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METHOD DEVELOPMENT AND VALIDATION OF REMDESIVIR BY USING RP HPLC METHOD

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Abstract:
A simple, precision and accuracy HPLC method was developed the estimation of Remdesivir analysis of formulation, consisting of an Methanol: water (60: 40 % v/v). The chromatographic condition was set at a Flow rate of 1 ml/min with the UV detector at 240 nm. The above method was optimized with a view to develop an assay method for Remdesivir. Several mobile phase compositions were tried to resolve the peaks of Remdesivir. The optimum mobile phase containing methanol: water (60: 40 % v/v) was selected because it was found ideal to resolve the analyte peaks of the drug. Quantification was achieved with UV detections at 240 nm based on peak area and absorbence. As per USP requirements system suitability studies were carried out and freshly prepared standard solutions of Remdesivir.

Keywords: *Remdesivir, RP-HPLC, Method development, Validation*

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INTRODUCTION:

Remdesivir is indicated for the treatment of adult and pediatric patients 28 days of age and older and weighing at least 3 kg for coronavirus disease 2019 (COVID-19) infection requiring hospitalization. It is also indicated for the treatment of non-hospitalized patients with mild-to-moderate COVID-19, who are at high risk for progression to severe COVID-19, including hospitalization or death. 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). IUPAC name is 2-ethylbutyl (2S)-2-{[(S)-{[(2R,3S,4R,5R)-5-{4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl}-5-cyano-3,4-dihydroxyoxolan-2 yl]methoxy}(phenoxy)phosphoryl]amino}propanoate. Molecular formula $C_{27}H_{35}N_6O_8P$. Molecular Weight is 602.5.

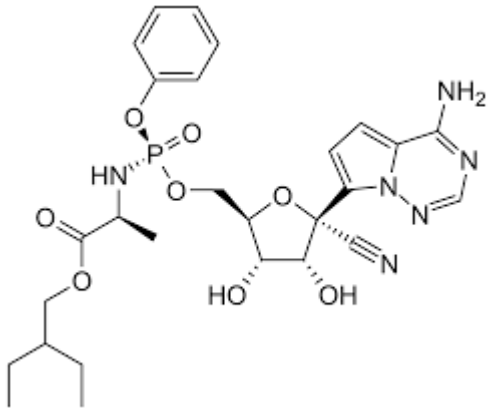

Figure 1: Structure of Remdesivir

The literature survey revealed that There are very few methods reported in the literature for analysis of Remdesivir alone or in combination with other drugs in the pure form and pharmaceuticals formulations by RP-HPLC.[3-7] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Remdesivir estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Remdesivir. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the estimation of Remdesivir in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Remdesivir were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 240 nm with column Symmetry C_{18} (4.6 X 150 mm; 5µm Waters), dimensions at Ambient temperature. The optimized mobile phase consists of methanol: water (60:40%v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Preparation Mobile phase:

Mix a mixture of above methanol (60%), 400 mL of HPLC water (40%) and degas in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.

Standard Solution Preparation

Accurately weigh and transfer 10 mg of Remdesivir working standard into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Sample Solution Preparation:

Accurately weigh and transfer equivalent to 368.0 mg of Remdesivir sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.3ml of Remdesivir of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Inject 20 µL of the standard, sample into the chromatographic system and measure the areas for Remdesivir peaks and calculate the %Assay by using the formulae.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 10 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into Symmetry C_{18} (4.6 X 150 mm; 5µm Waters), the mobile phase of composition methanol: water (60:40%v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Remdesivir in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: Remdesivir working standard solutions were prepared across the range of the analytical method with a minimum of 5 concentrations that are within the specified range (10-50 µg/ml) low level (10 µg/ml) and higher level (50µg/ml) for 5 replicating injections were taken and calculated the %RSD. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%,150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Remdesivir and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: The system precision of the test method was performed by injecting 5 replicate determination of standard preparation injections were injected and the % RSD was calculated. The %RSD for the area of five replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. The results are shown in table 6.

Robustness: Robustness of assay method was carried out with variation of flow rate. Standard preparation was prepared and performed analysis as per test method and evaluated the system suitability parameters. As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7,8.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 9.

$LOD = 3.3\sigma/S$ and

$LOQ = 10\ \sigma/S$, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION:

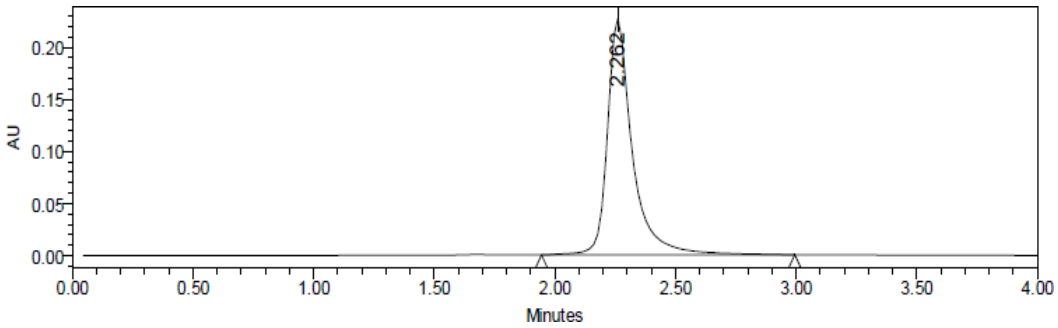

Figure 2: Standard chromatogram

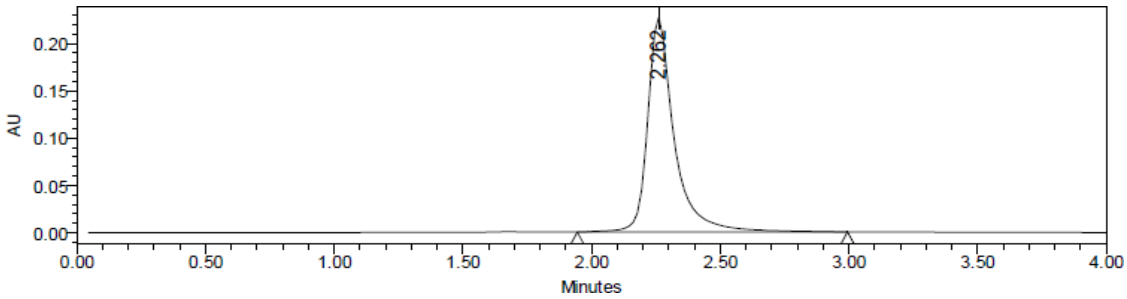

Figure 3: Sample chromatogram

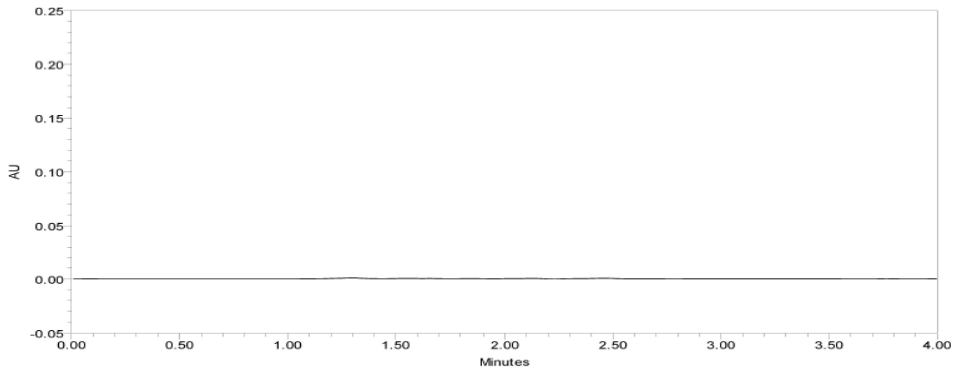

Figure 4: Blank chromatogram

Table 1: System suitability parameters

	Tailing factor	Theoretical Plates
Remdesivir	1.5	2804.8

Table 2: Assay results for Remdesivir

	Label Claim (mg)	% Assay
Remdesivir	10	99.6

Table 3: Linearity results of Remdesivir

S.No	Linearity Level	Concentration	Area
1	I	10ppm	682741
2	II	20ppm	1201305
3	III	30ppm	1627183
4	IV	40ppm	2180552
5	V	50ppm	2716958
Correlation Coefficient			0.999

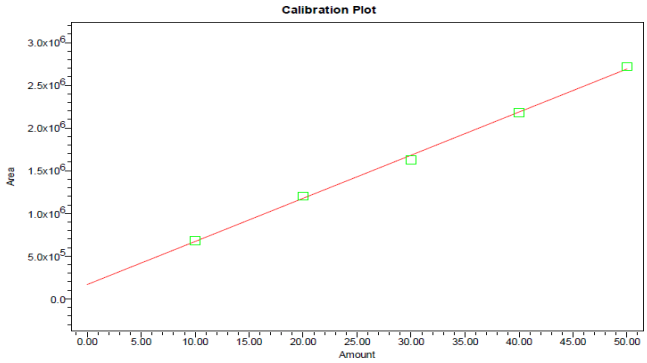

Figure 5: Linearity graph for Remdesivir

Table 4: Showing accuracy results for Remdesivir

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	823686.2	5.0	5.0	100.1%	99.5%
100%	1634793	10	9.93	99.3%	
150%	2451939	15.0	14.9	99.3%	

Table 5: Precision results for Remdesivir

Injection	Area
Injection-1	1631295
Injection-2	1630511
Injection-3	1636464
Injection-4	1628557
Injection-5	1635684
Average	1632502.2
Standard Deviation	3420.4
%RSD	0.2

Table 6. Ruggedness results of Remdesivir

Injection	Area
Injection-1	1639701
Injection-2	1645897
Injection-3	1640705
Injection-4	1637036
Injection-5	1638609
Average	1640389.4
Standard Deviation	3365.9
%RSD	0.2

Robustness results

Table 7: Flow variation results for Remdesivir

S.No	Flow Rate(ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	3353.0	1.5
2	1	2804.8	1.5
3	1.2	2384.0	1.4

Table 8: Change in organic composition results for Remdesivir

S.No	Change in Organic Composition in theMobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	2396.0	1.3
2	*Actual	2804.8	1.5
3	10% more	2218.0	1.4

Table 9: LOD, LOQ of Remdesivir

Drug	LOD	LOQ
Remdesivir	2.273	2.268

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Remdesivir in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Remdesivir in pure and its pharmaceutical dosage forms.

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