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Article

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Wavelets Synchronous Spectrofluorimetric Determination of Remdesivir in the Presence of Its Alkaline-Induced Degradation Product

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Abstract:

Fluorescence spectroscopy has lately emerged as a very promising analytical technique due to its exceptional sensitivity and environmentally friendly attributes. In pharmaceutical quality control, there is an increasing demand for analytical methods that are straightforward, swift, cost-effective, and able to deliver dependable results with high sample throughput. Remdesivir is a novel antiviral drug first formulated for the treatment of Ebola virus sickness. In 2020, the United States Food and Drug Administration (US-FDA) approved it for the treatment of COVID-19 infections caused by the SARS-CoV-2 virus. The primary objective of this study is to develop a sensitive, accurate, and exact wavelet synchronous spectrofluorimetric approach for the quantification of Remdesivir in bulk powder and pharmaceutical formulations, accounting for its alkaline-induced degradation product. The emission was documented at 248 nm following stimulation at 408 nm. The enhanced emission exhibited a strong linear association between wavelet synchronous fluorescence intensity and Remdesivir concentration, ranging from 100 to 1200 ng/ml, with a determination coefficient of 0.9997. The proposed approach was validated in accordance with ICH principles and was effectively utilized for the quantification of Remdesivir in bulk powder and pharmaceutical formulations, despite the presence of its alkaline-induced breakdown product.

Keywords: wavelets synchronous; Synchronous fluorimetry; pharmaceutical preparations; Alkaline-induced degradation product; Remdesivir.

1. Introduction:

Remdesivir (REM) Figure (1) 2-ethylbutyl (2S)-2-[[[(2R,3S,4R,5R)-5-(4-aminopyrrolo [2,1-f][1,2,4] The compound (triazin-7-yl)-5-cyano-3,4-dihydroxyoxolan-2-yl] methoxy-phenoxy phosphoryl]amino]propanoate is a singular diastereomer monophosphoramidate prodrug of a nucleoside analogue that disrupts viral replication. It is a nonhygroscopic solid that ranges from white to off-white or yellow. The molecular formula is C27H35N6O8P, with a molecular weight of 602.59 g/mol. It is essentially insoluble in water and soluble in methanol and ethanol. It is a broad-spectrum antiviral prodrug exhibiting significant in vitro antiviral efficacy against many RNA viruses, including Ebolavirus, MERS-CoV, and SARS-CoV. It is presently undergoing Phase III clinical studies for the treatment of COVID-19 in adults. It is an antiviral agent against

coronaviruses. It is a carboxylic ester, pyrrolotriazine, nitrile, phosphonamidite ester, C-nucleoside, and aromatic amine. It is functionally associated with GS-441524.[1].

In March 2020, the World Health Organization (WHO) designated COVID-19 as a global epidemic, and by May 2024, roughly 7 million fatalities had been documented globally. The swift proliferation and serious clinical consequences of the disease prompted substantial research initiatives to discover antiviral medicines capable of diminishing viral transmission and improving patient recovery. COVID-19 predominantly impacts the lower respiratory tract and can advance to severe acute respiratory syndrome, leading to elevated mortality in critical instances. Despite the development of several vaccinations, constraints in supply, viral mutations, and limited therapeutic options prompted the repurposing of FDA-approved medications such as lopinavir/ritonavir, remdesivir, and favipiravir.

If ongoing clinical trials result as expected, Remdesivir is considered to become an important tool to counter the effects of the COVID-19 pandemic. However, the literature review revealed that few studies have estimated Remdesivir simultaneously by UV visible Spectrophotometry [2-7], HPLC [8-13], TLC [14-17] and Spectrofluorimetry[18-21] . being simple, highly selective and low interference are the advantages of synchronous fluorescence spectroscopy [SFS] over conventional fluorescence spectroscopic methods [22]. SFS is a very simple and efficient process for quantitative estimation, as a consequence of its sharp and narrow spectrum [23].

The amplitude of derivative signal is inversely proportional to band width of the original spectrum, subsequently combination of SFS and differentiation is further advantageous than traditional emission spectrum [24, 25].

In the present study, wavelets synchronous spectrofluorimetric method for the selective quantitative determination of Remdesivir in bulk powder and pharmaceutical preparation in the presence of its alkaline-induced degradation product without previous separation.

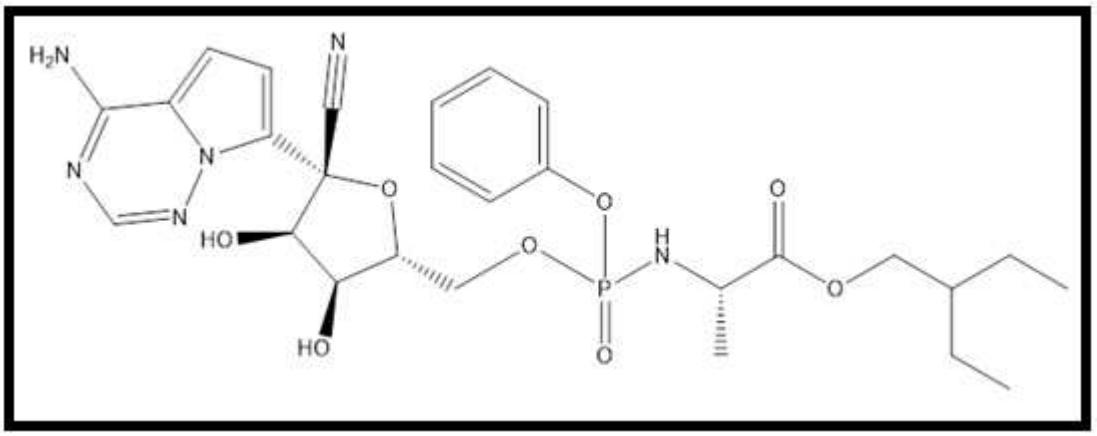


Figure (1): Chemical structure of Remdesivir

2. Experimental:

2.1.Apparatus:

- Jasco FP-6200 Spectrofluorometer (Japan), equipped with 150-Watt Xenon lamp. Slit widths for both monochromators were set at 10 nm. A 1 cm quartz cell was used. All measurements were done at medium sensitivity.
- MATLAB Software version MATLAB r2009a.
- Analytical balance (Precisa125A, Switzerland).
- Rotary evaporator (Scilogex, USA) with Buchi pump.
- Jenway, 3510 pH meter (USA).
- FT-IR, Nicolet IR 200 (Thermo electron corporation, USA).
- NMR, Gemini-300 BB (Agilent, USA)GCMS-QP-1000 EX mass
- spectrometer at70 ev (Shimadzu, Tokyo, Japan

2.2. Materials:

2.2.1. Pure sample:

Pure Remdesivir (99.1%), the purity was checked by applying the reported method, was kindly supplied by EVA Pharma Pharmaceutical Company, Cairo, Egypt.

2.2.2. Pharmaceutical preparation:

Remdesivir vials® labeled to contain 100 mg Remdesivir per vial, manufactured by EVA Pharma Pharmaceutical Company, Cairo, Egypt, purchased from the local market.

2.2.3. Chemicals and reagents:

- Methanol, analytical grade (Sigma–Aldrich, Germany).
- Hydrochloric acid and sodium hydroxide (El Nasr Co., Egypt).

- distilled water (Otsuka Pharmaceutical Company, Cairo, Egypt)
- Ethyl acetate, Ethanol analytical grade (Sigma–Aldrich, Germany)

2.3. Standard solutions:

2.3.1 Standard solution of intact Remdesivir:

Standard solutions of Remdesivir (100 μg/mL) were generated in 100-mL volumetric flasks by dissolving 10 mg of the drug powder in 50 mL of methanol and then diluting to the mark with the same solvent.

2.3.2. Standard solution of Remdesivir degradation product:

In a 100-mL round-bottom flask, 100 mg of pure Remdesivir powder was dissolved in 20 mL of methanol, followed by the addition of 50 mL of 1 M NaOH solution. The solution was subjected to reflux heating for one hour. Upon cooling, the solution was neutralized to pH 7 with 1 M HCl and subsequently evaporated to dryness under vacuum. The resultant residue was extracted using 25 mL of methanol, filtered into a 100-mL volumetric flask, and diluted with methanol to achieve a stock solution designated to include a degradation product at a concentration of 1 mg/mL of Remdesivir. A functional solution of the degradation product (100 μg/mL) was obtained by diluting the stock solution with methanol.

2.4. Procedures:

2.4.1. General procedures:

Aliquots of standard Remdesivir solution (0.1–12 μg) were dispensed into a series of 10-ml volumetric flasks, to which 1.5 ml of Britton Robinson buffer (pH 5) was then added. The solutions were diluted with methanol to a final volume of 10 ml and thoroughly mixed. The synchronized fluorescence spectra were obtained by scanning both monochromators at a fixed wavelength difference (Δλ) of 160 nm. The wavelets associated with each synchronous spectrum were documented using seven data points. The wavelets' peak amplitudes were recorded at 381 nm. A control experiment was conducted concurrently.

2.4.2.1. Optimization of experimental conditions

(i) Selection of optimum Δλ: The general procedure under **"2.4.1."** was repeated using a fixed amount of Remdesivir (2.5 μg) at different Δλ (100 - 300 nm), then comparing

the synchronous fluorescence intensity values.

(ii) Effect of diluting solvents: The general procedure under **"2.4.1."** was repeated using a fixed amount of Remdesivir (2.5 µg) and different diluting solvents, then comparing the synchronous fluorescence intensity values.

(iii) Effect of pH: The general procedure under **"2.4.1."** was repeated using a fixed amount of Remdesivir (2.5 µg) and different pH, then comparing the synchronous fluorescence intensity values.

(iv) Effect of Britton Robinson buffer volume: The general procedure under **"2.4.1."** was repeated using a fixed amount of Remdesivir (2.5 µg) and different volumes of Britton Robinson buffer (pH 5), then comparing the synchronous fluorescence intensity values.

3.3. Pharmaceutical applications:

i. Preparation of pharmaceutical sample:

10 mg of Remdesivir was precisely weighed from Remdesivir vials® and transferred to a 100-ml volumetric flask, with the volume adjusted to approximately 75 ml using methanol. The solution was agitated strongly for 15 minutes and thereafter sonicated for 30 minutes. The volume was adjusted to 100 ml using methanol, thereafter filtered to achieve a concentration of 100 µg/ml, and then diluted with the same solvent to reach a concentration of 1 µg/ml.

ii.Analysis of pharmaceutical sample:

The general procedure under **"2.4.1"** was repeated using aliquots covering the working concentration range. Remdesivir content of the vial was determined from the corresponding regression equation.

3.4. The reported method:

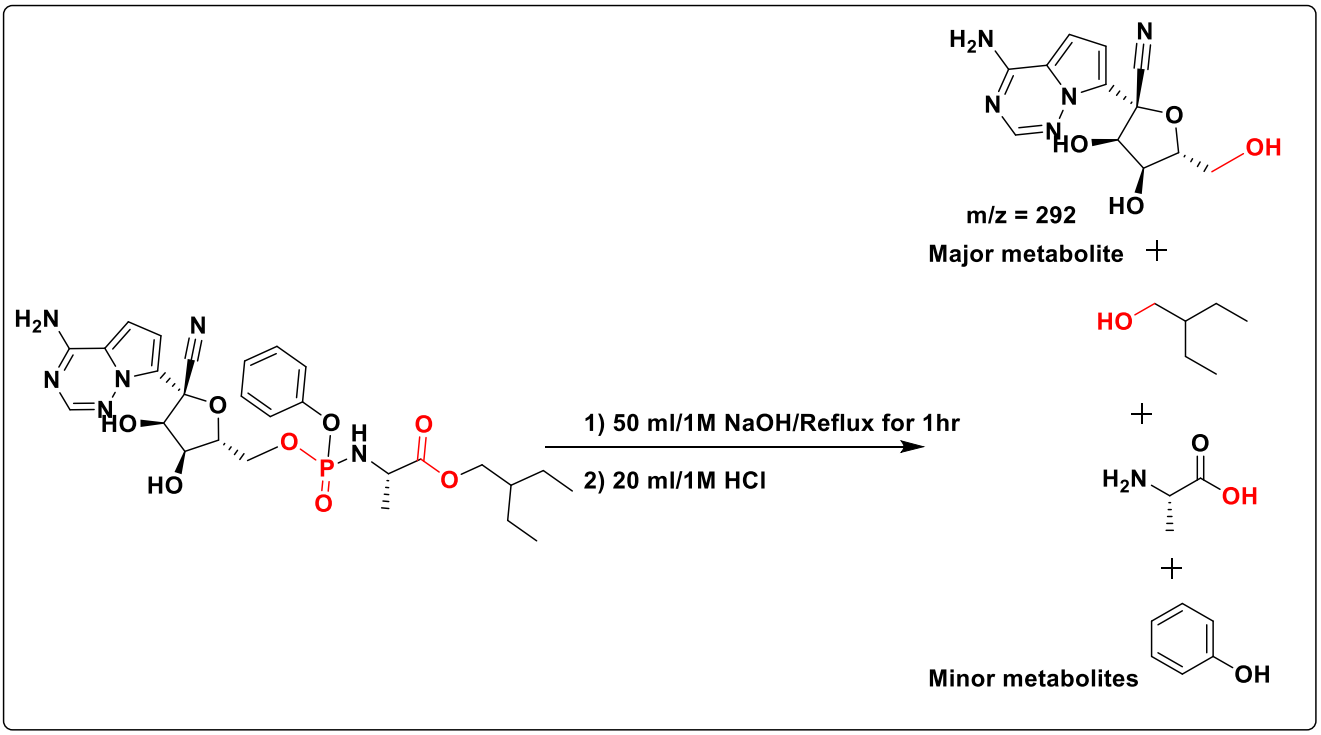
The reported method is based on the reaction of remdesivir with the computationally selected acid dye bromophenol blue to form a yellow ion-pair complex. The spectra showed absorption peaks at 418 nm[2].

3. Results & discussions

3.1. Degradation of Remdesivir:

Complete degradation of Remdesivir was achieved upon heating under reflux with 1

M NaOH for 1 hour, as shown in **Scheme (1)**.



Scheme (1): Suggested degradation pathway of Remdesivir affected by NaOH.

- **Confirmation of complete degradation using TLC technique:**

The duration for total degradation was assessed by analyzing TLC plates at 30-minute intervals with a mobile phase composed of ethyl acetate and ethanol in a 95:5 volume ratio. The total breakdown of Remdesivir was verified by the lack of secondary metabolite spots, with the exception of the principal pyrrolotriazine degradation product spot, which is soluble in methanol as a conjugated system. Other components are either soluble in methanol but not detectable in TLC (Rf 0.03 compared to the reference medication Remdesivir with Rf 0.42).

- **Confirmation of degradation product using IR, H^1 NMR, and Mass spectroscopy:**

Remdesivir contains two hydrolysis-prone functional groups: carboxylate ester and phosphamide. The IR spectrum of the alkaline-induced degradation product, **Figure (3),** showed the disappearance of two characteristic peaks observed in the Remdesivir spectrum, **Figure (2)**. These were the peaks at 1600–1700 cm−1 (C=O stretching) and 1200–1240 cm−1 (P=O stretching), suggesting cleavage of the phosphamide bond during hydrolysis.

The H^1 NMR of the intact Remdesivir in dimethyl sulfoxide (DMSO) **Figure (4)** showed a clear indication that the degradation of Remdesivir involves the cleavage of the purine nucleobase and possibly partial modification of the ribose and phosphoramidate moieties. This results in: A loss of aromatic signals, confirming nucleobase removal. Retention of aliphatic and phosphate-related peaks, suggesting partial stability of the sugar-phosphate scaffold. A more hydrophilic, polar degradation product with fewer complex splitting patterns and a simpler structure.

The Mass analysis revealed a molecular ion peak at m/z 603.6 for Remdesivir **Figure (6)** and m/z 292.3 for the pyrrolotriazine degradation product as major fragment **Figure (7)**, corresponding to [M-1] in negative ion mode. The disappearance of the 603 ion and phosphate fragments, together with emergence of 292, proves conversion to the nucleoside degradant and loss of P-centered atoms/bonds (P=O, P–N, P–O), while the nitrile-bearing base and ribose remain The combined IR , HNMR and MS findings indicate that Remdesivir undergoes hydrolysis via phosphamide bond cleavage rather than carboxylate ester hydrolysis. The proposed hydrolysis pathway is shown in **Scheme (1).**

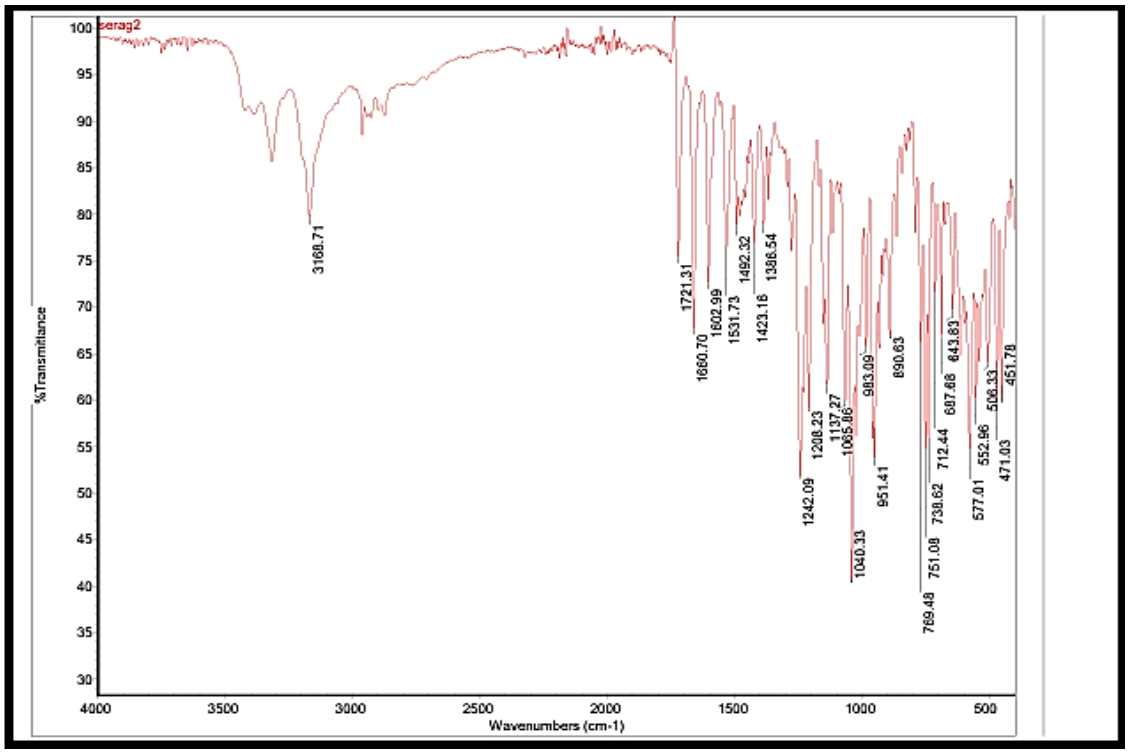


Figure (2): IR spectrum of intact Remdesivir.

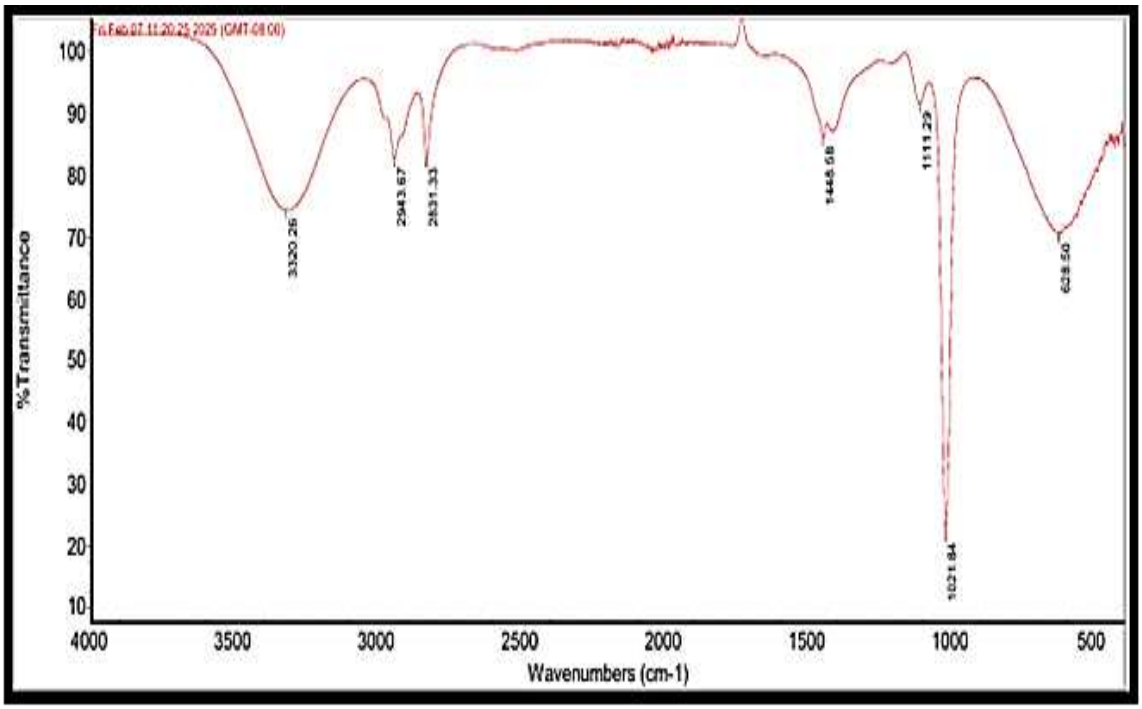


Figure (3): IR spectrum of Remdesivir degradation product.

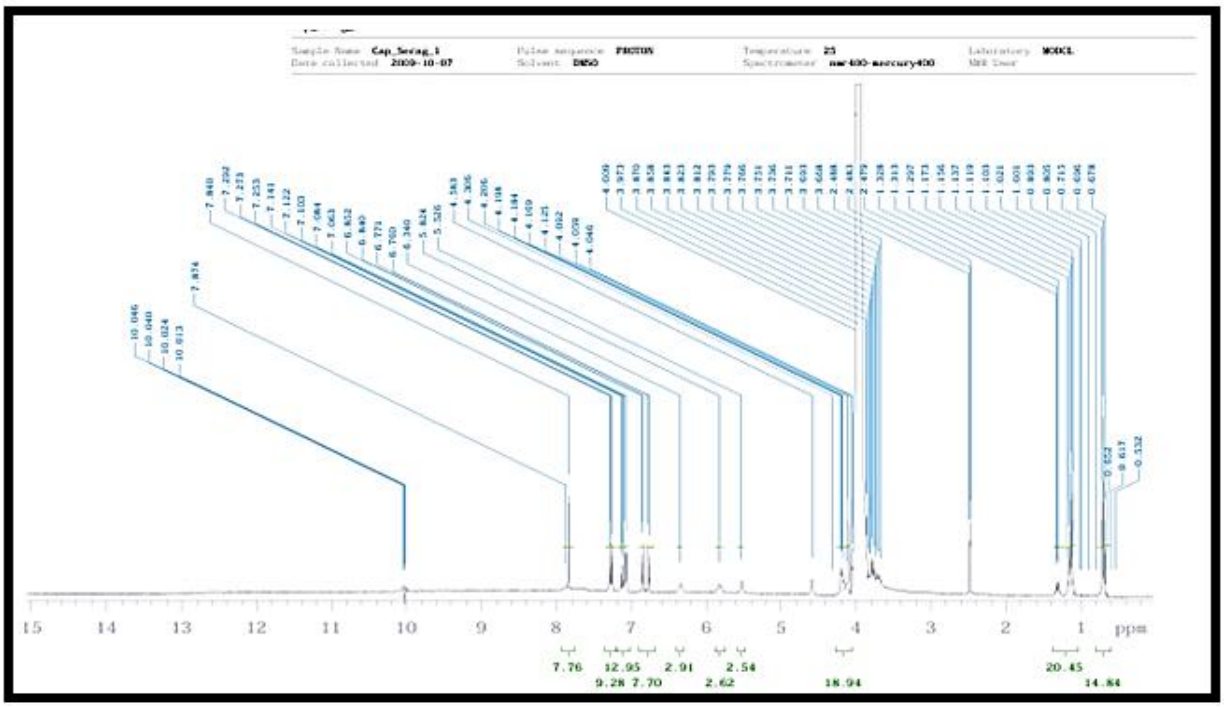


Figure (4): ^{1}H NMR spectrum of intact Remdesivir in (DMSO).

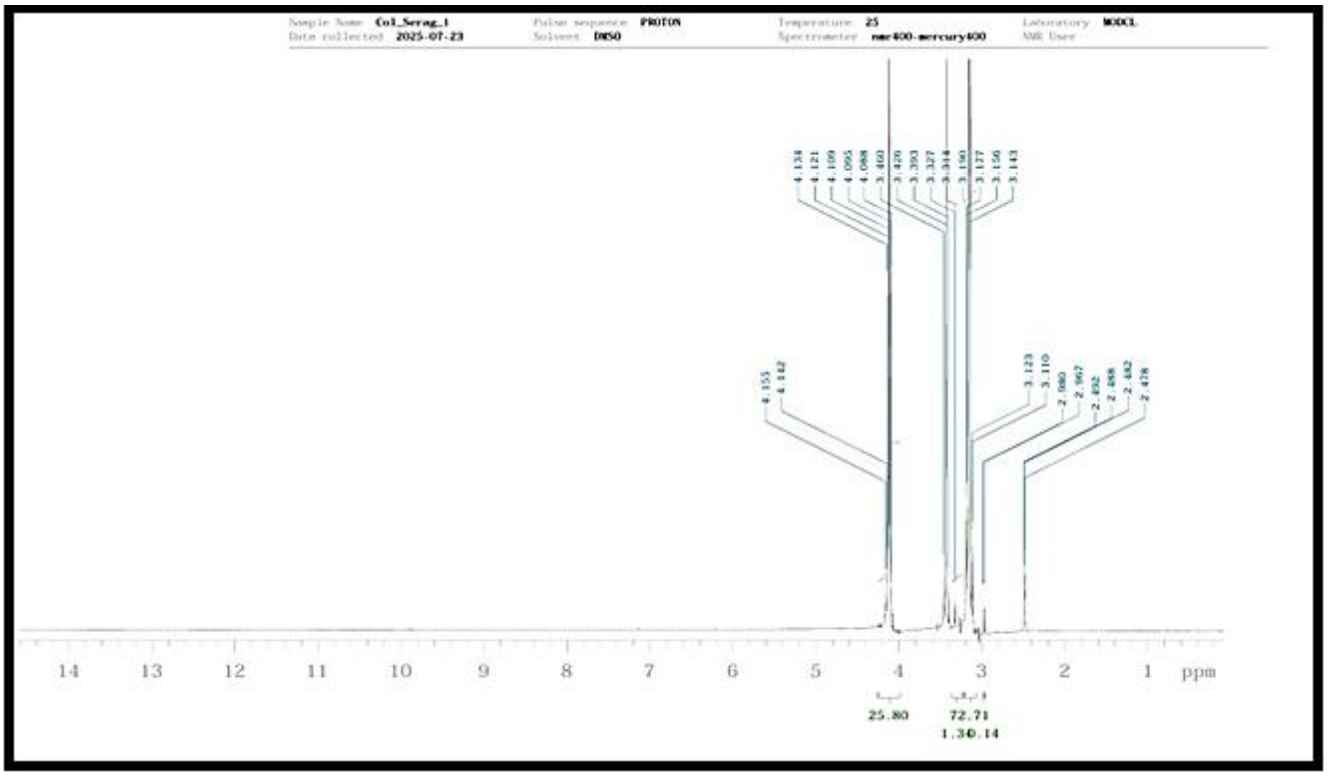


Figure (5): ^{1}H NMR spectrum of Remdesivir degradation product in (DMSO).

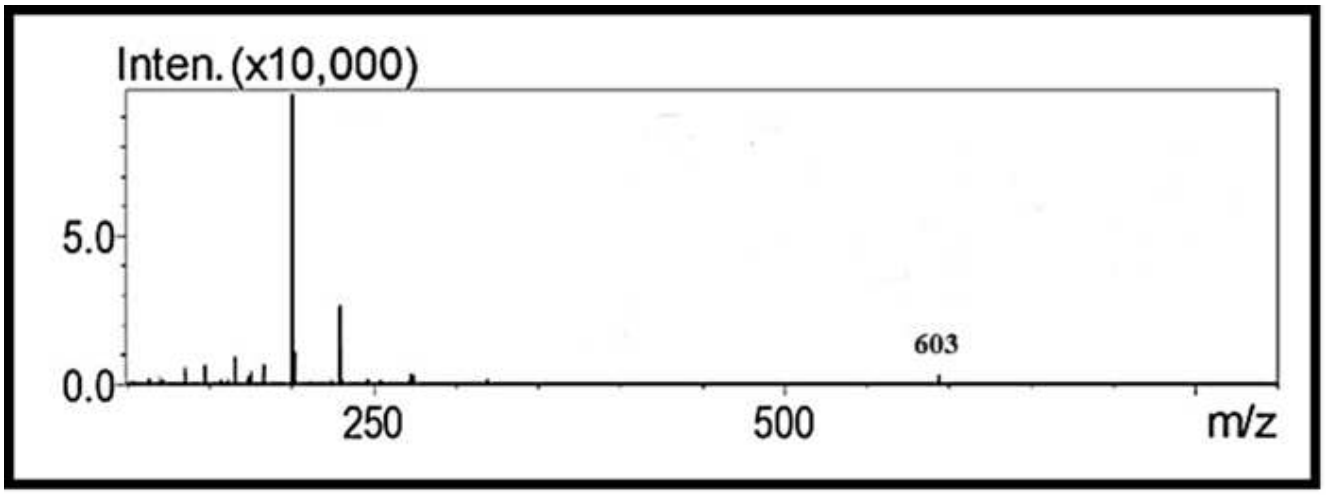


Figure (6): Mass spectrum of intact Remdesivir.

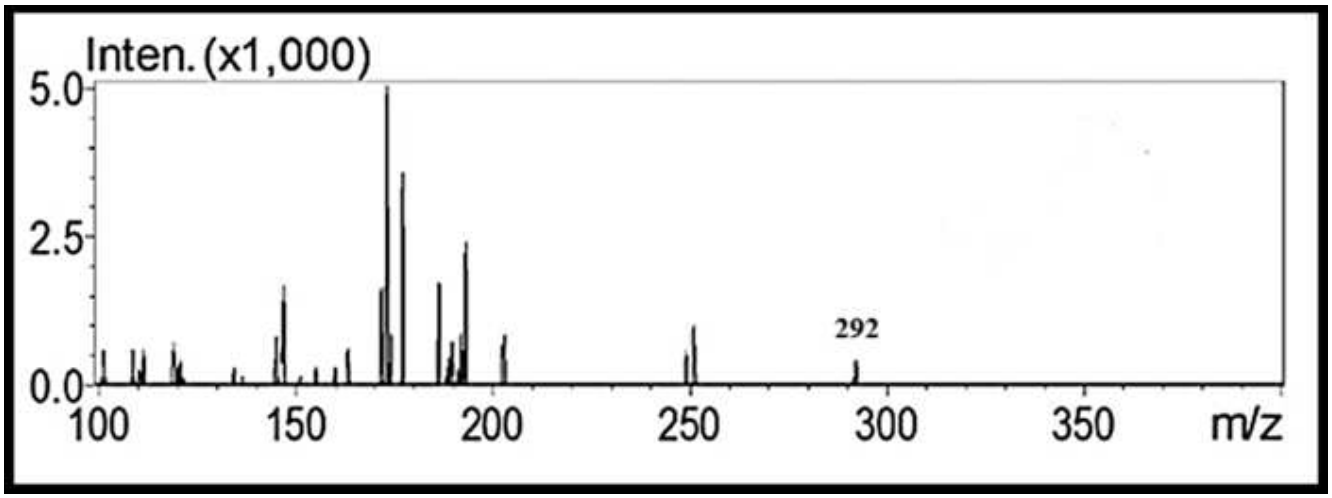


Figure (7): Mass spectrum of Remdesivir degradation product

3.2. Spectral characteristics:

Remdesivir and its alkaline degradation product display intrinsic fluorescence. The emission of Remdesivir may be quantified at 248 nm (λex. = 408 nm), and the excitation and emission spectra of Remdesivir utilizing 1.5 ml of Britton Robinson buffer (pH 5) are illustrated in Figure 8. The emission spectra of Remdesivir and its degradation product overlap, as illustrated in Figure 9, which obstructs the application of standard fluorescence for the quantification of Remdesivir in the presence of its degradation product. Furthermore, the derivatization of standard fluorescence spectra does not address the issue. The overlap can be addressed by detecting synchronous fluorescence at Δλ=160 nm, followed by the use of Continuous Wavelet Transform. The

synchronous fluorescence spectra of Remdesivir and its degradation product enable the quantification of Remdesivir in the presence of its degradation product at 381 nm, as illustrated in Figures (10-12).

Optimization of experimental conditions:

The many parameters influencing the synchronized fluorescence intensity of Remdesivir were meticulously examined and tuned. Certain factors were altered independently, while others remained constant. The criteria encompassed Δλ selection, pH, buffer volume, type of diluent, and duration.

The optimal Δλ value is crucial for executing synchronous fluorescence using the Continuous Wavelet Transform scanning technology, pertaining to its resolution, sensitivity, and characteristics. It can directly affect spectrum morphology, bandwidth, and signal amplitude. An ideal Δλ of 160 was selected for the separation, as it produced two distinct peaks with well-defined forms and minimized spectral interference from each molecule.

Methanol was determined to be the optimal diluting solvent. It provided the utmost sensitivity and the minimal blank reading with consistent results. Figure 13.

Maximum synchronous fluorescence intensity was achieved after using 1.5 ml of Britton Robinson buffer (pH 5) to adjust pH as shown in **Figures (14,15)**.

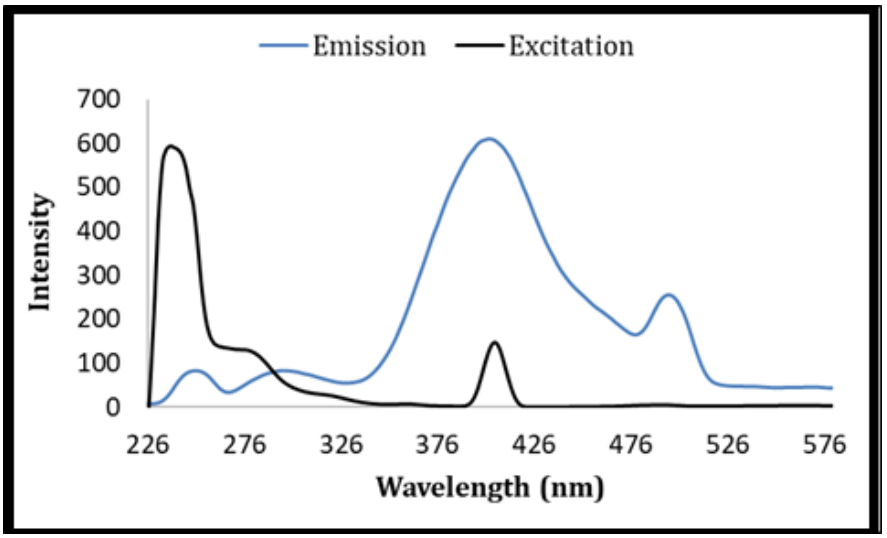


Figure (8): (A) Excitation, (B) emission spectra of Remdesivir (800 ng/ml).

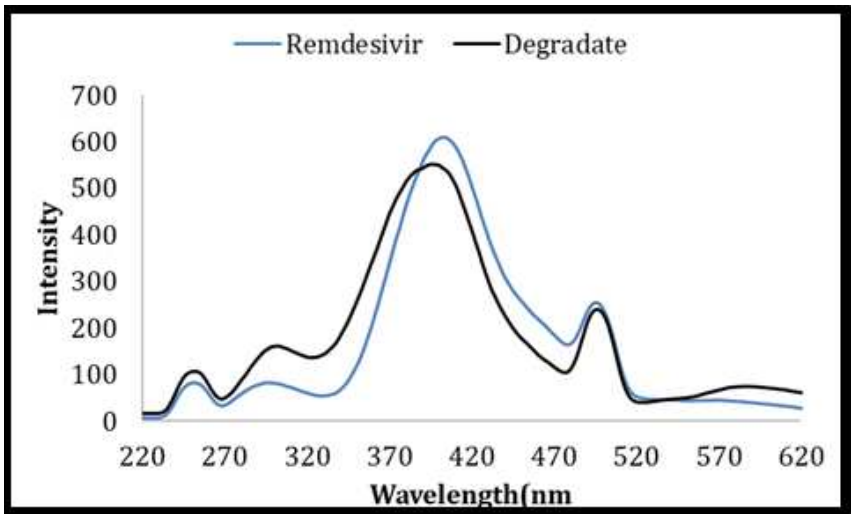


Figure (9): Emission spectra of (A) Remdesivir (800 ng/ml), (B) Remdesivir degradation product (800 ng/ml) .

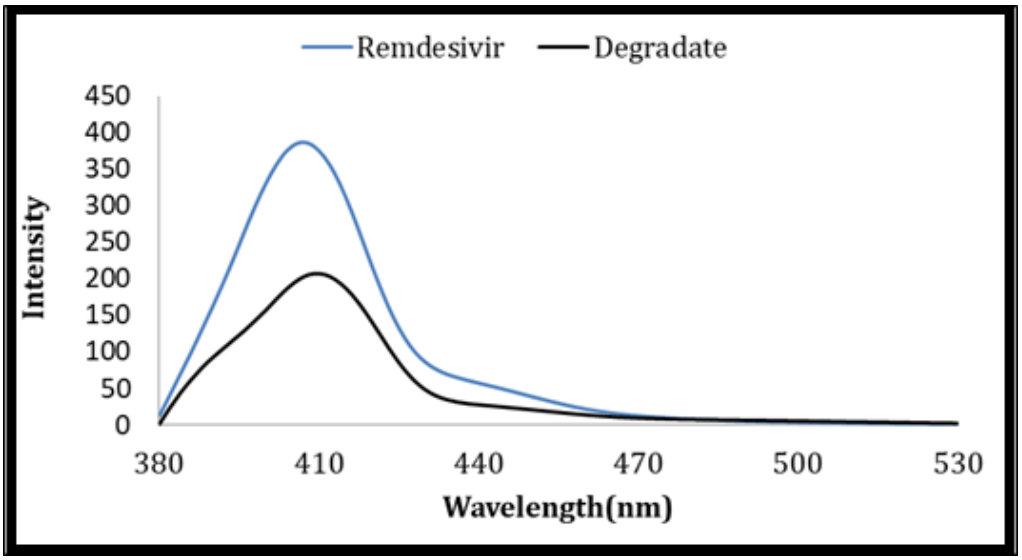


Figure (10): Synchronous fluorescence spectra of (A) Remdesivir (800 ng/ml), (B) Remdesivir degradation product (800 ng/ml) using Δλ=160 nm.

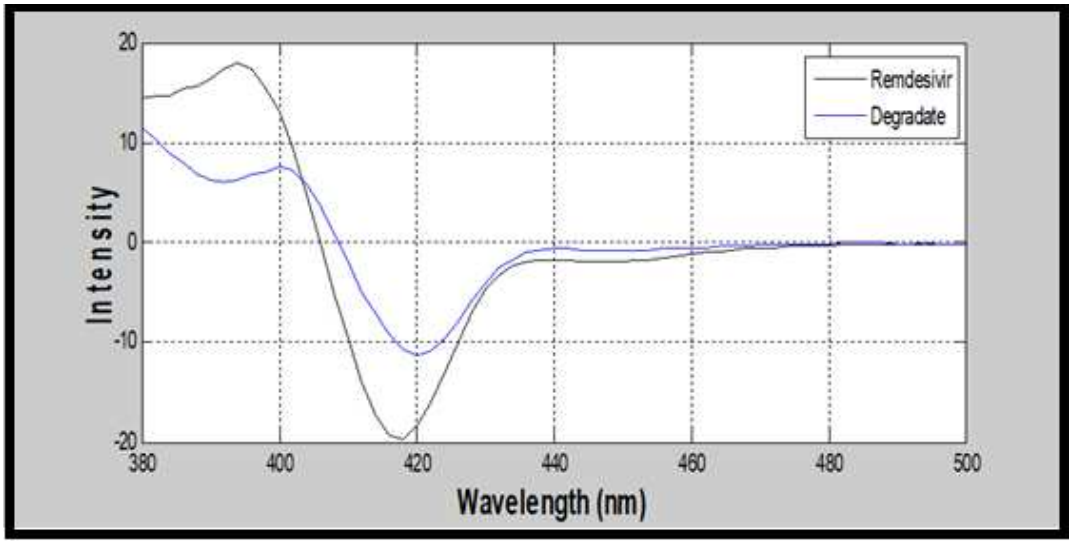


Figure (11): First derivative synchronous fluorescence spectra of (A) Remdesivir (800 ng/ml) and (B) Remdesivir degradation product (800 ng/ml).

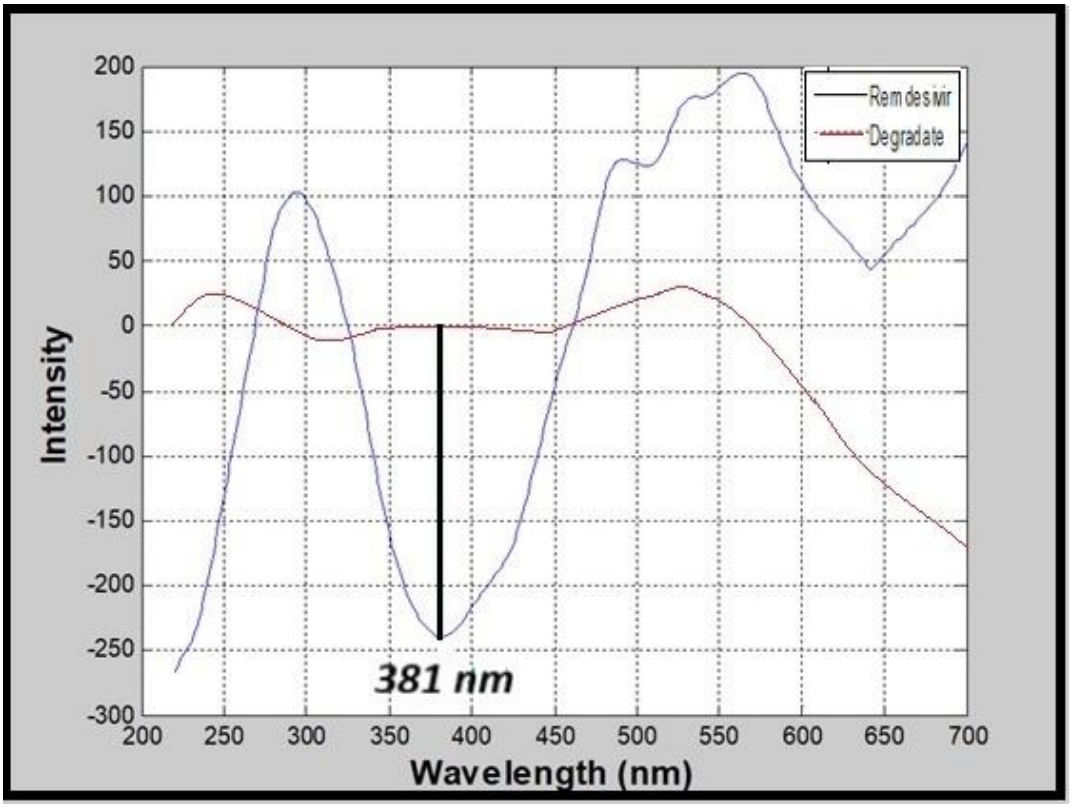


Figure (12): Wavelets synchronous fluorescence spectra of (A) Remdesivir (800 ng/ml) and (B) Remdesivir degradation product (800 ng/ml) .

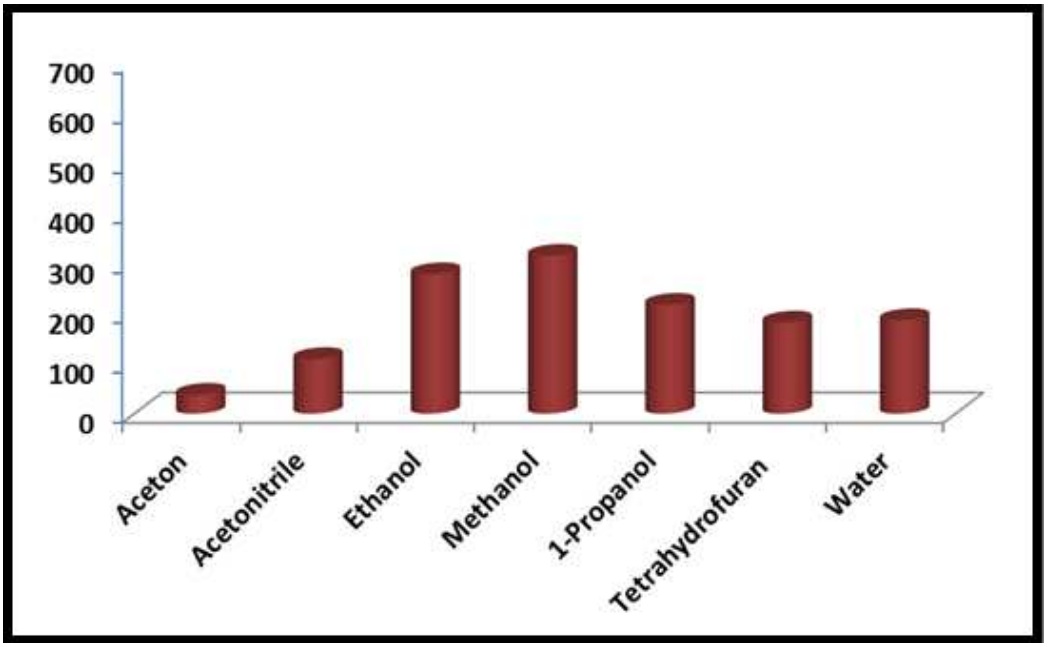


Figure (13): Effect of different diluting solvents on synchronous fluorescence intensity of Remdesivir (250 ng/ml).

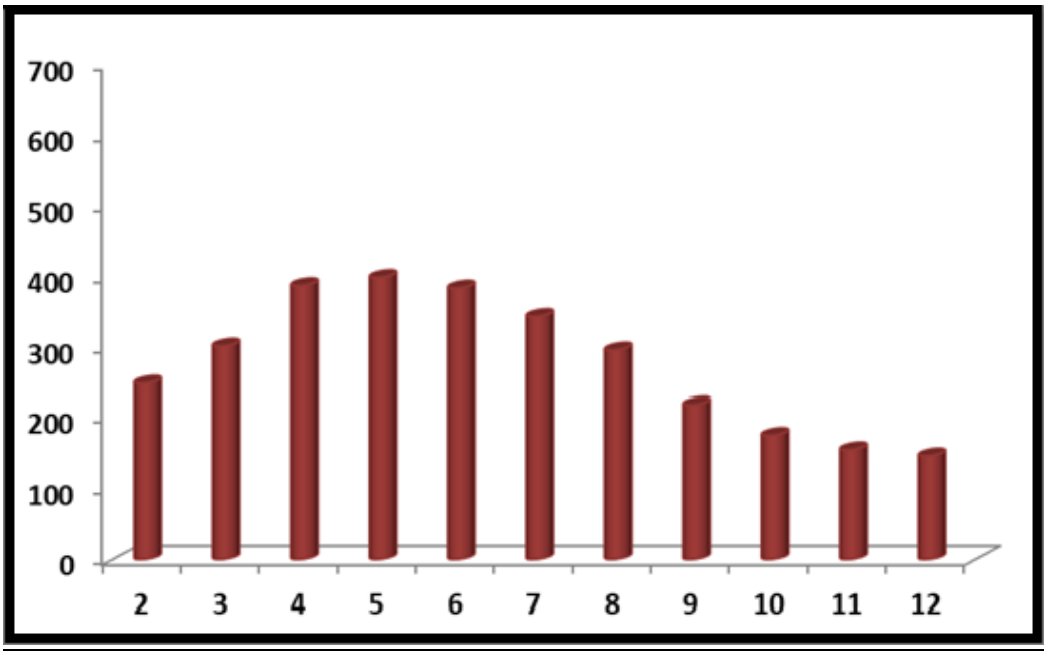


Figure (14): Effect of pH on synchronous fluorescence intensity of Remdesivir (250 ng/ml).

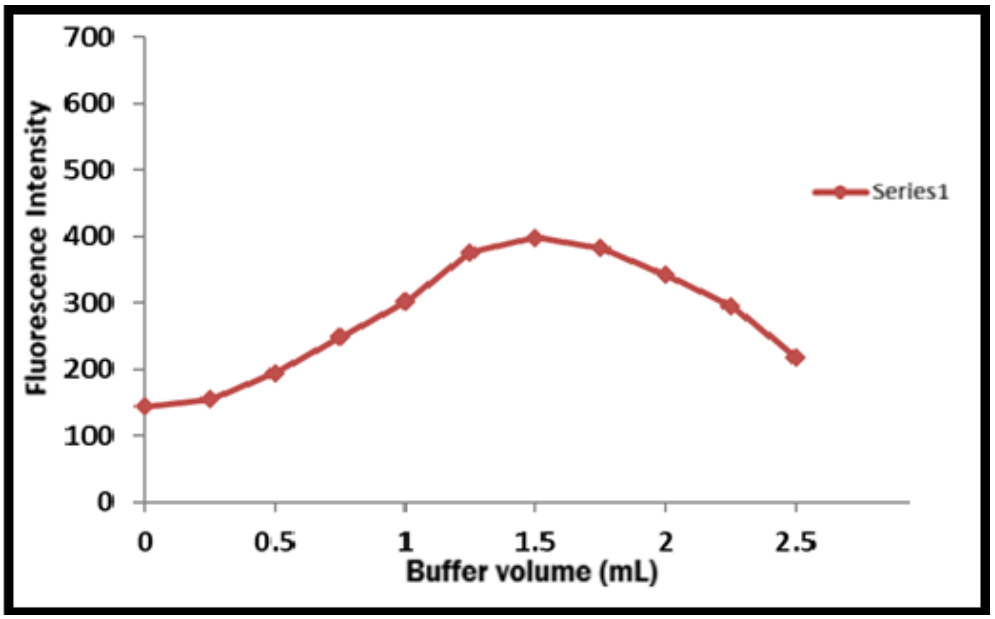


Figure (15): Effect of Britton Robinson buffer volume (pH 5) on synchronous fluorescence intensity of Remdesivir (800 ng/ml).

4. Method validation:

The methods were tested for linearity, range, limits of detection and quantitation, accuracy, precision and specificity as per the ICH guidelines[26].

- **Linearity and range:**

Under the described experimental conditions, the calibration graph for the method was constructed by plotting the amplitudes of the wavelets of the synchronous spectra versus drug concentrations in µg/ml. The regression plot was found to be linear over the range

of 100 - 1200 ng/ml, as shown in **Figure (16)**. The regression data were presented in **Table (1)**. The value of coefficient of determination indicated the good linearity of the calibration graph.

- **Limits of detection and quantitation:**

LOD and LOQ values were calculated, and the obtained results indicated the sensitivity of the proposed method for the analysis of the studied drug as shown in **Table (1).**

- **Accuracy and precision:**

Accuracy of the proposed method was indicated by the obtained good %R as shown in **Table (1).** While, the small values of %RSD indicated high precision of the method as shown in **Table (1)**.

- **Specificity:**

Synthetic mixtures containing different ratios of intact drug and its degradation product were prepared and mixed well. Then, the mixtures were analyzed using the previously described general procedure of the method. Good satisfactory results were obtained and listed in **Table (2).**

Specificity was also determined by the application of standard addition technique to check the effect of the matrix on the determination of Remdesivir . The obtained results, **Table (3)**, proved that the proposed method could selectively analyze the drug without any interference from any excipients.

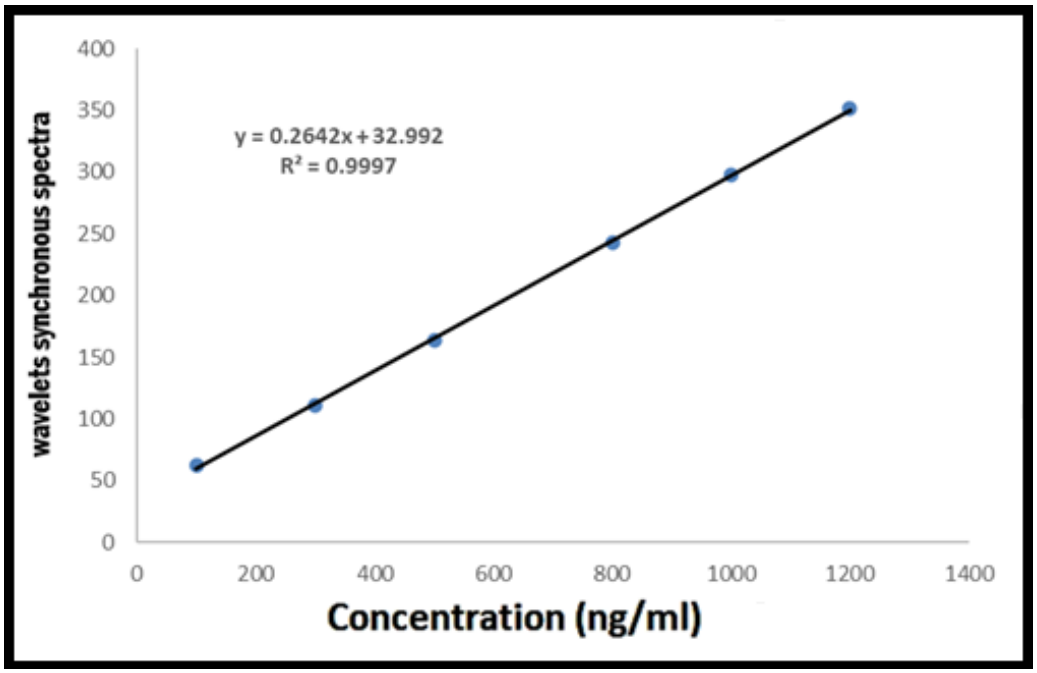


Figure (16): Calibration graph of Remdesivir by the proposed wavelets synchronous spectrofluorimetric method.

5. Pharmaceutical applications:

The proposed method was utilized for the quantification of Remdesivir in Remdesivir vials®. Satisfactory findings were achieved, aligning well with the label claim, suggesting no interference from excipients and additives, as validated by the standard addition technique results in Table (3). The results obtained were statistically compared to those derived using the reported approach[2]. No significant differences were identified by the use of Student's t-test and F-test at a 95% confidence level, demonstrating the correctness and precision of the suggested approach for analyzing the researched substance in its pharmaceutical dose form, as illustrated in **Table (4).**

Table (1): Regression and validation data for the determination of Remdesivir by the proposed wavelets synchronous spectrofluorimetric procedure:

Parameters	Proposed method
Wavelength (nm)	381
Linearity range (ng/ml)	100 ─ 1200
LOD (ng/ml)	28.234
LOQ (ng/ml)	93.172
Regression Equation - Slope (*b*) - Intercept (*a*)	$y^{a} = b\,x^{b} + a$ 0.2642 32.992
Coefficient of determination (r^2)	0.9997
Accuracy (% R)[c]	100.21
Precision[d] (% RSD) - Repeatability - Intermediate precision	 1.335 1.120

[a] The peak amplitude of the wavelets of synchronous fluorescence spectra.
[b] Concentration of Remdesivir in ng/ml.
[c] Average of nine determinations (three concentrations repeated three times).
[d] %RSD of nine determinations (three concentrations repeated three times).

[e] Average of three determinations.

Table (2): Determination of Remdesivir in mixtures with its degradation product by the proposed wavelets synchronous spectrofluorimetric procedure:

Intact (ng/ml)	Degradation product (ng/ml)	Intact found (ng/ml)	%Recovery of intact
1100	100	1098.57	99.87
900	300	895.32	98.48
700	500	694.33	99.19
300	900	294.18	98.06
100	1100	101.06	101.06
Mean ± %RSD			99.96±1.525

Table (3): Recovery study of Remdesivir by standard addition technique using the proposed wavelets synchronous spectrofluorimetric in Remdesivir® vials:

Pharmaceutical taken (ng/ml)	Pharmaceutical found[a] (ng/ml)	Pure added (ng/ml)	Pure found[b] (ng/ml)	Pure recovery (%R)
300	298.96	100	98.48	98.48
		300	300.27	100.09
		500	495.65	99.13
		800	789.36	98.67
Mean ± %RSD				99.09±0.723

[a] Average of five determinations.
[b] Average of three determinations.

Table (4): Determination of Remdesivir in Remdesivir® vials by the proposed wavelets synchronous spectrofluorimetric and reported methods:

Parameters	Proposed method	Reported method [2]
n*	5	5
Mean % recovery	98.96	99.89
% RSD	0.779	0.6942
Variance	0.595	0.482
Student's *t*-test**	2.00 (2.306)	——
F-value**	1.234 (6.388)	——

* No. of experiments.

** The values in parenthesis are tabulated values of "*t* " and "*F* " at (P = 0.05)

REFERENCES

1. Brayfield, A.a.C.C., *Martindale: The Complete Drug Reference. 41st and L. edition ed. 2024, UK: Pharmaceutical Press.*

2. Abdelazim, A.H. and S. Ramzy, *Spectrophotometric quantitative analysis of remdesivir using acid dye reagent selected by computational calculations.* Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 2022. **276**: p. 121188.

3. Bulduk, I. and E. Akbel, *A comparative study of HPLC and UV spectrophotometric methods for remdesivir quantification in pharmaceutical formulations.* Journal of Taibah University for Science, 2021. **15**(1): p. 507-513.

4. Batubara, A.S., et al., *Quantitative analysis of two COVID-19 antiviral agents, favipiravir and remdesivir, in spiked human plasma using spectrophotometric methods; greenness evaluation.* BMC chemistry, 2023. **17**(1): p. 58.

5. Noureldeen, D.A., et al., *Simultaneous Sensitive Spectroscopic Determination of Anti COVID-19 Agents in Raw Materials and Pharmaceuticals: In-Depth Green Assessment.* Available at SSRN 4354173, 2023.

6. Ibrahim, W.H. and H.S. Mahmood, *Remdesivir: Identification, Determination and Spectral Study.* Biomedicine and Chemical Sciences, 2023. **2**(2): p. 125-130.

7. Madbouly, E.A., et al., *Ecofriendly spectrophotometric methods for simultaneous determination of remdesivir and moxifloxacin hydrochloride as co administered drugs in corona virus treatment.* Scientific Reports, 2025. **15**(1): p. 1721.

8. Du, P., et al., *Quantitative HPLC-MS/MS determination of Nuc, the active metabolite of remdesivir, and its pharmacokinetics in rat.* Analytical and Bioanalytical Chemistry, 2021. **413**(23): p. 5811-5820.

9. Jitta, S.R., et al., *Development and validation of high-performance liquid chromatography method for the quantification of remdesivir in intravenous dosage form.* ASSAY and Drug Development Technologies, 2021. **19**(8): p. 475-483.

10. Hamdy, M.M., M.M. Abdel Moneim, and M.F. Kamal, *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. **35**(12): p. e5212.

11. Smerikarova, M., S. Bozhanov, and V. Maslarska, *Simultaneous quantitative determination of remdesivir, dexamethasone, rosuvastatin, and atorvastatin in human plasma using HPLC-UV analysis.* Pharmacia, 2025. **72**: p. 1-13.

12. Nassar, M.W., et al., *Development and validation of a RP-HPLC method for simultaneous determination of five COVID-19 antiviral drugs in pharmaceutical formulations.* Scientific reports, 2025. **15**(1): p. 25470.

13. Abd-Elmegid, O.A., M. Zaky, and K. Elgendy, *Rapid RP-HPLC-UV determination of*

molnupiravir, nirmatrelvir, and remdesivir alone, in pharmaceutical mixture and plasma. Discover Chemistry, 2025. **2**(1): p. 272.

14. Abo-Gharam, A.H. and D.S. El-Kafrawy, *Eco-friendly stability-indicating HPTLC micro-determination of the first FDA approved SARS-CoV-2 antiviral prodrug Remdesivir: Study of degradation kinetics and structural elucidation of the degradants using HPTLC-MS.* Sustainable chemistry and pharmacy, 2022. **29**: p. 100744.

15. Noureldeen, D.A., et al., *Novel environment friendly TLC-densitometric method for the determination of anti-coronavirus drugs "Remdesivir and Favipiravir": Green assessment with application to pharmaceutical formulations and human plasma.* Microchemical Journal, 2022. **174**: p. 107101.

16. Abd El-Fattah, M.H., et al., *A stability indicating TLC-densitometric method for the determination of Remdesivir in presence of its degradation products.* Zagazig Journal of Pharmaceutical Sciences, 2025. **34**(1): p. 56-69.

17. Nassar, M., et al., *Highly sensitive high-performance thin-layer chromatographic method for the simultaneous determination of Remdesivir, Nirmatrelvir and Ritonavir in their pure forms and pharmaceutical formulations.* Journal of Advanced Pharmacy Research, 2025. **9**(2): p. 83-90.

18. Attia, T.Z., et al., *Spectrofluorimetric determination of the anti-Covid 19 agent, remdesivir, in vials and spiked human plasma.* Luminescence, 2022. **37**(7): p. 1192-1199.

19. El-Awady, M., et al., *Insights on the quantitative concurrent fluorescence-based analysis of anti-COVID-19 drugs remdesivir and favipiravir.* Journal of fluorescence, 2022. **32**(5): p. 1941-1948.

20. El Sharkasy, M.E., et al., *Simultaneous spectrofluorimetic determination of remdesivir and simeprevir in human plasma.* Scientific Reports, 2022. **12**(1): p. 21980.

21. Aboelghar, S.M., M.A. Hegazy, and H.A. Wagdy, *Eco-Friendly Synchronous Spectrofluorimetric Method for Simultaneous Determination of Remdesivir and Acetyl Salicylic Acid in Spiked Human Plasma.* Journal of Fluorescence, 2025. **35**(7): p. 5059-5070.

22. Chen, G.-Z., et al., *The methods of fluorescence analysis.* Science, Beijing, 1990. **112**.

23. Poulli, K.I., G.A. Mousdis, and C.A. Georgiou, *Synchronous fluorescence spectroscopy for quantitative determination of virgin olive oil adulteration with sunflower oil.* Analytical and bioanalytical chemistry, 2006. **386**(5): p. 1571-1575.

24. Lloyd, J., *Nat Phys Sci 231: 64*. 2003.

25. Pulgarín, J.A.M., A.A. Molina, and P.F. López, *Simultaneous determination of atenolol, propranolol, dipyridamole and amiloride by means of non-linear variable-angle synchronous fluorescence spectrometry.* Analytica chimica acta, 1998. **370**(1): p. 9-18.

26. Guideline, I.H.T., *Validation of analytical procedures: text and methodology.* Q2 (R1), 2005. **1**(20): p. 05.

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Data Availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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