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QbD approach to HPLC method development and validation of Remdesivir

Sunil Kakad[1]*, Anagha Joshi[1], Vaishnavi Parlikar[2],

[1] Department of Pharmaceutical Chemistry**,** Shree Chanakya Education Society's Indira College of Pharmacy, Pune

[2] Department of Quality Assurance**,** Shree Chanakya Education Society's Indira College of Pharmacy, Pune

Corresponding author Email: sunil.kakad21@gmail.com

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Abstract

Background: Achieving a certain predicted quality with intended and predetermined parameters is known as quality by design (QbD). Due to the emphasis on risk assessment and management compared to a traditional or conventional approach, a quality-by-design approach to method development may result in a more robust or rugged method. Understanding dependent variables, different variables and their interaction effects by the desired set of trials on the responses that need to be studied is a crucial part of the QbD. The development and validation of a risk-based HPLC technique for Remdesivir in pharmaceutical dose form are described in the current work.

Remdesivir technique development with the mobile phase methanol and acetonitrile combined to water [(methanol + ACN): water] has not been reported in the literature review. Using this mobile phase, the developed method will also be economical. Also, there exists no report in the literature survey on method development of Remdesivir by QbD approach.

Results: An efficient experimental design based on 3 level factorial design of three key components of the RPHPLC method (mobile phase composition and flow rate) is presented. The chromatographic conditions were optimized with the Design Expert software 10.0 version, i.e., Acclaim ^{TM} Mixed-Mode WCX-1 column C18 (150mm × 4.6 mm) 120 Å, mobile phase used methanol and acetonitrile in combination (60:40) to water (60:40, v/v), and the flow rate was 0.8 ml/min with retention time 4.18 min. The developed method was found to be linear with r^2 = 0.996 for range of 10–30 µg/ml at 243 nm detection wavelength. The system suitability test parameters, tailing factor and theoretical plates, were found to be

1.05 and 7701 respectively. The % RSD for method precision and interday precision was found to be 1.00 and 1.26 respectively. The robustness values were less than 2%. The method validation parameters were in the prescribed limit as per ICH guidelines.

Conclusion: With the aid of the Design Expert 10.0 edition, the 3-level factorial design experimental design describes the interactions of mobile phase composition and flow rate at three different levels and the responses to be examined were retention time, tailing factor, and theoretical plates. Here, the elements that affect chromatographic separation are more understood, and the created HPLC method's capacity to achieve its goals is more confidently assessed. The QbD methodology was used to construct analytical methods and improve knowledge of method variables at various stages.

Keywords: Quality by design, HPLC, Remdesivir, Design approach

Background

Drug Profile

Name of the drug	Remdesivir
Molecular Formula	$C_{27}H_{35}N_6O_8P$
Molecular Weight	602.585 g/mol
IUPAC NAME	2-ethylbutyl (2*S*)-2-[[[(2*R*,3*S*,4*R*,5*R*)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxyoxolan-2-yl]methoxy-phenoxyphosphoryl]amino]propanoate
Category	Antiviral
Appearance	White to off-white crystalline powder

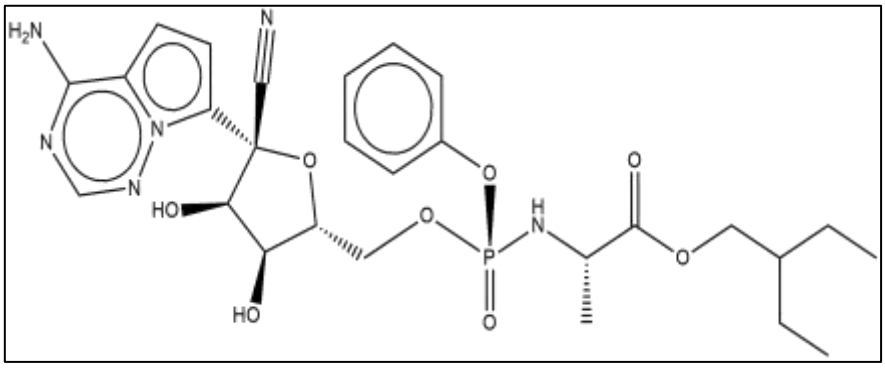

Fig. 1: Remdesivir

Spike protein, along with other nucleoproteins, polyproteins, and a membrane protein termed RNA polymerase, is a component of the distinctive structure of the coronavirus 2 (SARS-CoV-2). Remdesivir (CAS 1809249-37-3) is an antiviral nucleotide analogue that is used to treat severe new coronavirus illness (Covid-19) brought on by SARS-CoV-2 infection. Remdesivir is a prodrug of an adenosine triphosphate (ATP) analog, and after delivery it is converted to the active form GS441524. As an ATP analog, GS-441524 competes with ATP for incorporation into RNA, blocking the activity of viral RNA dependent RNA polymerase and causing RNA transcription to stop and viral RNA synthesis to decrease.

Key components of every pharmaceutical development program are the development and validation of analytical methods. To locate, measure, or purify desired substances, an HPLC analytical method is created. This technical brief will concentrate on activities for development and validation as they relate to pharmaceutical products. When techniques are developed effectively, laboratory resources are maximized and goals necessary for each phase of drug development are met. Method validation is the "process of proving that analytical procedures are suitable for their intended use," and it is needed by regulatory authorities at specific phases of the drug approval process [1-3].

The goal of the current effort is to use a quality-by-design methodology to create and improve the HPLC method for Remdesivir in pharmaceutical dosage form. According to the definition of QbD, it is "a systemic approach to method development that starts with predetermined goals and focuses product and process understanding and process control, guided by sound science and quality risk management." [4]. With a focus on product and process awareness, quality risk management, and controls, the QbD strategy leads to improved assurances of quality of the product, regulatory flexibility, and ongoing improvement. The ICH Q8 Pharmaceutical Development, ICH Q9 Quality Risk Management, and ICH Q10 Pharmaceutical Quality System guidelines served as the foundation for the QbD methodology [5-7].

The main goal of this study was to use the QbD methodology to the development and validation of an RP-HPLC method, as well as to establish a thorough knowledge of the method and incorporate quality into the method development process to guarantee optimal method performances over the course of the product.

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Materials and Methods

Instruments and reference standards

The HPLC WATERS-2487 with Detector-UV VIS Dual Absorbance Detector. Acclaim ™ Mixed-Mode WCX-1 C18 column (150mm × 4.6 mm) 120 Å was used at ambient temperature.

Reagents and Chemicals

Standard APIs were kindly gifted by Lupin, Pune and the marketed formulation (Lyophilized Powder) was procured from local market. HPLC grade Methanol, Water and Acetonitrile used in Mobile phase preparation were also procured from local market.

Chromatographic Conditions

Use was made of an Acclaim TM Mixed-Mode WCX-1 C18 column (150 mm 4.6 mm) 120 Å equilibrated with a mobile phase composed of methanol and acetonitrile in combination (60:40) to water (60:40, v/v) was used. The column was set at the ambient temperature, and the flow rate was maintained at 0.8 ml/min. The injection volume was 20 µl, and the detection was observed at 243 nm. With the aforementioned chromatographic conditions, an acceptable separation and peak symmetry for the medication were achieved. The mobile phase composition and flow rate, two variables at three different levels utilizing a 3 level factorial design, were two of the factors used to optimize the HPLC process for Remdesivir.

Preparation of reference standard solution

The 500 mg/ml standard stock solution was made by accurately combining 50 mg of remdesivir with 50 ml of methanol and 50 ml of distilled water to make 100 ml of diluent. The stock solution, 2 ml, was diluted to 100 ml with diluent to get the 10 g/ml solution.

Selection of mobile phase

Remdesivir was delivered as a pure medication into the HPLC device and then ran in several mobile phase systems. Among other mobile phases, we tried with water, acetonitrile, and methanol. The mixture of water, methanol, and acetonitrile was shown to yield outcomes that are suitable and pass the ICH Q2 (R1) guideline. As a result, the final mobile phase is made up of Methanol and Acetonitrile in combination (60:40) and Water in the ratio of 60:40.

HPLC method development by QbD approach

HPLC method development by Analytical QbD was as follows.

Selection of quality target product profile

The QTPP is crucial for figuring out the factors that influence the QTPP parameters. For the suggested HPLC method, theoretical plates, retention time, and tailing factor were identified as QTPP [8,9].

Determine critical quality attributes

The method variables that primarily affect the QTPP are the CQAs. Two crucial technique parameters that had to be kept under control in order to keep QTPP's response range within acceptable bounds were the flow rate and mobile phase composition [10].

Factorial Design

After the QTPP and CQAs were established, the 3-level factorial design was used to optimize and choose the two crucial elements of the flow rate and composition of the mobile phase of the HPLC process. The interaction effects and quadratic effects of the flow rate and mobile phase composition on theoretical plates, retention time, and tailing factor were investigated using a three-level factorial design.

Design Expert® (Version 10.0, Stat-Ease Inc., and M M), the most suitable answer for second-order polynomial exploring quadratic response surfaces, was utilized to create a 2-factor, mobile phase composition and flow rate at 3 separate levels, three-level design [9].

Evaluation of experimental results and selection of final method conditions

Methanol and acetonitrile were chosen as the mobile phase in combination to water. The study made use of a 20 µl injection volume. Drug detection was done using a UV detector. DOE was used to study and optimize three alternative mobile phase compositions, including 50:50, 60:40, and 70:30. Similar to this, the impact of the flow rate—namely, 0.8, 1.0, and 1.2 mL/min—was investigated, and the final flow rate was chosen based on DOE. The factorial design facilitates the investigation of the interactions and effects of independent and self-governing elements. Mobile phase composition (A) and flow rate (B) were considered as two independent parameters in the current study. For low, medium, and high factorial levels, respectively, the study employed the codes -1, 0 and +1. For the purpose of assessing the interaction between each level, the program recommended a total of 11 experimental runs, with the retention time (R1), tailing factor (R2), and theoretical plates (R3) being regarded as the response factors (dependent factors). The selected factors and various factor level values are displayed in Tables 1 and 2.

Table 1: 3^2 Factorial design with upper, middle & lower limits of all factors Statistical Optimization

2 Factors	3 Levels		
	Low (-1)	Middle (0)	High (+1)
Mobile phase composition (%)	50	60	70
Flow rate (ml/min)	0.8	1	1.2

Table 2: Optimization of parameters for analysis of Remdesivir

Run	Factor 1 Mobile phase composition	Factor 2 Flow rate	Response 1 Retention time	Response 2 Tailing factor	Response 3 Theoretical plates
1	50:50	1	5.92	1.09	5069
2	50:50	1.2	4.87	1.09	4596
3	70:30	1	2.4	1.17	3740
4	60:40	1	3.26	1.15	4433
5	60:40	1.2	2.68	1.12	4081
6	70:30	0.8	2.99	1.21	3812
7	60:40	1	3.26	1.15	4433
8	70:30	1.2	1.98	1.15	3322
9	60:40	1	3.26	1.15	4433
10	50:50	0.8	7.41	0.68	17098
11	60:40	0.8	4.03	1.16	4715

Method Optimization

The mobile phase was selected methanol and acetonitrile in combination to water. The UV detector was used for detection of drug and the best wavelength was found to be 243 nm showing highest sensitivity. A number of changes to the mobile phase's composition, flow rate, and column were undertaken in an effort to explore the potential for altering the chromatographic system's selectivity. These modifications included the change of the ratio of the organic modifier, flow rate and column.

Initially, no peaks were observed on Zorbax Aligent SB C-18 column (4.6 × 250, 5 µm) with the mobile phase ratio 70:30 and flow rate 1 ml/min. Therefore, column was replaced with Acclaim ^{TM} Mixed-Mode WCX-1 (150 × 4.6) 120 Å and mobile phase ratio and flow rate was same, then peaks were observed but retention time was 2.26. As retention time was low, therefore, mobile phase ratio was changed to 60:40 and flow rate was kept constant, then retention time was increased to 3.27. Therefore, from the above 11 runs, mobile phase ratio 60:40, flow rate 0.8 ml/min was selected. Acclaim ^{TM} Mixed-Mode WCX-1 (150 × 4.6) 120 Å was the most suitable one since it produced symmetrical peaks with better resolution.

To assess the impact of the variable on responses, measurements were made using the contour plot, polynomial equation, and 3D plots. Statistical analysis ANOVA and regression analysis were performed to determine the significant level for the model and to determine the best fit model.

Table 3: Obtained solution for optimized formulation

Mobile phase composition (%)	Flow rate (ml/min)	Retention time (min)	Theoretical plates	Tailing factor	Desirability
60:40	0.8	4.18	7701	1.05	1.000

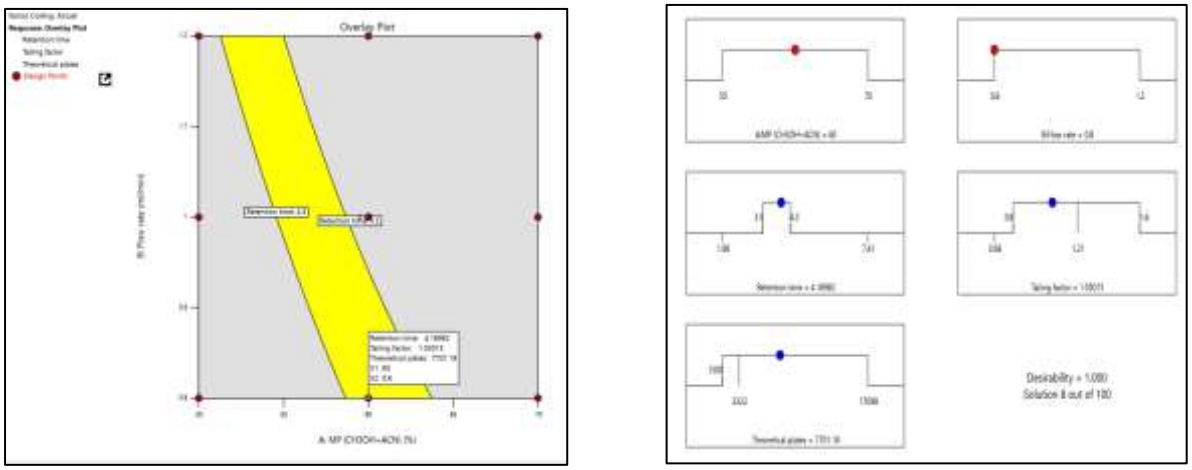


Table 4: Statistical ANOVA results for the responses

Results (ANOVA)	Retention time	Tailing factor	Theoretical plates
Model	Quadratic	2FI	2FI
Sum of squares	26.71	0.1461	1.091E+08
Degrees of freedom	5	3	3
Mean squares	5.34	0.0487	3.636E+07
F-value	390.62	5.54	5.91
p-value	< 0.0001	0.0289	0.0248
Lack of fit tests			
Sum of squares	0.0684	0.0615	4.311E+07
Degrees of freedom	3	5	5
Mean squares	0.0228	0.0123	8.621E+06

The best fit model was determined to be the quadratic model for retention time, the 2FI model for the tailing factor, and theoretical plates because its regression coefficient (r^2 = 0.99) is substantially higher (p 0.0001) than other models. Each model's ANOVA was assessed, and variables like the square root, p value, F value, and mean square for each response were also computed. The software created a contour and 3D plot of each response to show how numerous variables (integration effect) might have an impact on a response at once.

Table 5: Actual equation results for the responses

	Retention time	Tailing factor	Theoretical plates
Intercept	66.25728	-3.35152	1.22764E+05
MP (CH3OH+ACN)	-1.48807	0.069917	-1766.31667
Flow rate	-21.82149	3.78333	-1.01445E+05
MP (CH3OH+ACN) * Flow rate	0.191250	-0.058750	1501.50000
MP (CH3OH+ACN)^2	0.009303		
Flow rate^2	3.13158		

A: Mobile Phase, B: Flow rate

Effect of Independent factors

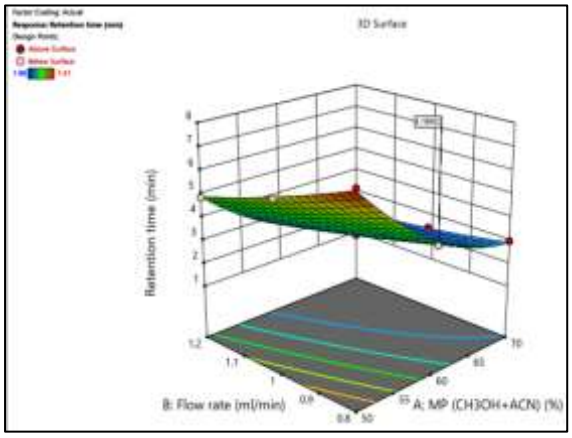
The 3D response, polynomial equation, and contour plot were used to express how an independent variable affected theoretical plates, retention time, and tailing factor.

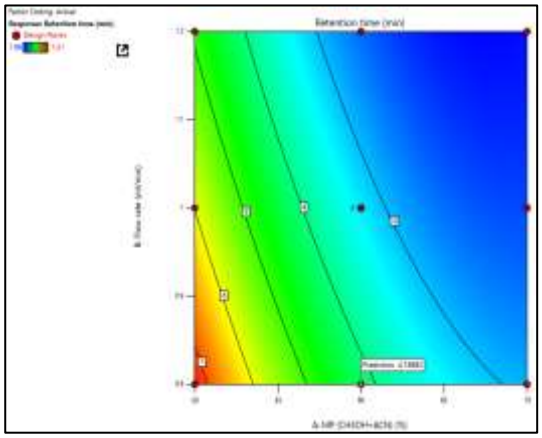
On Retention Time

The 2nd order quadratic polynomial equation for the response, retention time, is given as follows-

Retention time = +66.25728-1.48807A-21.82149B+0.191250AB+0.009303A^2+3.13158B^2,

it was concluded that as β1 negative coefficient (-1.48807) suggests that as the amount of organic modifier in the mobile phase (A) decreases and β2 negative coefficient (-21.82149) suggests that as flow rate (B) decreases, the value of retention time was increased.



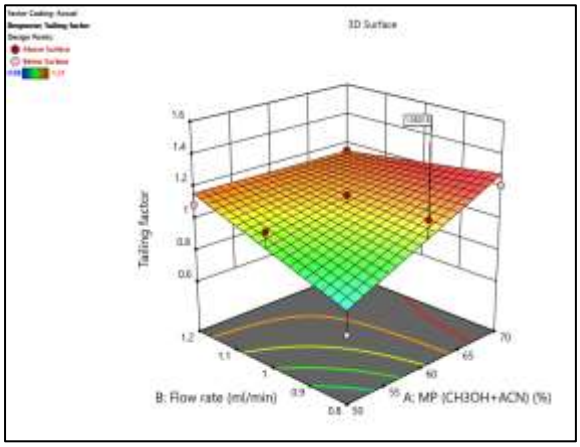


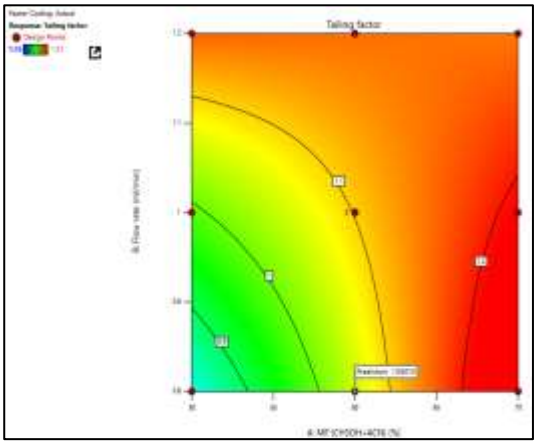
On Tailing factor

The 2nd order quadratic polynomial equation for the response, tailing factor, is given as follows-

Tailing factor = -3.35152+0.069917A+3.78333B-0.058750AB,

it was concluded that as β1 positive coefficient (0.069917) suggests that as the amount of organic modifier in the mobile phase (A) increases and β2 positive coefficient (3.78333) suggests that as flow rate (B) increases, the value of tailing factor was increased.





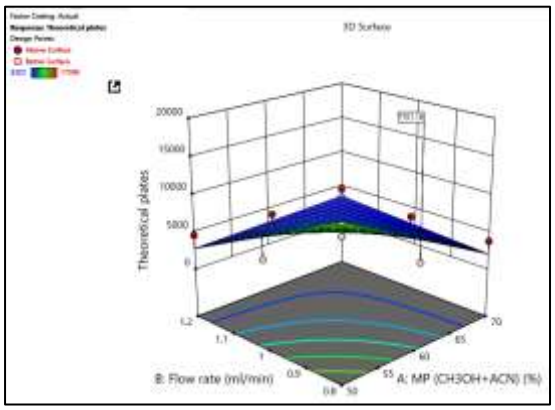
On Theoretical Plates

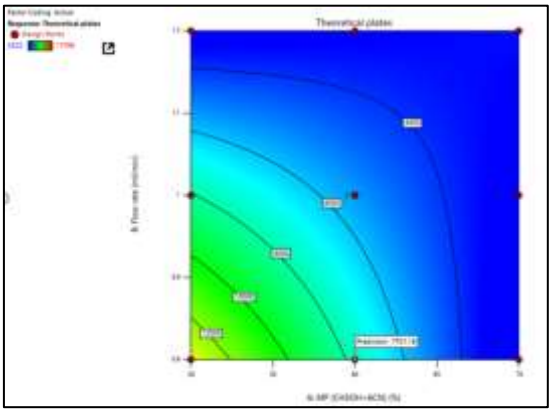
The 2nd order quadratic polynomial equation for the response, theoretical plates, is given as follows-

Theoretical plates = +1.22764E+05 -1766.31(A)-1.01445E+05(B) +1501.50000(AB),

it was concluded that as β1 negative coefficient (-1766.31) suggests that as the amount of organic modifier in the mobile phase (A) decreases and β2 negative coefficient

(-1.01445E+05) suggests that as flow rate (B) decreases, the value of theoretical plates was increased.





Method Validation

Method validation is a process that has been documented that gives a method a high degree of assurance that the procedure used to verify the analytical process is appropriate for its intended usage is suitable. According to ICH Q2 (R1) requirements, the devised HPLC technique for measurement of Remdesivir was verified [11].

Linearity

Linearity was assessed by examining 5 different levels of concentration over a 10–30 g/ml range. Peak area was plotted against concentration on the x-axis to create the calibration curve. Values for the correlation coefficient and regression line equation were calculated.

Method Precision

The standard solution was prepared at the test concentration and repeatedly injected six times to show the system's accuracy. The % RSD acceptability limit was no greater than two.

Accuracy

Calculating the recovery studies from the marketed formulation at three levels—50%, 100%, and 150% led to the determination of the method's accuracy. Recovery percentage was calculated. According to ICH rules, a recovery rate could not exceed 95–105%.

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LOD & LOQ

The detection limit (LOD) and the quantification limit (LOQ), respectively, are the lowest drug concentrations that can be quantified at the lowest concentration and that can be properly identified and distinguished from the background. In accordance with ICH recommendations, Limit of detection and Limit of quantification were measured using the following equation.

$LOD = 3.3 \times \sigma/SD$

$LOQ = 10 \times \sigma/SD$

where σ is the standard deviation of the y-intercept of the regression line, and SD represents slope of the calibration curve.

Robustness and ruggedness studies

By making a little change to the method's existing state, such as a change in pump flow rate or mobile phase composition, the robustness of the approach might be determined. In order to determine the toughness studies, the analyst was changed to an unimportant influencing component. The calculated %RSD of peak area's acceptable limit was less than 2.

System suitability studies

The suitability of the system was assessed using six replicate remdesivir studies. For standard solutions, theoretical plates, retention time, and tailing factor were computed.

Results

Initially a mobile phase tried was methanol and acetonitrile in combination (60:40) to water in the ratio of 70:30 v/v, the peak was observed at early retention time. The further mobile phase tried was methanol and acetonitrile in combination (60:40) to water in the ratio 60:40 v/v. This optimized mobile phase satisfied all the system suitability parameters. Further optimization of various parameters inside the design space was done using the 3-level factorial design.

HPLC method development by Qbd approach 12]

Quality target product profile

Retention time, tailing factor, and theoretical plates were chosen as the QTPPs for the improvement of HPLC chromatographic conditions.

Critical quality attributes

The mobile phase composition methanol and acetonitrile in combination (60:40) to water, 60:40, and flow rate adjusted to 0.8 ml/min were identified.

Factorial design

The development of the proposed HPLC method used the 3-level factorial design. Table 2 displays the optimization of several parameters.

Optimized condition obtained

Optimized HPLC settings and anticipated responses are displayed in Table 3 as a result of analysis of all responses under various experimental circumstances with the Design Expert 10.0 program. The observed value for responses was derived by conducting the HPLC chromatogram for the specified mobile phase and buffer pH, and the predicted and observed values were then compared to assess the percent prediction error.

Method Validation

System suitability

The suitability of the system was assessed using six replicate remdesivir studies. For standard solutions, theoretical plates, retention time, and tailing factor were computed. The working standard solution was injected six duplicate times, and the %RSD for the average area was computed (Table 6).

Table 6: System Suitability

Sr. No.	Concentration of Std. REM	Area of Std. REM	Retention time	Tailing factor	Theoretical plates
1	10	804864	3.86	1.17	4253
2	10	802727	3.86	1.17	4246
3	10	803416	3.87	1.17	4137
4	10	804939	3.86	1.17	4241
5	10	802643	3.86	1.17	4141
6	10	803425	3.87	1.17	4145
	Mean	803669			
	SD	922.362			
	%RSD	0.114769			

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Linearity

The remdesivir calibration curve that was created was linear for concentrations between 10-30 g/ml, as shown in Table 7. When a graph of peak area against concentration was created, the regression equation for the calibration curve was often found to be y = 95903x - 192272 with a 0.9963 correlation coefficient (Fig. 7).

Table 7: Linearity of remdesivir

Sr. No.	Concentration (µg/ml)	Peak area (mean ± SD)
1	10	815459 ± 77.92732
2	15	1218777 ± 1085.093
3	20	1686508 ± 703.94
4	25	2171613 ± 651.0567
5	30	2736627 ± 1940.813

Fig. 2: Chromatogram of 10 ppm

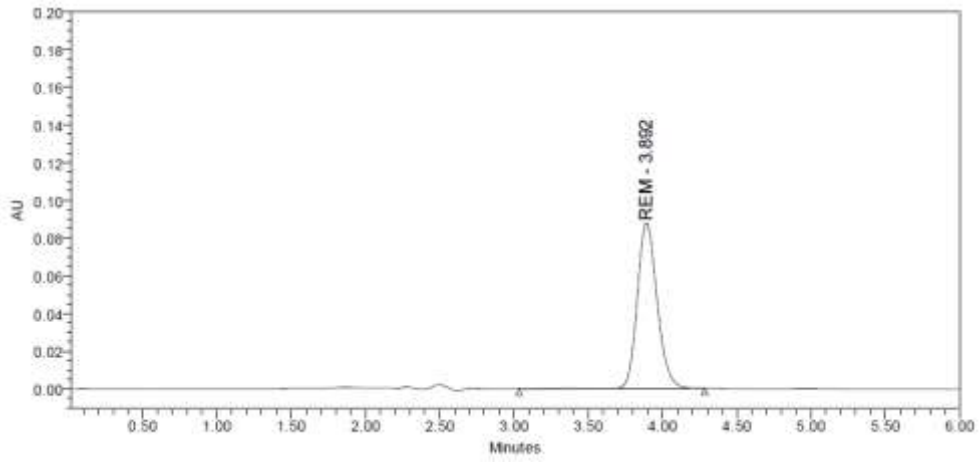


Fig. 3: Chromatogram of 15 ppm

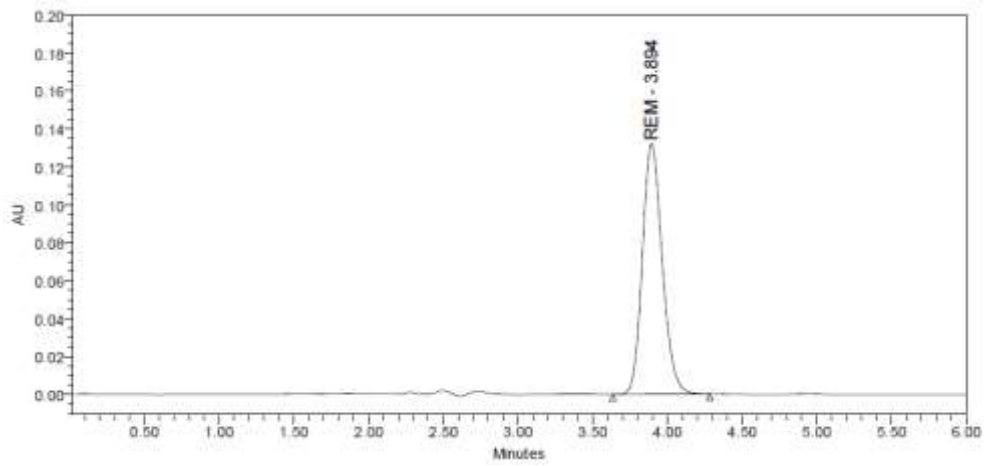


Fig. 4: Chromatogram of 20 ppm

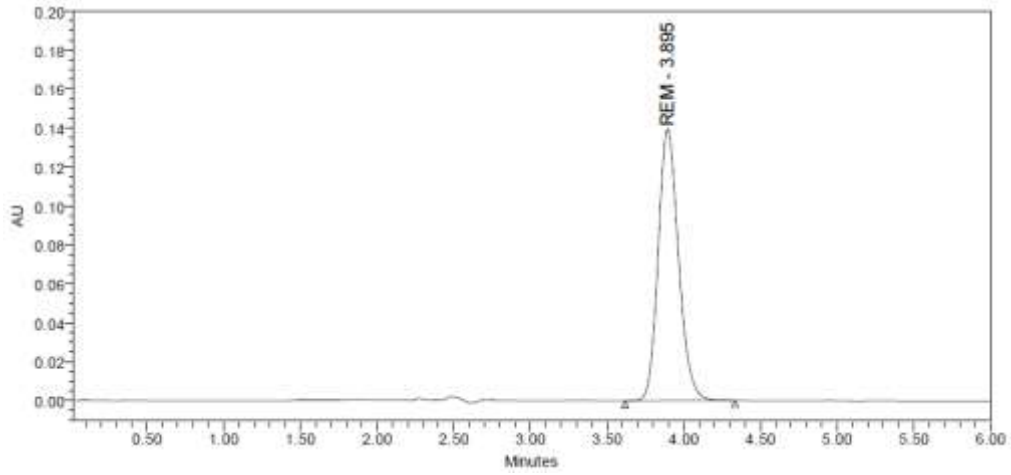


Fig. 5: Chromatogram of 25 ppm

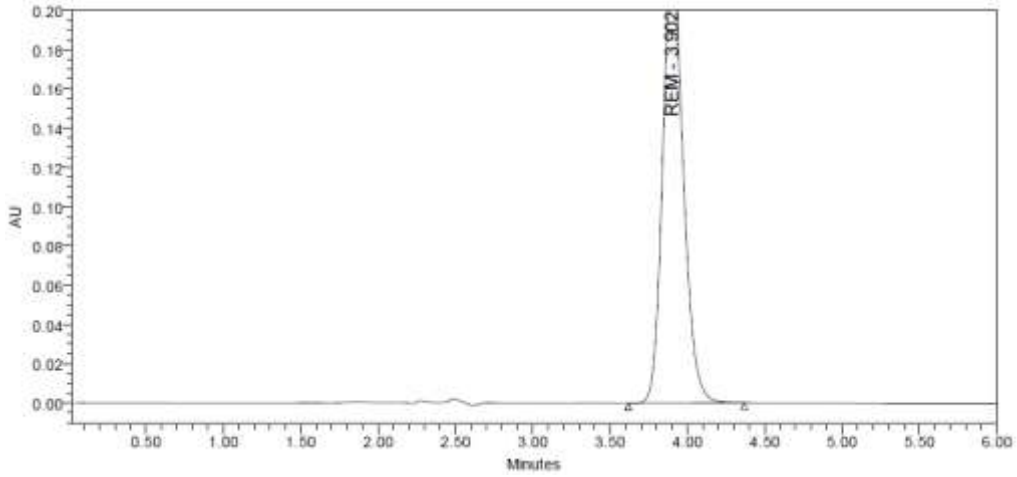


Fig. 6: Chromatogram of 30 ppm

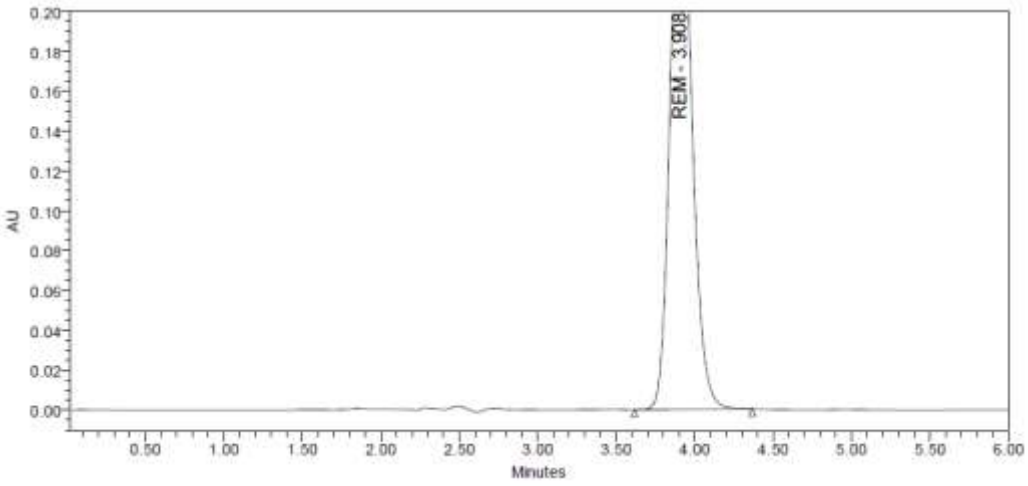
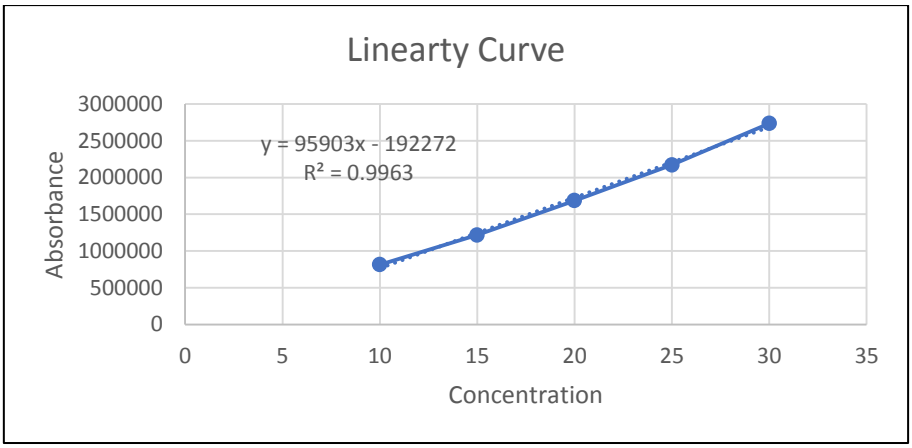


Fig. 7: Linearity Curve



Precision

Based on six measurements of the same concentration (10 g/ml), the repeatability percentage for remdesivir was found to be 0.927 (Table 10). Tables 8 and 9 demonstrate the method precision and interday precision, respectively. The constant area of the STD drug is 803669 (Table 6). The created approach was found to be accurate because the % RSD value was less than 2.

Weight of std. REM = 50 mg

Area of 10 mg REM = 803669

Sample wt. taken = 341 mg

Avg. wt. = 341 mg

Label claim = 100 mg REM

Formula - Sample area/ Std. area*Std. wt./100*2/100*100/Sample wt.*10/1*341/10

Table 8: Date for method precision of remdesivir

Sr. No.	Concentration (µg/ml)	Sample area
1	10	878521
2	10	887634
3	10	895226
4	10	885243
5	10	901956
6	10	903269
	Mean	891974
	SD	8971.418
	%RSD	1.00
	Assay	99.6

Table 9: Date for interday precision of remdesivir

Sr. No.	Concentration (µg/ml)	Sample area
1	10	862354
2	10	886541
3	10	873582
4	10	877632
5	10	897851
6	10	884712
	Mean	880445
	SD	11110.32
	%RSD	1.26
	Assay	98.3

Table 10: Date for repeatability of remdesivir

Sr. No.	Concentration (µg/ml)	Sample area
1	10	877569
2	10	885231
3	10	895219
4	10	886932
5	10	902547
6	10	896354
	Mean	890642
	SD	8258.846
	%RSD	0.92
	Assay	99.7

Accuracy

Recovery study was used to determine accuracy. Sample solutions were made by adding 3 different amounts of spiking, namely 50%, 100%, and 150%. Table 11 displays the% recovery data acquired using the suggested HPLC procedure. The devised approach was accurate in accordance with ICH Q2 (R1) requirements as evidenced by the recovery percentage of 95–105%.

Table 11: Date for % recovery of remdesivir

%	Area			Mean	SD	%RSD	Sample wt.(mg)	Assay
	Set 1	Set 2	Set 3					
50	406321	410362	411562	409415	2241.967	0.547603	170	96.7
100	887634	901956	895226	894938.7	5850.461	0.653728	341	99.9
150	1314758	1312569	1299845	1309057	6575.117	0.502279	510	104.2

Robustness and ruggedness studies

For experiments on robustness and roughness, remdesivir 10 g/ml solution was utilized. It was investigated by making a small but intentional adjustment to intrinsic process parameters such the mobile phase ratio, flow rate, and column temperature. In order to determine the toughness studies, the analyst was changed to an unimportant influencing component. The value of the %recovery was used to calculate the quantitative impact of the variables, and the permitted limits for the peak response and analyte retention times should be 2%. The computed RSD of the peak area's acceptable limit was below 2 %. By altering the ratio of mobile phase to flow rate, it was discovered that the % RSD for the peak area was less than 2. Results from the experiment's parameter optimization are utilized as a guide (Table 2). The findings above show that the procedure was remained reliable even though the chromatographic settings changed significantly.

LOD and LOQ

Based on the standard deviation of the slope and intercept, it was discovered that LOD and LOQ were 0.0306 g and 0.0929 g/ml, respectively.

Discussion

Analytical quality by design Remdesivir has been estimated using an HPLC technique in pharmaceutical formulations. For the HPLC analysis of remdesivir, the analytical target product profile included retention duration, tailing factor, and theoretical plates. The two factors, mobile phase composition and flow rate, were found to be the crucial quality characteristics that have an impact on the analytical target product profile. Using Design

Expert Software Version 10.0, the three-level factorial design was used for two components at each of three separate levels. When performing chromatographic separation, the variability of the column used, the instrument set up, and the injection volume were all maintained under control, while factors like the pH of the mobile phase, the flow rate, and the column temperature were given to a robustness study. The HPLC method for remdesivir was successfully developed using the quality-by-design methodology. Acclaim TM Mixed-Mode WCX-1 column C18 (150mm 4.6 mm) was used in the improved RP-HPLC method to measure remdesivir. 120 and the mobile phase is made up of a mixture of acetonitrile and methanol (60:40, v/v). Retention time was discovered to be 4.18 minutes. The approach had a 0.996 correlation coefficient and was linear in the 10-30 μg/ml range. The optimized approach was accurate, as seen by the less than 2% RSD for repeatability, intraday, and interday precision. The LOD and LOQ were, respectively, 0.0306 μg/ml and 0.0929 μg/ml. According to the ICH guidelines' acceptance criteria, the recovery rate of samples that had been spiked was determined to be between 95 and 104%. The approach was created in accordance with ICH principles.

Conclusion

It has been stated how to construct an HPLC method using a quality-by-design methodology. The analytical target product profile is used to clarify the method goals. The scouting of the primary HPLC process elements, such as the flow rate and mobile phase composition, is described in the experimental design. For the development of the HPLC method for Remdesivir, the analytical QbD concepts were expanded, and in order to identify the most effective system and the final design space, a multivariant analysis of a number of significant process parameters, including the combination of two factors, namely the flow rate and mobile phase composition at three different levels, was carried out. Utilizing a 3-level factorial design, their interrelationships were investigated and optimized at various levels. Here, the elements affecting chromatographic separation and the methods' ability to achieve their goals are well understood. This strategy provides real-world understanding that aids in the creation of a HPLC optimization that may be applied in the future. The acceptance criteria were determined to be met by every parameter that had been verified. The validated method for determining remdesivir was shown to be linear, exact, accurate, particular, robust, and tough. With a greater understanding of the method variables, there is a lower risk of failure during method validation and transfer thanks to the QbD approach to method creation. In comparison to human method creation, the automated QbD method development

methodology using the Design Expert software has produced a method that performs better and is more resilient in a shorter amount of time. Data analysis using statistical methods reveals that the procedure is reliable, accurate, and robust. The pharmaceutical sector will continue to employ this technology for routine analysis and quality monitoring.

Abbreviations

Qbd: Quality by design; API: Active pharmaceutical ingredient; QTPP: Quality target product profile; CQA: Critical quality attribute; HPLC: High-performance liquid chromatography; HPLC: High-performance liquid chromatography; RP HPLC: Reverse phase high-performance liquid chromatography; LOQ: Limit of quantitation; LOD; Limit of detection; RSD: Relative standard deviation; REM: Remdesivir; RT: Retention Time.

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