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Research Article

Quality by Design based Development and Validation of UV-spectrometric Method for the Determination of Remdesivir in Bulk and Marketed Formulation

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ABSTRACT

A simple and rapid UV spectrophotometric method was developed to quantify remdesivir in bulk and its dosage form used in the treatment of COVID-19. Analytical Quality by Design (AQbD) approach was implemented for method development and the relationship between variable input parameters and the method performance characteristics were designed into Ishikawa diagram. The critical parameters were optimized for solvent, wavelength, scan speed and sampling interval, determined by using principal component analysis and observation. The present method was validated according to ICH Q2 (R1) guideline. The method was found excellent linear ($r^2 \geq 0.999$) at concentration range of 2–12 µg/mL at maximum wavelength of 245.5 nm. The detection limit and quantitation limit were satisfactorily found to sensitive and precise. The intraday and interday precision were achieved consistently, and percentage relative standard deviation (%RSD) was ≤ 2%. The overall results of forced degradation studies such as acidic, basic, oxidative, thermal and photolytic conditions imply that the developed method could be well implemented as a stability indicating method for estimating remdesivir. The result confirmed that the method can be useful for the routine quality control of remdesivir in their pharmaceutical formulations.

Introduction

The (Coronavirus disease) COVID-19 pandemic is threatening the healthcare systems of several countries around the world. Despite the efforts being made to reduce the transmission of infection, they are still practically insufficient to control the outbreak of COVID-19. In response to this situation, there has been increasing interest in developing a treatment to reduce the pandemic's impact on human life. Whereas there is an urgent requirement for effective treatments, no drugs are approved for the purpose. Remdesivir administered by intravenous route (IV) is the only antiviral drug authorized by Food and Drug Administration (FDA) for treating adult and pediatric patients hospitalized with serious conditions.[1] Remdesivir is a broad-spectrum antiviral drug, which acts as a nucleotide analog inhibitor of RNA-dependent RNA polymerase,[2] developed by Gilead Sciences. It has been proven that remdesivir has antiviral activity against severe acute respiratory syndrome (SARS) and (Middle East respiratory syndrome) MERS RNA virus, and has caught significant attention for its potential application in the treatment for COVID-19.[3,4] Due to the therapeutic importance and escalation in the use of remdesivir, a proper assay method is needed for its determination in bulk and pharmaceutical formulations. The analytical investigation of bulk drug materials, drug formulation, impurities and degradation products are very important area of research in pharmaceutical product development. A literature study has published a few analytical techniques such as HPLC[5-7] method for determining remdesivir from bulk and formulation. and few bioanalytical approaches in human plasma

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using UHPLC-MS/MS.[8-11] The LC-MS-based approach for identifying and characterizing remdesivir degrading products has just been disclosed.[6] Remdesivir comes in a single-drug dosage formulation that doesn't require separation for testing when manufactured at the production site. Moreover, the chromatographic methods are time-consuming, required complex instrumentation and a trained person.

Therefore, the goal of the present investigation was to develop a simple, robust and accurate UV-spectrometric method for estimating remdesivir by using analytical quality by design (AQbD) approach. Unlike conventional methods, AQbD helps develop a robust and rugged analytical method applicable throughout the product's lifecycle, to facilitate regulatory flexibility in the analytical method. It means the freedom to change method parameters within a method's design space, referred to as the method operable design region (MODR).[12]

The analytical investigation of bulk drug materials, drug formulation, impurities and degradation products are very important areas of research in pharmaceutical product development. There have not been any reported attempts for the assay-based validated UV spectrophotometric method. The present work is aimed to develop a simple and precise UV-spectrophotometric method for remdesivir and fully validated as per International Council for Harmonization (ICH) guidelines.[13] The forced degradation behavior of remdesivir was also observed in ICH prescribed acidic, basic, oxidative, thermal and photolytic conditions in UV spectrophotometric method.

Materials and Methods

Reagents and Chemicals

The standard powder of remdesivir was collected from Graduate School of Pharmacy, Gandhinagar, India. Formulations of remdesivir, were purchased from the local pharmacy for research purposes. All other chemicals and reagents used in the experiment were of analytical grade and were purchased from Central Drug House (CDH) fine chemicals, India.

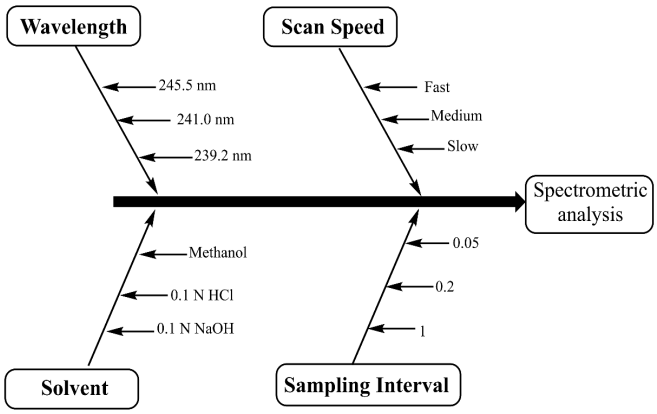

Fig. 1: Typical fish-bone diagram depicting cause effect relationship CMV and CAA

Instrumentation and Optical Characteristics

The absorbance was measured using a double-beam Shimadzu UV-Visible spectrophotometer (UV 1900, Shimadzu) having UV probe software version 2.70 with spectral bandwidth of 0.1-nm, wavelength accuracy ± 0.05 nm and a pair of 10 mm matched quartz cell cuvette. An analytical balance was used for weighing the reagents.

Method Development

Implementation of AQbD Approach in the Development of the Analytical Method

A typical fishbone diagram (Fig. 1) was generated, which represents the relationship between critical method variable (CMV) and critical analytical attribute (CAA). It was used for the subsequent Control-Noise-Experimental (C-N-X) analysis. C-N-X approach was undertaken for the identification of critical quality risk variables.[12] These variables were then assigned to scores and the total score was evaluated to identify the CMVs. The variables studied during the C-N-X analysis included variation in solvent used, wavelength detection, sampling interval, scanning speed etc.

Preliminary Selection of Solvent and Wavelength

The solvent selection is based on the maximum solubility and stability of the remdesivir over the period. To serve this purpose, the solubility of drug sample was tested in various solvents like methanol, water, 0.01 N HCl and 0.01 N NaOH, and the observed results were then compared. The remdesivir standard solution (10 µg/mL) was prepared in methanol, 0.01 N NaOH, and 0.01N HCl as a diluent. Solution was scanned using double beam UV-vis spectrophotometer between 200 to 400 nm to determine the maximum wavelength. Remdesivir shows λ_{max} of 245.5 nm, 241 nm and 239.2 nm in methanol, 0.01 N NaOH and 0.01 HCl, respectively (Supplementary Material 1). The absorbance of standard samples were monitored in different solvents and the stability of standard samples against different period (Fig. 2). The maximum solubility and stability of remdesivir were found in methanol hence the, methanol was selected as a solvent to determine the remdesivir at maximum wavelength 245.5 nm by UV spectrophotometer analytical method of remdesivir (Fig. 3). Stirring of methanol shows less significant change in the absorbance and linearity.

Selection of Instrumental Parameters

Center Composite Design (CCD) was employed to identify optimum method conditions and to ensure robustness. Face centered CCD was exercised with minimum five center points, producing total 13 experiments for optimizing CMVs such as sampling interval (A) and scanning speed (B), as obtained from the risk assessment studies.[14] The absorbance of standard remdesivir 10

µg/mL at 245.5 nm was determined and assessed as per CCD as the response variable. Sampling intervals of 0.05, 0.2, and 1-nm, as well as the scanning speed parameters - low, medium and high were evaluated for the design of experiment (DoE) purpose. The data obtained form this was incorporated in an appropriate mathematical model using Design Expert® version 13 software. Correlation between CMVs and CAA was determined by evaluation of contours as well as response surface plots. Further, the software optimized the method conditions via numerical and graphical mode.

Force Degradation Studies

The force degradation behavior of remdesivir was assessed under acidic, basic, oxidative, photolytic and thermolytic stress conditions. The standard solution of remdesivir (1-mg/mL) were directed with 0.1 N HCl, 0.1 N NaOH and 3% H_2O_2 at room temperature for 2 hours to analyze acidic, basic and oxidative degradation, respectively. The thermal and photolytic degradation of remdesivir was conducted in solid state. For the thermal stress testing, solid samples of drug substances were put into a controlled-temperature oven at room temperature and at 60°C for 24 hours; and for the photolytic degradation testing, the solid samples were placed under UV cabinet at 254 nm for 24 hours. A blank solution was prepared by applying it to all stressed conditions. The solutions were further processed to neutralize with relative acidic (0.1 N HCl) and basic (0.1 N NaOH) solution. The suitable aliquots were taken from these force degradation solutions and diluted with methanol to achieve 10 µg/mL and analyzed the sample against relative blank standard samples. The percent degradation was measured with the comparison against absorbance of standard solution and absorbance reduced.

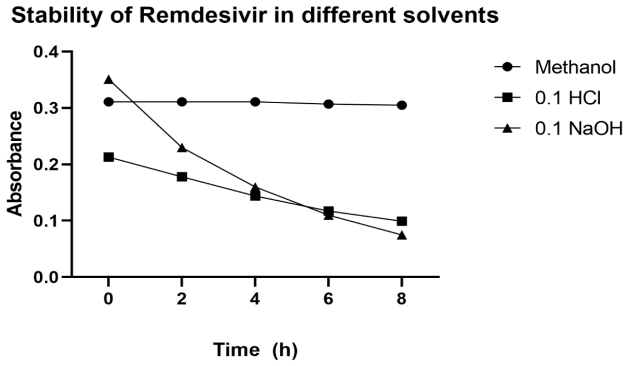

Fig. 2: Stability of remdesivir in different solvents

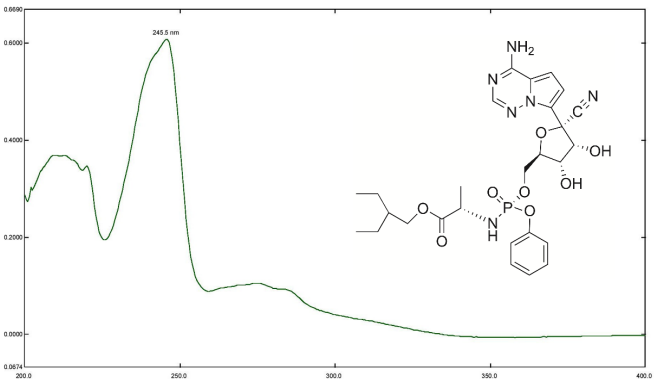

Fig. 3: UV-spectra of standard remdesivir solution in methanol

Method Validation

The specificity of the method was measured to observe interference of matrices present throughout the sample analysis. Therefore, the spectra were taken for methanol, water for injection, standard and test solution of remdesivir for any possible interference. The linearity was evaluated by constructing the three calibration curves with six concentrations of 2, 4, 6, 8, 10 and 12 µg/mL at 245.5 nm. The calculation of linear regression was employed by the least squares analysis method.

The determination of precision was realized through the replication of six determination of standard concentration solution (5 µg/mL) in the same day for intraday precision (repeatability) and on three consecutive days for interday precision (intermediate precision). The relative standard deviation (RSD) was determined.

The accuracy was calculated about percentage recovery by assay of the known added amount of remdesivir standard substance in the sample solutions using three concentrations level and three replicates of each concentration adding 80, 100 and 120% of the remdesivir concentration in the sample covering the specified range at 9, 10 and 11 µg/mL. The amount of drug present in recovery solutions was calculated using calibration curve. Robustness of the UV spectrophotometry analytical method was determined by the analysis of appropriate concentrations of remdesivir at different wavelengths (245.5 ± 2 nm) and at stirring the solvent with different time interval. The RSD less than 2% was set as an acceptable criterion for robustness.

Analysis of Marketed Formulation

The parenteral preparation of remdesivir injection (100 mg/20 mL) was used for the assay analysis. The 2 mL of injection solution equivalent to 10 mg of drug was diluted in to 10 mL of methanol and sonicated to make the final concentration of 1000 µg/mL. 1-mL of the solution mentioned above was taken into a 10 mL volumetric flask and was diluted up to 10 mL with methanol. The resultant solution having a concentration of 100 µg/mL remdesivir sample solution was determined using standard plot of remdesivir.

Results

Method Development

In this study, UV spectrophotometric methods was developed to determine the content of remdesivir in marketed formulation. Remdesivir was soluble in methanol, 0.01 N

HCl and 0.01 N NaOH. The standard stock solutions were prepared in different solvents and responses were measured at different time interval. The preliminary results with different solvents and time demonstrated (Fig. 2) that the remdesivir absorbance in methanol did not decrease in 245.5 nm for 72 hours, confirming the stability of the drug in these conditions. The stability of Remdesivir in methanol is higher compared to other solvents. Thus, methanol has been selected as a suitable solvent for further studies. The standard drug solution shows a sharp peak at 245.5 nm in methanol (Fig. 3).

The CMVs were obtained utilizing the QbD approach to develop final spectroscopic conditions. Ishikawa fish bone diagram and cause effect (C-E) risk assessment matrix (Table 1) were used to identify CMVs, employing the C-N-X approach. Total scores were calculated for different method variables and prioritized for DoE investigation.

3D response surface plots and 2D contour plots were used for response surface analysis. The 3D response surface for Y1 (Fig. 4a) displays a typical saddle system. The absorbance taken at 245 nm is much higher at low levels of sampling interval and scanning speed compared to other levels of both the factors. The 2D contour plot (Fig. 4b) exhibited a minimax system, which agrees with the three-dimensional response surface.

Further, the design space (Fig. 4c) for optimized conditions supports advancing the study with selected middle to high values, 0.2 nm sampling interval and Fast scan speed for both the factors.

Method Validation

The results met the system suitability requirements, indicating that the system was suitable for the analysis. The linearity plot of absorbance v/s concentration obeyed Beer Lambert's law in 2 to 12 µg/mL concentration range with a satisfactory correlation coefficient of 0.999 shown in Fig. 5.

The low RSD obtained for intraday and interday precision ranged from 0.57 to 1.92% showed good precision of the method. The accuracy of the method ranged from 91.93 to 93.73%. These values demonstrated the good accuracy of the proposed method. The method validation parameters and its values have been summarized in Table 2.

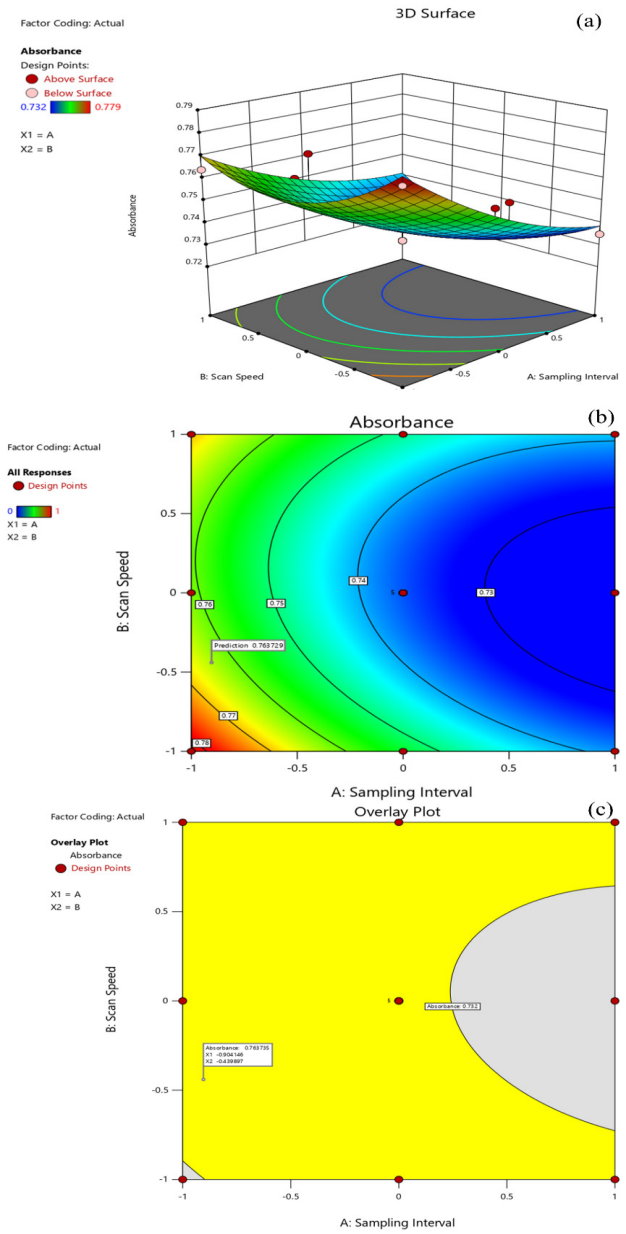

Fig. 4: (a) 3-D surface plot profilers (b) 2-D contour plot (c) Overlay plot, depicting analytical design space, obtained for response at 245.5 nm

Table 1: Cause Effect based risk assessment

Cause	*Effect of Risk level on absorbance*	*Total Score*	*C,N,X*	*Strategy*
Scanning Speed	10	100	X	DoE
Sampling Interval	10	100	X	DoE
Solvent	5	50	C	Controlled
Detection Wavelength	4	40	C	245.5 nm
Sample Purity	4	40	N	Quality Assessed
Sample Preparation	4	40	C	Controlled
Detector Equilibration	3	30	C	Controlled

C, N, X- Control, Noise, Experimental, Total Score = (Risk Level of CAA×10),
Score: 1 - Negligible risk, 5 - Low risk, 10 - High risk

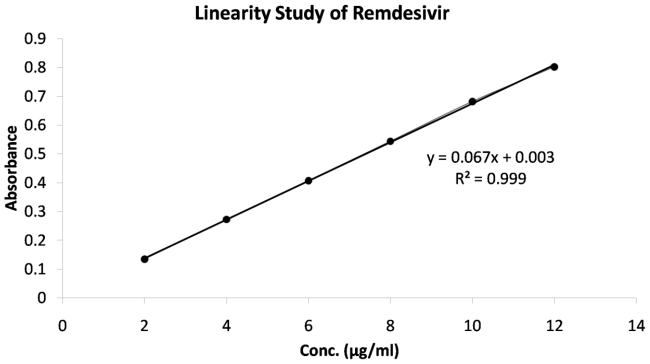

Fig. 5: Linearity curve of remdesivir

Table 2: Summary of validation parameters of remdesivir

Parameters	*Values*
Maximum wavelength (λmax)	245.5 nm
Linearity range (µg/mL)	2–12
Regression equation (y=mx+c) (n=3)	
Slope[a] (m)	0.065 ± 0.005
Intercept[a] (c)	0.005 ± 0.004
Correlation coefficient (*r*)	≥ 0.999
Precision (n=6) (RSD %)	
Repeatability	1.90
Intra-day[b]	1.16–1.84
Inter-day[b]	1.69–1.93
Accuracy (n=3) (%)	
Recovery range	91.09–94.18
LOD (µg/mL)	0.23
LOQ (µg/mL)	0.69
Robustness (n=6) (RSD %)	
Wavelength change[b] (± 2 nm)	0.45–1.84
With/without stirring	0.002–0.007
Assay (%)	95.07 ± 2.43

[a]Mean value ± SD, [b]Range of RSD (%), [c]Stirring measured at 0 and 8 min

The limit of detection limit (LOD) and limit of quantitation (LOQ) were determined following the ICH guidelines and their values were recorded to be 0.23 and 0.69 µg/mL, respectively.

Forced Degradation Studies

Table 3 represents the results of forced degradation studies of remdesivir by the developed UV spectrophotometry method using methanol as solvent. Remdesivir showed the degradation up to 15% and 25% under stressed acidic and basic condition applied for 2 hours. Under stressed oxidative conditions with 3% H_2O_2, degradation of remdesivir was found up to 12%. The least significant changes were seen when the drug was exposed in light and heat for 24 hours.

Table 3: Results of forced degradation studies of remdesivir by UV-spectrophotometry

Stress Conditions	*Concentration after degradation (µg/mL)*	*Recovery (%)*	*Degradation (%)*
Acid Induced	8.55 ± 0.03	85.46 ± 0.31	14.54 ± 0.31
base Induced	7.51 ± 0.02	75.05 ± 0.22	24.95 ± 0.22
Oxidative	8.82 ± 0.01	88.17 ± 0.10	11.83 ± 0.10
Thermal	9.36 ± 0.01	97.76 ± 0.11	2.24 ± 0.11
Photolytic	9.78 ± 0.01	93.62 ± 0.15	6.38 ± 0.11

Data represented as mean ± SD

Discussion

It was implemented to develop robust UV spectrophotometric methods for estimating remdesivir in bulk and its formulation QbD approach. For setting up the method control strategies and for continual improvement in method performance, two most CMV are employed, i.e., scanning speed and sampling interval. Another critical parameter that may affect purity, potency and safety of a drug product or a drug substance is stability. Variation in drug stability can put patient safety at risk either by forming a toxic degradation product(s) or by delivering a lower dose than anticipated. Hence, understanding of purity profile and behavior of the drug substance under various environmental conditions is crucial. The method for quantifying remdesivir by UV-spectrophotometry using methanol as solvent was successfully developed and validated as per the ICH guideline. The results suggest method novelty, simplicity, accuracy and preciseness.
Furthermore, the overall results obtained from forced degradation studies of remdesivir by the developed UV-spectrophotometric method in methanol demonstrate significant degradation of remdesivir in stress conditions. Preliminary results showed itself as stability indicating method, and can be further investigated by chromatographic techniques for the identification and quantification of degradation product/s. The QbD-based spectrophotometric method can be used flexibly and efficiently for quantitative estimation of remdesivir in bulk or in the dosage formulations.

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Abbreviations

AQbD: Analytical Quality by Design
C-E: Cause and Effect
C-N-X: Control-Noise-Experimental
CAA : Critical Analytical Attribute
CCD : Central Composite Design
CMV: Critical Method Variable
DOE: Design of Experiment

MODR: Method Operable Design Region
RSD : Relative Standard Deviation

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