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Stress Degradation Study Of Remdesivir Using RP-HPLC.

Monali Yerne, Shubhangi Vidhate, Krishna Gupta*, and Milind Umekar.

Department of Pharmaceutical Chemistry, Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee, Nagpur, Maharashtra, India.

ABSTRACT

The focus of this work was on the drug degradation kinetics and behavior of Remdesivir in the in-solution state. The study's design includes the choice of an RP-HPLC method for drug estimation, evaluation of the kinetics of drug degradation, estimation of shelf life, and method validation. The Shimadzu HPLC was used to assess the stress degradation of remdesivir in lyophilized powder injection dosage form. With an injection volume of 20µl and a flow rate of 0.8 ml/min, the analysis was conducted using an Agilent ZORBAX SB-C8 (4.6×150×5µm) column with a mobile phase consisting of acetonitrile:water in a ratio of 95:05 v/v at a wavelength of 246 nm. The suggested method was demonstrated to be linear over the concentration range of 10 to 50 µg/mL. The findings demonstrated that remdesivir's degradation behaviors followed first-order kinetics and that temperature, ionic strength, and oxidation all had a significant impact on Remdesivir's stability. Remdesivir did not remain stable at higher temperatures, neutral or alkaline environments. The suggested approach proved to be reliable, robust, and specific in its assay, drug degradation (stress testing), and degradation kinetics in solution state.

Keywords: Remdesivir, RP-HPLC, drug degradation, shelf-life Solution stability

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Corresponding author

INTRODUCTION

The term "forced degradation" describes the breakdown of novel drug compounds and drug products under circumstances more demanding than those found in accelerated conditions. The molecule's chemical stability is demonstrated by these investigations, which facilitates the development of stable formulations under suitable storage circumstances. Forced degradation studies provide a way for the pharmaceutical industry to analyse the stability of drug samples [1]. Studies on forced degradation conducted in a range of environments, including light, acidic, basic, hydrolysis, oxidation, pH, dry heat, and others. ICH guidelines mandate that they be followed. In order to assess how the quality of a drug substance or drug product varies over time and in response to different environmental factors, the FDA and ICH guidelines require forced deterioration [2,3]. For forced degradation studies, ICH Q1A, Q1B, and Q2B are applied [1, 3, 4]. The main objective of stability studies is to ascertain the drug product's shelf life. Any dosage form degradation product is anticipated to stabilize and not increase to a point where the patient would be concerned before the prescribed storage period has elapsed. Based on this period, the product's shelf life or expiration date is determined [5].

Stress testing is a preformulation procedure used by pharmaceutical companies to identify degradation products found during manufacture and/or stability studies of the new drug product, potential pathways for degradation of the new drug product, and contaminants resulting from interactions with excipients or the immediate container closure system.

The medication Remdesivir is a broad-spectrum antiviral that is injected into the vein. This prodrug is intended to facilitate the intracellular delivery of GS-441524 monophosphate and its subsequent biotransformation into GS-441524 triphosphate, a ribonucleotide-like inhibitor of viral RNA polymerase. Nausea is the most frequent side effect in COVID-19 patients. Remdesivir Soluble in ethanol and DMSO; very slightly soluble in water. Molecular weight: 602.585 g/mol; molecular formula: $C_{27}H_{35}N_6O_8P$ (Fig. 1).

Literature survey reveals that very few methods of analysis reported for the estimation of remdesivir drugs in single dosage form by using HPLC. But there are no official reports are available on estimation of Remdesivir in single dosage form and stressed degradation studies of Du P. reported Quantitative HPLC-MS/MS determination of Nuc, the active metabolite of remdesivir, and its pharmacokinetics in rat [6]. Also reveals that Development of UHPLC/MS [7], LC-MS/MS in human plasma [8], accelerated stability study using RP-HPLC method [9], comparative study of HPLC and UV spectroscopic method is reported for quantification of remdesivir [10]. But no HPLC method is reported for degradation kinetics study. Further no methods have reported about shelf-life determination and characterization of degraded samples using hyphenated techniques. Therefore, it was deemed valuable to develop a RP-HPLC method for evaluation of Stress degradation study and degradation kinetics along with Shelf-life determination.

So, the scope of developing and validating a method is to ensure a suitable strategy for a particular analyte which is more specific, accurate, simple and precise. The validation of this method is carried out according to the ICH guidelines [Q1A (R2)].

MATERIALS AND METHODS

Materials

Marketed Formulation was obtained as a gift sample from Mylan Laboratories Ltd. Remdesivir was obtained from Lupin Pharmaceutical Ltd. Acetonitrile (HPLC Grade), Methanol (HPLC Grade), GR Grade Hydrochloric Acid, Sodium Hydroxide and Hydrogen Peroxide etc. used during the analysis. In-house double distilled water was used during whole experiments.

Instrumentations

The Shimadzu-HPLC series 1100 equipped with isocratic pump LC-10ADVP, PDA - SPD M20A detector was used for stress degradation analysis of Remdesivir. The analysis was performed using Agilent ZORBAX SB-C8 (4.6×150×5µm) column, Acetonitrile in the ratio of 95:05 as mobile phase; wavelength selected for analysis was 246 nm with the flow rate of 0.8 mL/min at which drug showed sharp peak. The analysis was performed on the isocratic pump mode; with the injection volume of 20µl. As a diluent, the

mobile phase is being used.

System suitability

After equilibration of column with mobile phase, working standard solution of about 50µg/ml, was injected through the manual injector five times and the chromatograms were recorded and the peak area was measured.

Linearity

Aliquots of standard stock solution were in range 1.0 to 5.0 mL in 10 mL volumetric flask with diluent (methanol) and volume was made up to mark with diluent to obtained concentration ranging from 10 – 50 µg/mL of Remdesivir`. The mobile phase was allowed to equilibrate with stationary phase till steady baseline was obtained. Chromatograms were recorded after each final solution was injected and separated. The graph plotted between concentrations vs AUC.

Stressed degradation studies

Kinetic samples Preparations: Accurately weighed quantity of Remdesivir (~10 mg) was transferred to a series of different 10.0 mL volumetric flasks. To each flask 5.0 mL diluent was added and then 5.0 mL of reagent (acid, alkali, 3% hydrogen peroxide and distilled water) were added. After that the flasks were placed in the oven at room temperature and 40ºC, samples and standards were taken out after 60 and 180 minutes, respectively. After filtering the material, 1.0 ml of the filtrate was diluted with diluent to make 10.0 ml. The final concentration of solution was 50 µg/ml of Remdesivir.

The amount of drug undegraded of sample and standard was calculated using the formula.

$$\text{Drug undegraded} = \frac{\text{Au(exposeds)}}{\text{As(unexposeds)}} \times Cs \quad \text{----------(1)}$$

Where,

Au = peak area of sample (exposed), As = peak area of standard (unexposed) and
Cs = Concentration (10mg)

Kinetics of Degradation

After having submitted to various hydrolytic conditions like acidic, alkali, oxidative and neutral at two different temperatures, a reduction of drug concentration in marketed formulation according to the time of exposure to stress could also be observed.

From the order of reaction, the half-life and shelf life of the marketed formulation was calculated by using formula (2), (3) and (4)

For zero-order reaction ----------(2)

Half- life equation: t 1/2 = C0 /2K
Shelf-life equation: t 0.9 = 0.1C0/K

For first-order reaction ----------(3)

Half- life equation: t 1/2 = 0.693/ K
Shelf-life equation: t 0.9 = 0.105/K

For second-order reaction ----------(4)

Half- life equation: t 1/2 = 1/ KC0
Shelf-life equation: t 0.9 = 0.11/KC0

From the observation and results of degradation of various hydrolysis at room temperature and

40^0C in oven, the graph was plotted and regression coefficient was observed and the order of kinetics was decided.

Preparation of Stock Solutions

Standard stock Solution: A standard stock solution of 1000 µg/ml was prepared in methanol.

Working standard solution: The working stock solution was appropriately diluted with methanol get the final concentration of 50µg/ml.

Diluent: Methanol [Use mobile phase as a diluent.]

Selection of wavelength

The working standard solution of Remdesivir (50µg/mL) was scanned in the range of 400-200 nm in 1.0cm cell against solvent blank (Methanol) and the spectra was recorded.

Accuracy

The accuracy was evaluated by conducting recovery tests using the standard addition technique at three concentrations that were 80%, 100%, and 120% of the target level of remdesivir (40 µg/mL). Trials were conducted three times at each level, and the percentage of recoveries was calculated. The percentage recovery calculated for each concentration [11].

Precision

Intraday variations (repeatability achieved by testing a standard solution on the same day) and interday variations (repeatability performed by analyzing a standard solution on three successive days) were taken into account when determining precision.

Linearity

The linearity of the remdesivir HPLC technique was tested at five different concentration levels: 80, 90, 100, 110 and 120 µg/mL. To determine the quantitative concentration of remdesivir, peak areas were measured. The coefficient of determination (r2) values better than 0.999 ($r2 \geq 0.999$) were used as a criterion for linearity.

Robustness

An analytical method's robustness indicates how reliable it is under typical operating conditions and is able to endure purposeful, modest changes in its parameters. Perform experiments by changing conditions such as temperature (±5°C), change in wavelength (±5nm), and ionic strength of buffers, level of activities to mobile phase.

Ruggedness

Ruggedness is a measurement of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst.[12]

Limit of detection (LOD) and Limit of Quantification

In the ICH guidelines, several approaches for determining the detection (LOD) and quantification (LOQ) limits are described. 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 by

$$LOD = 3{:}3*s/m$$
$$LOQ = 10*s/m$$

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RESULT AND DISCUSSION

Preliminary optimization of mobile phase and other chromatographic conditions

The chromatographic conditions were set and various mobile phase composition was tried and some of the trials are mentioned below. Trial no 3 was found to be the best composition which showed sharp and well-defined peak. The observation are shown in Table 1.

The mobile phase was allowed to equilibrate with the stationary phase as indicated by a steady baseline once the chromatographic conditions were adjusted according to the optimal parameters. Using a manual injector, 20µL of solution was injected, and a chromatogram was recorded. A mobile phase containing ACN: Distilled water (95:05%v/v) gave well-resolved peak and reasonable retention time as shown in Figure 2.

System suitability Analysis

Six replicate estimations of standard solution of remdesivir were injected under the optimized chromatographic conditions mentioned above in Table 2.

Linearity

Inject each level into the HPLC chromatographic system and measure the peak area. Plot the peak area versus concentration on the X- and Y-axis (peak area versus concentration), then the correlation coefficient was determined and is shown in Figure 3

The correlation coefficient was found to be 0.9993.

Stressed Degradation Studies [1]

It was performed by placing standard and sample of marketed formulation with 0.1N HCL solution (Acid hydrolysis), 0.1N NaOH (Alkali Hydrolysis), 3% H2O2 (Oxidative Hydrolysis), water (Neutral Hydrolysis) in oven at 40ºC and room temperature for a period of 60 min and 180 min.

The marketed sample and standard solution were prepared as per procedure described earlier. The sample and standard solution were injected and chromatographed separately using optimized chromatographic conditions.

The chromatograms of marketed formulation and standard Remdesivir under various hydrolytic conditions like 0.1N HCL solution (Acid hydrolysis), 0.1N NaOH (Alkali Hydrolysis), 3% H_2O_2 (Oxidative Hydrolysis), water (Neutral Hydrolysis) at room temperature and 40^0C were recorded and amount undegraded of standard (exposed) and Sample was calculated. The results of Acid hydrolysis, Alkali hydrolysis, Oxidative hydrolysis and Neutral hydrolysis are shown in Table 4.

Kinetics of Solution State Degradation Studies

For all hydrolytic conditions, the kinetics of degraded sample was evaluated. Obtained the plot of regression coefficient (r) and the best fit observed indicates the order of degradation reaction.

zero- order kinetics= Values of concentration against time

first- order kinetics=Log of concentration verses time

second-order kinetics =Reciprocal of concentration verses time

From the order of reaction, the half-life and shelf life of the marketed formulation was calculated by using formulas**.** The results are shown in Table 5

Peak Purity

Peak purity analysis was done to ascertain the purity of signal obtained such that the peak produced in the chromatogram is solely because of the analyte and no other peak is contributing in it. Peak purity analysis data is shown in Table 6.

Application of Proposed Method For Assay Of Marketed Formulation

Preparation of sample

Lyophilized powder of Remdesivir injection were weighed. A quantity of remdesivir powder equivalent to 10.0 mg weighed and transferred to 10.0 mL of volumetric flask, sonicated for 15 min with sufficient quantity of diluent (mobile phase) and diluent was used to adjust the volume to the mark. The content of flask was filtrate through 0.45 µm filter paper. A 1.0 mL portion of the filtered was further diluted to 10.0 mL with diluent (100µg/mL). After that 5.0 mL portion of the dilution was further diluted to 10.0 mL with diluent (50µg/mL). After equilibration of stationary phase, five sample solutions were injected separately and chromatogram were recorded. The content of Remdesivir in each sample was calculated by comparing the peak area of sample with that standard. Results of estimation are shown in Table 7.

Validation Of Proposed Method [13]

The proposed method was validated in accordance with ICH guidelines for various parameters mentioned below:

Accuracy

The percent RSD of the recovery study should not be more than 2%. Results of estimation are shown in Table no 8.

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Precision

The sample was prepared as per procedure described under marketed formulation and analyzed at intervals of 3h upto 6h for intraday study and on 1st, 3rd, 7th and 10th day for interday study. The content of Remdesivir was calculated.

Precision of proposed method is expressed as SD and %RSD of series of measurements. Percent RSD should not be more than 2. Results of study are shown in Table 9

Linearity and Range

The recorded area at five different concentration was used to construct the linearity graph. The plot of concentration vs AUC so plotted was found to be linear and is shown in Figure 4.

Robustness

The results of the deliberate changes in made parameters where found to be within limits, hence the method was found to be robust and reliable. Results of study are shown in Table 10.

Ruggedness

Analyst to analyst study

The samples were analyzed using proposed method by two different analysts and the sample preparation was done as per the general procedure described earlier. The results are shown in Table 11.

Detection Limit [DL] and Quantification Limit [QL]

The LOD and LOQ were determined using the calibration curve slope and the standard deviation

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of the Y-intercept. DL was found to be 1.63 µg and DQ was found to be 4.95 µg.

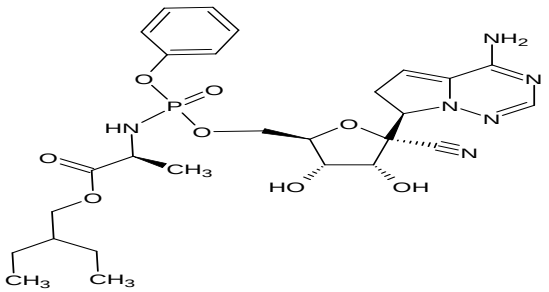

Figure 1: Structure of Remdesivir

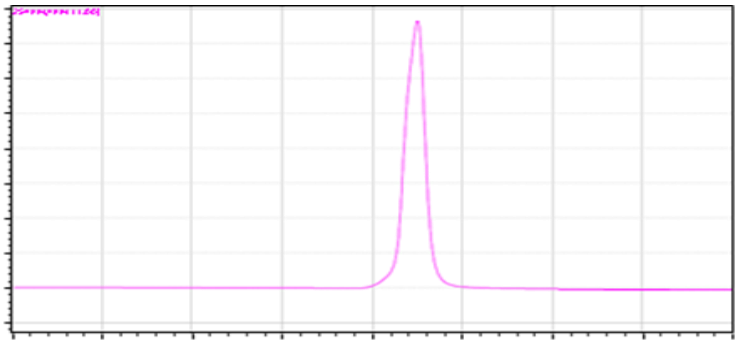

Figure 2: Standard chromatogram of Remdesivir

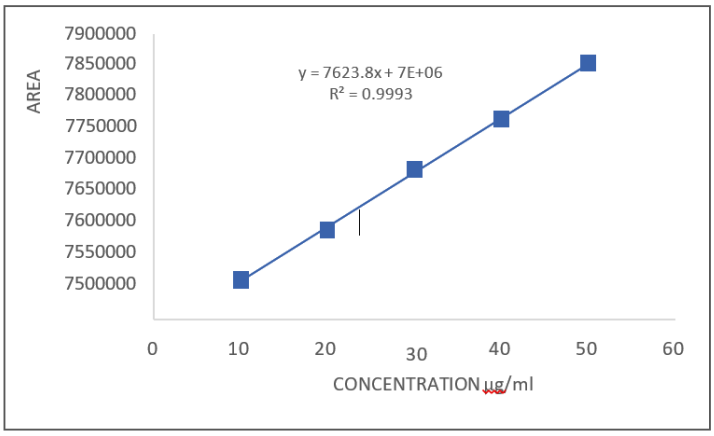

Figure 3: Calibration curve of Remdesivir

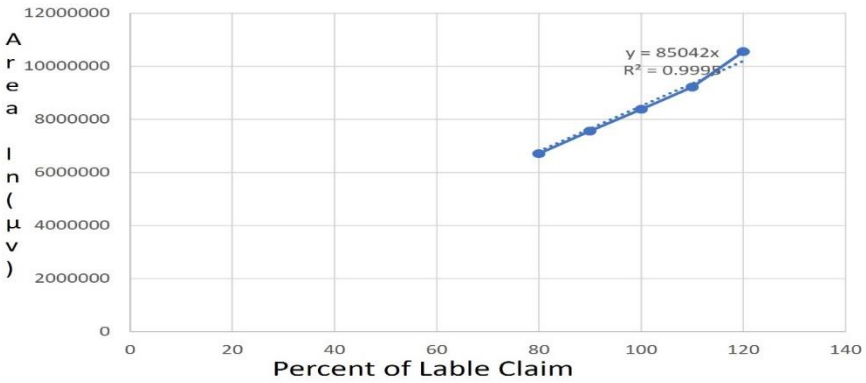

Figure 4: Plot of linearity and range of remdesivir

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Table 1: Selection of mobile phase

Trial	Mobile phase (%V/V)	Remarks
I	ACN: Methanol(80:20v/v)	Peak shape was not proper, Broadpeak
II	ACN: Methanol(90:10v/v)	Slightly Broad peak was observed
III	**ACN: Methanol (95:05v/v)**	**Sharp peak, R.T. is 4.49**

Table 2: Optimized chromatographic parameters

Parameters	Condition
Column	Agilent ZORBAX SB-C8(4.6×150×5µm)
Mobile Phase	ACN: Distilled water (95:05)
Flow rate	0.8 mL/min
Injection volume	20µl
Detection wavelength	246 nm
Column temperature	30-35^{0}C
Pump mode	Isocratic

Table 3: Results of System suitability Analysis

Sr. No.	Peak Area (µV)
1	7283511
2	7284241
3	7284789
4	7285251
5	7287587
6	7299812
Mean	**7287532**
±SD	**6172.966**
%RSD	**0.08**
Retention time (Mean)	**4.45**
Tailing Factor	**1.209**
HETP	**3403.41**

Table 4: Observation and result of hydrolytic condition of standard and sample at room temperature and 40^{0}C.

Condition	% drug undegradedstandard				% drug undegraded sample			
	RT		40^{0}C		RT		40^{0}C	
	60 min	180 min	60 min	180 min	60 min	180 min	60 min	180 min
Acid hydrolysis	--	29.59	79.32	48.55	--	44.42	60.55	33.40
Alkali hydrolysis	--	46.55	53.64	24.54	--	44.66	56.35	88.86
Oxidative hydrolysis	--	48.48	97.84	19.24	--	40.15	71.17	25.29
Neutral hydrolysis	--	67.30	88.12	39.59	--	76.14	99.61	28.26

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Table 5: Observation and result of Half-life and Self life

Sr No.	Conditions	Order (40°)		Rate Constant (k) (40°)		Half Life (t1/2) (40°) (MIN)		Shelf Life (t0.9) (40°) (MIN)	
		Std	Sample	Std	Sample	Std	Sample	Std	Sample
1	0.1N HCl	Zero	Zero	2.67×10-2	6.53×10-2	200.85	436.7	40.13	87.34
2	0.1N NaOH	First	First	3.95×10-3	1.17×10-3	750.05	203.8	150.01	30.88
3	3% H_2O_2	First	First	9.1×10-4	1.18×10-2	88.84	92.63	13.46	14.04
4	Neutral	Zero	First	2.42×10-2	7.35×10-3	175.4	182.3	26.58	27.63

Table 6: Observation of peak purity analysis

Sr. no	Condition	Purity angle		Purity threshold	
		Sample	Std	Sample	Std
1	Unexposed	0.5501	0.6703	0.8542	0.9917
2	Acidic	0.8038	0.6106	0.9998	0.9995
3	Alkaline	0.8337	0.4072	0.9970	0.9461
4	Oxidative	0.9995	0.9732	0.9998	0.9998
5	Neutral	0.9306	0.7272	0.9978	0.9920

Table 7: Results for estimation of Remdesivir in marketedformulation

Sr.No	Wt. of sample taken (mg)	AUC Sample (µV)	% Labelled claim
1	10.1	3102624	98.38
2	10.2	3112366	99.69
3	10.1	3149846	100.89
4	10.1	3112177	100.33
5	10.2	3155908	101.09
		Mean	**100.07**
		±S.D.	**1.093**
		%RSD	**1.092**

Table 8: Observation and result of Accuracy

Sr. No.	Wt. of sample taken (mg)	Amt. of Pure Drug added (mg)	AUC(µV) Sample	Total Amount Recovered(mg)	% Recovery
1	10.1	5.0	4748413	15.03	100.7
2	10.2	10.0	6287952	19.91	99.12
3	10.1	15.0	7822654	24.77	98.48
Mean					**99.43**
±SD					**1.14**
%RSD					**1.15**

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Table 9: Result of Intraday and Interday study

Time/ Days	Wt. of tablet powder taken (mg)	% labelled claim
Intraday precision		
0 h.	~ 10	99.97
3 h.		100.26
6 h.		101.51
Mean		**100.58**
±S.D.		0.81
%RSD		0.81
Inter day precision		
Day 1	~ 10	99.89
Day 3		100.19
Day 7		100.29
Mean		**100.12**
±S.D.		0.20
%RSD		0.20

Table 10: Results of robustness study

Sr. No.	Deliberate condition	Retention time (min)	Theoretical plate	Tailing factor
1	Standard Condition	4.442	3403.41	1.209
2	Organic Phase change (+10%)	4.488	3355.24	1.206
3	Organic Phase change (-10%)	4.597	3745.94	1.227
4	Wavelength 241	4.533	3784.19	1.213
5	Wavelength 251	4.483	3840.94	1.204
6	Flow rate (1 ml/min)	3.584	3824.09	1.204
7	Flow rate (0.6 ml/min)	5.968	3825.05	1.200
			Mean	1.209
			±SD	0.008
			%RSD	0.73

Table 11: Observation of ruggedness study

Sr. No.	% Label Claim	
	Analyst-I	**Analyst-II**
1	99.97	99.89
2	100.26	100.19
3	101.51	100.29
Mean	**100.58**	**100.12**
±SD	**0.81**	**0.20**
%RSD	**0.81**	**0.20**

CONCLUSION

From the results obtained by RP-HPLC and Kinetics study of the samples it can be concluded that the proposed method was successfully applied for its assay, degradation (stress testing) of drug and degradation kinetics in solution state. The method was found to be accurate, precise, rugged and robust. The results indicate that the temperature, ionic strength and oxidation greatly influence the stability of Remdesivir and the degradation behaviour of remdesivir followed first-order kinetics. Remdesivir was not stable in neutral, alkaline, high temperature conditions. Investigating of suitable storage conditions for remdesivir should consider the influence of temperature, ionic strength and oxidation. This study was a typical example of the method development and degradation kinetics of drug following the recommendations of ICH guidelines. The information obtained from the degradation kinetics will be helpful for understanding the stability and shelf life of remdesivir and providing a reference for further studies of remdesivir clinical applications.

REFERENCES

[1] Blessy M., Patel RD, Prajapati P, and Agrawal YK. Development of Forced Degradation and Stability Indicating Studies of Drugs A Review. Jr of Pharma Anal. 4(3); 2014:159– 165.

[2] Singh S.S., and Bakshi M. Development of Validated Stability Indicating Assay Method, Critical Review. J. Pharm. Biom. Anal., (28); 2002:1011-1040.

[3] FDA, International Conference on Harmonization: Stability Testing of New Drug Substance and Product. Federal Register (ICH Q1A). 59(183); 1994:48753-48759.

[4] Baertschi, S.W., Alsante, K.M., & Reed, R.A. (Eds.). (2005). Pharmaceutical Stress Testing: Predicting Drug Degradation (1st ed.). CRC Press,2005,504.

[5] Panda A, Kulkarni S, Tiwari R, Stability Studies: An Integral Part of Drug Development Process, Int Jr of Pharm Res and Bio-Sci. 2013; 2:69-80.

[6] Du P., Wang G., Yang S., Pengfei L. and Lihong L. "Quantitative HPLC-MS/MS determination of Nuc, the active metabolite of remdesivir, and its pharmacokinetics in rat" Analytical and Bioanalytical Chemistry 2021: 413:5811-5820.

[7] Avataneo V., Nicolo A., Cusato J., Antonucci M., Manca A. and Palermiti 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" Journal of Antimicrobial Chemotherapy 2020: 75: 1772-1777.

[8] Nguyen R., Goodell J. C., Shankarappa P. S. and Zimmerman S. "Development and validation of a simple, selective, and sensitive LC-MS/MS assay for the quantification of remdesivir in human plasma", Journal of Chromatography B: 1171 (2021) 122641.

[9] Hamdy M., Moneim M. and Kamal M., "Accelerated stability study of the ester prodrug remdesivir: Recently FDA-approved Covid-19 antiviral using reversed phase-HPLC with fluorimetric and diode array detection", Biomedical Chromatography, 2021: e5212.

[10] 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, 15(1), 2021: 507-513.

[11] Kashyap Thummar *, Malay Pandya, Nisarg Patel and Foram Rawat; an RP-HPLC method for the quantitative determination of remdesivir in intravenous dosage form,2023, vol. 14(3): 1273-1279.

[12] Heyden YV, Smith SW, Nijhuis A, Smeyers-Verbeke J, Vandeginste BG, Massart DL. Guidance for robustness/ ruggedness tests in method validation. J Pharm Biomed Anal 2001; 24:723-53.

[13] Shabir G. HPLC Method Development and Validation for Pharmaceutical Analysis. Pharmaceutical Technology Europe. 16(3), 2004. (Accessed 12 January 2020.

[14] Naveed S., Basheer S. and Qamar F. Stability of a Dosage Form and Forced Degradation Studies. Jr of Bioequi & Bioavail. 8(4); 2016:191-193.

[15] Barron M. D., "Implementing successful Stability Testing Operations", Pharm. Tech., 2003, 2: 14-21.

[16] Trisel L., "Stability Indicating HPLC method for drug analysis" Pharmaceutical Press, 2nd Edn., Benin, 2003, 01.

[17] Singh S.S. and Bakshi M., "Guidance on Conduct of Stress Test to Determine Inheritant Stability of The Drug", Pharm. Tech., 2000, 4: 01-14.

[18] Brahmankar D. M. & Jaiswal S. B. Biopharmaceutics and Pharmacokinetics a Treatise. First edition, Vallabh Prakashan, Delhi, 2005,215-219.

[19] Hayes S. Remington: The Science and Practice of Pharmacy, volume I and volume II. Twenty-second

edition. Journal of the Medical Library Association: JMLA, 102(3),2014, 220–221.
[20] Leon Lachman and Herbert A. Lieberman. The Theory and Practice of Industrial Pharmacy. Special Indian Edition 2009, 761-764.
[21] Martin A. N. (1993). Physical pharmacy: physical chemical principles in the pharmaceutical sciences (4th ed.). Williams & Wilkins,1993,284-290.
[22] Kapil Kalra. Method Development and Validation of Analytical Procedures. Quality Control of Herbal Medicines and Related Areas, Prof. Yukihiro Shoyama (Ed.), 2011. InTech,
[23] ICH Validation of Analytical Procedures: Text and Methodology. Q2(R1), Current Step 4 Version, Parent Guideline. 1994, 630.
[24] Bajaj S., Singla D. and Sakhuja N. Stability Testing of Pharmaceutical Products, Journal of Applied Pharmaceutical Science 2(3); 2012:129-138.
[25] Surabhi SR, Jaina N. Validated stability indicating method for determination of umifenovir-remdesivir in presence of its degradation products. International Journal of Development Research, 2021;11:46227-32.
[26] Santhosh Illendula, K. Sai Sneha, Rajeswar Dutt; A new RP HPLC method for the simultaneous estimation of Atazanavir and Ritonavir in its pure and pharmaceutical dosage form as per ICH guidelines, WJPPS 08(09) 2019, 1018-1033.
[27] K. Monika Raasi1, U. Spandana2, Sk. Abdul Rahaman1, Analytical Method Development and Validation of Remdesivir in Bulk and Pharmaceutical Dosage Forms Using Reverse-Phase-High Performance Liquid Chromatography, International Journal of Pharmaceutical Sciences and Clinical Research 2021; 1(2):282-329.
[28] Srinivas Reddy Jitta 1, Salwa 1, Lalit Kumar 1, Puja K Gangurde 1, Ruchi Verma 2, Development and Validation of High-Performance Liquid Chromatography Method for the Quantification of Remdesivir in Intravenous Dosage Form, Assay Drug Dev Technol, Nov-Dec 2021; 19(8):475-483.
[29] 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).
[30] 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.
[31] 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.
[32] 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.
[33] 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.