	No precision in retention time. Good peak shape	Use of buffer, acetonitrile, and methanol required.

Table 3: Design summary for screening studies

Critical method parameters	*Type*	*Low level*	*Medium level*	*High level*
Buffer pH	Numeric	4	5	6
Organic phase (% acetonitrile)	Numeric	10	20	30
Organic modifier (methanol)	Numeric	10	20	30

Selection of CQAs

CQAs are the responses that are measured to judge the quality of the developed analytical methods. So, the CQAs selected for the study are retention time and tailing factor. These responses were monitored during the experimental trials.

Method Development as per the Experimental Design

By the factor screening studies, the selection of the CMPs affecting the method performance was optimized using a three-factor at two equidistant levels, i.e., low (-1) and high (+1) levels. Table 4 summarizes the design matrix as per the split-plot design with 12 experimental runs along with quintuplicate studies of the center point (0, 0) runs. A standard concentration of 10 µg/mL was used for all experimental runs, which were analyzed for method CAAs, i.e., retention time, peak tailing, and theoretical plates.

Data Analysis and Model Validation

The responses obtained after carrying out the above trial runs were fed back to Design Expert software and plots like 3D-response surface plots and graph plots were plotted. These plots revealed the influence of critical method parameters on the selected quality attributes. The analysis of these plots was used to estimate as to which method parameter gave the most acceptable responses. Thus, based on these observations, the final critical method parameters of the method were determined and the optimized chromatographic conditions were finalized.

Moreover, the evaluation of statistical analysis tool, like analysis of variance (ANOVA) for each individual response was used to determine the significance of each method parameter selected for the study using the p value (probability).

Method Validation

For confirming the suitability of the method for its intended purpose, method validation is carried out as per ICH guidelines for assessing system suitability, linearity, accuracy, and recovery, LOD, LOQ, intra-day precision, inter-day precision, and robustness.

System Suitability

System suitability testing is an integral part of any analytical procedure. System suitability testing was carried out by injecting six replicates of 10 µg/mL standard ROSU solution. In this test, system suitability parameters, like retention time, number of theoretical plates, and tailing factor were evaluated.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solution of ROSU using the developed HPLC method. This was done until a signal to noise ratio of NLT 3:1 and NLT 10:1 is maintained for LOD and LOQ, respectively.

Specificity

The specificity of the method was determined by recording the chromatogram of standard stock solution of ROSU (10 µg/mL) and blank chromatogram (only diluent). Specificity signifies the identification of analyte, interference from other peaks, and peak purity.

Linearity and Range

The linearity of the method was evaluated in the range of 50 to 150% of the working concentration level, i.e., 10 µg/mL for ROSU. The linearity of response was determined by preparing different concentrations of standard solution, i.e., 5 µg/mL (50%), 8 µg/mL (80%), 10 µg/mL (100%), 12 µg/mL (120%), and 15 µg/mL (150%). Then, each level was injected six times into HPLC, chromatograms were recorded and peak area was recorded for all the peaks. The calibration graphs were plotted as peak area of the analyte against the concentration of the drug in µg/mL.

Precision and Accuracy

The precision is reported in terms of relative standard deviation (RSD) over the range of quantitation for a single experiment, in which standards are assayed in replicate (intraday) and for a series of experiments, in which standards are assayed in over several experiments (interday). The precision of the developed analytical method was tested by injecting three replicate injections of concentration 5, 10, and 15 µg/mL (50, 100, and 150% of the working level).

Table 4: Experiments suggested by 3-factor 12-run 2-level factorial design for screening of method variables and process parameters

Run	*Factor 1:pH*	*Factor 2:% ACN*	*Factor 3: Methanol*	*Response 1: Retention time*	*Response 2: Tailing factor*
1	6	10	20	72	1.3
2	6	20	30	3.6	1.89
3	6	20	10	3.1	1.79
4	3	30	20	2.52	1.95
5	3	20	10	2.2	1.8
6	4.5	30	30	2.7	2.42
7	3	10	20	6.7	1.9
8	4.5	10	30	6.3	2.3
9	6	30	20	2.3	2.19
10	4.5	30	10	2.8	1.99
11	3	20	30	2.5	2.1
12	4.5	10	10	5.9	1.8

Intraday and interday precision study was carried out by estimating the corresponding responses for the solutions of above three concentration levels on the same day and on three different days, respectively. Accuracy was calculated for the same solutions which were injected for intraday precision.

Analysis of Marketed Formulation

10 units of Galvus tablets containing ROSU were weighed and finely powdered. An accurately weighed amount of the powder equivalent to 50 mg of ROSU was transferred into a 10 mL volumetric flask and sonicated for 20 minutes with 7 mL of distilled water (diluent). The resulting suspension was filtered through Whatman 1 filter paper and diluted up to 10 mL with diluent. A suitable aliquot of this filtrate was diluted with diluent in order to obtain a final concentration of 10 µg/mL. A 20µL of the obtained solution was chromate graphed.

RESULTS AND DISCUSSION

Choice of Column

Due to the heterocyclic nature of ROSU, reverse phase chromatography is the best choice. The efficiency of two different reverse phase columns C8 and C18 were evaluated. C18 column being hydrophobic was preferred for separation of drug because drug retention was a problem on C8 column. Moreover, use of water-acetonitrile and water-methanol lead to poor precision in retention of analyte. This clearly indicated that use of buffer is required in order to control the ionization of analyte.

Development and Optimization of new RP-HPLC Method for ROSU using QbD Approach

A QbD with design of experiments (DoE) approach to the development of an analytical method mainly involves two phases as follows:

Screening Phase

The first phase of the method development involves the screening of the major effectors of selectivity and peak shape, primarily the buffer pH, organic mobile phase, and organic modifier. This screening phase was carried out using Design Expert 9 software. In this software, Box-Behnken statistical screening design was chosen to optimize the CMPs, wherein all the parameters were varied simultaneously, unlike the conventional one factor at a time (OFAT) approach. The responses obtained after carrying out the 12 experimental trial runs under Box-Behnken design was fed back to DoE software.

Statistical Analysis and Final Optimization

Statistical analysis was used to identify the significant influential chromatographic factors and their interaction impact on the two responses, i.e., retention time and tailing factor. The analysis of 3D-response surface plots and graph plots were used to estimate as to which method parameter gave the most acceptable responses.

The statistical analysis tool, like ANOVA was evaluated for each individual response to determining the most influential chromatographic parameter. Moreover, these statistical analysis tools was used to determine the significance of each method parameter selected for the study. The significance level for probability of null hypothesis was defined at $p \geq 0.05$. Null hypothesis indicates variation in all factors which has no influence on the responses. The two response variables, i.e., retention time and tailing factor, were statistically evaluated as follows:

Retention Time

Retention time is one of the critical quality attributes under experimental design. The effect of most influential chromatographic parameters on retention time was evaluated using different statistical analysis tools and plots which are described as follows:

Analysis of Variance (ANOVA)

The statistical inference from ANOVA reveals that the p value is significant at alpha (a) = 0.05. If the value obtained is less than 0.05, indicating that the model explains a significant portion of variability. Hence, the null hypothesis can be rejected, i.e., the factors have a significant effect on the responses. The ANOVA result for retention time is tabulated in Table 5 and 6.

The ANOVA result data reveals that 'p value' for the model is 0.0055, which indicates this model explains significant variability. Also, the model F-value of 9.28 implies the model is significant. The "pred R-squared" of 0.4979 is in reasonable agreement with the "adj R-squared" of 0.6932. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 7.304 in the model indicates an adequate signal.

Table 5: ANOVA result for retention time

	Model	*Factor 1: pH*	*Factor 2: % ACN*	*Factor 3: % Methanol*
Sum of squares	31.93	40.1	31.13	0.15
Mean squares	10.64	40.1	31.13	0.15
F value	9.28	53.5	27.15	0.13
p value	0.0055	0.0053	0.0008	0.7258
Std. deviation	1.07	-	-	-
R-squared	0.7769	-	-	-
Adj R-squared	0.6932	-	-	-
Predi R-squared	0.4979	-	-	-
Adequate precision	7.304	-	-	-

Moreover, the p value for pH and % acetonitrile is 0.0053 and 0.0008, respectively, i.e., less than 0.05, which indicates that both these parameters have a significant effect on retention time.

Graph Plots

The three graph plots indicate the values of retention time at different levels of pH, % organic phase, and % methanol. In Figure 2, the early retention times are neglected as there are chances of merging of early eluting analyte peak with the solvent peaks. Hence, peaks with retention time above 5 minutes are only considered. Figure 3 reveals that the retention time of the analyte peak is less than 3 minutes at 20 and 30% of organic modifier. Thus, 10% organic phase is suitable as it gives retention time of peak between 5 to 8 minutes.

3D Response-Surface Graph

The response surface graph is a 3D plot with pH and % organic phase on the X- and Y-axis, respectively, and retention time on Z-axis. As observed in Figure 3, the factor % organic modifier is kept constant as pH and % organic phase are the most influential parameters of retention time which were evident from the ANOVA result. The above graph plot indicates that the retention time is above 5 minutes at pH 6 and 10% organic phase and pH 3 and 10% organic phase. While, the retention time is less at other data points, due to which these points are not considered.

Tailing factor on Z-axis. As observed in Figure 3, The factor % organic modifier is kept constant to 20%. The above graph plot indicates that the tailing factor is closer to one when pH is six and the organic phase is 10%, while the tailing factor is more than two at other data points which is not within the acceptable limits. The predicted result for the response variables estimated with 95% continual improvement (CI) was found to be retention time as 7.21 minutes and tailing factor as 1.36.

Final Optimized Chromatographic Conditions

The final chromatographic conditions developed using QbD approach are tabulated in Table 7.

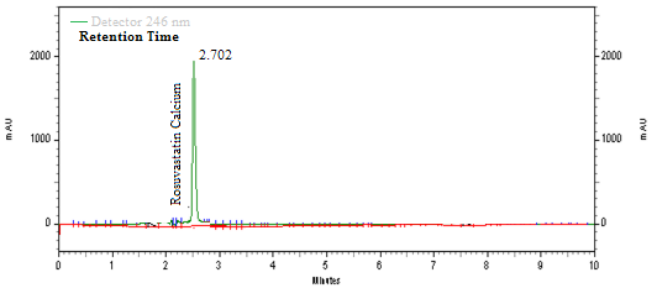

Figure 1: Chromatogram of sample run

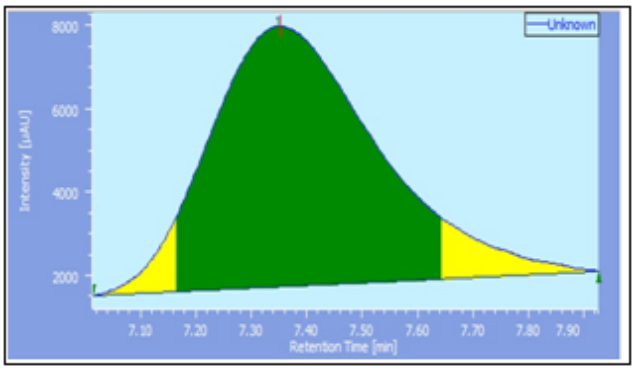

Figure 2: Peak purity for ROSU solution (10 µg/mL)

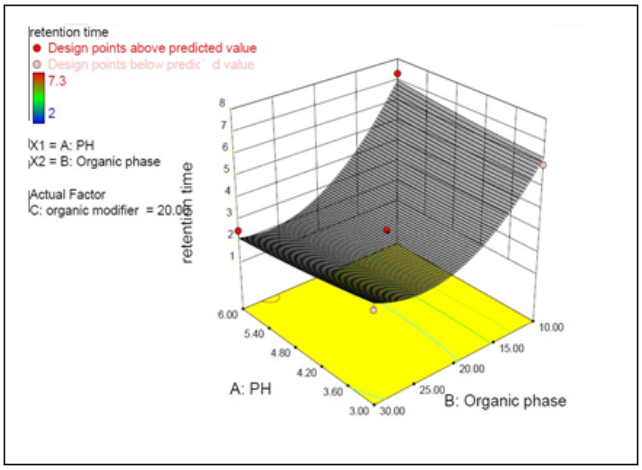

Figure 3: 3D response-surface graph

Table 6: ANOVA result for tailing factor

	Model	*Factor 1: pH*	*Factor 2: % ACN*	*Factor 3: % Methanol*
Sum of squares	40.23	0.042	0.2	0.22
Mean squares	14.82	0.042	0.2	0.22
F value	7.46	0.72	3.35	8.31
p value	0.0128	0.4201	0.1044	0.0471
Std. deviation	1.37	-	-	-
R-squared	0.6961	-	-	-
Adj R-squared	0.551	-	-	-
Predi R-squared	0.3962	-	-	-
Adequate precision	6.237	-	-	-

Validation of the Optimized Method

Once the chromatographic conditions were set, method validation was done on ROSU for system suitability, specificity, LOD, LOQ, linearity, range, accuracy, and precision.

Evaluation of System Suitability

The standard solution (10 µg/mL) was injected six times. The % RSD obtained from six replicate injections It was found to be 1.08. The tailing factor was less than. Theoretical plates were also found to be above 2,000. Thus, all the parameters evaluated for system suitability were found to be within the acceptance criteria and the system was suitable for analysis of ROSU.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were obtained by successively decreasing the concentration of ROSU as long as a signal to noise ratio of not less than 3:1 and 10:1 is maintained, respectively. The LOD of ROSU was found to be 200 ng/mL. The LOQ for ROSU was found to be 600 ng/mL. These values indicate that the method developed is sensitive.

Specificity

Blank (diluent) and standard solution (10 µg/mL) were injected. The method was quite selective for ROSU as there was no other interfering peak around the retention time of ROSU (Figure 2). Even the baseline did not show any significant peak (Figure 2). In Figure 5, the green part of the peak purity graph corresponds to high purity of 97.22%. Hence, the standard peak of ROSU was found to be pure at working concentration level. Representative chromatograms for specificity are shown in Figures 5.

Linearity and Range

Linearity was evaluated in the range of 50 to 150% of the working concentration level, i.e., 10 µg/mL (5–15 µg/mL) for ROSU. The linearity was confirmed in the range of 5 to 15 µg/mL. The coefficient of correlation (R2) was found to be 0.999 and the equation of the line was y = 73636x + 170.3, as is evident from the below calibration curve. Thus, the data shows that the response is found to be linear (Figure 5). This clearly indicates that an excellent correlation existed between the peak area and concentration of the analyte.

Table 7: Final optimization result

Critical method parameters	*Low level*	*Medium level*	*High level*	*Final level selected*
Buffer pH	3	4.5	6	6
Organic phase (% acetonitrile)	10	20	30	10
Organic modifier (methanol)	10	20	30	20

Table 8: Method precision (inter and intraday) studies for ROSU

Method precision by proposed method for ROSU	
Method precision (inter and intraday)	
Precision 1	6,361,263
Precision 2	6,361,248
Precision 3	6,361,269
Overall avg.	6,361,260
Overall std. dev.	10.81665383
Overall % RSD	0.000170039

Precision and Accuracy

Precision is reported in terms of RSD over the range of quantitation for a single experiment, in which standards are assayed in replicate (intraday) and for a series of experiments, in which standards are assayed in over several experiments (interday). The data for intraday and interday are shown in Tables 8 and 9, respectively. Accuracy was calculated for the same solutions which were injected for intraday precision.

The precision of the method for the standard solution of ROSU shows that RSD for both intraday and interday falls within the limits, i.e., within 2%. Moreover, the accuracy data shows that % mean recovery of ROSU at each level is within the acceptance criteria of 98 to 102%.

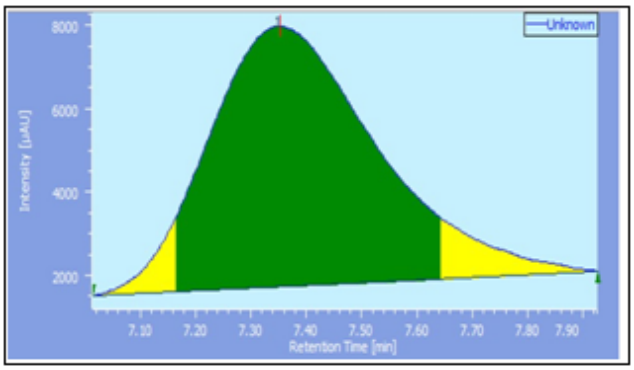

Figure 4: Specificity for ROSU solution (10 µg/mL)

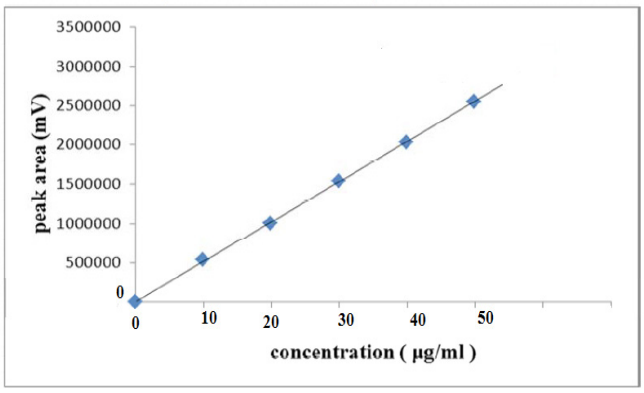

Figure 5: Standard linearity curve for ROSU

Table 9: Recovery studies for ROSU by the proposed method

Drug	*Spiked level (%)*	*Amount taken (µg/mL)*	*Amount found (µg/mL)*	*Percent recovery (% w/w) ± RSD*
ROSU	80	8	7.88	99.6 ± 0.456
	100	10	9.99	100.02 ± 0.487
	120	12	11.97	99.8 ± 0.434

Stability Indicating Assay of ROSU

The validated HPLC method was used to perform forced degradation studies on ROSU. Forced degradation studies done on ROSU indicated that the drug was degraded by 4.595, 10.326, and 25.497% when subjected to acid hydrolysis, base hydrolysis, and oxidation degradation, respectively. This shows that ROSU is susceptible to acid hydrolysis, base hydrolysis, and oxidation, while the drug was found to be stable after thermal degradation and photolytic degradation. The results of forced degradation studies reveal that all the degradation products were fully resolved and do not interfere with the analyte peak which indicates specificity of the method. Thus, the method can be employed for monitoring the stability of ROSU in bulk drugs.

Moreover, these studies also determine the physical and chemical stability of drug substance and drug products which may be further useful to determine the storage conditions for the drug product. Since ROSU is susceptible to oxidation at room temperature, ROSU tablets should be stored in a dry place as moisture is a catalyst of oxidation, and a low moisture environment may sometimes resolve the problem of oxidation. Another alternative is to use an oxygen scavenger that helps to control the oxygen level within the headspace of a drug's primary packaging. This may help to maintain the drug potency and other properties under extended and variable storage and shelf conditions.

Application on Marketed Formulation

The developed stability indicating RP-HPLC method was successfully applied for the estimation of ROSU from the marketed formulation which was found to contain 98.92% of the label claim.

CONCLUSION

A robust RP-HPLC method for ROSU was developed using a QbD approach on Design Expert 9 software. Three independent factors were used, such as, mobile phase ratio, buffer pH, and column type. Totally 12 experimental runs were suggested by the software for analyzing the interaction of each response, i.e., retention time, peak tailing, and number of theoretical plates were considered as dependent factors.

The method was validated according to ICH guidelines. Validation of the analytical QbD method corroborated excellent linearity, accuracy, precision, LOD, LOQ, system suitability, and robust and rugged for determination based on the knowledge of method obtained through the method development and the result of risk assessment. The approach can be successfully used in the laboratory to develop the RP-HPLC method for ROSU.

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