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Development and Validation of a Simple RP-HPLC Method for Quantitative Determination of Remdesivir in Bulk Drug and Pharmaceutical Dosage Form

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ABSTRACT

Background: Remdesivir is an antiviral nucleotide analogue used in the management of COVID-19. Reliable analytical procedures are essential for quantitative determination of remdesivir in pharmaceutical products and for routine quality-control analysis.

Objective: The present study aimed to develop and validate a simple, precise, accurate, specific, sensitive, robust and rugged RP-HPLC method for quantitative determination of remdesivir in bulk drug and pharmaceutical dosage form.

Methods: Chromatographic method development was performed using a reversed-phase C18 column. Different mobile-phase compositions and chromatographic conditions were evaluated to obtain satisfactory peak shape and chromatographic performance. The final validated method employed a C18 column (250 × 4.6 mm, 5 µm) with phosphate buffer (0.02 M, pH 3.5):acetonitrile (60:40, v/v) as an isocratic mobile phase at a flow rate of 1.0 mL/min. Detection was performed at 245 nm with an injection volume of 20 µL and a total run time of 6 min. The method was validated for system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), robustness and ruggedness.

Results: The developed method produced a well-defined remdesivir peak at a mean retention time of 5.82 min. System-suitability assessment demonstrated a mean theoretical plate count of 5467 and a mean tailing factor of 1.09, with retention-time %RSD of 0.12%. The method was linear over the concentration range of 5–50 µg/mL Mean recovery was 99.57%. Intra-day and inter-day precision showed %RSD values of 0.10% and 0.12%, respectively. LOD and LOQ were 0.095 and 0.287 µg/mL, respectively. No significant interference from blank or placebo was observed at the remdesivir retention region. Ruggedness studies using different analysts and instruments also demonstrated satisfactory reproducibility.

Conclusion: The validated RP-HPLC method demonstrated satisfactory specificity, linearity, accuracy, precision, sensitivity, robustness and ruggedness. The method is simple and suitable for routine quantitative determination of remdesivir in bulk drug and pharmaceutical dosage forms.

Introduction

Remdesivir is a nucleotide analogue phosphoramidate prodrug possessing antiviral activity and has been extensively investigated for the treatment of viral infections, including COVID-19. The increasing pharmaceutical use of remdesivir necessitates reliable analytical procedures capable of accurately determining drug content in active pharmaceutical ingredients and finished dosage forms [1].

Pharmaceutical analysis requires analytical methods that are sufficiently selective, accurate, precise and reproducible for their intended application. High-performance liquid chromatography (HPLC), particularly reversed-phase HPLC (RP-HPLC), is one of the most widely employed analytical techniques for pharmaceutical quality control because of its versatility, reproducibility and ability to separate analytes from formulation components and potential degradation products.

During development of an RP-HPLC method, chromatographic parameters including stationary phase, mobile-phase composition, pH, flow rate, detection wavelength and injection volume must be optimized to achieve satisfactory peak shape, retention and resolution. Following development, validation is required to demonstrate that the method is suitable for its intended analytical purpose [2].

In the present study, an RP-HPLC method was developed for quantitative estimation of remdesivir. Initial chromatographic optimization involved evaluation of different mobile phases. The thesis reports that acetonitrile:phosphate buffer at pH 3.5 produced satisfactory chromatographic characteristics during the preliminary optimization stage. Subsequently, the chromatographic procedure was further optimized, and a final validation protocol was established. The thesis explicitly identifies phosphate buffer (0.02 M, pH 3.5): acetonitrile (60:40, v/v), a flow rate of 1.0 mL/min, a C18 column and UV detection at 245 nm as the conditions used for the validation experiments [3].

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The objective of the present investigation was therefore to validate this final RP-HPLC procedure according to the validation parameters reported in the thesis and establish its suitability for routine quantitative determination of remdesivir.

Materials and Methods

Materials

Remdesivir active pharmaceutical ingredient was obtained as a gift sample from Asian Pharma, Baddi, Himachal Pradesh. A commercially available remdesivir injection was obtained from a local registered pharmacy for formulation analysis. Analytical- or HPLC-grade methanol, acetonitrile, water, orthophosphoric acid, potassium dihydrogen phosphate and triethylamine were used during the analytical work. Whatman 0.45 µm membrane filters were used for filtration.

Instruments

The thesis reports use of a Shimadzu LC-20AT/Agilent 1200 series HPLC system for chromatographic analysis, Shimadzu UV-1800 UV-visible spectrophotometer for wavelength assessment, a PCI Instruments ultrasonic bath for degassing and dissolution, an Eutech pH meter for mobile-phase pH adjustment, Shimadzu AY-220 analytical balance for weighing and a Millipore vacuum filtration unit for filtration.

Preparation of Mobile Phase

The method-development section describes preparation of phosphate buffer at pH 3.5 followed by mixing with acetonitrile. The mobile phase was filtered through a 0.45 µm membrane and degassed using an ultrasonic bath for 10–15 min.

Preparation of Standard Solution

Approximately 10 mg of remdesivir was accurately weighed and transferred to a 10-mL volumetric flask. Approximately 5 mL methanol was added, followed by sonication for 10 min, and the volume was made up with methanol to obtain a nominal stock concentration of 1000 µg/mL. Further dilutions were prepared to obtain working concentrations required for method development and validation.

Chromatographic Method Development

RP-HPLC was selected because of the chromatographic behaviour of remdesivir and the suitability of C18 stationary phases for pharmaceutical analysis. The thesis reports evaluation of different solvent systems

and flow rates. Methanol 50:50 produced a broad peak with poor resolution, whereas acetonitrile 60:40 produced a sharp peak but short retention. Acetonitrile buffer at pH 3.5 in a 70:30 ratio was reported during the optimization trials to provide a symmetrical peak with good resolution. A C18 column of dimensions 250 × 4.6 mm with 5 µm particle size was selected, and a flow rate of 1.0 mL/min was considered optimal during the development study [4,5].

Table 1: Mobile-phase optimization.

Trial	Mobile phase	Ratio (v/v)	Observation
1	Methanol	50:50	Broad peak, poor resolution
2	Acetonitrile	60:40	Sharp peak but short retention time
3	Acetonitrile buffer, pH 3.5	70:30	Symmetrical peak with good resolution

Final Chromatographic Conditions Used for Validation

The results chapter identifies the C18 column, phosphate buffer/acetonitrile mobile phase, 1.0 mL/min flow rate, ambient temperature, 20 µL injection volume and UV detection at 245 nm as the chromatographic protocol used for validation.

Table 2: Chromatographic protocol reported for the validation study.

Parameter	Condition reported in thesis
Stationary phase	C18, 250 × 4.6 mm, 5 µm
Mobile phase	Phosphate buffer pH 3.5, 60:40 v/v
Mode	Isocratic
Flow rate	1.0 mL/min
Column temperature	Ambient, 25 ± 2°C
Injection volume	20 µL
Detection wavelength	245 nm
Run time	6 min

Method Validation

The analytical method was evaluated using the validation parameters reported in the thesis.

System Suitability

System suitability was evaluated using six replicate injections of a 20 µg/mL remdesivir standard. Retention time, peak area, theoretical plates and tailing factor were assessed. The thesis reports mean theoretical plates of 5467 and a mean tailing factor of 1.09.

Table 3: System-suitability data.

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	5.82	982345	5450	1.09
2	5.83	984112	5472	1.11
3	5.81	979875	5480	1.10
4	5.84	981642	5461	1.08
5	5.83	983520	5475	1.09
6	5.82	980914	5468	1.11

Mean Rt: 5.82 min; Rt %RSD: 0.12%; mean theoretical plates: 5467; mean tailing factor: 1.09.

The low variation in retention time and favourable theoretical plate number and tailing factor indicate satisfactory chromatographic performance under the conditions reported in the validation dataset.

Specificity

Specificity was evaluated using blank, placebo and standard remdesivir solutions. The thesis reports no peak from the blank at the remdesivir retention region

and no interference from formulation excipients. A sharp remdesivir peak was observed in the standard solution [6]. The project further reports forced-degradation assessment involving acidic/basic hydrolysis, oxidative, thermal and photolytic conditions. Degradation products were reported at retention times different from remdesivir, with resolution from the closest impurity greater than 2.0 and peak-purity index greater than 0.999.

Linearity and Range

Linearity was studied over 5–50 µg/mL. The calibration response increased proportionally with concentration. The regression equation reported:

Area = 49602 × Concentration + 3208.5

with $R^2 = 0.9994$.

Table 4: Linearity data.

Remdesivir concentration (µg/mL)	Mean peak area
5	246532
10	489320
20	985460
30	1487342
40	1975683
50	2480920

The correlation coefficient demonstrates a strong linear relationship between remdesivir concentration and chromatographic response within the investigated range [7].

Accuracy

Accuracy was evaluated using recovery studies at 80%, 100% and 120% concentration levels. The reported recoveries were 99.31%, 99.75% and 99.67%, respectively, giving a mean recovery of 99.57% [8].

Table 5: Accuracy/recovery data.

Level	Added amount (µg/mL)	Found amount (µg/mL)	Recovery (%)	RSD (%)
80%	16	15.89	99.31	0.82
100%	20	19.95	99.75	0.64
120%	24	23.92	99.67	0.58

Mean recovery: 99.57%.

The recovery values were close to 100%, indicating good agreement between the measured and nominal concentrations and suggesting the absence of significant systematic error under the studied conditions.

Precision

Repeatability was assessed by six replicate injections of approximately 20 µg/mL standard solution on the same day. The thesis reports a %RSD of 0.10%.

Table 6: Precision data.

Precision parameter	Result
Intra-day precision	0.10% RSD
Inter-day precision	≈0.12% RSD
Acceptance criterion	≤2.0% RSD

Intermediate precision was assessed on different days by different analysts. The reported day-wise %RSD values were 0.10% and 0.15%, with an overall reported %RSD of approximately 0.12%.

The low %RSD values indicate excellent repeatability and intermediate precision of the analytical response [9].

Limit of Detection and Limit of Quantification

The thesis reports an LOD of 0.095 µg/mL and LOQ of 0.287 µg/mL.

These values indicate that the developed procedure possesses adequate sensitivity for quantitative determination within the studied analytical application.

Robustness

Robustness was assessed by deliberate changes in flow rate, detection wavelength and mobile-phase composition. The thesis reports %RSD values between 0.18% and 0.25% for the investigated changes and classifies the method as robust [10].

Table 7: Robustness data.

Parameter change	Mean Rt (min)	%RSD of peak area	Observation
Flow rate 0.9 mL/min	5.96	0.21	Robust
Flow rate 1.1 mL/min	5.71	0.18	Robust
Wavelength 238 nm	5.83	0.24	Robust
Wavelength 242 nm	5.82	0.19	Robust
Mobile phase 58:42	5.84	0.22	Robust
Mobile phase 62:38	5.79	0.25	Robust

Ruggedness

Ruggedness was investigated using different analysts and instruments on different days. The thesis reports %RSD values of 0.22% and 0.28% for the two analysts / instrument combinations, supporting reproducibility of the procedure under altered laboratory conditions.

Table 8: Ruggedness data.

Analyst	Instrument	Mean area	%RSD
Analyst 1	Waters HPLC 2695	982341	0.22
Analyst 2	Shimadzu LC-20AD	981754	0.28

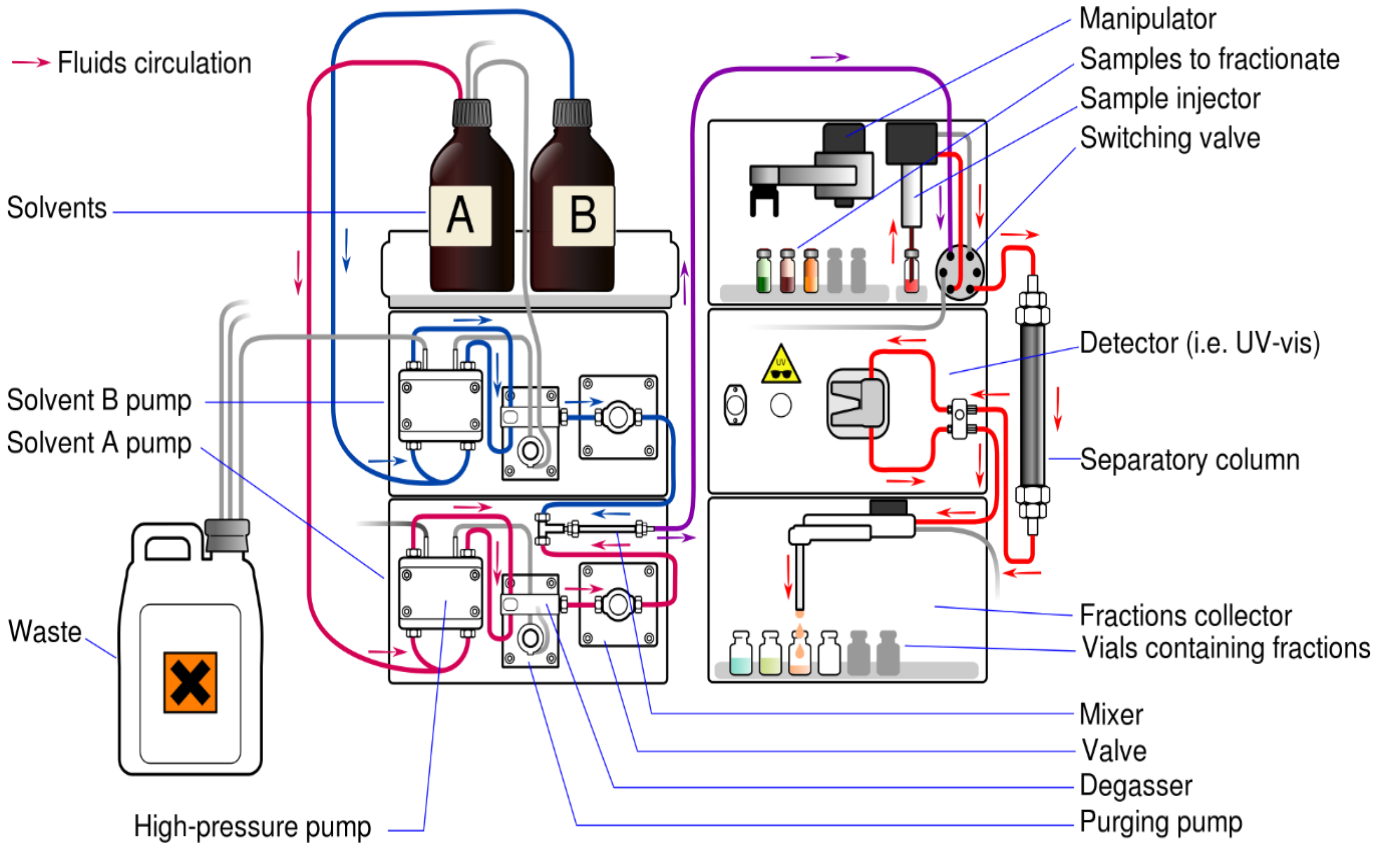

Figure 2: Diagram of used High-Performance Liquid Chromatography System.

Results and Discussion

The present work was directed toward development of a straightforward RP-HPLC procedure for quantitative determination of remdesivir. The method-development experiments indicated that the chromatographic behaviour of remdesivir was influenced by mobile-phase composition and flow rate. The thesis reports that methanol at 50:50 generated a broad peak with poor resolution, while acetonitrile at 60:40 generated a sharper peak but with short retention. Acetonitrile with phosphate buffer at pH 3.5 was subsequently reported to provide a symmetrical peak with good resolution. Selection of a C18 stationary phase was consistent with the intended reversed-phase separation [11]. The C18 column provided the retention and peak shape required for quantitative analysis. The flow-rate investigation identified 1.0 mL/min as the preferred condition during method development. System-suitability evaluation demonstrated reproducible chromatographic performance. Six replicate injections showed low variation in retention time, with a reported %RSD of 0.12% [12,13]. The mean theoretical plate count of 5467 indicated satisfactory column efficiency, while the mean tailing factor of 1.09 demonstrated a relatively symmetrical chromatographic peak. The calibration study showed excellent linearity over the concentration range of 5–50 µg/mL. The R^2 value of 0.9994 demonstrates close agreement between concentration and peak-area response. This range is relevant to the quantitative assay [14].

Accuracy studies produced recoveries between 99.31% and 99.75%, with a mean recovery of 99.57%. The closeness of the results to 100% indicates satisfactory analytical accuracy. The reported intra-day and inter-day precision values were substantially below the 2% criterion, indicating that random analytical variation was low [15].

The reported LOD and LOQ of 0.095 and 0.287 µg/mL, respectively, demonstrate sensitivity of the procedure. The thesis compares these characteristics with reported RP-HPLC procedures and notes that the proposed method provides suitable sensitivity for routine assay and stability-related applications [16].

Specificity studies demonstrated the absence of interference from blank and placebo at the reported remdesivir retention region. The thesis additionally describes forced degradation under acidic, basic, oxidative, thermal and photolytic conditions and reports separation of degradation products from the remdesivir peak, with resolution greater than 2.0 [17].

Robustness studies demonstrated that small deliberate variations in flow rate, wavelength and mobile-phase composition did not cause significant changes in analytical response. The reported %RSD values remained below 0.25%. Ruggedness assessment using different analysts and HPLC instruments also produced low %RSD values, supporting reproducibility of the method [18].

Table 9: Overall validation summary.

Validation parameter	Result	Acceptance criterion/status
Linearity	5–50 µg/mL; R^2 = 0.9994	Passed
Accuracy	99.57% mean recovery	Passed
Precision	0.10–0.15% RSD	Passed
LOD	0.095 µg/mL	Reported
LOQ	0.287 µg/mL	Reported
Specificity	No interference; degradants resolved, Rs >2	Passed
Robustness	Minor variations tolerated	Passed
Ruggedness	%RSD <0.5% in reported study	Passed

Comparison with Previously Reported Methods

The thesis compares the developed procedure with previously reported RP-HPLC methods. According to the reported comparison, previously described methods commonly used ACN or ACN systems, retention times of approximately 6–8 min, linearity ranges around 10–100 µg/mL and detection wavelengths around 245–248 nm. The thesis reports a 5–50 µg/mL linearity range, R^2 of 0.9996 in its comparison section, recovery around 99.46%, and %RSD below 1%.

Table 10: Comparison with reported methods.

Parameter	Reported RP-HPLC methods	Developed method reported in thesis
Mobile phase	ACN (70:30) or ACN	ACN (60:40)
Retention time	6–8 min	4.82 min
Linear range	10–100 µg/mL	5–50 µg/mL
Detection wavelength	245–248 nm	245 nm
R^2	0.998–0.999	0.9996
Recovery	98.5–101.0%	99.46%
%RSD	<2.0	<1.0

The project therefore emphasizes simplicity, relatively short analysis time, reproducibility and suitability for routine quality-control laboratories as major advantages of the proposed approach.

Conclusion

A reverse phase high performance liquid chromatographic method for quantitative determination of remdesivir was developed and evaluated using the experimental and validation data reported. The procedure demonstrated satisfactory chromatographic performance and produced a strong linear response over the concentration range of 5–50 µg/mL with an R^2 value of 0.9994. Accuracy was demonstrated by a mean recovery of 99.57%, while intra-day and inter-day precision produced low %RSD values of 0.10% and approximately 0.12%, respectively. The reported LOD and LOQ were 0.095 and 0.287 µg/mL. Specificity studies showed absence of interference from blank and placebo, while the thesis reported separation of degradation products under forced-degradation conditions. Robustness studies demonstrated that deliberate variations in chromatographic parameters did not significantly affect analytical performance, and ruggedness studies supported reproducibility between analysts and instruments. Collectively, the reported validation results support the potential use of the RP-HPLC procedure for routine quality-control analysis of remdesivir in bulk drug and pharmaceutical dosage forms. The principal advantages described in the thesis are simplicity, good analytical precision, satisfactory accuracy, sensitivity, reproducibility and suitability for routine laboratory application.

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None.

Conflict of Interest

None.

Author Contributions

All the authors contributed to the study.

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