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Spectrophotometric Estimation of Remdesivir in the Pharmaceutical Preparation "COVIFOR™" Using the Oxidative Coupling Reaction with Nicotinic Acid in an Alkaline Medium.

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Abstract: A simple and selective spectrophotometric method has been developed for the estimation of the drug Remdesivir (Rem). The method is based on the oxidation of Rem with Sodium Nitroprusside (SNP) as oxidizing agent in an alkaline aqueous medium, followed by coupling of Rem with the reagent Nicotinic Acid (NCA) to form a complex (yellow-colored) with maximum absorption at a wavelength of 394 nm. The method exhibits linearity in the range of 1-25 µg/mL , with a detection limit of 0.0139 µg/mL, and a quantitation limit of 0.0422 µg/mL. The molar absorptivity is 8556.778 L/mol·cm, and the Sandell's sensitivity value is 0.0704 µg/cm². The recovery values ranged from 98.9787-100.1690%, with a relative standard deviation not exceeding 0.3086% for Rem. The method was successfully applied for the estimation of pharmaceutical preparations using both direct and standard addition methods.

Keywords: Spectrophotometric Estimation, Oxidative Coupling, Remdesivir, Nicotinic Acid, Sodium Nitroprusside.

Introduction

The IUPAC systematic name for Remdesivir 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-dihydroxytetrahydrofuran-2-yl]methoxyphosphoryl]amino]propanoate). Its molar mass is 602.59 g/mol, and its molecular formula is C27H35N6O8P, as illustrated in Figure 1 [1,2].

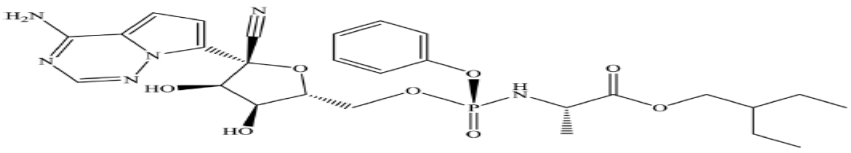

FIGURE 1: Structural Formula of Remdesivir[3].

Remdesivir is a crystalline solid drug that does not absorb moisture and comes in various colors, including white, off-white, or yellow[4]. It is soluble in ethanol and methanol but has limited solubility in water. Its solubility in water depends on the pH, with increased solubility at lower pH levels[5].

Remdesivir was discovered and introduced as an antiviral agent due to its broad-spectrum activity against RNA viruses. This led to its approval by the U.S. Food and Drug Administration (USFDA) on October 22, 2020, under the name Remdesivir. Its usage is subject to close monitoring due to potential side effects[6,7], the most common of which include nausea, decreased albumin levels, decreased potassium levels, reduced red blood cell count, decreased platelet count, increased bilirubin levels, elevated liver enzyme levels, electrocardiogram abnormalities, vomiting, sweating, tremors, and slowed heart rate acceleration[8-13)]

Despite these side effects, Remdesivir is considered a promising candidate for treating various RNA viruses due to its unique structural characteristics [14]. Given its global significance. Various analytical techniques have been employed to quantify Remdesivir, including spectroscopy [15-17] and high-performance liquid chromatography (HPLC)[18-20]. The objective of this study is to develop a new spectroscopic method for the quantitative analysis of Remdesivir in pharmaceutical preparations by oxidizing it using sodium nitroprusside as an oxidizing agent and subsequently coupling it with the reagent nicotinic acid.

- ## Chemicals and Equipment

High-purity pharmaceutical compounds and reagents from reputable international companies in Germany, England, etc., were used. The following laboratory equipment was employed: UV-Vis Spectrophotometer Double Beam – SHIMADZU - Japan, Ultrasonic Water Bath Sensitive – LabTech - Korea, Analytical Balance with four decimal places – Radwag - Poland, and Shaking Water Bath - MemRemt - Germany.

PREPARATION OF STANDARD SOLUTIONS

- **Remdesivir Standard Solution (100 µg/mL)**

The standard solution was prepared by dissolving 0.1 gm of the standard substance (Remdesivir) in a sufficient amount of triple-distilled water in a 100 mL volumetric flask. It was then placed in an ultrasonic water bath for 5 min until complete dissolution, and the volume was adjusted to the mark with the same solvent. Working solutions were prepared as needed.

- **Nicotinic Acid Solution (0.001 M)**

The solution was prepared by dissolving 0.024 gm of the reagent in a specific volume of triple-distilled water in a 100 mL volumetric flask. The volume was then adjusted to the mark with the same solvent.

- **Sodium Nitroprusside Oxidizing Agent Solution (0.001 M)**

The solution was prepared by dissolving 0.012 gm of the substance in a specific volume of triple-distilled water in a 100 mL volumetric flask. The volume was adjusted to the mark with the same solvent.

- **Sodium Hydroxide Solution (Approximately 1 M)**

The solution was prepared by dissolving 4 gm of the base in a specific volume of triple-distilled water in a 100 mL volumetric flask. The volume was adjusted to the mark with triple-distilled water to obtain a stock solution. From this stock solution, diluted solutions were prepared as needed.

- **COVIFOR™ Pharmaceutical Preparation Solution (100 µg/mL)**

COVIFOR™ (Injection 1000mg) pharmaceutical preparation from Hetero Healthcare Limited, India, was used. It was dissolved in a 100 mL volumetric flask using triple-distilled water to obtain a stock solution containing 10,000 µg/mL of the active substance. From this stock solution, a final solution with a concentration of 100 µg/mL was prepared.

These chemicals and solutions were used in the analytical procedures.

RESULTS AND DISCUSSION

- **Preparation of the Oxidative Coupling Complex of Remdesivir**

When 1 mL of 0.001 M SNP solution was added to 2 mL of 100 µg/mL Remdesivir solution and the reaction medium was made alkaline by adding 0.5 mL of 1 M NaOH, followed by the addition of 1 mL of 0.001 M NCA reagent, a yellow soluble complex was formed, and the complex exhibited a new peak at 394 nm.

OPTIMAL CONDITIONS

- **Selection of the Best Coupling Reagent**

Several reagents, including Sitagliptin, Nicotinic acid, Pseudoephedrine HCl, were used to determine the best reagent that would yield the highest absorption for the formed complex. Nicotinic acid reagent was found to be the most suitable as it produced a colored complex with the highest wavelength. Therefore, it was chosen for subsequent experiments.

- **Selection of the Best Oxidizing Agent**

To select the best oxidizing agent, various oxidizing agents with a concentration of 0.001M were used. 1mL of each oxidizing agent was added to 2 mL of 100 µg/mL Remdesivir solution, followed by the addition of 0.5 mL of 1M NaOH and 1mL of NCA reagent. The results showed that sodium nitroprusside (SNP) provided the highest absorption, making it the best oxidizing agent for further experiments, as shown in Table 1.

TABLE 1: Selection of the best oxidizing agent for the Rem oxidative coupling complex.

Oxidizing Agent (0.001M)	λ_{max}(nm)	Absorbance
Sodium nitrite	354	74100.
Sodium thiosulfate	316	60.024
Sodium nitroprusside	394	7780.3

- **Effect of NCA Reagent Volume**

To determine the optimal volume of the NCA reagent with a concentration of 0.001M, various volumes ranging from 0.2 - 2.5 mL were used. Each volume was added to a series of 10 mL volumetric flasks containing 2 mL of 100 µg/mL Remdesivir solution, 1mL of SNP reagent, and 0.5 mL of 1M NaOH. The solutions were then diluted to the mark with distilled water, and the absorption was measured against a blank solution. The results indicated that the optimal volume of NCA reagent was 1.8mL of 0.001M. This volume was adopted for subsequent experiments, as shown in Table 2.

TABLE 2: Effect of NCA Reagent Volume on the Absorption of the Formed Complex of Remdesivir.

V(ml) of NCA (0.001M)	Absorbance	V(ml) of NCA (0.001M)	Absorbance
0.2	0.0715	1.6	0.4177
0.2	0.0715	1.6	0.4177
0.4	0.1454	1.8	0.4329
0.6	0.2105	2	0.4144

0.8	0.2950	2.2	0.4379
1	0.3776	2.4	0.4154
1.2	0.3771	2.5	0.3924
1.4	0. 3845		

- **Effect of Oxidizing Agent Volume**

The effect of the volume of the oxidizing agent on the proposed method was studied using a solution of sodium nitroprusside (SNP) with a concentration of 0.001 M as the oxidizing agent. Volumes ranging from 0.2 -3 mL were tested, and it was found that 2 mL of sodium nitroprusside was the optimal volume, providing the highest absorption. Therefore, 2 mL of sodium nitroprusside was used in subsequent experiments, as shown in Table 3.

TABLE 3: Effect of Oxidizing Agent Volume on the Absorption of the Formed Complex of Remdesivir.

V(ml) of SNP (0.001M)	Absorbance	V(ml) of SNP (0.001M)	Absorbance
0.2	0.2215	1.8	0.4949
0.4	0.3754	**2**	**0.5244**
0.6	0.3948	2.2	0.5179
0.8	0.4015	2.4	0.5041
1	0.4326	2.6	0.4842
1.2	0.4376	2.8	0.4519
1.4	0.4428	3	0.4227
1.6	0.4772		

- **Effect of Oxidation Time**

The oxidation time for the proposed method was studied by adding a fixed volume of 2 mL of 100 µg/mL Remdesivir solution to a series of 10 mL volumetric flasks. Then, 2 mL of 0.001 M SNP reagent and 0.5 mL of 1 M NaOH were added, followed by waiting for different time intervals ranging from (0 - 50 min) to ensure complete oxidation. Afterward, 1.8 mL of 0.001 M NCA reagent was added, and the solution was diluted to the mark with distilled water. The absorption was measured at a wavelength of 394 nm. The results showed that a 10min oxidation time was sufficient for the complete oxidation of Remdesivir, and it was adopted in subsequent experiments, as shown in Table 4.

TABLE 4: Effect of Oxidation Time on the Absorption of the Formed Complex of Remdesivir.

Time (min)	Absorbance	Time (min)	Absorbance
Immediately	0.5243	30	0.4742
5	0.5417	35	0.4709
10	**0.5593**	40	0.4628
15	0.5270	45	0.4465
20	0.5180	50	0.4377
25	0.4965		

- **Effect of Acid Addition**

The effect of acid on the absorption of the colored solution was studied, and it was observed that the addition of 0.1 mL of Hydrochloric acid solution instead of the base led to the disappearance of the color of the complex and the peak. Therefore, acid addition was excluded from further experiments.

- **Effect of base type**

To determine the effect of the type of base on the absorption spectrum of the colored complex, various bases including NaOH, $NaHCO_3$, Na_2CO_3, KOH, and $Ca(OH)_2$ were tested. Each base was added in a fixed volume of 0.5 mL at a concentration of 1 M. It was found that NaOH yielded the highest absorption, and it was subsequently used in further experiments, as shown in Table 5.

TABLE 5: Effect of Base Type on the Absorption of the Formed Complex of Remdesivir.

Type of Base (0.001M)	**λ_{max}(nm)**	**Absorbance**
Na_2CO_3	394	0.04546
$NaHCO_3$	394	0.03240
NaOH	394	0.5593
$Ca(OH)_2$	394	0.38113
KOH	394	0.28784

- **Effect of Base Volume**

To determine the optimal base volume that provides the best absorption, various volumes of 0.001 M NaOH solution were added to a series of 10 mL volumetric flasks containing 2 mL of 100 µg/mL Remdesivir solution, 2 mL of 0.001 M SNP reagent, and 0.5 mL of 1 M NaOH. After waiting for 10 minutes, 1.8 mL of 0.001 M NCA reagent was added, and the solution was diluted to the mark with distilled water. The absorption of the resulting colored solution was measured at a wavelength of 394 nm. It was found that adding 0.8 mL of 0.001 M NaOH solution significantly increased the absorption of the formed complex. Further increases in base volume led to a decrease in absorption. Therefore, 0.8 mL of 0.001 M NaOH solution was adopted for subsequent experiments, as shown in Table 6.

TABLE 6: Effect of Base Volume on the Absorption of the Formed Complex of Remdesivir.

V(ml)NaOH (0.001M)	**Absorbance**	**pH**	**V(ml)NaOH (0.001M)**	**Absorbance**	**pH**
0.1	0.1901	9.92	0.7	0.6197	11.88
0.2	0.3094	10.35	**0.8**	**0.6473**	12.2
0.3	0.4396	10.57	0.9	0.6129	12.53
0.4	0.5095	10.92	1	0.5900	12.85
0.5	0.5591	11.24	1.1	0.5740	13.17
0.6	0.5931	11.56	1.2	0.4506	13.49

- **Sequence of addition**

Several sequences of addition were studied to determine the sequence that provides the best absorption values for the formed colored complex. Based on the optimal conditions and measuring the absorption of the resulting color at the wavelength of 394 nm, it was found that the best additive sequence is sequence number (1), which achieved the highest absorption for the colored product. Therefore, it was adopted in subsequent experiments, as shown in Table 8.

TABLE 8: Effect of additive sequence on the absorption of the formed complex for Remdesivir.

No	**Order of addition**	**Absorbance**
1	**D + O + B + R**	**0.6473**
2	D + R + O + B	0.6145
3	R + B + D + O	0.5939

4	R + O + D + B	0.5904
5	B + O + D + R	0.5932
6	O + R + D + B	0.5941

D=Drug, O =Oxidant, B = Base, R = Reagent

- **Effect of Temperature**

To select the optimal temperature that gives the highest absorption for the resulting complex, the effect of temperature on the complex formed at a wavelength of 394 nm was studied, covering a temperature range from (10 - 60 C°). It was found that the maximum absorption occurred at room temperature, while an increase in temperature resulted in a decrease in absorption. Therefore, room temperature was adopted in subsequent experiments, as indicated in Table 9.

TABLE 9: Effect of temperature on the oxidative coupling complex of Remdesivir.

°Temperature C	**Absorbance**	**°Temperature C**	**Absorbance**
10	0.5718	40	0.6083
15	0.5908	45	0.6043
20	0.6176	50	0.5986
25	**0.6471**	55	0.5829
30	0.6194	60	0.5783
35	0.6105		

- **Effect of Time on Complex Stability**

A study was conducted to assess the stability and persistence of the complex formed after establishing the optimal conditions. This was done by taking 2 mL of a Remdesivir solution with a concentration of 100 µg/mL, adding 2 mL of the oxidizing agent SNP with a concentration of 0.001 M, and 0.8 mL of NaOH solution with a concentration of 0.001 M to a 10 mL volumetric flask. After waiting for 10 min, 1.8 mL of the NCA reagent with a concentration of 0.001 M was added, and the volume was completed to the mark with distilled water. The absorption of the complex was then measured at different times. It was found that the complex remained stable for at least an hour, which is sufficient for conducting multiple analytical measurements, as shown in Table 10.

TABLE 10: Effect of time on the stability of the formed complex for Remdesivir.

Time (min)	**Absorbance**	**Time (min)**	**Absorbance**
Immediately	0.6473	35	0.6474
5	0.6474	40	0.6476
10	0.6475	45	0.6471
15	0.6479	50	0.6479
20	0.6474	55	80.647
25	0.6473	60	40.647
30	0.6472	65	650.64

- **Effect of Solvent**

The effect of the type of solvent used for dilution on the spectral properties of the resulting complex was studied. Various organic solvents were used in addition to distilled water. Acetone provided the highest

absorption, but water was retained as the solvent due to its availability, low cost, and safety in subsequent experiments, The results are shown in Figure 2 and Table 11.

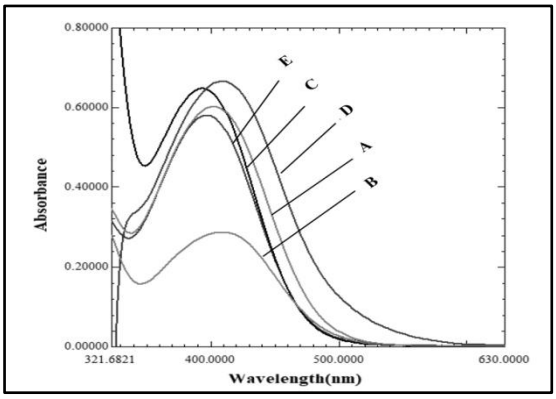

FIGURE 2: The effect of solvents on the absorption spectrum of the complex produced by the oxidative coupling reaction.

TABLE 11: shows the spectral properties of the complex formed with different solvents.

Solvent	Absorbance	λ_{max}(nm)
Ethanol(A)	0.6025	402
Methanol(B)	0.2876	408
Water(C)	950.64	394
Acetone(D)	10.666	410
2-Propanol(E)	20.581	396

- **Final Absorption Spectrum**

Under the optimal conditions obtained, the final absorption spectrum of the Remdesivir complex was recorded against the reference solution to confirm the formation of the product. 2 mL of Remdesivir solution with a concentration of 100 µg /mL were taken, along with 2 mL of the oxidizing agent SNP with a concentration of 0.001 M and 0.8 mL of NaOH solution with a concentration of 0.001 M. After waiting for 10 min, 1.8 mL of the NCA reagent with a concentration of 0.001 M was added to a 10 mL volumetric flask, and the volume was completed to the mark with distilled water. The absorption of the yellow-colored product was measured against the reference solution, and a new peak for the complex was observed at the wavelength of 394 nm, while the reference solution showed no absorption in this region, as illustrated in Figure 3.

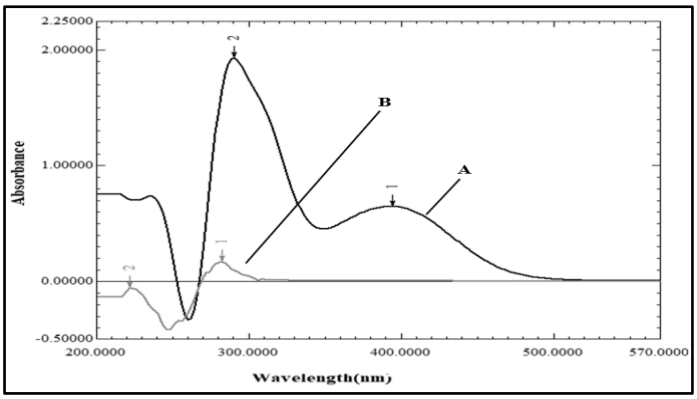

FIGURE 3: Final absorption spectra for the proposed reaction A: Absorption spectrum of the complex versus the photo solution B: Absorption spectrum of the reference solution versus the solvent.

- **Calibration Curve for the Complex**

Under the optimal conditions, a calibration curve was prepared by adding increasing volumes of the standard solution of Remdesivir with a concentration of 100 µg /mL to a series of 10 mL volumetric flasks, with volumes ranging from 0.05 to 5 mL. Fixed volumes of the oxidizing agent sodium nitroprusside (2 ml, 0.001 M) and sodium hydroxide (0.8 mL, 0.001 M) were added to these flasks. After waiting for 10 min, 1.8 mLof the NCA reagent with a concentration of 0.001 M was added, and the solutions were diluted to the mark with triple-distilled water to obtain concentrations ranging from(0.5 - 50)µg /mL of the drug. Under the previously established optimal conditions, the absorbance of the solutions was measured against the reference solution at a wavelength of 392 nm, and the standard calibration curve was plotted by drawing the relationship between absorbance and concentration. The method exhibited linearity over the concentration range of 1-25 µg /mL, with a detection limit of 0.0139 µg/mL and an estimation limit of 0.0422 µg/mL. The M absorption coefficient was calculated as 8556.778 L/mole.cm, and the Sandell's sensitivity value was 0.0704 µg /cm^2, as shown in Figure 4.

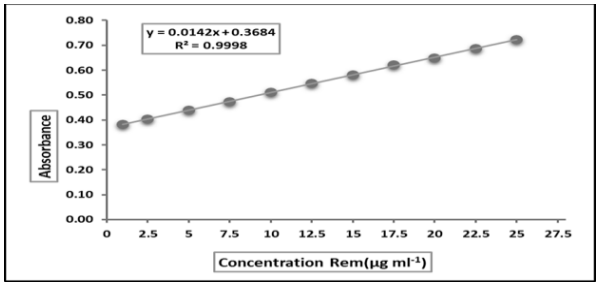

FIGURE 4: Standard calibration curve for the Remdesivir complex.

- **Accuracy and Precision**

Based on the optimal conditions obtained, the accuracy and precision of the proposed method for Remdesivir solution were tested. Three concentrations were taken with five readings for each of the concentrations that obeyed Lambert-Beer's law, which were 20, 12.5, and 5 µg /mL for Remdesivir solution. The results in Table 12 indicate that the recovery values ranged from 98.9789% to 100.1690%, indicating high accuracy, and the RSD% values did not exceed 0.3086%, indicating high precision.

TABLE 12: Accuracy and precision of the proposed method for estimating REM.

Drug	**Concentration (µg.ml ^{1})**			**%Rec**	**%*RSD**
	Taken	**Absorbance**	**Found**		
Rem	5	0.4393	4.9929	99.8592	0.3086

	12.5	0.5462	12.5211	100.1690	20.266
	20	0.6495	19.7958	98.9789	0.2781

*Average of five readings

- **Binding Ratio of the Complex**

Using the continuous variation method (Job's method), a study was conducted to determine the binding ratio of the drug to the reagent in the oxidative coupling method. Solutions with equal M concentrations of the drug and the reagent (0.001 M) were used, along with the oxidizing agent SNP and NaOH. Increasing volumes of the standard drug solution (0.1-0.9 mL) were added to 10 ml volumetric flasks, and opposite volumes of the standard reagent solution (0.9-0.1 mL) were added to these flasks. The volume was then completed to the mark with triple-distilled water, and the absorbance values of the resulting colored product were measured against the reference solution, as shown in Table 12.

TABLE 12: Binding ratio by Job's method.

V_D	V_R	Absorbance	$V_D/(V_D+V_R)$
0.1	0.9	0.4044	0.1
0.2	0.8	0.4206	0.2
0.3	0.7	0.4328	0.3
0.4	0.6	0.4395	0.4
0.5	**0.5**	**0.4954**	**0.5**
0.6	0.4	0.3761	0.6
0.7	0.3	0.3477	0.7
0.8	0.2	0.3009	0.8
0.9	0.1	0.2745	0.9

Job's method revealed that the colored product formed under the optimal conditions consists of an equivalent molar ratio of the drug and the reagent, with a ratio of 1:1, as illustrated in Figure 5.

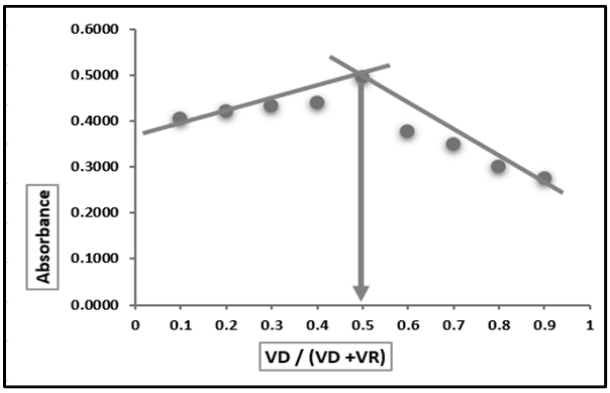

FIGURE 5: Binding ratio by Job's method.

To further confirm the binding ratio of Remdesivir to the NCA reagent, the mole ratio method was employed. Solutions with equal M concentrations of the drug and the reagent (0.001 M) were prepared and increasing

volumes of the NCA reagent (0.5-4.5 mL) were added to 1 mL of the Remdesivir solutions, in the presence of the oxidizing agent SNP and NaOH. The volume was completed to the mark with triple-distilled water, and the absorbance was measured against the reference solution, as shown in Table 12.

TABLE 12: Binding ratio by mole ratio method.

V_D	V_R	Absorbance	V_R / V_D
1	0.5	0.2838	0.5
1	1	0.3728	1
1	1.5	0.4216	1.5
1	2	0.4835	2
1	**2.5**	**0.5265**	**2.5**
1	3	0.5084	3
1	3.5	0.4996	3.5
1	4	0.4784	4
1	4.5	0.4486	4.5

The mole ratio method confirmed that the colored product formed under the optimal conditions consists of an equivalent ratio of the drug and the reagent, with a ratio of 1:1, as illustrated in Figure 6.

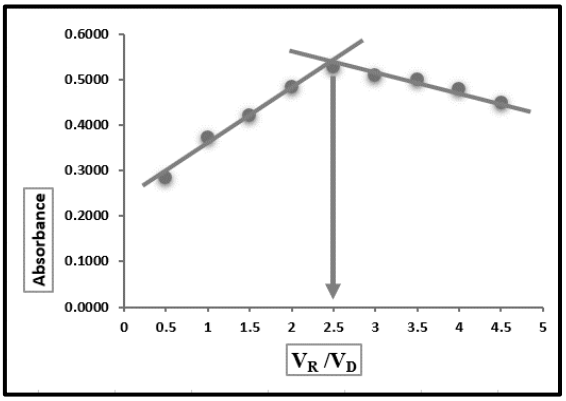

FIGURE 6: Binding ratio by mole ratio method.

Application of Method

- **Direct Application of the Method**

The proposed method was applied to the pharmaceutical preparation COVIFOR™. Different volumes of 1.5, 1, and 0.5 mL were withdrawn from the COVIFOR™ solution with a concentration of 100 µg /mL. Then, 2 mL of the oxidizing agent SNP with a concentration of 0.001 and 0.8 mL of 1 M NaOH were added. After waiting for 10 minutes, 1.8 mL of the NCA reagent with a concentration of 0.001 M was added to a 10 mL volumetric flask. The volume was then completed to the mark with triple-distilled water to obtain concentrations of 15, 10, and 5 µg/mL of the pharmaceutical preparation. Following the same procedures used for the calibration curve, the absorbance was measured at a wavelength of 394 nm, and the concentration of the active substance in each tablet was determined using the linear equation. The precision of the results was expressed using Rec% and the results' agreement using RSD%. The results are shown in Table 14.

TABLE 14: Results of direct application of the oxidative coupling method for estimating Remdesivir in pharmaceutical preparations.

Samples	λ_{max} (nm)	Concentration (µg.ml^{-1})			Rec%	* RSD%
		Taken	Absorbance	Found		
Rem (COVIFOR™)	394	5	0.4406	5.0845	101.6901	0.3964
		10	0.5102	9.9859	99.8592	0.5531
		15	0.5819	15.0352	100.2347	0.1441

*Average of five readings

The results above indicate that the values of Rec% ranged from 99.8592- 101.2347%, and the relative standard deviation (RSD%) did not exceed 0.5531%, demonstrating the success of the proposed method in estimating Remdesivir.

Analytical Characteristics of the Remdesivir Estimation Method under Optimal Conditions are shown in Table 15.

TABLE 15: Analytical characteristics of the proposed method for estimating Remdesivir.

Drug	λ_{max} (nm)	Linearity (µg.ml^{-1})	Regression equation	R^2	Slope	LOD (µg.ml^{-1})	LOQ (µg.ml^{-1})
REM	394	25-1	y = 0.0142x + 0.3684	0.9998	0.0142	0.0139	0.0422

- **Multiple Standard Additions Method**

The multiple standard additions method was employed to demonstrate the efficiency and lack of interference from additives in the proposed method for the pharmaceutical preparation COVIFOR™. A set of 10 mL volumetric flasks was used, each containing 0.3 mL of the pharmaceutical preparation COVIFOR™ with a concentration of 100 µg/mL. Increasing volumes of the standard solution of Remdesivir with a concentration of 100 µg /mL were added, ranging from 0 -2 mL, for ten volumetric flasks. Using the optimal conditions, the samples were treated in the same manner as the calibration curve, and the volume was completed to the mark with the solvent. Concentrations of Remdesivir ranging from 0-20 µg/mL were obtained, and a concentration vs. absorbance curve was plotted at a wavelength of 394 nm, as shown in Figure 7.

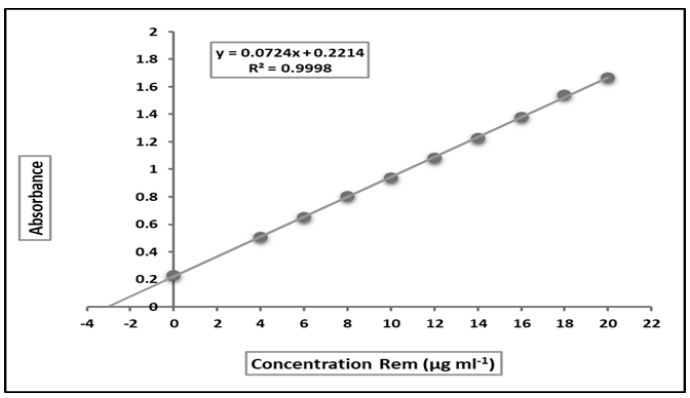

FIGURE 7: Multiple standard additions curve for Remdesivir.

The results demonstrated the efficiency and success of the method, as evidenced by Rec% of 101.9337% and RSD% of 0.8486%, indicating good accuracy and precision. There was no significant interference observed from additives, as shown in Table 16.

TABLE 16: Multiple standard additions method for Remdesivir.

Samples	**λ_{max} (nm)**	**Concentration ($\mu g.ml^{-1}$)**		**Rec%**	*** RSD%**
		Taken	**Found**		
Rem (COVIFOR™)	394	3	3.058	101.9337	0.8486

*Average of five readings

- **Method Comparison**

A comparison of the analytical variables of the proposed method with other spectrophotometric methods is presented in Table 17.

TABLE 17: Comparison of the proposed method with other spectrophotometric methods.

Parameters	**Present Method**	**Other Method [21]**
Type of Medium	Water (Base)	Water (acid)
Type of Medium	Water (Base)	Water (Base)
Reagent	Nicotinic acid	NNDPH
Reagent Oxidant	Sodium nitroprusside	chloride ferric
Color	yellow	Green - Blue
λ_{max} (nm)	394	680
Linear range ($\mu g.ml^{-1}$)	1-25	4-22
Correlation coefficient	0.9998	0.9991
LOD ($\mu g.ml^{-1}$)	0.0139	0.015
LOQ ($\mu g.ml^{-1}$)	0.0422	-
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	8556.778	5540
Sandall's sensitivity ($\mu g.cm^{-2}$)	0.0704	0.038
Rec%	100.1690 – 98.9787	100.7-101.5
RSD%	30860. ≤	0.9-2.25
Regression equation	y = 0.0142x + 0.3683	Y = 0.0272x − 0.0115

CONCLUSION

A simple and accurate spectrophotometric method for the estimation of Remdesivir by the oxidative coupling method in an alkaline aqueous medium without the need for heating, organic solvents, or extraction has been developed. The method relies on the oxidation of Remdesivir by adding 2 mL of sodium nitroprusside (0.001 M) as an oxidizing agent and 0.8 mL of 1 M sodium hydroxide as a base. Subsequently, 1.8 mL of the NCA reagent (0.001 M) was added, resulting in a yellow-colored product. The method exhibited linearity over the concentration range of 1-25 µg /mL, with the highest absorbance at a wavelength of 394 nm, and the complex remained stable for an hour, allowing multiple measurements. The method was successfully applied to estimate Remdesivir in the pharmaceutical preparation COVIFOR™ (Injection 100 mg), with a relative standard deviation of less than 0.6% and a recovery rate of 100.5947%, indicating the success of the proposed method.

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