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STABILITY INDICATING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR QUANTITATIVE ESTIMATION OF REMDESIVIR ORAL SOLUTION DOSAGE FORM

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ABSTRACT

The aim was to develop a simple, accurate, precise, and reproducible Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) method for the quantitative estimation of Remdesivir in oral solution dosage form. The chromatographic Separation was performed on the Inert Sustain C18 (4.6 mm × 100 mm i.e., 3 µm particle size) column, and a mobile phase comprising of 0.1 % Triethylamine in Water (pH 3) adjusted with OPA and Acetonitrile in the ratio of (60:40 v/v) was used. The diluent consisting of water and acetonitrile in the ratio of (60:40 v/v) was used. The flow rate was kept at 1 ml/min and detection was carried out at 245nm on UV. The retention time of Remdesivir was found to be about 4.5 min. The validation parameters such as accuracy, precision, linearity, ruggedness, robustness were used for validating the developed method according to ICH guidelines. The method was linear over a concentration range of 10-90 µg/ml with a regression coefficient of 0.9997. The percentage RSD of every parameter was found within the limit. The stress testing studies were executed to give degradation products by exposing the drugs to hydrolytic, photolytic, oxidative, acid, alkali, and thermal degradation conditions. The acquired data showed that the degradation product successfully separated without any intrusion, which establishes stability-indicating the nature of a developed method. The reliable, simple, precise, accurate, and easy for RP-

HPLC method has been successfully developed and validated. The developed method was applied for routine quality control analysis of Remdesivir in oral solution dosage forms.

KEYWORDS: Remdesivir, RP-HPLC, Oral Solution, Method Development, Validation parameters, Force Degradation Studies.

INTRODUCTION

The Corona virus first appeared on a small scale in November 2019 with the first large cluster appearing in Wuhan, China in December 2019. There have been numerous investigations to determine the origins of SARS-CoV-2 but none has been definitive. Coronaviruses behind Middle East respiratory syndrome (MERS) and severe acute respiratory syndrome (SARS) was developed from bats. It was first thought SARS-CoV-2 made the jump to humans at one of Wuhan, China's open-air "wet markets." Later theories voiced concern that it may have originated as a biological weapon in a lab o China.[1,2] Remdesivir is the nucleotide prodrug of an adenosine analog. It binds to the viral RNA-dependent RNA polymerase and inhibits viral replication by conclude RNA transcription untimely. Remdesivir has demonstrated in vitro activity against SARS-CoV-2.1 In the rhesus macaque model of SARS-CoV-2 in contagion, remdesivir treatment was initiated soon after vaccination. Intravenous remdesivir was approved by the Food and Drug Administration (FDA) for the treatment of COVID-19 in adult and pediatric patients (aged ≥12 years and weighing ≥40 kg). It is approved for the treatment of mild to moderate COVID-19 in high-risk, non-hospitalized patients (i.e., a 3-day course initiated within 7 days of symptom onset) and for the treatment of hospitalized patients with COVID-19 (i.e., a 5-day course).[3,4]

Remdesivir is an antiviral nucleotide analogue used for therapy of severe novel coronavirus disease 2019 which is (COVID-19) caused by severe acute respiratory syndrome (SARS) coronavirus 2 (CoV-2) infection. The chemical name of Remdesivir is 2-ethylbutyl (2*S*)-2-[[[(2*R*,3*S*,4*R*,5*R*)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl)-5-cyano-3,4-dihydroxyoxolan-2-yl]methoxy-phenoxyphosphoryl]amino]propanoate and molecular formula is $C_{27}H_{35}N_6O_8P$.[5]

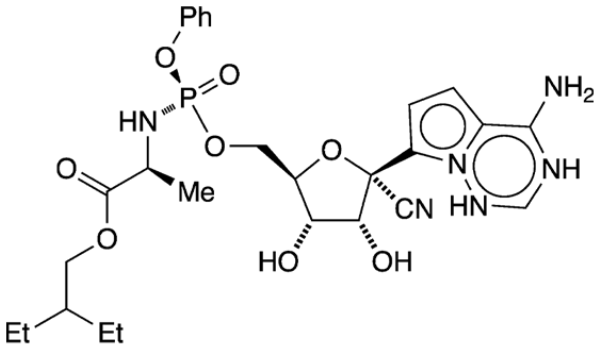

Fig. 1: Structure of Remdesivir.

IP, BP, USP and other pharmacopeia has no monograph of remdesivir as well as no chromatographic method was found during the literature survey for analysis of Remdesivir in oral dosage form.[6] Hence, attempts were made to develop a simple, rapid, precise, and accurate reverse phase chromatographic method to estimate Remdesivir in oral solution dosage form. This method has been successfully used for Quality control analysis of Remdesivir formulation. According to International Conference on Harmonization (ICH) guidelines this method was optimized and validated.[7]

The objective is to give an overview of the mechanism of Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) of Remdesivir and explain the basis of the retention mechanism and achieve high-speed separation without any loss of reproducibility.[8]

MATERIAL AND METHODS

Chemical and reagents

An analytically pure Remdesivir working standard was procured from the Central Drug Testing Laboratory Mumbai with defined potency [99.1 % as is basis]. Remdesivir oral solution containing 100 mg/5ml marketed by Lupin formulation was used for analysis. Acetonitrile (HPLC grade) from Merk life science, triethylamine AR Grade from Merck, Ultra-purified HPLC grade distilled water was obtained from the Milli-Q®.[9]

Instrumentation

Thermo Scientific Ultimate 3000 system equipped with chromeleon 7.4.2 software was used to perform quantitative detection. For all Spectrophotometric measurements Perkin Elmer UV/VIS spectrometer lambda 25 equipped with software Perkin Elmer UV win lab was used. Analytical weighing balance, vacuum filter pump, millipore filtration kit, sonicator, Water bath, sample filtration assembly and different type of glassware's were used throughout the

experiment. Forced degradation studies were carried out on Waters HPLC system equipped with Photo Diode Array Detector-2996.[10]

Chromatographic Conditions

The chromatographic separation was performed by using Inert Sustain C18 (4.6 mm × 100 mm i.e., 3 µm particle size) column and a mobile phase comprising of 0.1% trimethylamine in water (pH 3) adjusted with OPA and acetonitrile in the ratio of (60:40 v/v) was used. The diluent consisting of water and acetonitrile in the ratio of (60:40 v/v) was used. The flow rate was kept at 1 ml/min. The injection volume of 10 µl and detector wavelength at 245 nm was selected.[11]

Determination of wavelength of maximum Absorbance

Remdesivir standard 25 mg weighed accurately transferred to the 50 ml volumetric flask and the volume was make up to the mark with diluent i.e., 500ppm. From stock solution further dilution were made to get a concentration of 40ppm. Then the solution was scanned in UV visible Spectrophotometer in the range of 400.0 nm to 200.0 nm. Remdesivir showed maximum absorbance at 245 nm as shown in Fig. 2 Hence the same wavelength selected for the analysis of Remdesivir.

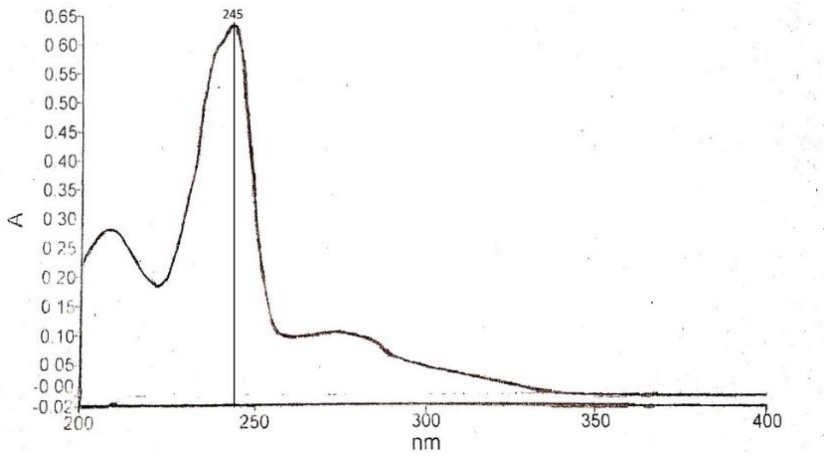

Fig. 2: UV spectra of Remdesivir.

Selection of Solvent (Diluent)

Based on the molecular structure and chemical nature of Remdesivir, water and acetonitrile in the ratio of (60:40 v/v) was selected as a diluent for preparation of standard and sample solutions.

Preparation of Mobile Phase

0.1% Triethylamine buffer adjusted pH 3.0 with OPA and Acetonitrile in the ratio of 60:40 % v/v was used as a mobile phase for the present study. The mobile phase was sonicated and filtered through 0.45µm nylon filter.

Preparation of standard solution

A standard solution of concentration 40 µg/ml of Remdesivir was prepared using a diluent.

Analysis of marketed formulation

1 ml (20mg/ml) of Remdesivir oral solution was dissolved in diluent and sonicated. Further dilutions were made to set a concentration 40 µg/ml of Remdesivir in diluent.

Method Optimization

The chemical structure of Remdesivir shows that it is basic and non-polar in nature with (pKa value 10.23: strongest acidic, pKa 0.65: strongest basic). Initially base ineffectual column was used. The Inert Sustain C18 (4.6 mm × 100 mm i.e., 3 µm particle size) column was selected for the study. The different mobile phase ratios were tried including Water: Acetonitrile (50:50 % v/v), (60:40 % v/v) and also Methanol and Water of different compositions but poor peak shape was found. Further trials were started with the addition of TEA adjusted pH 3 with OPA. Some good peak shape with with accurate SST parameters. The flow rate was kept at 1 ml/min and detection was carried out at 240nm on UV and column oven temperature maintained at 30˚C.

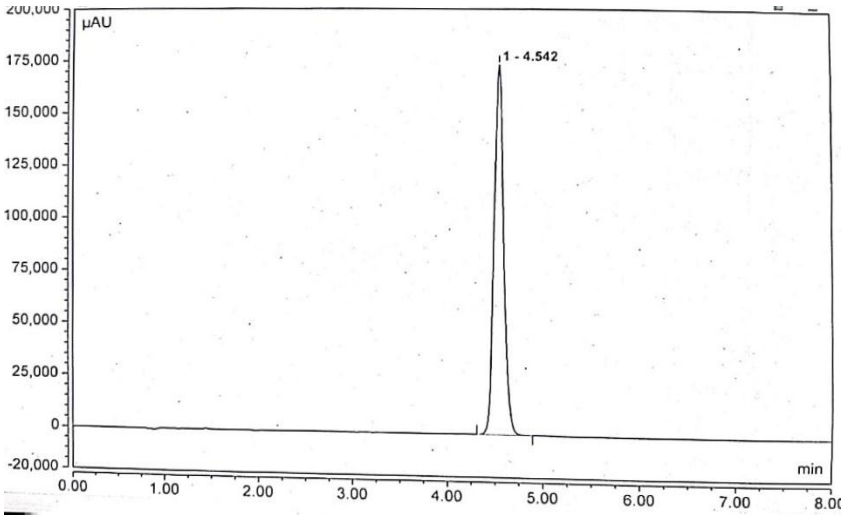

Fig. 3: Chromatogram of Remdesivir standard solution.

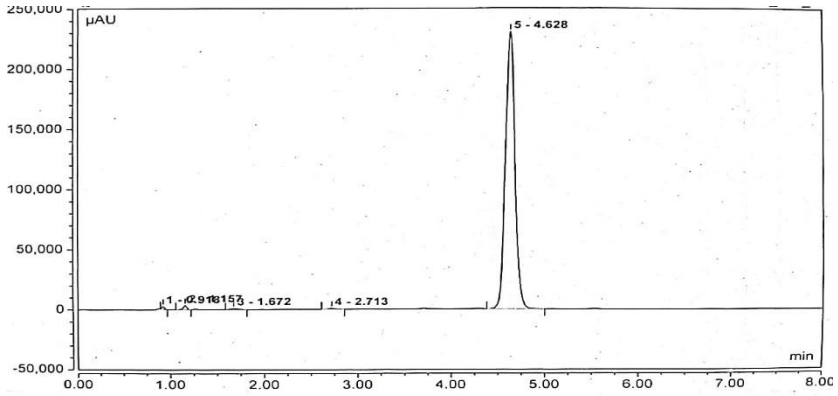

Fig. 4: Chromatogram of Remdesivir sample solution.

Method Validation

Validation of developed RP-HPLC method was done for parameters such as specificity, linearity, precision, accuracy and recovery and robustness as per ICH guidelines.[12]

Linearity

Appropriate aliquots from standard Remdesivir stock solutions were prepared to obtain concentrations of 10 - 90µg/ml. Linearity was determined over the range of (10-90 µg/ml) for the Remdesivir. Regression equation obtained was y = 5160.7x + 47.042. The method is having good linearity (r^2 = 0.9997). The results established that the analyte response is proportional to the analyte concentration in the selected concentration range. The calibration for Remdesivir is done as shown in Table no. 2 and Figure no. 5.

Precision

Precision of System was ascertained by six replicate analysis of Standard solution of Remdesivir. Precision of method (Repeatability) was ascertained by six replicate analyses of homogeneous sample of oral solution at concentration 40 µg/ml. The Intermediate precision was studied by injecting freshly prepared working standard solution of Remdesivir on two different days (Interday) and on same day but at three different time (Intraday).

System precision

The % RSD values were found to be within the limit that is less than 2 %. The results are briefed in Table No 3.

Method Precision

The mean assay percentage results are briefed in Table No. 4 and were found to be within the limit.

Intermediate Precision

The % assay, average, SD, % RSD for Day-1 and Day- 2, and HPLC-1 were found to be not more than 2 %. The results are brief in Table No. 5

Accuracy

Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. A known amount of Remdesivir (110, 120, and 130%) of standard solution was added to the pre analysed solution of formulation. This solution was analysed as previously described. The assay was repeated over 3 injections of each concentration to obtain data. The resultant % RSD for this study was found to be < 2.0 % with a corresponding percentage recovery value. Accuracy results at various levels of concentration are summarized in Table No.6. From the results, it can be seen that the percent mean recovery is 100.12 % which is within the limit, hence the method is accurate. The results are brief in Table No. 6

Robustness

The Robustness of the method was established by deliberate change in detection wavelength by ±2 nm, change in the temperature by ± 2°C and flow rate by ± 0.2 ml in the estimation of Oral solution .The reproducible results were obtained which proves that method is robust. Robustness was performed and by analysing, resulted values were found to be within the limit that is less than 2 %, thus the developed method was proved to be robust. The results are brief in Table No. 8.

Specificity

Specificity of the method was carried out by forced degradation studies at different conditions like acidic, alkaline, hydrolytic, oxidative and photolytic conditions.

Force Degradation Study

Force degradation is also known as stress testing and a drug is degraded forcefully by applying artificial methods. It is useful tool to predict the stability of any Active Pharmaceutical Ingredient (API) or formulation product. It helps to know about the impurity's development during the storage of drug products in various environmental conditions. PDA detector was used to evaluate the peak purity of Remdesivir in each condition and the detection was carried out at different wavelengths to confirm the stability indicating nature of the proposed method.

Acid degradation

Acid degradation was performed by adding 1 ml of 0.1 N HCl to 10 ml of Remdesivir stock solutions and kept at room temperature for 15 mins. Later the solution was cooled and neutralized to pH 7 with 0.1 N NaOH and diluted to volume with diluent to obtain 40 µg/ml solutions. The same solution was injected into the HPLC system and represented the chromatograms.

Alkaline degradation

Alkaline degradation was performed by adding 1 ml of 0.1 N NaOH to 10 ml of Remdesivir stock solutions and kept at room temperature for 15 mins. Later the solution was cooled and neutralized to pH 7 with 0.1 N HCL and diluted to volume with diluent to obtain 40 µg/ml solutions. The same solution was injected into the HPLC system and represented the chromatograms.

Water degradation

Water degradation was carried out by adding 3 ml of water to 10ml of Remdesivir standard stock solutions and heated it on boiling water bath at 100 °C for 2 hrs. Later the solution was cooled and diluted to volume with diluent to obtain 40 µg/ml solutions. The same solution was injected into the HPLC system and represented the chromatograms.

Oxidation degradation –Oxidation degradation was carried out by adding 1 ml of 3 % hydrogen peroxide to 10 ml of Remdesivir stock solutions and heated it on boiling water bath at 100 °C for 2 hrs. Later the solution was cooled and diluted to volume with diluent to obtain 40 µg/ml solutions. The same solution was injected into the HPLC system and represented the chromatograms.

Photolysis degradation

Photolysis degradation was carried out by adding 10ml of Remdesivir standard stock solutions were exposed to UV light at 245 nm for 24hrs. Later the solution was cooled and diluted to volume with diluent to obtain 40 µg/ml solutions. The same solution was injected into the HPLC system and represented the chromatograms.

RESULT AND DISCUSSION

Novel and simple RP HPLC method have been developed for the determination of Remdesivir in Oral Solution dosage form. The chromatographic conditions were optimized

by taking into consideration the chemical structures of Remdesivir. % RSD for the six replicate injections was found to be less than 2 and ensured that entire testing system and chemicals used could generate precise and accurate outcome. All the SST parameters like theoretical plates were observed greater than 10040 of Remdesivir drug. This result are briefed in **Table 1**

The optimized chromatographic conditions were found satisfactory to yield well retained, sharp and symmetrical peak at 4.5 min. The results of linearity studied over the concentration range 10 - 90µg/ml showed the linear detector response with correlation coefficient of 0.9997 and the regression equation of y = 519.48x + 106.26. **Table 2 and Fig 5**

Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. Percent Recovery was observed to be 100% representing the accuracy of the method. **Table 6**

Replicate estimations (n=6) of Remdesivir in standard solution and oral solution by proposed method have yielded % RSD of 0.15% respectively indicating substantially high precision of system and method (repeatability). **Table 3,4**

The intermediate precision study was ascertained on the basis of intra-day and inter-day data obtained by analysing Remdesivir Oral Solution by proposed method and it is found to be very much reproducible with minimum % RSD. i.e. 0.32% and 0.29%. **Table 5**

The method was sufficiently robust for normally expected variations in chromatographic conditions such as wavelength, temperature and flow rate and mobile phase. **Table 8**

The number of Average theoretical plates was 10005 and tailing factor was 1.1 for Remdesivir which indicates *efficient* performance of the column.

Chromatograms of acidic and oxidative degradation showed extra peaks indicating mild degradation i.e. 1.1 % degradation in acidic condition and 0.39 % degradation due to oxidative degradation. Most significant degradation was observed under alkaline condition i.e. 40.05 %. Remdesivir was found to be stable to water hydrolytic stress conditions and hence no degradation was observed. Under each condition, peak purity of main peak was found to be 100.00 % as the peaks of degradation products were successfully separated and

resolved from the main peak of Remdesivir without any interference. This confirms the stability indicating nature of the proposed method. **Table 9**

Table 1: System suitability studies of Remdesivir.

Sr. No.	Peak Area	Retention Time
1	20138.5	4.542
2	20207.1	4.54
3	20227.3	4.543
4	20128.3	4.543
5	20130.6	4.542
6	20143.9	4.542
Average	20162.61667	4.542
S.D.	43.12152208	0.001095445
%R.S.D.	0.21386868	0.024118122
Limits	NMT 2.0%	NMT 1.0%

*Average Mean of Six determination, SD = Standard Deviation, % RSD = Percentage relative standard deviation, NMT = Not more than, NLT = Not less than

Table 2: Linearity data of Remdesivir.

Linearity level	Concentration	Area
1	10	5283.1
2	20	10361.1
3	30	15511.1
4	40	20229
5	50	25818.2
6	60	31026.4
7	80	41675.2

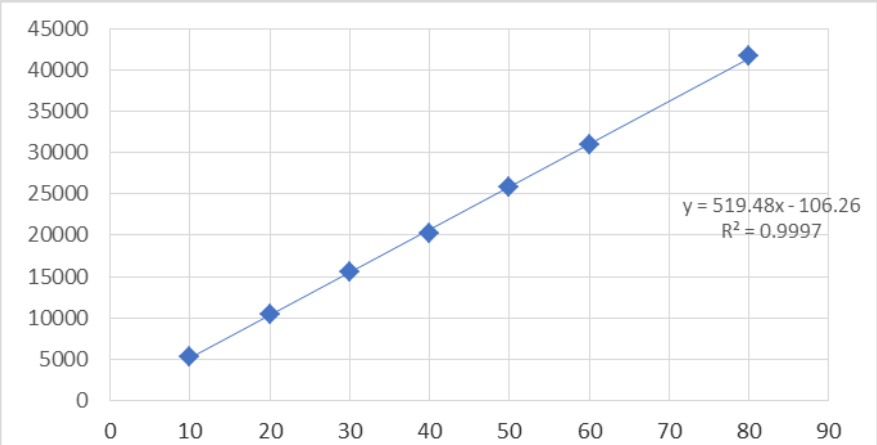

Fig. 5: Linearity graph of Remdesivir.

Table 3: System Precision (Standard).

Sr. No.	Area
1	20128.3
2	20130.6
3	20143.9
4	20059
5	20121.9
6	20098
Mean	20113.6
SD	30.7359
% RSD	0.15281
Limit	NMT 2.0 %

SD= Standard Deviation. %RSD= Percentage relative standard deviation. NMT= Not more than.

Table 4: Method Precision (Sample).

Sr. No.	Analyst A
	Day 1 %
1	100.83
2	100.71
3	100.98
4	100.88
5	100.91
6	100.1
Mean	100.735
SD	0.323898132
% RSD	0.321534851

Table 5: Intermediate Precision/ Interday Precision.

Sr. No.	Analyst A	Analyst B
	Day 1 %	Day 2 %
1	100.83	100.35
2	100.71	100.63
3	100.98	100.26
4	100.88	100.03
5	100.91	100.06
6	100.1	100.77
Mean	100.735	100.35
SD	0.323898132	0.299799933
% RSD	0.321534851	0.298754293

Table 6: Accuracy studies of Remdesivir.

% level	STD spiked (µg/ml)	Amount recovered (mg)	% amount recovered	% recovery	Mean % recovery
110	4	110.42	99.88	100.38	100.21
120	8	120.14	120.14	100.12	
130	12	130.17	130.17	100.13	

Table 7: Assay results of Remdesivir.

Sample no.	Weight of standard (mg)	Sample Weight	Mean Area of standard at 245nm	Area of sample at 245nm	% Assay
1	25.12	0.5026	20750	27760	100.7
2		0.5236		27725	100.6
3		0.5623		27800	100.9
4		0.5123		27771	100.8
5		0.5126		27780	100.8
6		0.5412		27559	100
Mean				27732.5	100.63

Table 8: Robustness studies of Remdesivir.

Parameter	Change in parameter (±)			% Estimation			Mean	SD	% RSD
Wavelength (± 2 nm)	242	245	247	100.38	100.14	100.24	100.2533333	0.120554275	0.12025
Mobile Phase composition (± 2 nm)	62:38	60:40	58:42	100.18	100.14	100.17	100.1633333	0.02081666	0.020783
Flow rate (± 0.02 ml/min)	0.98	1	1.02	100.1	100.14	100.26	100.17	0.08326664	0.083128
Temperature±2^0c	28	30	32	100.35,	100.14,	100.26	100.25	0.105356538	0.105094

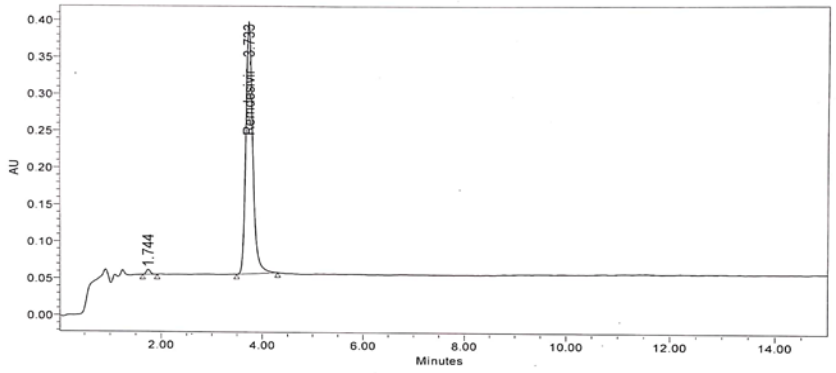

Fig. 6: Chromatogram of Acid degradation of Remdesivir.

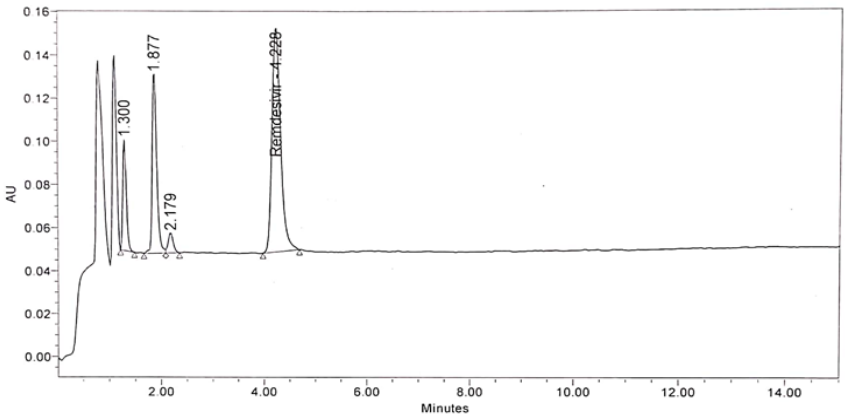

Fig. 7: Chromatogram of Alkali degradation of Remdesivir.

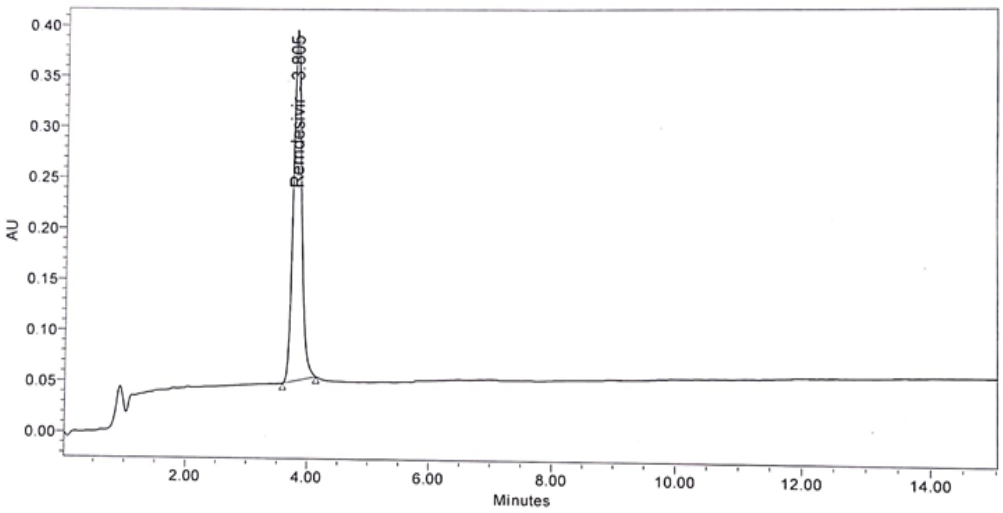

Fig. 8: Chromatogram of Water hydrolysis of Remdesivir.

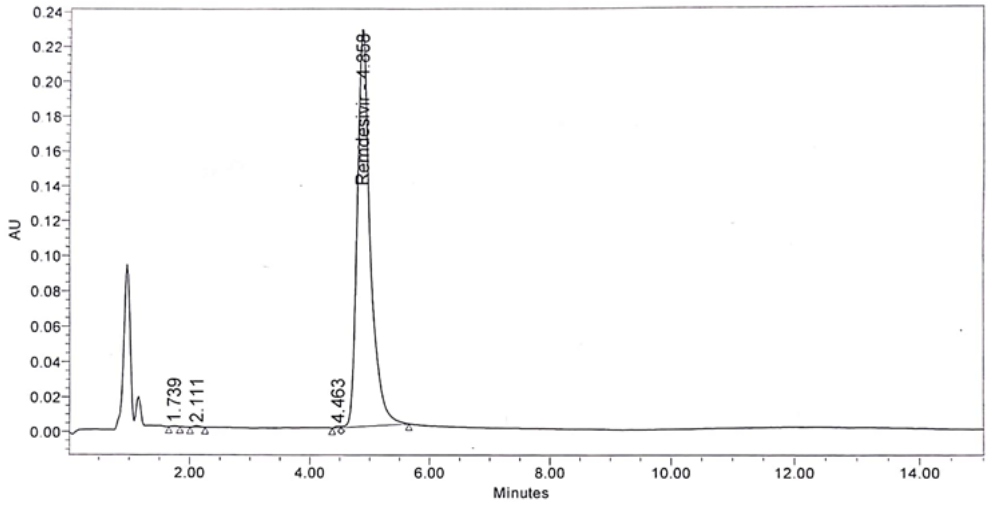

Fig. 9: Chromatogram of Oxidative degradation of Remdesivir.

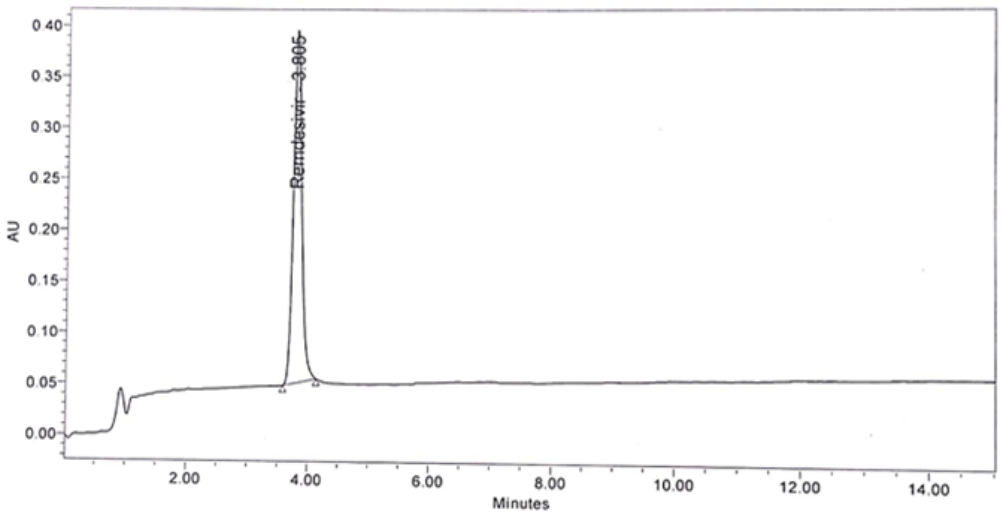

Fig. 10: Chromatogram of Photolytic degradation of Remdesivir.

Table 9: Data for Forced Degradation studies of Remdesivir.

Sr. no.	Degradation condition	Temperature conditions	Retention time of degradation products (Min)	% Residual drug	% degradant	Observed peak purity (%)
1	Acid degradation: 1ml of 0.1 N HCL	at room temperature for 15	1.744	98.9	1.1	100
2	Alkali degradation: 1 ml of 0.1 N NaOH	at room temperature for 15 min	1.300, 1.877, 2.197	59.95	40.05	100
3	Water hydrolysis: 3ml od water at 100° C	for 2 hrs	_	100	_	100
4	Oxidative degradation: 1ml of 3% H2O2	at 80° C for 15 min	1.739, 2.111, 4.463	99.61	0.39	100
5	Photolytic degradation: UV lamp (245nm)	for 24 hrs	_	100	_	100

CONCLUSION

The RP-HPLC method development of Remdesivir oral solution was found to be simple, rapid, specific, and can generate accurate and precise results. Moreover, the duration of analysis time is less as well as lesser mobile phase consumption confirmed that the method is rapid and economical. Validation parameters that is linearity, accuracy and recovery, precision, specificity, robustness, assay is successfully validated as per ICH Q2 (R1) guidelines. The successful separation of the forced degradation products from the active pharmaceutical ingredients without any interference confirmed the stability-indicating nature of the developed method. According to ICH guidelines the parameters were within the range. Hence the proposed RP-HPLC technique can be used for routine analysis and quality control of Remdesivir oral solution.

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