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VALIDATED STABILITY INDICATING METHOD FOR DETERMINATION OF UMIFENOVIR- REMDESIVIR IN PRESENCE OF ITS DEGRADATION PRODUCTS

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ABSTRACT

A simple stability indicating reverse-phase high performance liquid chromatographic (RP-HPLC) method has been developed for the determination of Umifenovir and Remdesivir in its tablet dosage form. The chromatographic solution was optimized by using the standard solution. The chromatographic method was used by Zorbax SB C18 column of dimensions 150x4.6 mm, 3.5 microns, using isocratic elution with a mobile phase of acetonitrile and water with a 50:50 ratio was used for the chromatographic separation and was monitored at a wavelength 230 nm PDA detector with flow rate 1 ml/min. The total run time was 10 min. According to the ICH guidelines, the developed approach was validated. The calibration charts plotted were linear with a regression coefficient of R2 > 0.999, means the linearity was within the limit. Recovery, specificity, linearity, accuracy, robustness, ruggedness were determined as a part of method validation and the results were found to be within the acceptable range. All the degradation products generated during the stress conditions are well separated and peaks have been well resolved with an acceptable retention period indicating that the proposed method was fast, easy, feasible and affordable.

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INTRODUCTION

Umifenovir, marketed under the brand name Arbidol, is an antiviral drug used in Russia and China to treat influenza (*Lampejo T*; Kalil AC). Umifenovir is a drug that comes in the form of pills, capsules, and syrup. Umifenovir has an indole centre that is functionalized with different substituents in all but one location. In trials, the drug was found to prevent viral entry into target cells while also stimulating the immune response (Bonilla FA; Sarma J). Umifenovir is available in the form of pills, capsules, and syrup. Umifenovir prevents influenza virus (Leneva IA) membrane fusion. Umifenovir acts as a barrier between the virus and the cells that it infects. Fusion between the viral envelope (which surrounds the viral capsid (Luque A; Krupovic M; Newcomb WW)) and the target cell's (Halfdanarson T. R) cell membrane (Budin I; Zeidi) is prevented. As a result, viral penetration into the target cell is prevented, and the cell is protected from infection (Boriskin YS). Remdesivir, also known as veklury, is a broad-spectrum antiviral drug (Irwin K; Hayden FG) developed by Gilead Sciences, a biopharmaceutical company. It's administered as a vein injection (Kienle). Remdesivir was first developed to treat hepatitis C (Kim A; Webster DP), then screened for Ebola virus disease (Swetha) and Marburg virus infections before being looked into as a COVID-19 post-infection treatment. Raised blood levels of liver enzymes are the most common side effect in healthy volunteers (Kwo). (a sign of liver problems). Nausea is the most common side effect in people who have COVID 19. Liver inflammation and an infusion-related reaction with nausea, low blood pressure (Arnold), and sweating (Robertshaw D) are possible side effects.

MATERIALS AND METHODS

Chemicals: Merck India Ltd, Mumbai, India, provided acetonitrile, HPLC-grade Tri-ethyl amine (TEA), and water. Merck India Ltd, Mumbai, provided APIs of Remdesivir, Umifenovir, and their impurities as reference standards.

The Instrumentation: This study used a Waters alliance liquid chromatography (model 2695) with an empower 2.0 data handling

device and a Zorbax SB C18 (150x4.6mm, 3.5) and a photo diode array detector (model 2998).

Chromatographic conditions: The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and water 50:50 ratio and Zorbax SB C_{18} (150x4.6 mm, 3.5 micron) column with a flow rate of 1 ml /min.

Diluents: Mobile phase was used as diluent.

Preparation of standard stock solution: To make a normal stock solution, combine 100 mg Umifenovir and 50 mg Remdesivir in a 100 mL volumetric flask with 70 mL diluents and sonicate for ten minutes to fully dissolve the contents, then top up with diluents.

Preparation of standard solution: 1 mL of the above normal stock solution is transferred to a 10mL volumetric flask and diluted to the desired concentration with the diluent.

Preparation of sample solution : In a 100ml volumetric flask, place 240mg of Umifenovir and 100mg of Remdesivir of the sample drug, add 70ml of diluent, and sonicate for ten minutes to fully dissolve the contents, then make up to the mark with diluent. The solution is filtered into a device using a 0.45 nylon syringe in a vial.

Validation procedure: The ICH Q2 (R1) guidelines accepted systematic boundaries such as device suitability, precision, specificity, consistency, linearity, robustness, LOD, LOQ, forced deterioration, and steadiness.

System suitability: System suitability criteria have been calculated in order to assess the system's presentation. The parameters USP plate count, USP tailing, and percent RSD can all be calculated and found to be within the cap.

Specificity: Specificity refers to the ability to analyse the analyte unequivocally in the presence of other factors such as impurities and excitements that may be believed to be present in the sample and norm solutions. It was examined using a blank sample as well as samples spiked with Umifenovir and Remdesivir.

Accuracy: Accuracy refers to how similar the test results produced by the process are to the true value. The recovery trails were carried out at three separate concentration levels. A minimum of three injections were given at each stage, with the amount of medication present, the percentage of recovery, and the associated standard deviation all being recorded.

Precision: The degree of agreement between individual test results is the precision of the analytical process. A multiple sampling analysis of a homogeneous sample was used to assess the precision of the current procedure in terms of repeatability, intraday, and interday variations. The sample was examined on the same day as well as on several days at various times.

Linearity: The empirical method's linearity refers to its ability to produce findings within a specified framework. For linearity spectrum evaluation, the peak area was directly proportional to the concentration of analytes in the sample: six series of standard solutions were chosen. The regression equations were determined using the peak area relative to the normal solution concentration, which was plotted on the calibration curve. The system of least squares was used to calculate the slope, coefficient, and intercept of the correlation.

LOD and LOQ: LOD stands for the smallest analyte quantity in a sample that can be defined, while LOQ stands for the smallest analyte quantity in a sample that can be measured with acceptable precision and accuracy. LOD and LOQ were calculated separately using calibration curves.

According to ICH guidelines, the LOD and LOQ were estimated to be 3.3s/n and 10 s/n, respectively, where s/n stands for signal to noise ratio.

Robustness: The robustness of an analytical method is a measure of its ability to remain unaffected by small but deliberate changes in the system's process parameters, as well as an indication of its performance in routine use. The robustness analysis was performed by injecting the standard solution into the HPLC system and adjusting the flow rate (0.3 ml/min), Organic step (20%) of chromatographic conditions. By evaluating the impact of the changed parameters and peak symmetry, the separation factor, retention time, and peak symmetry were determined.

Stability: Analytical solution was prepared and injected into the HPLC system at regular intervals ranging from 6 hours to 24 hours, depending on the instrument used and the injection series.

Stress degradation: There should be no interference between the peaks obtained for a chromatogram of preparations after stress degradation. Stress degradation experiments were carried out in accordance with ICH guidelines. The deterioration peaks should be spaced widely apart, the resolution between them should be at least 2.0, and the peak purity of the main peaks must pass. Various forms of stress conditions were used in forced degradation experiments to achieve a degradation of about 20%.

RESULTS AND DISCUSSION

The main analytical challenge during development of a new method was to separate active pharma ingredients from their impurities. In order to provide a good performance the chromatographic conditions were optimized.

Method optimization: Different ratios of phosphate buffer and acetonitrile were checked with isocratic and gradient mode in the mobile phase in order to optimize the chromatographic conditions. In each trail, however the mobile step composition was altered to improve the resolution and also to achieve reasonable retention times. Finally, buffer is water and isocratic elution acetonitrile was selected because it results in a greater response of the active pharma ingredient and the isocratic elution. During the optimization of the method various stationary phases such as C_8, C_{18} phenyl and amino columns were tested. From these trials the peak shapes were relatively good with a column of ZorBax SB C_{18} 150x4.6mm, 3.5 µ with a PDA detector. The mobile phase flow rate has been done at 230nm in order to obtain enough sensitivity. By using above conditions we get retention times of Remdesivir and Umifenovir were about 4.360 and 7.746 min with a tailing factor of 1.03 & 1.09. The number of theoretical plates for Umifenovir and Remdesivir were 4851, 5763 which indicate the column's successful output the % RSD for six replicate injections was less than 2% the proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Method validation: The optimized RP-HPLC validated method according to ICH guidelines in terms of system suitability, linearity, consistency, precision and robustness.

System suitability: USP plate count, USP tailing, and percent RSD have all been evaluated as device suitability parameters. The results of system suitability are shown in Table 1, and the system suitability chromatogram of Umifenovir and Remdesivir is shown in Figure 2.

Specificity: To assess the interference, sample and regular solutions were tested separately using the placebo test process. The active ingredients were well isolated from the blank and their excipients, as seen in the diagram below, and there was no interference from the placebo with the principal peak. As a result, the procedure is special. Figure 3 depicts a blank chromatogram.

Table 1. Results of system suitability

System suitability parameter	Acceptance criteria	Drug name	
		Umifenovir	Remdesivir
USP plate count	NLT 2000	4851	5763
USP Tailing	NMT 2.0	1.08	1.06
USP resolution	NMT 2.5	-	7.627
%RSD	NMT2.0	0.73	0.61

Table 2. Linearity of Umifenovir and Remdesivir

Sno	Umifenovir Conc.µg/ml	Umifenovir area count	Remdesivir Conc.µg/ml	Remdesivir area count
1	10	353112	5	219579
2	25	971058	12.5	628451
3	50	1942116	25	1207682
4	75	2913173	37.5	1836133
5	100	3884231	50	2415364
6	125	4855289	62.5	3043815
7	150	5826347	75	3845129
Correl coef		0.9999		0.9999
Slope		38961.783		50223.540
Intercept		11615.523		29830.579

Table 3. Results of accuracy

S. No	% Level	Umifenovir % Recovery	Remdesivir % Recovery
1	50	99.8	100.3
2	100	99.9	100.7
3	150	99.9	100.1

Table 4. Intraday precision results Remdesivir and Umifenovir

Umifenovir				Remdesivir		
S No	Umifenovir Conc.(µg/ml)	Area counts	% assay as is	Remdesivir Conc.(µg/ml)	Area count	% assay as is
1	100	3845751	100.642	50	2484231	100.245
2		3840854	100.845		2480341	100.549
3		3835952	100.546		2475854	100.542
4		3830874	100.245		2410951	100.642
5		3828471	100.697		2465874	100.753
6		3825745	100.243		2460541	100.854
%RSD		0.200	0.244		1.095	0.209

Table 5. Inter-day outcomes of accuracy of Umifenovir and Remdesivir

Umifenovir				Remdesivir		
S. No	Umifenovir Conc.(µg/ml)	Area counts	% assay as is	Remdesivir Conc.(µg/ml	Area count	% assay as is
1	100	2645864	100.841	50	3284201	100.845
2		2640785	100.694		3281569	100.745
3		2635865	100.245		3275845	100.256
4		2650147	100.521		3271894	100.456
5		2655012	100.623		3269612	100.856
6		2644126	100.845		3261547	100.541
% RSD	0.255			0.253		

Table 6. LOD and LOQ for Umifenovir and Remdesivir

Umifenovir				Remdesivir			
LOD		LOQ		LOD		LOQ	
Conc	S/n	Conc	S/n	Conc	S/n	Conc	S/n
0.125 µg/ml	8	0.413µg/ml	26	0.063µg/ml	6	0.208µg/ml	21

Table 7. Robustness data of Umifenovir and Remdesivir

Parameter name	% RSD	
	Umifenovir	Remdesivir
Flow minus (0.8 ml/min	0.64	0.72
Flow plus (1.2 ml/min)	0.38	0.68
Organic minus (-10%)	0.59	0.29
Organic plus (+10%)	0.52	0.64

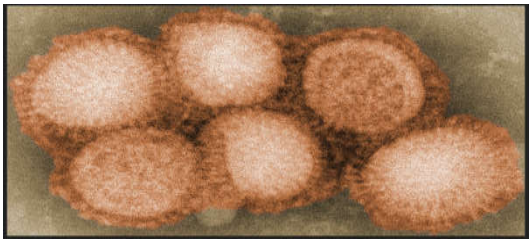


Figure 1. Structure of Influenza

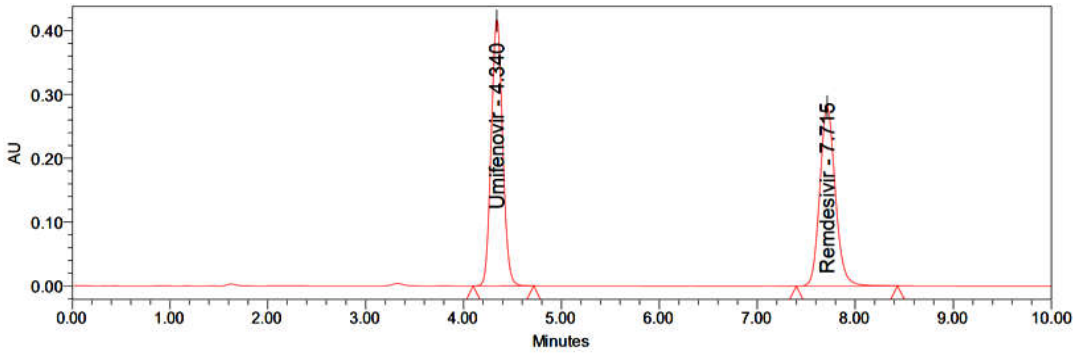


Figure 2. Chromatogram of system suitability

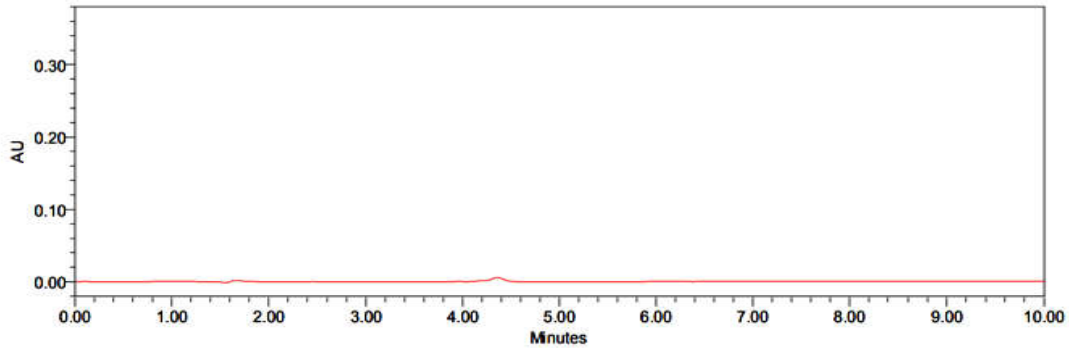
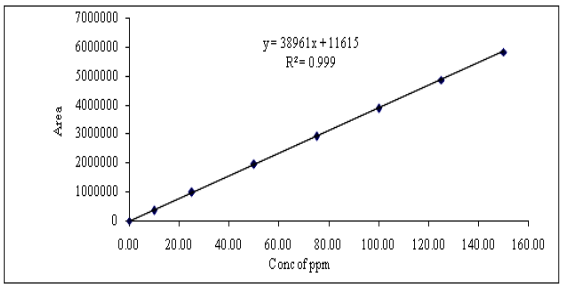
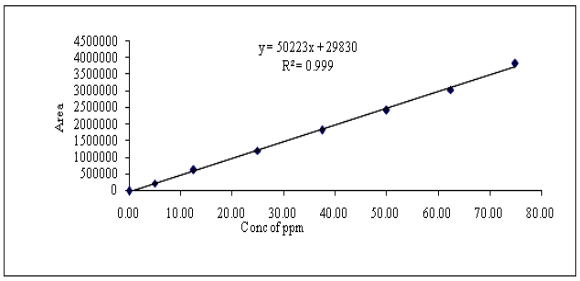


Figure 3. Chromatogram of blank



a



b

Figure 4. Calibration plots of (a) Umifenovir (b) Remdesivir

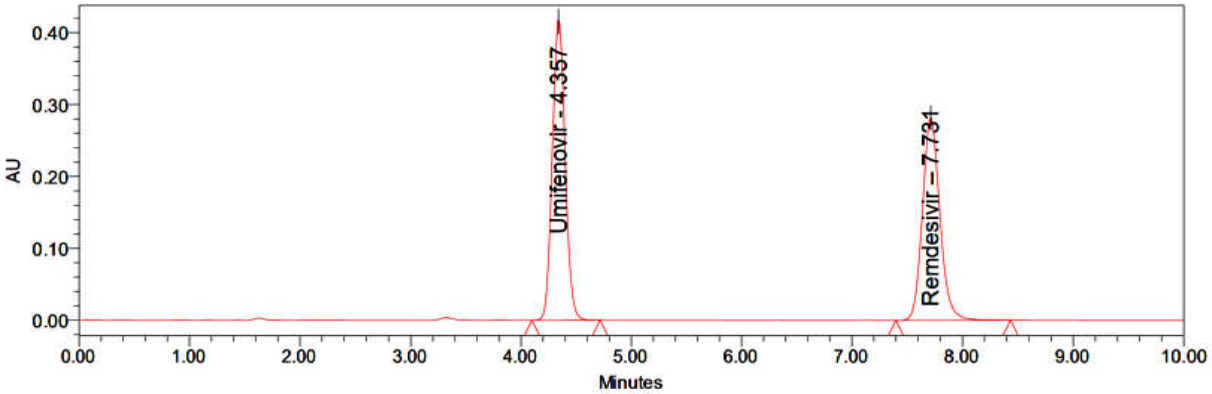


Figure 5. Chromatogram of sample

Table 8. Stability results of Umifenovir and Remdesivir

Stability	Umifenovir		Remdesivir	
	Purity	% of deviation	Purity	% of deviation
Initial	99.81	0.19	99.94	0.06
6 Hrs	99.72	0.28	99.86	0.14
12 Hrs	99.65	0.35	99.75	0.25
18 Hrs	99.54	0.46	99.68	0.32
24 Hrs	99.41	0.59	99.51	0.49

Table 9. Forced degradation results of Umifenovir and Remdesivir

Degradation condition	Umifenovir		Remdesivir	
	% assay	%Deg	%assay	%Deg
Acid degradation	86.284	13.716	85.284	14.716
Alkali degradation	86.358	13.642	85.469	14.531
Peroxide degradation	85.241	14.759	84.751	15.249
Reduction degradation	87.361	12.639	85.928	14.072
Thermal degradation	99.924	0.146	99.981	0.549
Hydrolysis degradation	99.952	0	99.981	0

Linearity: The area of the linearity peak versus different concentrations has been evaluated for Umifenovir, Remdesivir as 10,25,50,75,100,125,150 percent respectively. The linear regression plots are with the concentration data versus peak area. The correlation coefficients of regression, percent, y- intercept and slope of the calibration curves were calculated. The correlation coefficients succeeded 0.9999 for all. Table 2 gives the results of linearity and the calibration plots were shown in figure 4.

Accuracy: Accuracy was conducted in triplicate by analyzing active pharma ingredient sample solution at three kinds of concentration levels of 50, 100 and 150% of each at a specified limit. For all impurities, percentage recoveries were measured and found to be within the limit. Results of accuracy were shown in table 3.

Precision: Method precision was investigated by the analysis of six separately prepared samples of an equivalent batch. From these six separate samples, solution was injected and therefore the peak responses obtained wont to calculate mean and percentage RSD values. This technique was observed to be precise and RSD was 2.0%. The results were represented in table 4 and the sample chromatogram was shown in figure 5.

Intermediate precision: Six replicates of the sample solution were studied by different analysts on different days, and different instruments were tested. The peak areas that were used to calculate the mean percent RSD values were calculated. Table 5 shows the intermediate precision results.

LOD and LOQ: The calibration curve approach was used to calculate LOD and LOQ separately. Using an established RP-HPLC process, the compound's LOD and LOQ were calculated by injecting progressively lower concentrations of standard solution. Umifenovir has a LOD concentration of 0.125 g/ml and a s/n value of 8, while Remdesivir has a LOD concentration of 0.063 g/ml and a s/n value of 6. Umifenovir's LOQ concentration is 0.413 g/ml, and its s/n value is 26, while Remdesivir's LOQ concentration is 0.208 g/ml, and its s/n value is 21. LOD and LOQ values are mentioned in Table 6.

Robustness: The experiment's conditions were created to measure the robustness of an existing framework that had been deliberately changed, such as flow rate and mobile phase under organic percentage, in all of these different conditions. The resolution between active pharmaceutical ingredients and impurities was unaffected, and the time of retention, plate count, and tailing factor were all unaffected. As a result, this approach was reliable. Table 7 shows the Umifenovir and Remdesivir robustness results.

Stability: The standard and sample solution was kept at room temperature and at 2-8^{o}C up to 24 hours. Then these solutions were pumped into the device and calculate the % of deviation from initial to 24 hrs. There was no significant deviation observed and confirmed that the solutions were stable up to 24 hrs during the analysis. Results of stability were shown in table 8.

Degradation studies: The Umifenovir and Remdesivir samples were subjected to different conditions of forced degradation in order to partially degrade the compound. Forced degradation experiments have been carried out to find that the process is acceptable for degradation materials.

Furthermore, the studies include descriptions of the situations under which the medication is unstable in order to ensure that steps are always taken to prevent possible instabilities during formulation. In a 100ml volumetric flask, place 240mg of Umifenovir and 100mg of Remdesivir of the sample drug, add 70ml of diluent, and sonicate for ten minutes to fully dissolve the contents, then make up to the mark with diluent. This is the stock solution for a sample.

Acid degradation: 1 ml of sample stock solution is transferred to a volumetric flask of 10 ml, along with 1 ml of 1N HCl, and left to degrade for 15 minutes. After 15 minutes, apply 1 ml of 1N NaOH and dilute to the desired strength with diluent.

Alkali degradation: 1 ml of sample stock solution was put in a 10 ml volumetric flask, along with 1 ml of 1N NaOH, and left for 15 minutes. After 15 minutes, apply 1 ml of 1N HCl and dilute to the desired concentration with diluent.

Peroxide degradation: 1 ml of sample stock solution was moved to a 10 ml volumetric flask, along with 0.3 ml of 30% hydrogen peroxide and diluent to make up to the mark.

Reduction degradation: 1 ml sample stock solution was moved to a 10 ml volumetric flask, along with 1 ml 30 percent sodium bi sulphate solution, and made up to the mark with diluent.

Thermal degradation: It was achieved by placing the sample solution in a 105°F oven for 6 hours. The resulting solution was then injected into HPLC for analysis.

Hydrolysis degradation: 1 ml of sample stock was transferred to a volumetric flack of 10 ml, 1 ml of water was added, and the mixture was diluted to the desired concentration. The degradation results of Umifenovir and Remdesivir are shown in Table 9.

CONCLUSION

In this article, we present simple, selective, validated and well defined stability that illustrates the methodology of gradient RP-HPLC for the quantitative determination of Umifenovir and Remdesivir. All the degradation products produced during the stress conditions and the related active pharmaceutical ingredients are well separated and peaks have been well resolved and separated with an acceptable retention period indicating that the proposed method in RS condition is quick, easy, feasible and affordable .

Therefore the developed method during stability tests, it can be used for routine analysis of production samples and to verify the quality of drug samples during stability studies.

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