Biomedicine and Chemical Sciences 2(2) (2023) 125-130

9-BCS-1934-444

Content lists available at:
www.journals.irapa.org/index.php/BCS/issue/view/23

Biomedicine and Chemical Sciences

Journal homepage: https://www.journals.irapa.org/index.php/BCS

Remdesivir: Identification, Determination and Spectral Study

Wallada H. Ibrahim[1] & Hana Sh. Mahmood[2*]

1 Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul – Iraq
2 Department of Chemistry, College of Science, University of Mosul, Mosul – Iraq

ARTICLE INFO

Article history:
Received on: January 18, 2023
Revised on: March 1, 2023
Accepted on: March 12, 2023
Published on: April 01, 2023

Keywords:
Determination
Identification
Remdesivir
Spectral study

ABSTRACT

Corona viruses resist many antiviral agents which reduces the antiviral therapies. Remdesivir is an antiviral drug efficient against single-stranded RNA viruses. Analytical determinations of this drug are very benefit in selecting the correct dose and getting real information and formulas of this compound. In this work, remdesivir has been identified at 239 nm in aqueous alkaline (1M NaOH), at 245 nm in ethanol, and at 246 nm. in methanol, it has also been followed at 239 nm using the alkaline medium and determined in injection, the linearity range was from 1to 60 µg/ml with molar absorptivity 2.036×10^4 l.mol^{-1}.cm^{-1}, and Sandell's sensitivity index is 0.0295 µg.cm^{-2}, LOD is 0.2613 and LOQ is 0.871 µg/mL. I. R spectrum of solid remdesivir shows band at 1639.96 cm $^{-1}$ due to the carbonyl group and at 3350.79 cm^{-1}refer to the hydroxy group adjacent to NH_2 group which appears at about 3300 cm^{-1}, the aromatic was identified at 1660-2000 cm^{-1} and C-O of ester at 1153cm^{-1}. These bands in the alkaline medium were slightly shifted to be 1637 cm $^{-1}$ due to carbonyl group and at 3318 cm^{-1}refer to hydroxy group adjacent to NH_2 group which appeared at about 3264 cm^{-1}, the aromatic was identified at 2131 cm^{-1}, and C-O of ester at 1379cm^{-1}.

1. Introduction

Remdesivir (RMR) is a pro-drug of an adenosine nucleotide analogue, it is an antiviral agent with broad-spectrum activity against viruses from several families, it was developed for the treatment of Ebola virus disease followed by other developments for treating RNA-based viruses cause in the global pandemic (Eastman, et al., 2020; Lamb, 2020). RMR is 2-ethylbutyl(2S)-2-[[[(2R,3S,4R,5R)-5-(4-aminopyrrolo[2,1-f][1,2,4]triazin-7-yl) -5-cyano-3,4-dihydroxyoxolan-2-yl] methoxy-phenoxy phosphoryl] amino] propanoate with the chemical formula $C_{27}H_{35}N_6O_8P$ and molecular weight 602.585 g/mol (Al-Tannak, et al., 2020).

RMR has been used for treatments respiratory caused by coronaviruses, a simple mechanism may be represented by bio-activated transformation to monophosphate of GS-441524 the pro-drug of RMR, this phosphorylation of nucleosides is the rate- determining step, the monophosphate derivative, is highly polar, inter, trap the cell and transfer by another phosphorylation step to form nucleoside triphosphate in the host cell, and this triphosphate derivative used by the viral as a substrate leading to prevent its replication (Eastman, et al., 2020). Figure (1) shows The Chemical and biological transformation of RMR.

*Corresponding author: Hana Sh. Mahmood, Department of Chemistry, College of Science, University of Mosul, Mosul – Iraq

E-mail: hanashukermahmood@uomosul.edu

How to cite:

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

DOI: https://doi.org/10.48112/bcs.v2i2.444

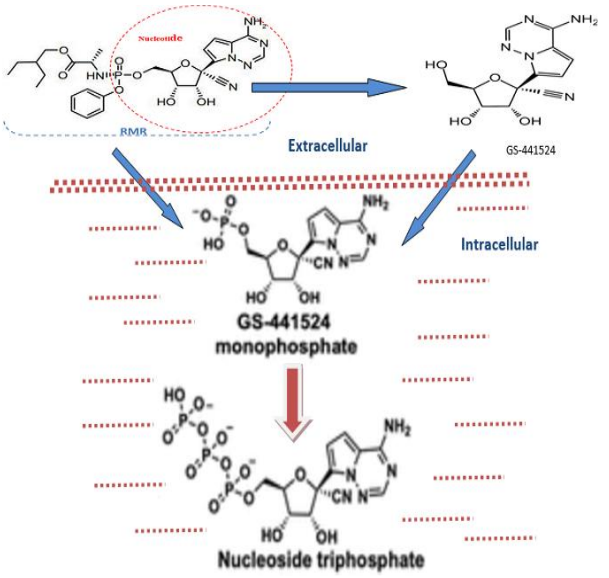

Fig. 1. The Chemical and biological transformation of RMR (Yan & Muller, 2020)

RMR is crystalline white to off- white powder, soluble in dimethyl sulfoxide (DMSO), 0.339 mg RMR can be dissolved in a liter of water (Sahakijpijarn, et al., 2020). Many chromatographic methods have been used to analyze RMR, UHPLC – MS/MS is more accurate than TLC (Avataneo, et al., 2020; Noureldeen, et al., 2022). RMR in biological samples such as plasma of hamsters, plasma of cats, and plasma of humans, RMR was first isolated from protein by methanol/Zinc sulphate (1M), improve selectivity by micellar formation, attached to C_{18} column relatively non polar package, eluted by polar mobile phase, and detected by dual detectors, diode array detector and fluorometric detector (Sahakijpijarn, et al., 2021; Murphy, et al., 2018; Attia, et al., 2022; Alvarez, et al., 2020; Pashaei, 2020; Emam, et al., 2022; Abdel Moneim, et al., 2021; Eastman, et al., 2020). Some of the chromatographic methods have been determine RMR in both biological and pharmaceutical samples (Rizk, et al., 2022). One spectrophotometric method is available for the determination of RMR based on a charge transfer complex between RMR with chloranilic acid in methanol to form (1:1) complex measured at 530 nm.

The molar absorptivity was 3.33×10^3 L mol^{-1} cm^{-1} and the association constant of the complex was 1.11×10^9 L mol^{-1}. The RMR-chloranilic acid complex was synthesized in the solid state, and identified by UV-visible, mass, FT-IR, and ^{1}H NMR spectroscopy (Darwish, et al., 2022). A spectrophotometric method studied by computer using density functional theory program B3LYP/6-31G(d) has been followed to choose the best acid dye that may produce a selective colorimetric reaction for the determination of RMR. According to theoretical calculations, the affinity of bromophenol blue to match remdesivir is higher than other acid dyes because the energy of interaction is higher as calculated (Skaggs, et al., 2022). Native fluorescence of RMR at pH 4 and was appeared and measured at wavelengths of 244/405 nm. with the linearity range of 1.0–65.0 ng mL^{-1}. limits of detection and limit of quantification were 0.287 and 0.871 ng ml^{-1} respectively, the method consider the first assay fluorescent method for RMR (Elmansi, et al., 2021).

There is no available study of RMR in the literatures about the identification, spectral and structural behavior of it in different solvents, this article offers an identification of RMR by UV, I.R, Mass, and H^1 NMR spectrum at a variety of conditions as well as it offers a determination procedure of it at UV region in alkaline solution.

2. Material and Methods

Instruments

Absorption spectra were measured on a double-beam Jasco V- 630 spectrophotometer with 1.0 cm matched glass cells, and pH measurements were measured on HANNA 301 pH meter. I.R. spectrum has been measured using BRUKER (ALPHA II, PLATINUM-ATR).

Chemicals

High percent of purity of all chemicals and reagents were used. Sodium hydroxide (Fluka). Ethanol and methanol (Fluka) DMSO (Fluka). NaOH (0.1M) : solution was prepared by diluting the ampoule(10 mi of 1molar) with distilled water then complete the volume to 100 ml in a volumetric flask. Remdesivir was provided from India (RMR) working solution (100 µg ml^{-1}). The stock solution of RMR was prepared by dissolving 0.0100 g in 10 ml of 0.1 M NaOH solution and completed to 100 ml with 0.1 M NaOH solution.

Method

Absorption Spectrum at UV

Three series, of 20,40,60,80, and 100 µg/ml of RMR were prepared in methanol, ethanol, and 10,20,40,60,80,100 µg/ml of RMR was prepared in 1M of NaOH, 1 M of Na_2CO_3 and scanned at wavelength from 200 to 400 nm. Figure 2 shows no distinguished peak at high level of RMR concentration which dissolved in methanol, this may be due to the amphoteric behavior of methanol in which it acts as an acid against RMR (classified as amine), ethanol in the other side exhibits more acidic effect towards RMR and give clearer peak at 246 nm (Camões & Anes, 2019).

Determination Method

0.01 g of remdesivir powder has been dissolved in ml of 1M sodium hydroxide, and 1, 2 ,4 ,6,8,10 ml of the solution has been further diluted by 1M solution of sodium hydroxide, diluted to 10 ml in calibrated flasks to prepare 10,20,40,60,80, and100 µg/ml, measured at 239 nm.

I.R. Spectrum

Absorption spectrum of RMR in solid state, in alkaline NaOH solution, in methanol, and in DMSO by using BRUKER (ALPHA II, PLATINUM-ATR) spectrometer.

3. Results and Discussion

Absorption Spectrum at UV

The spectrum of RMR in the base and in basic salt sodium carbonate shows different absorption bands and clearer details; therefore, it has been used for subsequent estimation of RMR in injection.

Determination Method

Figure 3 shows the absorption spectrum of prepared standards of RMR in sodium hydroxide. Figure 4 shows the high correlation between absorbances and concentrations.

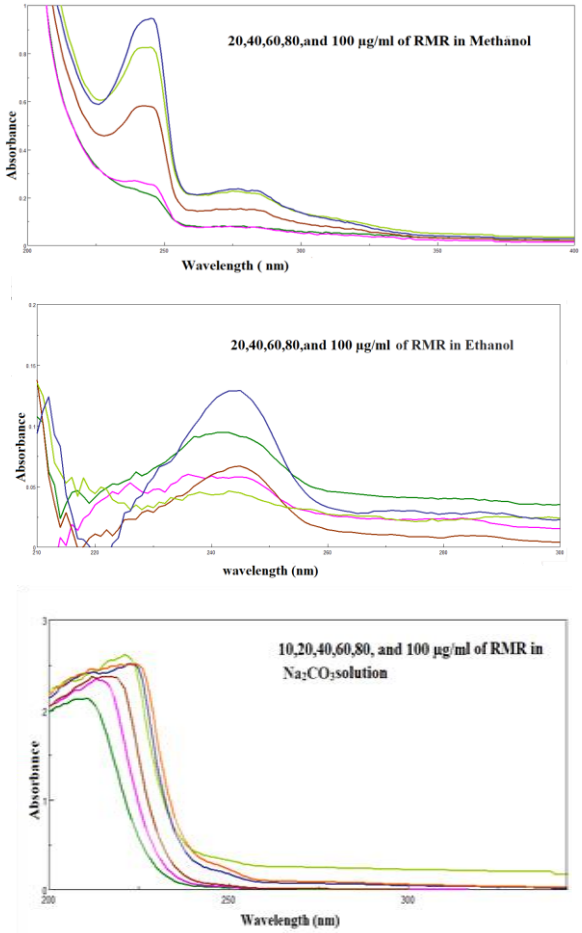

Fig. 2. The absorption spectra of increasing concentrations of RMR in ethanol, methanol and selected alkaline solution

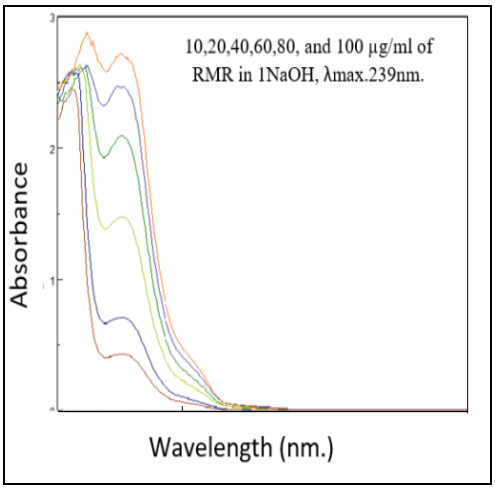

Fig. 3. The absorption spectrum of RMR in 1 M NaOH solution

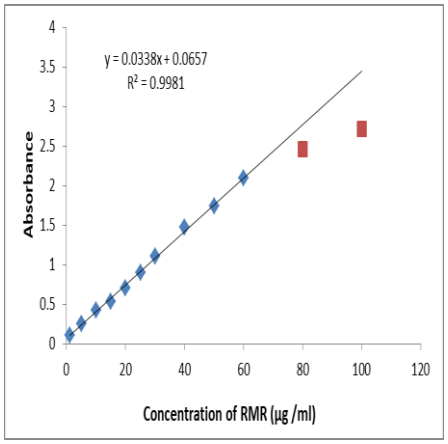

Fig. 4. The calibration curve for the determination of RMR according to the suggested new method

Table 1
The calculated and derived values from the calibration curve

$\lambda_{Max.}$(nm.)	Linearity(µg/ml)	LOD	LOQ	R^2	ε (l. mol.-1cm.-1)	Sandell's index(µg.cm^{-2})
239	1-60	0.2613 µg/mL	0.871 µg/mL	0.9981	2.036×10^4	0.0295

Application and Validation of the Method

The present method has been applied for determination of RMR in Pandovir (100mg) IV injection by preparing three concentrations 10,30 and 50 µg/ml, under the selected optimum conditions, each one replicate for five times. The results in Table 2 show high recovery percentage (98.72-99.65%), with relative standard deviation range between 0.168 to 0.549. as well as the "t" value test the confidence of the proposed method, and for it calculation, Five determined, and cheeked for t-test by applying the following mathematical relationship (Christian, et al., 2013).

$$\pm t = (\bar{x} - \mu)\frac{\sqrt{N}}{S}$$

Where $\bar{x}$ the percentage recovery rate, µ the amount of standard remdesivir, and N is the number of readings of the proposed method, and S is the standard deviation that can be calculated by applying the following relationship:

$$S = \sqrt{\frac{\sum d^2}{N-1}}$$

Table 2
The validation of the method

	Remdesivir(µg/ml)		Recovery%	RSD%	t-exp.
	Taken	Found			
Pandovir (100mg) (BEACON- Spain)	50	49.82	99.65	0.168	1.37
	30	29.82	99.41	0.385	1.24
	10	9.87	98.72	0.549	1.21

I.R. Spectrum

The identified essential bands in I. R spectrum of solid remdesivir shows band at 1639.96 cm $^{-1}$ due to the carbonyl group and at 3350.79 cm^{-1}refer to the hydroxy group and merged with the NH of NH_2 group at about 3300 cm^{-1}, the aromatic was identified at 1660-2000 cm^{-1} and C-O of ester at 1153cm^{-1}. These bands in the alkaline medium were appeared with less details;1637 cm $^{-1}$ due to carbonyl group and at 3318 cm^{-1}refer to hydroxy group adjacent to NH_2 group which appeared at about 3264 cm^{-1}, the aromatic was identified at 2131 cm^{-1}, and C-O of ester at 1379cm^{-1}. While bands in other medium were slightly shifted in such positions and merged in others. Table 3 summarizes the position of the base peaks. Figure 5 shows I.R spectra of RMR in different media.

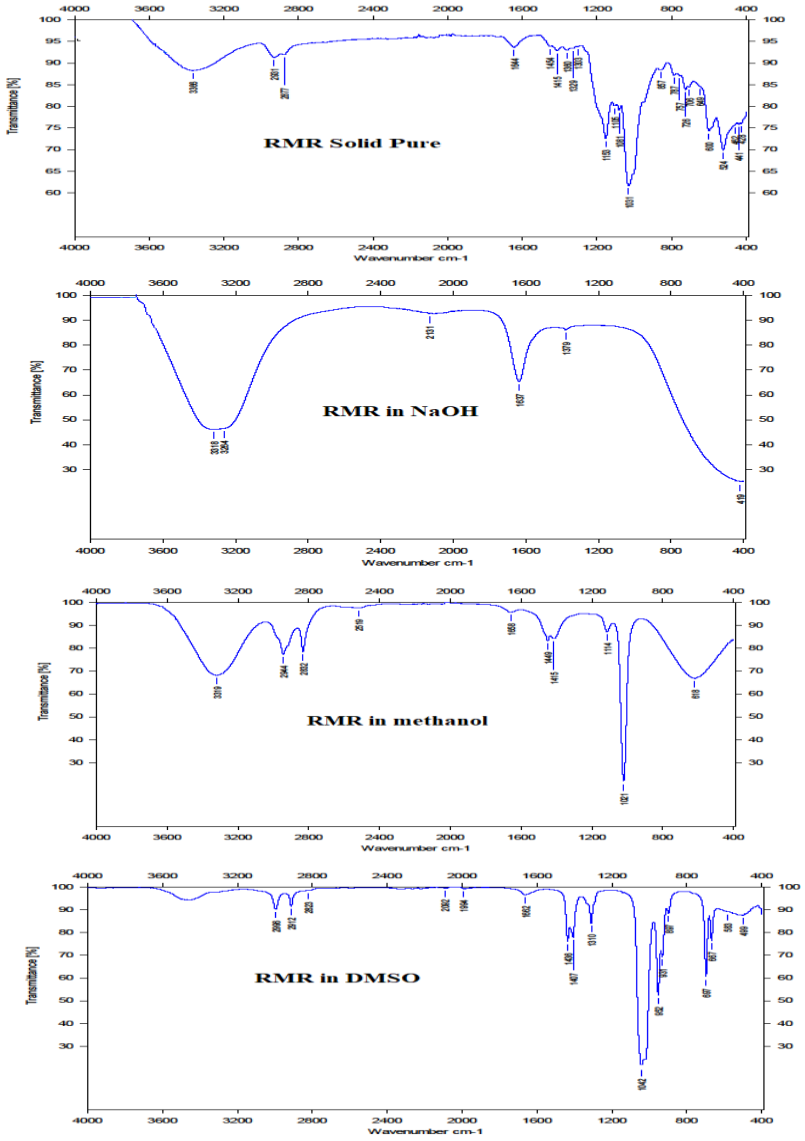

Fig. 5. I.R spectra of RMR in different media

Table 3
Band positions of functional groups result from I.R spectra of RMR in different media

Band	Position in different media (cm^{-1})			
	Solid pure	NaOH	Methanol	DMSO
Aromatic	1600 -2000	2131	2519	2092
C=O	1639.96	1637	1658	1662
C-O	1153	1379	1114	1310
NH2	3350.79	3264	2944	3445
OH		3318	3319	3478

4. Conclusion

Remdesivir can be easily identified at 1 M NaOH and determined at 239nm in its intravenous vial with good linearity range of 1-60 µg/ml with high Determination cofficient 0.9981 and high sensitivity (molar absorptivity 2.036×10^4 $l.mol^{-1}.cm^{-1}$), (Sandell's sensitivity index is 0.0295 $\mu g.cm^{-2}$), low value of LOD is 0.2613 and LOQ is 0.871 µg/mL. I.R spectrum shows 1639.96 cm^{-1} due to the carbonyl group and at 3350.79 cm^{-1}refer to the hydroxy group adjacent to the NH_2 group which appear at about 3300 cm^{-1}, the aromatic was identified at 1660-2000 cm^{-1} and C-O of ester at 1153cm^{-1}. These bands were slightly shifted at different aqueous and organic mediums.

Acknowledgment

The authors would like to thank the University of Mosul, the college of science, and the department of chemistry for offering the facilities to do this work.

Competing Interests

The authors have declared that no competing interests exist.

References

Abdel Moneim, M. M., Kamal, M. F., & Hamdy, M. M. (2021). Rapid sensitive bioscreening of remdesivir in COVID-19 medication: Selective drug determination in the presence of six co-administered therapeutics. Reviews in Analytical Chemistry, 40(1), 323-333. **https://doi.org/10.1515/revac-2021-0141**

Al-Tannak, N. F., Novotny, L., & Alhunayan, A. (2020). Remdesivir—bringing hope for COVID-19 treatment. Scientia Pharmaceutica, 88(2), 29. **https://doi.org/10.3390/scipharm88020029**

Alvarez, J. C., Moine, P., Etting, I., Annane, D., & Larabi, I. A. (2020). 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), 58(9), 1461-1468. **https://doi.org/10.1515/cclm-2020-0612**

Attia, T. Z., Boushra, J. M., Abdel Hakiem, A. F., Lashien, A. S., & Noureldeen, D. A. (2022). Spectrofluorimetric determination of the anti-Covid 19 agent, remdesivir, in vials and spiked human plasma. Luminescence, 37(7), 1192-1199. **https://doi.org/10.1002/bio.4274**

Avataneo, V., De Nicolò, A., Cusato, J., Antonucci, M., Manca, A., Palermiti, A., ... & D'Avolio, A. (2020). 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, 75(7), 1772-1777. **https://doi.org/10.1093/jac/dkaa152**

Camões, M. F., & Anes, B. (2019). Hydrated Protons. Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. **https://doi.org/10.1016/B978-0-12-409547-2.14298-2**

Christian, G. D., Dasgupta, P. K., & Schug, K. A. (2013). Analytical chemistry. John Wiley & Sons.

Darwish, I. A., Khalil, N. Y., Darwish, H. W., Alzoman, N. Z., & Al-Hossaini, A. M. (2022). Synthesis, spectroscopic and computational characterization of charge transfer complex of remdesivir with chloranilic acid: Application to development of novel 96-microwell spectrophotometric assay. Journal of Molecular Structure, 1263, 133104. **https://doi.org/10.1016/j.molstruc.2022.133104**

Eastman, R. T., Roth, J. S., Brimacombe, K. R., Simeonov, A., Shen, M., Patnaik, S., & Hall, M. D. (2020). Remdesivir: a review of its discovery and development leading to emergency use authorization for treatment of COVID-19. ACS central science, 6(5), 672-683. **https://doi.org/10.1021/acscentsci.0c00489**

Elmansi, H., Ibrahim, A. E., Mikhail, I. E., & Belal, F. (2021). Green and sensitive spectrofluorimetric determination of Remdesivir, an FDA approved SARS-CoV-2 candidate antiviral; application in pharmaceutical dosage forms and spiked human plasma. Analytical Methods, 13(23), 2596-2602. **https://doi.org/10.1039/D1AY00469G**

Emam, A. A., Abdelaleem, E. A., Abdelmomen, E. H., Abdelmoety, R. H., & Abdelfatah, R. M. (2022). Rapid and ecofriendly UPLC quantification of Remdesivir, Favipiravir and Dexamethasone for accurate therapeutic drug monitoring in Covid-19 Patient's plasma. Microchemical Journal, 179, 107580. **https://doi.org/10.1016/j.microc.2022.107580**

Lamb, Y. N. (2020). Remdesivir: first approval. Drugs, 80, 1355-1363. **https://doi.org/10.1007/s40265-020-01378-w**

Murphy, B. G., Perron, M., Murakami, E., Bauer, K., Park, Y., Eckstrand, C., ... & Pedersen, N. C. (2018). The nucleoside analog GS-441524 strongly inhibits feline infectious peritonitis (FIP) virus in tissue culture and experimental cat infection studies. Veterinary microbiology, 219, 226-233. **https://doi.org/10.1016/j.vetmic.2018.04.026**

Noureldeen, D. A., Boushra, J. M., Lashien, A. S., Hakiem, A. F. A., & Attia, T. Z. (2022). 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, 174, 107101. **https://doi.org/10.1016/j.microc.2021.107101**

Pashaei, Y. (2020). Analytical methods for the determination of remdesivir as a promising antiviral candidate drug for the COVID-19 pandemic. Drug Discoveries & Therapeutics, 14(6), 273-281. **https://doi.org/10.5582/ddt.2020.03097**

Rizk, M., Sultan, M. A., Tawfik, B. M., & El-Eryan, R. T. (2022). Highly Sensitive and Selective Sensing Probe for Determination of Anti-Covid-19 Remdesvir: Application to Pharmaceutical Dosage Form and Biological Fluids. Journal of The Electrochemical Society, 169(2), 026522. **https://doi.org/10.1149/1945-7111/ac53ca**

Sahakijpijarn, S., Moon, C., Koleng, J. J., Christensen, D. J., & Williams Iii, R. O. (2020). Development of remdesivir as a dry powder for inhalation by thin film freezing. Pharmaceutics, 12(11), 1002. **https://doi.org/10.3390/pharmaceutics12111002**

Sahakijpijarn, S., Moon, C., Warnken, Z. N., Maier, E. Y., DeVore, J. E., Christensen, D. J., ... & Williams III, R. O. (2021). In vivo pharmacokinetic study of remdesivir dry powder for inhalation in hamsters. International Journal of Pharmaceutics: X, 3, 100073. **https://doi.org/10.1016/j.ijpx.2021.100073**

Skaggs, C., Zimmerman, H., Manicke, N., & Kirkpatrick, L. (2022). Development and validation of a paper spray mass spectrometry method for the rapid quantitation of remdesivir and its active metabolite, GS-441524, in human plasma. Journal of Mass Spectrometry and Advances in the Clinical lab, 25, 27-35. **https://doi.org/10.1016/j.jmsacl.2022.06.001**

Yan, V. C., & Muller, F. L. (2020). Advantages of the parent nucleoside GS-441524 over remdesivir for Covid-19 treatment. ACS medicinal chemistry letters, 11(7), 1361-1366. **https://doi.org/10.1021/acsmedchemlett.0c00316**