IJPSR (2023), Volume 14, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 July 2022; received in revised form, 21 August 2022; accepted 01 September 2022; published 01 March 2023

AN RP-HPLC METHOD FOR THE QUANTITATIVE DETERMINATION OF REMDESIVIR IN INTRAVENOUS DOSAGE FORM

Kashyap Thummar *, Malay Pandya, Nisarg Patel and Foram Rawat

Graduate School of Pharmacy, Gujarat Technological University, Ahmedabad - 382424, Gujarat, India.

Keywords:

Covid-19, Remdesivir, HPLC, ICH

Correspondence to Author:
Kashyap Naranbhai Thummar

Assistant Professor,
Graduate School of Pharmacy,
Gujarat Technological University,
Ahmedabad - 382424, Gujarat, India.

E-mail: kasu_patel5@yahoo.co.in

ABSTRACT: The worldwide epidemic of Coronavirus disease 2019 (COVID-19), caused by a new virus known as severe acute respiratory syndrome (SARS) coronavirus 2, has posed a growing threat to public health (SARS-CoV-2). The only antiviral drug authorized by the FDA for treating adult and pediatric patients hospitalized with a severe disease is remdesivir, which is given intravenously (IV). Although only a few methods for estimating remdesivir in pharmaceutical formulations using high-pressure liquid chromatography (HPLC) have been described, its determination still requires an accurate, precise, quick, and easy analytical methodology. The main goal of this study was to develop and validate a reliable and accurate HPLC method for quantitative estimation of remdesivir in its intravenous dosage formulation. The separation was performed on a C18 (4.6 mm x 150 mm, 5.0 µm) column with a flow rate of 0.7 mL/min and a total run duration of 6 minutes using a simple isocratic mobile phase of acetonitrile and 0.1 percent formic acid. The method was validated for the system suitability, linearity, precision, accuracy, robustness, and others as per the International Council for Harmonization (ICH) Q2 (R1) guideline. The results show that the method for measuring remdesivir using HPLC is simple, quick, sensitive, accurate, precise and robust. The described approach was successfully used to quantify remdesivir in a commercially available pharmaceutical formulation.

INTRODUCTION: The COVID-19 epidemic threatens healthcare systems in several countries throughout the world, perhaps resulting in a global health calamity. As a result of this situation, there has been a surge in interest in developing a treatment to mitigate the epidemic's consequences on human life. As our understanding of the disease grows, so does the majority of the approach to patient management. New information is supporting us in making sound COVID-19 management decisions [1]. Newer antivirals, such as remdesivir and favipiravir, have been demonstrated in clinical studies to improve patient recovery and reduce mortality [2]. Remdesivir **Fig. 1**, administered intravenously (IV), is the only antiviral medicine approved by the FDA for the treatment of adult and pediatric patients hospitalized with a serious illness [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(3).1273-79

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.14(3).1273-79

Remdesivir is a broad-spectrum antiviral agent that works as a nucleotide analogue inhibitor of RNA-dependent RNA polymerase. It has been demonstrated that remdesivir has antiviral efficacy against the SARS and MERS RNA viruses, and it has sparked considerable interest in its possible application in the treatment of COVID-19 [4].

Increasing Covid-19 cases led to an increase in demand for antivirals and vaccinations, which led to widespread use, stockpiling, and black marketing, resulting in artificial shortages and price increases across the globe [5]. Because of remdesivir's medicinal potential and rising use, an appropriate and quick assay approach for its determination in bulk and pharmaceutical formulations is required to identify remdesivir by both qualitative and quantitative assessment.

According to a study of the literature, few analytical techniques such as HPLC [6-8] method for determining remdesivir from bulk and formulation, have been published, and few bioanalytical approaches in human plasma using UHPLC-MS/MS [9-12]. The LC-MS-based approach for identifying and characterizing remdesivir degrading products has just been disclosed [7].

Therefore, the current effort aims to develop and fully validate a simple, rapid and precise high-pressure liquid chromatographic (HPLC) technique for remdesivir per International Council for Harmonization (ICH) guidelines [13].

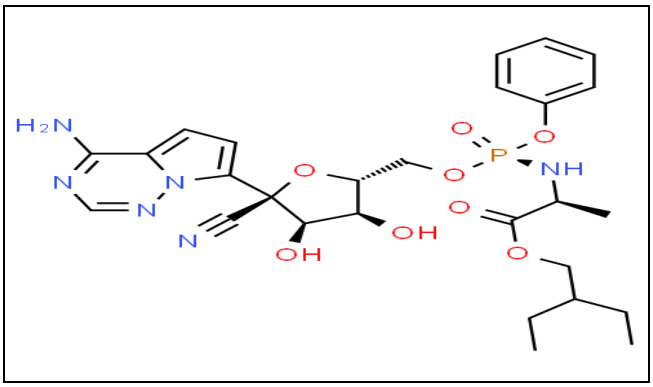


FIG. 1: MOLECULAR STRUCTURE OF REMDESIVIR

MATERIALS AND METHODS:

Chemicals and Reagents: HPLC grade acetonitrile and methanol from Centre Drug House (CDH, India) fine chemicals as well as Emplura grade formic acid from Merck were utilized. HPLC grade water were used throughout the study from Merck Millipore. Remdesivir Active Pharmaceutical Ingredient (API) powder was procured from Cadila Healthcare, Gujarat, India.

Preparation of Standard Solution: Accurately weighed 50 mg equivalent of remdesivir API was transferred to a 50 mL volumetric flask and dissolved in around 30 mL of methanol, sonicated for 5 minutes, and the final volume was brought up to 50 mL to achieve 1 mg/mL of standard stock solution. Suitable aliquots were taken and diluted up to 10 mL with methanol to generate ten linearity standards at concentrations of 2, 4, 6, 8, 10, 20, 40, 60, 80, and 100 µg/mL.

Instrumentation and Chromatographic Optimization: The chromatographic analysis was performed on a Zorbax Eclipse plus C18 (4.6 mm x 150 mm, 5.0 µm) column utilizing an Agilent 1260 Infinity II with a 600 bar HPLC system. Various organic solvents, including acetonitrile, methanol, and buffer solutions such as ammonium formate, formic acid, and ammonium acetate, have been tested for the formulation of the mobile phase. The mobile phase, composed of acetonitrile and 0.1 % formic acid in water (45:55, v/v), achieves the desired result with optimum efficiency. The mobile phase was degassed by an ultrasonic bath and filtered through a 0.45μm membrane filter under vacuum. The flow rate was optimized at 0.7 mL/min, and column has been thermostated at 30 °C. The detection wavelength was set at 245 nm in photodiode array (PDA) detector. The chromatographic data were recorded using OpenLab CDS software in the computed system.

Method Validation: The analytical technique was validated in accordance with the ICH standards for Validation of Analytical Procedure: Q2 (R1). Specificity, linearity, accuracy, precision, the limit of detection (LOD), quantification (LOQ), robustness, and system suitability tests were all addressed.

Solution Stability: The stability of the sample and standard solutions was monitored for 24 hours. This was accomplished by injecting standard solutions into the system thrice over eight hours and measuring the peak area and retention time. Standard solutions were held at 2-8 °C for the stability investigation.

Specificity / Selectivity: The ability of an analytical method to produce a response for the analyte in the presence of extraneous interference is referred to as selectivity. The method's selectivity was determined by comparing the chromatograms obtained for diluents, placebo (Water for Injection), remdesivir standard sample solutions.

The parameters retention time and tailing factor were obtained to indicate that the methodology used was unique.

Linearity: The linearity of the remdesivir HPLC technique was tested at ten different concentration levels: 2, 4, 6, 8, 10, 20, 40, 60, 80 and 100 µg/mL. Peak areas were measured to determine the quantitative concentration of remdesivir. The method's linearity was confirmed using a least squares linear regression analysis of peak area vs concentration data. The coefficient of determination (r^2) values better than 0.999 ($r^2 \geq 0.999$) were used as a criterion for linearity.

Precision: Precision was determined by considering intraday (repeatability obtained by analysing a standard solution on the same day) and interday variations (repeatability carried out by analyzing a standard solution on three consecutive days). The precision study was conducted by injecting six duplicates of standard solution at 40 µg/mL on the same day and three days in a row.

Accuracy: The method's accuracy was assessed by performing recovery tests at three concentrations that were 80 %, 100 %, and 120 % of the target level of remdesivir (40 µg/mL) using the standard addition technique. At each level, the trials were repeated in triplicate and % recoveries were determined. For each concentration, the % recovery and percentage relative standard deviation (%RSD), were computed.

Limit of Detection and Limit of Quantification: Several approaches for determining the detection (LOD) and quantification (LOQ) limits are described in the ICH guidelines. In this study, the LOD and the LOQ were based on the response (s) standard deviation, and the slope of the regression line (m) were calculated following equations.

$$LOD = 3{:}3*s/m$$

$$LOQ = 10*s/m$$

Robustness: A robustness study was conducted to assess the impacts of minor but consistent changes in chromatographic settings. Variable mobile phase flow rates (0.65 and 0.75 mL/min), acetonitrile ratio in the mobile phase (53 and 57%) and column temperatures are among the modifications (27 and 33 °C). The system suitability parameters were examined after each change and the results were compared to those in the original chromatographic conditions.

Analysis of Commercial Formulation: In a 50 mL volumetric flask, 10 mL of sample (Covifor™ Injection, Hetero Healthcare, 5 mg/mL) solution containing 50 mg of remdesivir drug was dissolved in around 30 mL of methanol. After sonicating the mixture for 5 minutes, it was diluted to volume using methanol to get the final concentration (1mg/mL). Before diluting further, the resultant solution was filtered using a 0.2 µm nylon syringe filter. The final test solution was created by properly measuring 0.4 mL of the previously produced sample solution into a volumetric flask of 10 mL and filling the mark with methanol to reach a theoretical concentration of 40 µg/mL.

RESULT AND DISCUSSION:

Method Development and Optimization of Chromatographic Conditions: The remdesivir stock solution remained stable in methanol for more than 24 hours [14]. Therefore, it was chosen as the diluent. In the UV spectrum, the remdesivir has a maximum absorbance (λmax) of 245 nm in methanol and same has been chosen as the detecting wavelength. Several chromatographic trials were taken on a Zorbax Eclipse plus C18 (4.6 mm x 150 mm, 5.0 µm) column (Agilent Technologies Pvt. Ltd.).

Initially, methanol and water were combined with volatile buffers, the drug was not held well, and the drug was asymmetrically shaped. Then, a trial with acetonitrile and 0.1% formic acid in water (50:50 %, v/v) on the same column at 1 mL/min flow rate resulted in an early elution at 2.25 minutes with a poor peak shape. Additional studies were conducted in the same mobile phase systems with 10 percent reduction in the acetonitrile component and an increase in the same formic acid percentage. We observed that the acetonitrile component higher than 45 % with 1 mL/min flow rate was leads to the early elution of the drug near column dead volume (~Rt 2.5 min). Then a 40:60 percent v/v solution was tested, which gave the drug elution at a longer Rt (Rt > 6 min) with less than 0.8 peak tailing. Then, at a flow rate of 0.7 mL/min, a mixture of acetonitrile and 0.1% formic acid in water (45:55 %, v/v) provided optimal drug elution. A sharp

peak, rapid elution and good intensity of peak were found in isocratic mode of elution. The system suitability was tested, and the findings were confirmed to be within limitations. The standard and test solutions were set to inject 10 µL only to minimize column loading and, if any, carryover. In the case of remdesivir, the column temperature was fixed at 30 °C, and altering the temperature from 25 to 35 °C had no significant effect on drug elution or system suitability metrics. **Table 1** summarizes the optimized HPLC settings for remdesivir.

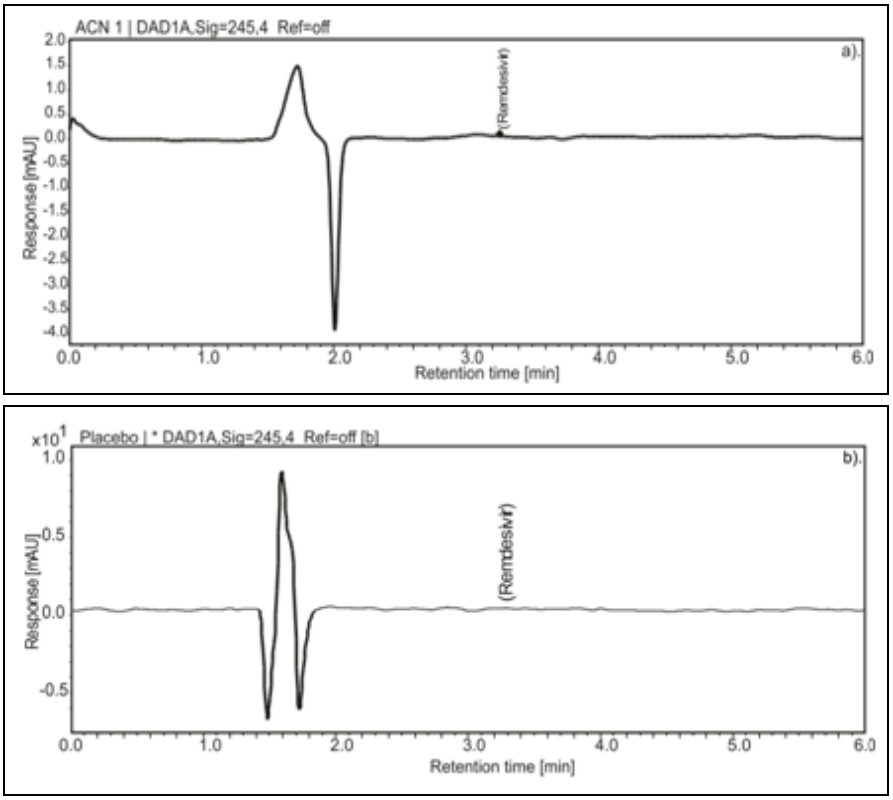
TABLE 1: RP-HPLC OPTIMIZED CONDITION

Parameter	Chromatographic Condition
Elution mode	Isocratic
Mobile Phase	Acetonitrile and 0.1 % Formic Acid in Water (45:55, v/v)
Column	Zorbax Eclipse plus C18 (4.6 mm x 150 mm, 5.0 µm)
Flow Rate	0.7 mL/min
Detection Wavelength	245 nm
Injection Volume	10 µL
Temperature	30 °C
Retention Time (± SD)	3.296 ± 0.001 min
Run Time	6 min
USP Plates (± SD)	7884 ± 211
USP Tailing (± SD)	1.11 ± 0.01

Method Validation:

Solution Stability: No significant changes in standard concentrations have been observed over a period of 24 h. The % RSD for peak area (n = 3) was 0.78 % and the value for retention time (n = 3) was 0.6 % for standard solution.

Specificity: To demonstrate that the approach used was specific, the parameters tailing factor, retention time, and theoretical plates were measured. The mean peak tailing factor, retention time, and theoretical plate number have all been 1.12, 3.28, and 7884, respectively. The values were within the acceptable range, and any other component or excipient did not affect the remdesivir chromatogram **Fig. 2.**



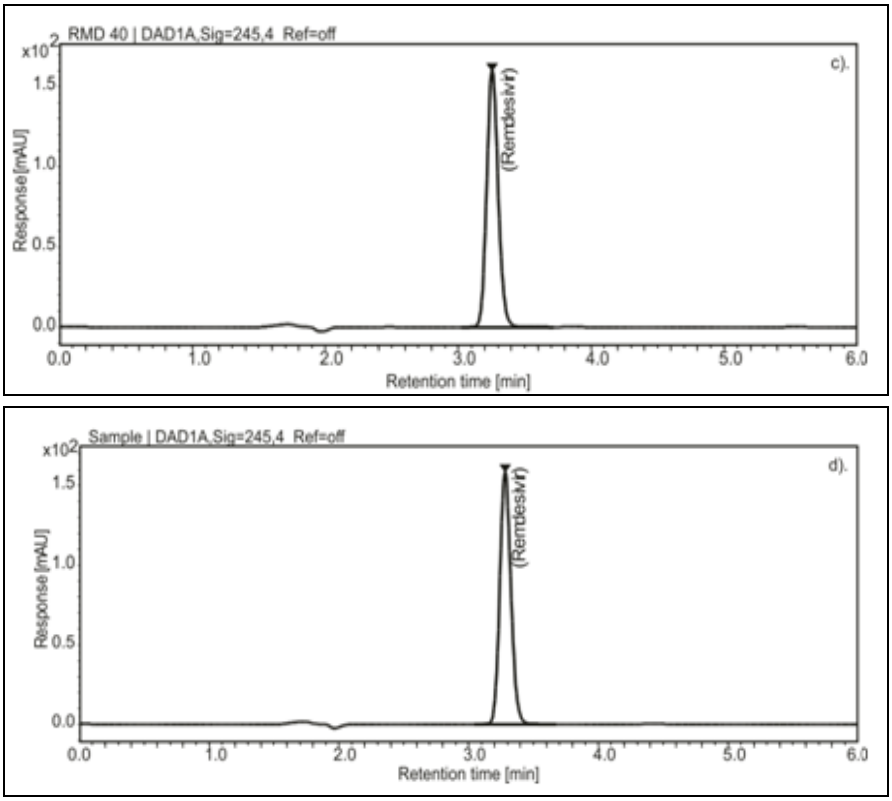


FIG. 2: HPLC CHROMATOGRAM OF (A) BLANK SAMPLE (DILUENT) (B) PLACEBO (C) REMDESIVIR STANDARD AT 40 µG/ML (D) REMDESIVIR SAMPLE AT 40 µG/ML

Linearity, Precision and Accuracy: The calibration curve was developed by plotting the average peak area versus the standard concentration of remdesivir **Fig. 3**. The average slope and intercept values were 23.57 ± 0.29 and 0.91 ± 4.088, respectively. The average coefficient of determination (r^2) was achieved to 0.999 ± 0.0002. A precision study of intraday and interday was performed to determine the precision of the developed method. The % RSD value for interday and intraday precision was found to be 0.571 and 0.579, respectively. Hence, the method is precise and can be used for our intended purpose.

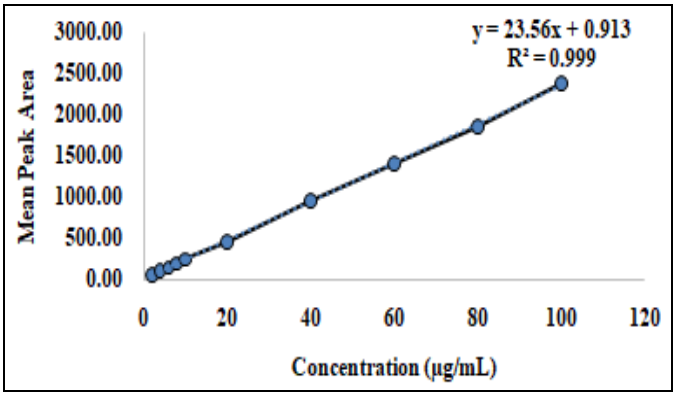


FIG. 3: STANDARD CALIBRATION CURVE OF REMDESIVIR (MEAN PEAK AREA V/S CONCENTRATION) (N=3)

The mean recovery data from the study of remdesivir was 99.47%. The % RSD values for all analyses were less than 1%, indicating that excipients found in remdesivir intravenous dosage formulation do not interfere and analytical method is very accurate. The linearity, precision, and accuracy data were given in **Table 2.**

TABLE 2: SUMMARY OF ANALYTICAL METHOD VALIDATION PARAMETERS OF

Parameters	values
Linearity range (µg/mL)	2 – 100
Regression equation (y = mx ± c) (n=3)	
Slope[a] (m)	23.57 ± 0.295
Intercept[a] (c)	0.914 ± 4.088
Correlation coefficient (*r*)	≥ 0.999
Precision (n=6) (RSD %)	
Repeatability	0.995
Intra-day[b]	0.571 – 0.995
Inter-day[b]	0.441 – 0.684
Accuracy (n=3) (%)	
Recovery range (%)	98.4 – 100.6
LOD (µg/mL)	0.57
LOQ (µg/mL)	1.73
Robustness (n=6) (RSD %)	
Flow rate (± 0.1 mL)	0.87 - 1.12
Mobile phase composition [b] (± 2 mL, v/v)	0.81- 1.24
Column Oven Temperature[b] (± 5 °C)	1.55-1.90
Assay (%)	98.07 ± 1.23

[a]Mean value ± SD, [b]Range of RSD (%)

Robustness: The results showed that the change in flow rate and mobile phase concentration had little effect on the chromatographic behavior of remdesivir.

The small change in the mobile phase flow rate and acetonitrile content has a small impact on the retention time of remdesivir. The change in the column temperature did not significantly affect the method. The results of this study, expressed as % RSD, were presented in **Table 2.**

Application of the Method to the Marketed Formulation: The developed and validated method has been successfully applied to determine remdesivir in pharmaceutical formulations. The result of the assay of the marketed intravenous dosage formulation of remdesivir was shown in **Table 2**.

The results obtained are closely related to the amount indicated on the labels of the formulations. This shows that the method will be useful for the quantitative evaluation of remdesivir in their pharmaceutical dosage form.

CONCLUSION: The results of our study indicate that the proposed RP-HPLC method is simple, rapid, precise, and accurate. The developed HPLC method was found suitable for the determination of remdesivir in bulk drugs and in intravenous dosage formulations without any interference from the excipients. Statistical analysis proves that the method is repeatable and selective for the analysis of remdesivir. Therefore, it can be concluded that the method can save much time and money and can be used in small laboratories with very high accuracy and a wide linear range. This method has a slower flow rate (0.7 mL/min) and volatile organic buffer (0.1% formic acid); this approach is suitable with mass spectrometric analysis for further analysis of degradation products or bioanalysis of remdesivir. Therefore, the method has wide applicability of routine quality control of remdesivir assay in its pharmaceutical dosage form and has a future scope of further analysis extension.

ACKNOWLEDGEMENT: The authors greatly acknowledge the funding provided by Gujarat Technological University's Seed Money Scheme for PG Scholars and faculties enabling this work to be concluded.

CONFLICTS OF INTEREST: It is declared that the authors do not have any conflict of interest.

REFERENCES:

1. Dixit SB, Zirpe KG, Kulkarni AP, Chaudhry D and Govil D: Current Approaches to COVID-19: Therapy and Prevention. Indian Journal of Critical Care Medicine 2020; 24(9): 838-846.
2. Gilead. COVID 19: Gilead's Response to COVID-19. Gilead Sciences, Inc, USA. Available from: https://www.gilead.com/purpose/advancing-global-health/covid-19. [Accessed on 20th January, 2022].
3. USFDA. FDA NEWS Release. FDA Approves First Treatment for COVID-19. FDA Office of Media Affairs, USA. Available from: https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-covid-19. [Accessed on 4th November, 2021].
4. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institute of Health, USA. Available from: https://www.covid19treatmentguidelines.nih.gov/antiviral-therapy/remdesivir/. [Accessed on 4th December, 2021].
5. Dawoud D, Chalkidou K, Sullivan R, Ruiz FJ and Adler A: USA stockpiling of remdesivir: How should the world respond? J Comp Eff Res 2020; 9(18): 1243-1246.
6. Bulduk I and Akbel E: A comparative study of HPLC and UV-spectrophotometric methods for remdesivir quantification in pharmaceutical formulations. Journal of Taibah University for Science 2021; 15(1): 507-513.
7. Dadinaboyina SB, Yerra NV, Adimoolam BM, Parsa S and Bathini NB: Identification and characterization of degradation products of Remdesivir using liquid chromatography/mass spectrometry. New Journal of Chemistry 2021; 45(16): 7217-7224.
8. Jitta SR, Salwa L. Kumar, Gangurde PK and Verma R: Development and Validation of High-Performance Liquid Chromatography Method for the Quantification of Remdesivir in Intravenous Dosage Form. Assay and Drug Development Technologies 2021; 19(8): 475-483.
9. Alvarez JC, Moine P, Etting I, Annane D and Larabi IA: Quantification of plasma remdesivir and its metabolite GS-441524 using liquid chromatography coupled to tandem mass spectrometry. Application to a Covid-19 treated patient. Clinical Chemistry and Laboratory Medicine (CCLM) 2020; 58(9): 1461-1468.
10. Avataneo V, de Nicolò A, Cusato J, Antonucci M and Manca A: Development and validation of a UHPLC-MS/MS method for quantification of the prodrug remdesivir and its metabolite GS-441524: a tool for clinical pharmacokinetics of SARS-CoV-2/COVID-19 and Ebola virus disease. J Antim Chemo 2020; 75(7): 1772-77.
11. Du P, Wang G, Yang S, Li P and Liu L: Quantitative HPLC-MS/MS determination of Nuc, the active metabolite of remdesivir and its pharmacokinetics in rat. Analytical and Bioanalytical Chemistry 2021; 413(23): 5811-5820.
12. Habler K, Brügel M, Teupser D, Liebchen U and Scharf C: Simultaneous quantification of seven repurposed COVID-19 drugs remdesivir (plus metabolite GS-441524), chloroquine, hydroxychloroquine, lopinavir, ritonavir, favipiravir and azithromycin by a two-dimensional isotope dilution LC–MS/MS method in human serum. J of Pharma and Biomedical Analysis 2021; 196: 113935.
13. International Council for Harmonization Validation of Analytical Procedures: Text and Methodology Q2 (R1). 2005: Geneva 1-17.

14. Thummar K., Pandya M, Patel N, Rawat F and Chauhan S: QbD based Development and Validation of UV Spectrophotometry Method for the Determination of Remdesivir in Bulk and Marketed Formulation. Int J of Pharmaceutical Sciences and Drug Research 2022; 14(4).

How to cite this article:
Thummar K, Pandya M, Patel N and Rawat F: A RP-HPLC method for the quantitative determination of remdesivir in intravenous dosage form. Int J Pharm Sci & Res 2023; 14(3): 1273-79. doi: 10.13040/IJPSR.0975-8232.14(3).1273-79.