Original Article

Method Development And Validation For The Simultaneous Estimation Of Remdesivir In Bulk And Pharmaceutical Dosage Form And Stability Studies By Uplc

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Abstract

An Easy, sensitive, specific and precise UPLC method for the pharmaceutical dose estimation of Remdesivir in tablet dosage form. Chromatogram was run through BEH (2.1 x 50mm, 1.7µm) Mobile phase containing 0.01N Kh2po4: Acetonitrile taken in the ratio 60:40 was pumped through column at a flow rate of 0.3ml/min. Buffer used in this method was 0.01N Potassium dihydrogen phosphate buffer. Temperature was maintained at 30°C. Optimized wavelength selected was 252.0nm. Retention time of Remdesivir were found to be 1.565 min. %RSD of the Remdesivir were and found to be 0.3. %RSD of Repeatably precision of Remdesivir were found to be 0.3. %Recovery was obtained as 99.87% for Remdesivir. %Assay was obtained as 99.58% for Remdesivir. LOD, LOQ values obtained from regression equation of Remdesivir were 0.33, 1.00. Regression equation of Remdesivir is y = 22602x + 1936. Retention times were decreased and that run time was decreased, so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

Key Words: Remdesivir, UPLC,

INTRODUCTION

Remdesivir Chemical name is 2-ethylbutyl (2S)-2-{[(S)-{[(2R,3S,4R,5R)-5-{4-aminopyrrolo [2,1f][1,2,4] triazin7yl} 5cyano3,4dihydroxyoxolan2yl]methoxy}(phenoxy)phosphoryl]amino}propanoate.This compound belongs to the class of organic compounds known as alpha amino acid esters. These are ester derivatives of alpha amino acids. REMDESIVIR is a nucleoside analog used to inhibit the action of RNA polymerase.[3] The duration of action is moderate, as it is given once daily.[16] Due to much higher selectivity of mammalian DNA and RNA polymerases, including human mitochondrial RNA polymerase, for ATP over REMDESIVIR triphosphate, REMDESIVIR is not a significant inhibitor of these enzymes, which contributes to its overall tolerability and safety profile.[10,18] Despite this, REMDESIVIR carries risks for hypersensitivity reactions, including anaphylaxis and other infusion-related reactions, elevated transaminase levels, and potential decreased efficacy when combined with hydroxychloroquine or chloroquine.COVID-19 is caused by the positive-sense RNA virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Replication of the viral genome is a key step in the infectious cycle of RNA viruses, including those of the Filoviridae, Paramyxoviridae, Pneumoviridae, and Coronaviridae families, and is carried out by viral RNA-dependent RNA polymerase (RdRp) enzymes or enzyme complexes.9,10 For both SARS-CoV and SARS-CoV-2, the RdRp comprises nsp7, nsp8, and nsp12 subunits under physiological conditions, although functional RdRp complexes can be reassembled in vitro that incorporate only the nsp8 and nsp12 subunits, similar to the Middle East respiratory syndrome coronavirus (MERS-CoV).

In reported RP-HPLC method the separation was done by using a Symmetry C18 (250mm x 4.6 mm, 5µ) column, a high-performance liquid chromatographic method for quantifcation of Remdesivir in active pharmaceutical ingredients was developed and validated. The mobile phase is made up of Buffer, pH 5.0: Acetonitrile (30:70)and The fow rate was kept constant at 1.0 ml/min, and detection was accomplished through absorption at 253 nm with a photodiode array detector. After detailed studies no method was reported to estimate Remdesivir by Ultra Performance Liquid Chromatography (UPLC); hence our present plan is to develop a new, sensitive, economical method for its analysis in bulk and formulation and validated as per ICH norms.

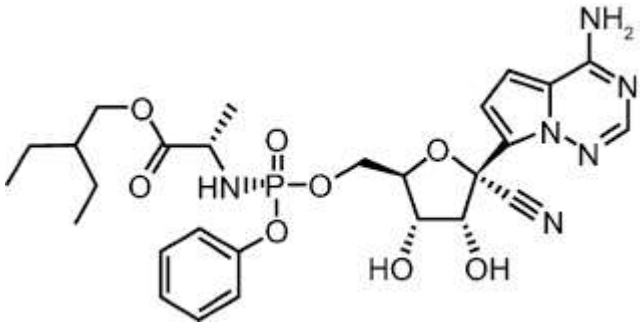


Figure 1. Structure of Remdesivir

MATERIALS AND METHODS

Materials

Remdesivir pure drug (API), Remdesivir Injection, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Instruments

WATERS ACQUITY UPLC SYSTEM 2965 equipped with Binary pumps, TUV detector and Auto sampler integrated with Empower 2 Software.UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz cells integrated with UV win 6 Software was used for measuring absorbances of Remdesivir solution. Sonicator (Ultrasonicator-BVK enterprises), P^H meter (Thermo scientific), Micro balance (Sartorius), Vacuum filter pump (Welch) are the other instruments used for this study.

Analytical methodology

Preparation of buffer(0.01N KH_2PO_4 Buffer)

Accurately weighed 1.36gm of Potassium dihyrogen Ortho phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.PH adjusted to 5.4 with dil. Orthophosphoric acid solution.

0.1%OPA Buffer: 1ml of ortho phosphoric acid was diluted to1000ml with UPLC grade water.

0.1% formic acid buffer: 1ml of formic acid solution was diluted to 1000ml with UPLC grade water.

0.01N NA_2HPO_4 buffer: Accuratel y weighed 1.42gm of disodium phosphate or sodium hydrogen phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Standard/ Working solution preparation

Preparation of Standard stock solutions: Accurately weighed 25mg of Remdesivir is transferred to 50ml volumetric flask. 3/4th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (500µg/ml of Remdesivir).

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (50µg/ml of Remdesivir).

Preparation of Sample stock solutions: Pippete out 20ml of Remdesivir injection sample into a 100 volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters. (1000µg/ml Remdesivir).

Preparation of Sample working solutions (100% solution): 0.5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (50µg/ml Remdesivir).

Diluent: Based up on the solubility of the drugs, diluent was selected, Methanol and Water taken in the ratio of 50:50

Linearity

Linearity solutions are prepared such that 0.25, 0.5, 0.75, 1, 1.25, 1.5ml from the Stock solutions of Remdesivir are taken in to 6 different volumetric flasks and diluted to 10ml with diluents to get 12.5ppm, 25ppm, 37.5ppm, 50ppm, 62.5ppm, 75ppm of Remdesivir .

Precision

Accurately weighed 25mg of Remdesivir is transferred to 50ml volumetric flask. 3/4th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (500µg/ml of Remdesivir).1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with

diluent. (50µg/ml of Remdesivir).

Accuracy

Accurately weighed 25mg of Remdesivir is transferred to 50ml volumetric flask. 3/4 Th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (500µg/ml of Remdesivir). From this solution 0.25, 0.5 and 0.75ml was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent to produce 50, 100, 150% of spiked solution respectively.

Validation Procedure [24]

The analytical method was validated as per ICH Q2(R1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ) and forced degradation.

System Suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to bewithin the limits.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value andthe value found. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and the amount of the drug present, percentage of recovery and related standard deviation were calculated.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the present method was assessed in terms ofrepeatability, intra-day and inter-day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

Linearity and range

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample within a given range. The six series of standard solutions were injected for assessing linearity range. The calibration curve was plotted using peak area with concentration of the standard solution and the regression equations were calculated.

LOD and LOQ

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were separately determined based on the calibration curve. The LOD and LOQ of Remdesivir determined by injecting progressively low concentrations of standard solutions by using the developed method. The LOD and LOQ were calculated as 3.3s/n and 10s/n respectively as per ICH guidelines, where s/n indicates signal-to-noise ratio.

Stress degradation

Stress degradation should be no interference between the peaks obtained for thechromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q1A (R2).The degradation peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions (acid, alkali, oxidation, thermal, UV, water) to obtain the degradation of about 20%.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected bysmall, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the UPLC system and altered chromatographic conditions such as Flow minus, Flow plus, mobile phase minus, mobile phase plus, temperature minus and temperature plus.

The separation factor, retention time and peak asymmetry were calculated by determining theeffect of the modified parameters.

METHOD DEVELOPMENT

Optimized method

Trials were performed for the method development by using different column like X Bridge,BEH C18, HSS C18, Hibar

C18 etc., and the best peak were eluted at 1.542min with good resolution. Plate count and tailing factor was very satisfactory. Optimized chromatographic conditions were shown in Table 1 and optimized chromatogram was shown in figure 2.

Table 1. Chromatographic conditions

Mobile phase	Acetonitrile: 0.01N Kh2po4 (60:40 v/v)
Flow rate	0.3 ml/min
Column	BEH (2.1 x 50mm, 1.7µm)
Detector wavelength	Acquity TUV252nm
Column temperature	30°C
Injection volume	1.00µL
Run time	10min
Diluent	Water and Acetonitrile in the ratio 50:50

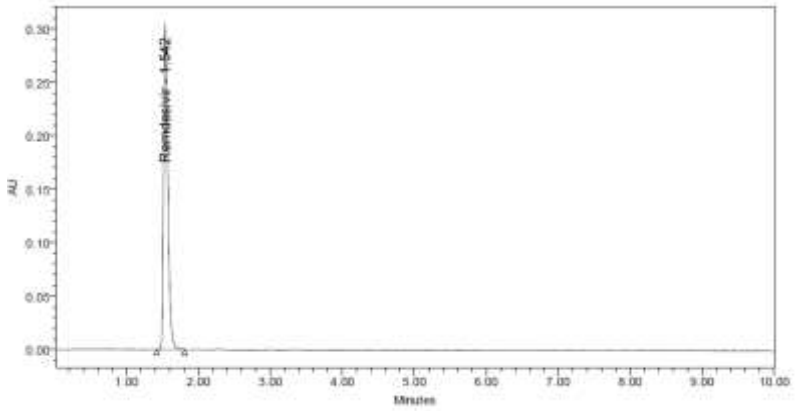


Figure 2. Optimized chromatogram

System suitability

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. System suitability parameters were shown in table 2 and chromatogram was shown in figure 3.

Table 2. System suitability parameters of Remdesivir

S. No.	**REM**		
Injection	RT(min)	USP Plate Count	Tailing
1	1.552	3196	1.38
2	1.554	3250	1.33
3	1.556	3010	1.36
4	1.558	2999	1.37
5	1.564	3298	1.36
6	1.565	3424	1.37

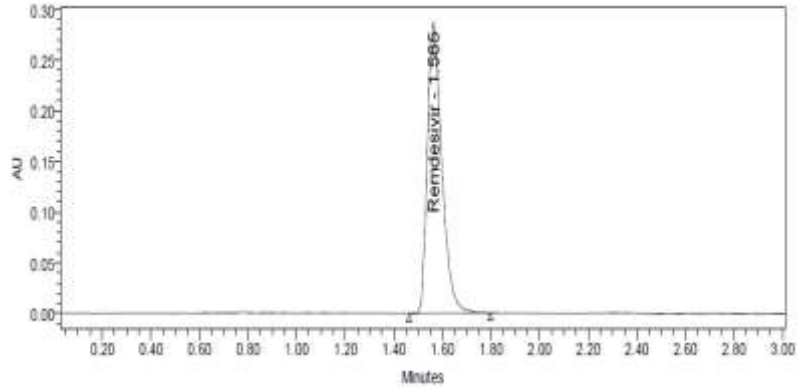


Figure 3. System suitability Chromatogram

METHODS FOR VALIDATION

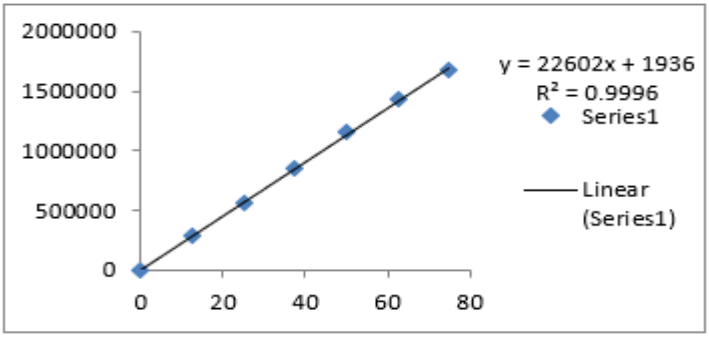
Linearity

Six linear concentration of **Remdesivir** (12.5-75µg/ml) were injected in a duplicate manner. Average areas were mentioned above and linearity equations obtained for Remdesivir was y = 22602x + 1936. Correlation coefficient obtained was 0.999 for the two drugs.

Table 3. Linearity table of Remdesivir

REMDESIVIR	
Conc (µg/mL)	**Peak area**
0	0
12.5	283153
25	563931
37.5	843799
50	1150325
62.5	1425382
75	1679920

Figure 4. Calibration curve of Remdesivir



1.1. Precision

From a single volumetric flask of working standard solution six injections were given and the obtained areas were mentioned above. Average area, standard deviation and % RSD were calculated % RSD obtained as 0.3% for Remdesivir. As the limit of Precision was less than "2" the system precision was passed in this method.. System precision values were shown in Table 4.

Table 4. System precision table of Remdesivir

S. No	**REMDESIVIR**	
	Peak area	**Day-Day precisionPeak area**
1.	1119225	1101212
2.	1115550	1100447
3.	1124768	1104442
4.	1124005	1107216
5.	1118581	1103477
6.	1119987	1086592
Mean	1120353	1100564
S.D	3476.9	7258.4
%RSD	0.3	0.7

Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 99.87% for Remdesivir.. Recovery study values were shown in Table 5.

Table 5. Recovery studies of Remdesivir

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Mean %Recovery**
50%	**25**	**50**	**99.42**	**99.87%**
	25	**50**	**99.31**	
	25	**50**	**100.03**	
100%	**50**	**50**	**100.00**	
	50	**50**	**99.95**	

	50	**50**	**100.09**
150%	**75**	**50**	**100.44**
	75	**50**	**99.93**
	75	**50**	**99.62**

Robustness

Robustness conditions like Flow minus (0.27ml/min), Flow plus (0.35ml/min), mobile phase minus (65B:35A), mobile phase plus (55B:45A), temperature minus (25°C) and temperatureplus(35°C) was maintained and samples were injected in duplicate manner. System suitabilityparameters were not much affected and all the parameters were passed. %RSD was within thelimit. Robustness data were shown in table 6.

Table 6. Robustness data of Remdesivir

S.no	Condition	%RSD of Remdesivir
1	Flow rate (-) 0.25ml/min	0.8
2	Flow rate (+) 0.35ml/min	0.8
3	Mobile phase (-) 65B:35A	0.8
4	Mobile phase (+) 55B:45A	1
5	Temperature (-) 25°C	1
6	Temperature (+) 35°C	0.1

LOD and LOQ

LOD and LOQ were estimated from the signal-to-noise ratio. The LOD of Remdesivir were found to be 0.33 µg/ml and the LOQ were 1.00µg/ml respectively. LOD and LOQ values were shown in table 7. LOD and LOQ Chromatograms were shown in figure5 & 6 .

Table 7. Sensitivity table of Remdesivir

Molecule	**LOD**	**LOQ**
Remdesivir	0.33	1.00

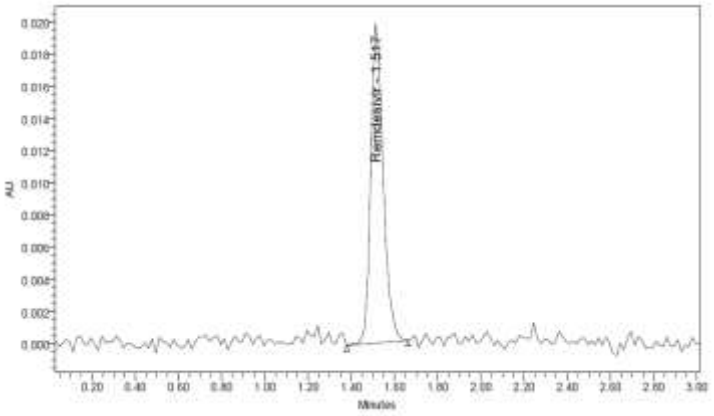


Figure 5. LOD Chromatogram of Remdesivir

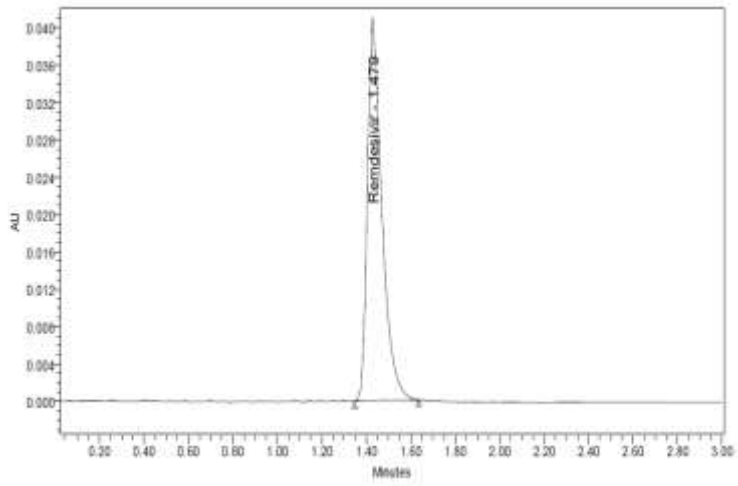


Figure 6. LOQ Chromatogram of Remdesivir

Degradation Data

Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits ofdegradation. Degradation values were shown in table 8.

Table 8. Degradation data of Remdesivir

Type of degradation	Remdesivir		
	AREA	% RECOVERED	% DEGRADED
Acid	1115012	94.60	5.40
Base	1122214	95.54	4.46
Peroxide	1121622	92.55	7.45
Thermal	1118974	91.36	8.64
Uv	1113560	97.24	2.76
Water	1115685	99.36	0.64

Assay of marked formulation

COVIFOR bearing the label claim Remdesivir 100mg.Assay was performed with the above formulation. Average % Assay for Remdesivir obtained was 99.58%. Assay Data of Marked Formulation were shown in table 9.

Table 9. Assay Data of Marked Formulation of Remdesivir

S.no	Standard Area	Sample area	% Assay
1	1119225	1115012	99.32
2	1115550	1122214	99.97
3	1124768	1121622	99.91
4	1124005	1118974	99.68
5	1118581	1113560	99.19
6	1119987	1115685	99.38
Avg	1120353	1117845	99.58
Stdev	3476.9	3624.4	0.32
%RSD	0.3	0.3	0.32

RESULTS AND DISCUSSION

Literature review depicts that no UPLC analytical method reported for Remdesivir. Up-to-date only very few different methods were reported for the analysis of Remdesivir in bulk and formulations by LCMS and FTIR[8], RP-HPLC-MS/MS[9] and other methods.[10-12] The aim and objectives of the present study was to develop a new UPLC method for rapid, simple and simultaneous quantification, validation and stability studies of Remdesivir. The present method was developed with trials and error method by using different mobile phases and different columns likeX Bridge,BEH C18, HSS C18, Hibar C18 etc., The mobile phase containing Acetonitrile and 0.01N Kh2po4 (60:40 v/v) produced the optimized separation chromatogram (Fig. 2) using BEH (2.1 x 50mm, 1.7µm) column. The developed method was validated as per ICH guidelines. The validation parameters such as specificity, linearity (R^2 as 0.999), precision (0.3%), accuracy (99.87%), robustness and system suitability results were achieved and were within the ICH guidelines [13-15]. The retention time was

showed in this proposed method were eluted at1.542 min . The calibration curve was linear over the concentration range of Remdesivir (12.5-75µg/ml)). The LOD of Remdesivir were found to be 0.33µg/ml and the LOQ were 1.00 µg/ml respectively. For the assay of marked Formulation, the average % Assay forRemdesivir obtained was 99.58±1.25% is under the limits. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method. Hence it was concluded that the RP-UPLC method developed was very much suit for routine analysis.

CONCLUSION

In the present investigation, from the above experimental results it was concluded that, the newly developed RP-UPLC method was simple, specific, accurate and precise. The method was effectively validated in terms of system suitability, linearity, precision, accuracy, range, LOD, LOQ and robustness and stability indicating studies according to ICH guidelines. Hence the developed method can use for estimation of Remdesivir in quality control departments of pharmaceutical industries and testing laboratories.

CONFLICTS OF INTEREST

The authors have no conflict of interest.

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