Analytical methods for the determination of remdesivir as a promising antiviral candidate drug for the COVID-19 pandemic

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SUMMARY   Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is undoubtedly the most challenging pandemic in the current century. A total of 73,953,702 confirmed cases of COVID-19 and 1,644,416 deaths were reported globally up to December 17, 2020. Therefore, in the absence of a safe and effective vaccine, it is urgent to identify a novel antiviral drug to effectively treat patients with COVID-19. On October 22, the U.S. Food and Drug Administration approved remdesivir, a nucleotide analog prodrug with broad antiviral activity, for adults and children (12 years of age and older and weighing at least 40 kg) who need to be admitted to hospital for covid-19 treatment. In order to monitor the optimization of patient clinical response profile, as well as address the challenges associated with remdesivir metabolism, highly sensitive, selective and accurate analytical methods are necessary. This review clearly covers all the analytical methods developed for the identification and quantitative determination of remdesivir and its metabolites in biological matrices, which helps the researchers in developing new methods for the analysis of remdesivir by considering the pros and cons of the previously reported methods.

Keywords   Remdesivir, antiviral, COVID-19, SARS-CoV-2, analytical methods

1. Introduction

Coronaviruses are large, enveloped, positive-strand RNA viruses that can cause diseases ranging from the common cold to severe acute respiratory syndrome (SARS). The virus causing coronavirus disease 2019 (COVID-19) is a novel β-coronavirus which is now named as SARS-CoV-2 (*1*). This virus has four essential structural proteins including the small envelope (E) glycoprotein, membrane (M) protein, nucleocapsid (N) protein, and spike (S) glycoprotein, and also three accessory (non-structural) proteins including papain-like protease (PLpro) and 3Chemotrypsin-like protease (3CLpro, also known as the main protease-Mpro), which are responsible for cleavage of viral polypeptide into functional units; and RNA-dependent RNA polymerase (RdRp), which is critical for viral replication and transcription (*2*). SARS-CoV-2 penetrates the host cell *via* the binding of its S-protein with the angiotensin converting enzyme II (ACE-2) receptor, which is found in virtually all human organs in varying degrees (*3*). In general, S protein, PLpro, 3CLpro, RdRp and ACE-2 are the most attractive targets for the development of new antiviral drugs against COVID-19 (*4*).

Although this disease is asymptomatic to mild in most people (approximately 80%), in the most severe cases, it can lead to pneumonia, acute respiratory distress syndrome, sepsis and septic shock, multi-organ failure, and even death (*5*). Despite global containment measures to fight the current pandemic, the incidence of COVID-19 has continued to rise, with over 73.9 million confirmed-cases and over 1.6 million deaths worldwide as of 17 December 2020 (*6*). COVID-19 poses a serious threat to global public health and a broadly effective therapeutic strategy could provide a key means of overcoming this crisis (*7*). Unfortunately, there is currently no known effective treatment for COVID-19. Thus, drug repurposing (*i.e.*, testing the efficacy of existing drugs used previously to treat other diseases) is a basic goal in order to develop a fast therapeutic approach for patients with COVID-19 (*8*).

One of these drugs is remdesivir, an RdRP inhibitor, which shows a broad spectrum of antiviral activity against many RNA viruses like Ebola virus, Marburg, MERS-CoV, SARS-CoV, respiratory syncytial virus and Nipah virus *in vivo* and *in vitro* studies, and thus it is being studied for post-infection treatment for COVID-19 (*9-12*). Human studies of the pharmacokinetic-pharmacodynamics relationship of remdesivir and

its metabolite appeared necessary in the context of COVID-19. Despite these study needs, to the best of our knowledge, only four studies have been reported for the analysis of remdesivir and its metabolites in biological samples. Therefore, there is an urgent need to improve the robustness of the available analytical methods and to establish new standardized methods, which must be fast, more sensitive, more accurate and more specific, to determine remdesivir and its metabolites in biological matrices (*e.g.*, urine, serum, plasma, intracellular matrix, tissues).

On the other hand, the maximum information (physical and chemical properties) about the drug is important and necessary to dispose of a starting point to develop the analytical method. For instance, the structures (Figure 1), acid/basic activity and hydrophobicity are useful to elucidate the composition of the mobile phase (*13*). These parameters are listed in Table 1. To the best of our knowledge, up until now, no systematic report summarizing the analytical methods applied to remdesivir analysis has been achieved in the literature. This review article highlights the analytical methods used for the quantification and identification of remdesivir in biological matrices.

2. Remdesivir

Remdesivir (Veklury®; GS-5734) is a novel antiviral drug developed by Gilead Sciences, originally for the treatment of Ebola virus disease and Marburg virus infections (*14*). Remdesivir, 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-phenoxyphosphoryl]amino]propanoate (Figure 1A), is a single diastereomer monophosphoramidate prodrug of a nucleoside analog that perturbs viral

replication. It is a white to off-white or yellow non hygroscopic solid, practically insoluble in water and soluble in ethanol (*15*). The physicochemical properties of remdesivir are summarized in Table 1.

2.1. Remdesivir mechanism of action

Remdesivir has a complex activation pathway (see Figure 1 for further details). Briefly, upon diffusion of remdesivir (Figure 1A) into the respiratory epithelial cell, it is first metabolized into an intermediate alanine metabolite (GS-704277; Figure 1B), which is further processed into nucleoside monophosphate derivative (GS-441524; Figure 1C), the major circulating metabolite of remdesivir, *via* a phosphoramidase-type enzyme. Ultimately, GS-441524 is rapidly converted by intracellular kinases to the pharmacologically active nucleoside triphosphate analog (GS-443902; Figure 1D), a final product of remdesivir activation, which has a prolonged intracellular half-life ($T_{1/2}$ ~40 hours). Overall, remdesivir inhibits the RdRp activity of SARS-CoV-2 *via* non-obligate termination of RNA chains, after being activated to a triphosphate (*16,17*).

2.2. Efficacy of remdesivir against SARS-COV-2

Antiviral actions of remdesivir against SARS-CoV-2 have been evaluated in both cultured cells and animal models. Pruijssers *et al.* (*18*) reported that remdesivir potently inhibited SARS-CoV-2 replication in human lung cells and primary human airway epithelial cultures with a half maximal effective (EC_{50}) concentration of 0.01 μM. Remdesivir was also found to have an EC_{50} of 0.77 μM against SARS-CoV-2 in Vero E6 cells (*19*). Moreover, *in vivo* studies in a rhesus macaque model infected with SARS-CoV-2 was found to prevent
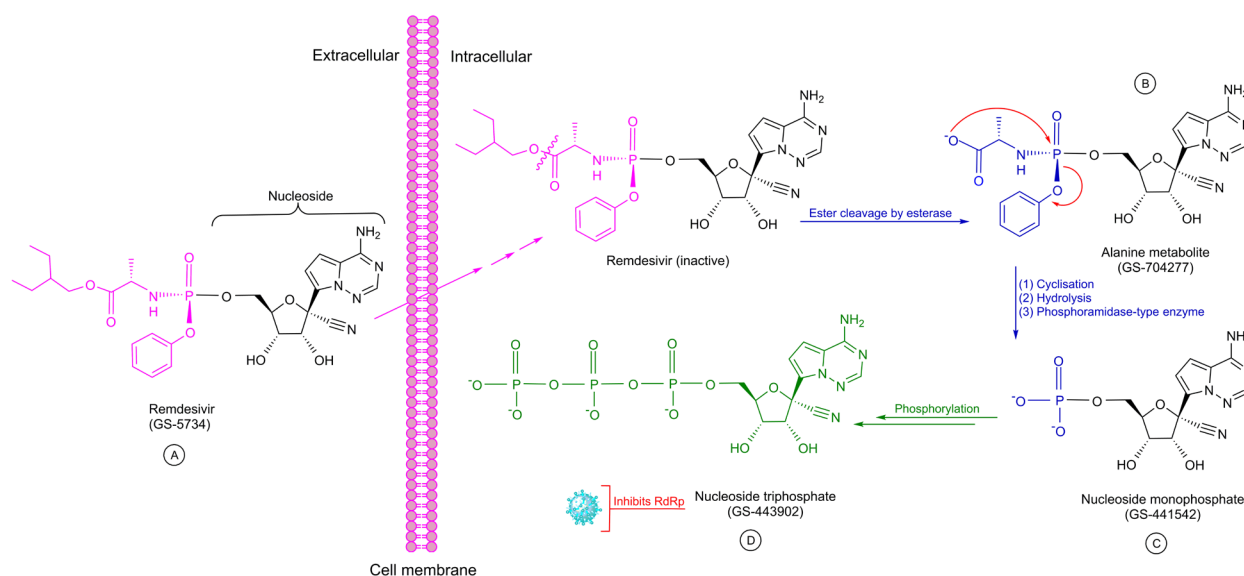

Figure 1. Remdesivir and its intracellular conversion.

Table 1. Salient features of the studied drug

Generic Name/Trade Name	Remdesivir (GS-5734)/Veklury
Drug Class	Antiviral (Small Molecule)
Therapeutic Area	Coronavirus Infections
Molecular Formula	$C_{27}H_{35}N_6O_8P$
Molecular Weight	602.59 g/moL
Exact Mass	602.22539 Da
Water Solubility	0.339 mg/L
pKa (strongest acidic/basic)	10.23/0.65
Log Po/w	2.01
Charge at pH 7	0
Route	Intravenous (*i.v.*)
Dose	200 mg *i.v.* loading dose over 30 min on day 1, then 100 mg *i.v.* daily over 30 min on days 5 to 10
C_{max} (ng/mL) (*i.v.* 100 mg once daily)	2229 and 145 (GS-441524)
EC_{50}	ND
AUC_{tau} (ng/hour/mL) (*i.v.* 100 mg once daily)	1585 and 2229 (GS-441524)
C_{trough} (ng/mL)	ND (24-hr post dose) and 69.2 (GS-441524)
Bioavailability (%)	0
T_{max} (hour)	0.67-0.68 and 1.51-2.00 (GS-441524)
$T_{1/2}$ (hour)	~1 and ~27 (GS-441524)
Toxicity	ND
Protein Binding (%)	88-93.6% and 2% (GS-441524)
Metabolic pathway(s)	CES1 (80%), Cathepsin A (10%), CYP3A (10%)
Major route of elimination	Metabolism (for remdesivir) and Glomerular filtration and active tubular secretion (for GS-441524)
Extraction	Urine: 10% and 49% (GS-441524) Feces: ND and 0.5% (GS-441524)

ND: not detected; C_{max}: maximum plasma concentration; EC_{50}: half maximal effective concentration; $T_{1/2}$: plasma half-life; Tmax: time post-dose of maximum plasma concentration; AUC: the integrated area under the plasma concentration-time curve.

disease progression with remdesivir (*20*). These initial studies demonstrate that remdesivir is potently active against SARS-CoV-2 virus infection *in vitro* and *in vivo*, supporting its further clinical testing for treatment of COVID-19.

There are several randomized control trials currently being conducted to assess the efficacy and safety of this drug in patients with COVID-19 (*https://clinicaltrials. gov*). Some evidence suggests that compassionate use of remdesivir may cause some clinical improvement in patients with mild or moderate, or severe COVID-19 disease (*21-24*). But, before making any conclusive statement about the efficacy of remdesivir for COVID-19 treatment, more randomized, placebo-controlled clinical trials and other scientific validation need to be performed.

2.3. Remdesivir pharmacokinetics

Table 1 summarizes the data on the pharmacokinetic properties of remdesivir for SARS-CoV-2. Remdesivir is administered *via* an intravenous (*i.v.*) infusion, in a total volume of up to 250 mL 0.9% saline over 30 to 120 min, with a loading dose of 200 mg once daily in patients $\geq$ 12 years old and weighing $\geq$ 40 kg, followed by a maintenance dose of 100 mg once daily for 5 to10 days (*25*). This dose is also being evaluated in multicenter randomized trials. Remdesivir is not recommended for patients with an estimated glomerular filtration

rate of $\leq$ 30 mL/min, or for patients with an alanine aminotransferase level $\geq$ 5 times the upper limit of normal (*26*). The most common adverse effects include gastrointestinal distress, elevated transaminase and bilirubin levels, and infusion site reactions (*27*).

Due to poor hepatic stability, remdesivir should not be given orally as bioavailability is expected to be low. Remdesivir is unstable in plasma and is widely distributed in many tissues, including the kidney, kidney medulla, and liver, but does not cross the blood-brain barrier (*28*). After *i.v.* administration, maximum plasma concentrations (C_{max}) of remdesivir and its main metabolite (GS-441524) were 2,229 ng/mL and 145 ng/mL, respectively. Plasma $T_{1/2}$ of remdesivir and GS-441524 were 1 and 27 hours, respectively. Remdesivir is widely bound to human plasma proteins (88-93.6%). By contrast, metabolites GS-704277 and GS-441524 exhibit low plasma protein binding (< 2% bound). Remdesivir is a substrate for CYP2C8 (minor), CYP2D6 (minor), and CYP3A4 (minor), and is a substrate for organic anion transporting polypeptides 1B1 and P-glycoprotein transporters (minor). The majority of the remdesivir dose recovered in urine is GS-441524 (49%), while 10% is recovered as the unmetabolized parent compound (*24,29*).

3. Therapeutic drug monitoring

Therapeutic drug monitoring (TDM), which is defined as

a valuable tool to individualize and optimize drug dosage in order to obtain drug concentrations associated with the highest therapeutic efficacy with a reduced risk of concentration-dependent adverse effects, is already well-established in many different infectious diseases (*30,31*) and may be useful in the issue of COVID-19 therapy (*32*). This approach involves determining drug concentration in a human biological matrix (*e.g.*, serum, plasma, or whole blood) and interpreting these concentrations in terms of relevant clinical parameters.

In general, determination of drug concentration is an inherent part of preclinical and clinical investigation of new therapeutic agents because no pharmacokinetic studies can be carried out without it. It is necessary to investigate drug-effect or drug-toxicity relationship and can be also used to understand drug mechanism of action (*33*). Therefore, both for pharmacokinetic-pharmacodynamic studies and for possible future TDM, there is an imperative and urgent need to develop a highly sensitive, rapid, and high-throughput analytical method for the quantitative determination of remdesivir and/or its metabolite (GS-441524) in biological matrices. Despite these study needs, to our knowledge, only a few methods have been reported for the qualitative and quantitative analysis of remdesivir and its metabolites (*34-37*). The instrumental and analytical properties of each reported method are described in detail and tabulated for easy access (see Table 2 for further details).

4. Analytical methods

In 2016, Warren *et al.* (*9*) developed a liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the pharmacokinetic study of remdesivir in uninfected male rhesus monkeys (*Macaca mulatta*). The MS instrument was operated in positive ion electrospray ionization (ESI) mode with multiple reaction monitoring (MRM). Remdesivir was formulated in solution at 5 mg/mL with 12% sulfobutylether-β-cyclodextrin in water, pH 3.5-4.0, and 2 mL/kg was administered by slow bolus (~ 1 min) for a final dose of 10 mg/kg. An aliquot of plasma sample was spiked with 20 nM 5-(2-aminopropyl)indole solution as internal standard (IS), extracted with 90% methanol and acetonitrile mixture (1:1, v:v) and 10% water, evaporated to dryness at 40°C under a gentle stream of nitrogen, reconstituted in a mixture containing 1% acetonitrile and 99% water with 0.01% formic acid, and assayed. A Phenomenex Synergi Hydro-RP 30A (75 × 2.0 mm, 4.0 μm) column was used as a stationary phase, and mobile phase was chosen as a binary mobile phase gradient (A: 0.2% formic acid in 99% water and 1% acetonitrile and B: 0.2% formic acid in 95% acetonitrile and 5% water) at flow rate of 0.26 mL/min. Unfortunately, the details of the validation process are not described.

In 2020, Alvarez *et al.* (*34*) determined remdesivir

and its metabolite GS-441524 in human plasma using an LC-MS/MS method with the help of a simple protein precipitation (PP) step using a mixture of methanol and Zinc sulphate ($ZnSO_4$, 1 M). $ZnSO_4$ reduces protein stability by altering the isoelectric points and by replacing protons on proteins, thereby lowering of the solution's pH. Therefore, the use of $ZnSO_4$ makes the PP thoroughly and enhances the detection sensitivity of remdesivir at low concentration. When $ZnSO_4$ is used as the sole reagent for extraction, a lot of inorganic salts move into the supernatant to contaminate the MS sources. The addition of methanol or acetone/methanol mixture to the precipitation step successfully prevents water and water-soluble salt into the supernatant, making the reconstituted samples definitely cleaner (*13*). In this work the authors used deuterated remdesivir-13C6 as an IS. The system was operated in positive (+) ESI mode, and the following MRM transitions were used: 603.3→200.0 and 603.3→229.0 for remdesivir, 292.2→173.1 and 292.2→147.1 for GS-441524, and 609.3→206.0 for IS. The authors used a Kinetex® Polar C18 column (100 × 2.1 mm I.D., 2.6 μm) to separate analytes. The elution was performed with a gradient of 10 mM sodium formate buffer in 0.1% formic acid (A) and acetonitrile (B) starting from 0% of (B) to 100% in 2 min, at flow rate of 0.50 mL/min, and the total run time was 5 min per sample. The method linearity was over the range of 1-5,000 ng/mL for remdesivir and 5-2,500 ng/mL for GS-441524, with limit of detection (LOD), as the lower concentration with a signal/noise (S/N) ratio higher than three, of 0.3 and 2 ng/mL and lower limit of quantitation (LLOQ) of 1 and 5 ng/mL, for remdesivir and GS-441524, respectively. The major advantages of the method were the requirement for small plasma volume and simple sample preparation procedure, while the main limitation of study was slightly higher LLOQ value obtained for GS-441524 (5 ng/mL). After the optimization and validation according to European Medicines Agency guidelines, the method was successfully applied to a pharmacokinetic study in a COVID-19 patient after a single dose of remdesivir (200 mg *i.v.*).

Humeniuk *et al.* (*35*) also used an LC-ESI(+)-MS/MS method to determine plasma remdesivir concentrations. Quantification was performed using MRM of the transitions m/z 603.3→402.2 and m/z 606.3→402.2 for remdesivir and an isotopically-labeled IS (GS-829143), m/z 441.1→150.1 and m/z 444.1→150.1 for metabolite GS-704277 and an isotopically-labeled IS (GS-829466), m/z 292.2→202.2 and m/z 295.2→205.2 for metabolite GS-441524 and an isotopically-labeled IS (GS-828840), respectively. The method was linear over the range of 4-4,000 ng/mL for remdesivir, 2-2,000 ng/mL for GS-704277 and 2-2,000 ng/mL for GS-441524, respectively. Interassay precision, based on coefficient of variation for remdesivir, GS-704277 and GS-441524, ranged

from 2.1% to 5.3%, and accuracy, based on interassay percent relative error for remdesivir, GS-704277 and GS-441524, ranged from – 9.8% to 9.5%. This study has some drawbacks, such as the lack of analysis of selectivity, sensitivity, robustness, and stability. However, these parameters are reported as fundamental performance characteristics for a method to be considered as validated.

Recently, Avataneo *et al.* (*36*) described a simple, rapid and sensitive UHPLC-ESI(+)-MS/MS, an fast technique (total run time < 2.5 min) which has the advantage of high sensitivity and high sample throughput over conventional LC-MS systems, method for the quantification of remdesivir and its main metabolite, GS-441524, in spiked human plasma. The MRM transitions were set at 603.15>200 (m/z), 292>163 (m/z) and 313.2>78.05 (m/z) for remdesivir, GS-441524 and 6,7-dimethyl-2,3-di(2-pyridyl) quinoxaline (as IS), respectively. After a PP procedure with a mixture of methanol:acetonitrile (50:50, v/v), the chromatographic separation of the analytes was achieved on an Acquity® HSS T3 column (50 × 2.1 mm I.D., 1.8 μm) with gradient elution of the mobile phase (A: water/formic acid 0.05% and B: acetonitrile/ formic acid 0.05%) at flow rate of 0.40 mL/min. Retention times were 0.98, 1.67 and 1.72 min for GS-441524, remdesivir and IS, respectively. The LLOQ value for both the analytes was 0.98 ng/mL while the LOD values (S/N > 3) were 0.24 ng/mL for remdesivir and 0.98 ng/mL for GS-441524. The recoveries ranged from 87-118% (remdesivir) and 81-102% (GS-441524). The established method was shown to be accurate, precise, sensitive, and linear. However, it is still necessary to develop a more sensitive method to measure the concentrations of remdesivir in human plasma for advanced pharmacokinetic profiles in low dose remdesivir. According to the authors, this method could be a useful tool for studying remdesivir and GS-441524 clinical pharmacokinetics, particularly during the current COVID-19 outbreak.

The same UHPLC system was then employed by Tempestilli *et al.* (*37*) for the pharmacokinetic evaluation of remdesivir and GS-441524 in two critically ill patients who recovered from COVID-19. They used a PP technique (600 μL of methanol/ acetonitrile, 50:50, v/v) for pretreatment and cleanup of plasma samples. Using this method remdesivir and GS-441524 were simultaneously measured with a LOQ of 5.86 ng/mL for remdesivir and 1.96 ng/mL for GS-441524. Since most studies, including *in vivo* pharmacokinetics, have a large number of samples to analyze, run time per sample can be very important. The main advantage of the developed methods is the lack of a laborious sample preparation step, which results in a shortening of the analysis time. Although recoveries obtained by LC-MS/MS (*34,35*) and UPLC-MS/MS (*36,37*) were similar, UHPLC gave significantly better

precision. The details of these methods are summarized in Table 2.

5. Conclusions and future perspectives

COVID-19, a highly infectious respiratory disease, is undoubtedly the most challenging pandemic in the current century. It has been shown that remdesivir is highly effective in stopping the replication mechanism of the coronavirus that causes COVID-19. This review provides for the first time a simplified and thorough evaluation of the analytical methods for the analysis of remdesivir from 2000 up to 2020.

A survey of the literature reveals that only LC-MS methods have been introduced for remdesivir determination in biological samples, which increases analysis costs to a considerable extent. To date, all the studies reported in literature have been performed in biological fluids, particularly in plasma samples. The sample clean-up procedure is a mandatory step in the whole analytical process, due to the low concentration of remdesivir and interference of complex matrix in biological samples. Surprisingly, all the authors have used conventional PP technique for the extraction of remdesivir and its metabolites. PP is a rapid, low cost and convenient extraction technique; however, it is nonselective and does not remove many of the matrix interferences. In addition, PP is not as rugged and reproducible sample perpetration procedure with LC-MS quantification due to the strong and inconsistent matrix effect. On the other hand, it is difficult to choose the ideal precipitating agents to remove interfering proteins from biological samples. Hence, to overcome such drawbacks, it is recommended that future trends should focus on the development of modern and more effective sample preparation techniques.

Qualitative and quantitative determination plays an important role in ensuring the safety and efficacy of drugs in different matrices. To the best of our knowledge, no stability indicating method has been reported for the estimation of remdesivir impurities and degradation products present in pharmaceutical formulation. Thus, it is felt necessary to carry out forced degradation studies as per International Conference on Harmonization guidelines and design a selective and validated stability-indicating HPLC method. According to the literature data, it can be concluded that both LC-MS/MS and UPLC-MS/MS methods provide acceptable analytical performance for remdesivir measurement but UPLC exhibited shorter analysis time, higher efficiency with better resolution and better precision. In all cases, the MS instrument was operated in the positive polarity. Furthermore, the protonated molecular ion [M+H]$^+$ was chosen as a precursor ion for quantitation in all developed methods. Based on the cited literature, ESI is the most widely used ion source in the analysis of remdesivir and its metabolites in biological matrices by means of LC-

Table 2. Summary of liquid chromatographic methods for the analysis of remdesivir and its metabolites

Analyte(s)	Chromatographic conditions	Validation parameters	Key assay findings	Ref.
Remdesivir and its metabolite GS-441524	System: LC-MS/MS, using a TSQ Endura triple-quadrupole mass spectrometer (Thermo Fisher) equipped with an ESI source set in a positive mode with ion spray potential at +3.5 kV. Mass spectrometric detection: MRM transitions were set according to the following m/z values: 603.3→200.0 (35%) and 603.3→229.0 (23%) for remdesivir, 292.2→173.1 (24%) and 292.2→147.1 (29%) for GS-441524, and 609.3→206.0 for (33%) remdesivir-$_{13}$C^6. Matrix: human plasma. Column: Kinetex® 2.6 Polar C18 100A LC (100×2.1 mm, 3 μm), preheated at 30 °C. Mobile phase: 10 mM sodium formate buffer in 0.1% formic acid (A) and acetonitrile (B) starting from 0% of (B) to 100% in 2 min. Sample volume: 50 μL. Extraction: a one-step protein precipitation (PP): 50 μL of samples were precipitated with 75 μL methanol containing IS and 5 μL of ZnSO$_4$ 1M. After vortex mix for 15 s, samples were left at +4°C for 10 min, and then centrifuged. Supernatants were collected and injected to system. Internal standard: remdesivir-$_{13}$C^6 (20 μL). Injection volume: 20 μL. Flow rate: 0.5 mL min^{-1}. Total run time: 5 min Retention time: 1.9, 2.41 and 2.42 min for GS-441524, remdesivir and IS, respectively.	Regression type: linear fit with weighting factor (1/x). Linearity: 1-5,000 ng/mL for remdesivir and 5-2,500 ng/mL for GS-441524, with coefficient of determination r^2 = 0.998 and r^2 = 0.997, respectively. Precision and Accuracy: inter- and intra-day precision (%CV) ranged from 2.6-5.6 and 2.5-7.3%. Inter- and intra-day accuracy (as percent of the nominal value) was 89.6–100.4 and 88.9-108.9%. Limit of detection (LOD): 0.3 and 2 ng/mL (S/N>3) for remdesivir and GS-441524, respectively. Lower limit of quantification (LLOQ): 1 and 5 ng/mL for remdesivir and GS-441524, respectively. Selectivity: no interference was observed from the six different blank matrices on the retention times and ion channel of both compounds and IS. Carry-over: a replicate of blank sample was analyzed immediately after the highest CS, and there was no significant carry-over. Matrix effect: for remdesivir, GS and IS, matrix factor was similar, ranging from 72 to 84%. A slight matrix effect was observed, compensated by IS. Stability: remdesivir and GS-441524 were stable in whole blood stored at +4°C for 24 h but not at ambient temperature. Stability for remdesivir was adequate in NaF-plasma when frozen at −20°C. On Li-heparin-plasma, a 50-60% decrease was observed even if plasma was frozen at −20°C. GS is stable in both plasma when frozen at −20°C. After two frozen/thaw cycles, GS appeared to increase in Li-heparin-plasma. Remdesivir was stable at 8°C for 4 h and 48 h when kept in the autosampler.	▲ A simple, rapid and accurate LC method, according to the European Medicines Agency (EMA) guidelines, was developed and fully validated for the measurement of plasma concentrations of remdesivir and its active metabolite, GS-441524. ▲ Higher stability of remdesivir and metabolite was observed on NaF-plasma tubes. ▲ This method was successfully applied to a pharmacokinetic study in a patient suffering from COVID-19.	34
Remdesivir, GS-704277 and GS-441524	System: LC system with ABSciex 4000 Q-trap MS/MS with an ESI source in positive mode. Mass spectrometric detection: as follows: m/z 603.3→402.2 and m/z 606.3→402.2 for remdesivir and GS-829143 (as an isotopically-labeled IS), m/z 441.1→150.1 and m/z 444.1→150.1 for GS-704277 and GS-829466 (as an isotopically-labeled IS), m/z 292.2→202.2 and m/z 295.2→205.2 for GS-441524 and GS-828840 (as an isotopically-labeled IS), in MRM mode. Matrix: human plasma.	Calibration range: 4-4,000 ng/mL for remdesivir, 2-2,000 ng/mL for GS-704277 and 2-2,000 ng/mL for GS-441524, respectively. Precision and Accuracy: interassay precision (%CV) for remdesivir, GS-704277 and GS-441524 ranged from 2.1% to 5.3%, and accuracy, based on interassay percent relative error for remdesivir, GS-704277 and GS-441524, ranged from −9.8% to 9.5%.	▲ This study reports results of first-in-human single- and multiple-dose studies conducted to evaluate safety and pharmacokinetics of solution and lyophilized formulations of remdesivir in healthy volunteers. ▲ Overall, remdesivir exhibited favorable safety and pharmacokinetic profiles that supported once-daily dosing.	35

Table 2. Summary of liquid chromatographic methods for the analysis of remdesivir and its metabolites (continued)

Analyte(s)	Chromatographic conditions	Validation parameters	Key assay findings	Ref.
Remdesivir and its metabolite GS-441524	System: Perkin Elmer LX-50VR UHPLC system coupled with a Triple Quadrupole QSight 220®, equipped with an ESI source in positive mode. Mass spectrometric detection: MRM traces (m/z) were quantified as: 603.15→200 for remdesivir, 292→163 for metabolite GS-441524 and 313.2→78.05 for 6,7-dimethyl-2,3-di(2-pyridyl) quinoxaline (QX; as IS). Matrix: human plasma. Column: Acquity® HSS T3 C18 (2.1 × 50 mm, 1.8 μm; Waters Corp), The column temperature was 40°C. Mobile phase: water/formic acid 0.05% (A) and acetonitrile/formic acid 0.05% (B) with linear gradient elution. Sample volume: 50 μL. Extraction: several-step PP technique; plasma samples were precipitated with 600 μL of methanol/acetonitrile (50:50 v/v) containing IS. After being vortexed for 30 s, 300 μL of the supernatant reconstituted in 600 μL of pure water, and then injected into system. Internal standard: QX. Injection volume: 8 μL. Flow rate: 0.4 mL min⁻¹. Total run time: 2.5 min. Retention time: 0.98, 1.67 and 1.72 min for GS-441524, remdesivir and IS, respectively.	Regression type: linear fit with weighting factor (1/x). Precision and Accuracy: intra–day and inter–day precision (RSD%) were in the range from 1-10% and 6-12% (for remdesivir), 6-9% and 3-14% (for GS), respectively. Accuracy was 87-118% and 81-102%% for remdesivir and GS. Recovery: mean recovery was 71% with %RSD = 6% for remdesivir and 102% with %RSD = 7% for GS. Extraction: mean extraction was 67% with %RSD = 9% for remdesivir and 105% with %RSD = 10% for GS. LOD: (S/N >3) 0.24 ng/mL for remdesivir and 0.98 ng/mL for GS. LLOQ: 0.98 ng/mL for both the analytes. Specificity and Selectivity: blank plasma, alone and spiked with antiretroviral drugs, presented no interfering peaks at the analyte retention times. Matrix effect: 6% with %RSD = 4% for remdesivir and −2% with %RSD = 12% for GS. Stability: both remdesivir and GS remained stable in QCs conserved at −80°C for over 4 months. Remdesivir, when dissolved in plasma, was found to be unstable at ambient temperature and 4°C, even for 24 h; in contrast, in extracted plasma samples, remdesivir was stable for up to 7 days in the autosampler (10°C).	▲ Validation data showed that the assay for remdesivir is sensitive, selective, fast, and reproducible. ▲ This method represents a useful tool for studying remdesivir and GS-441524 clinical pharmacokinetics, particularly during the current COVID-19 outbreak.	36

MS(/MS) methods. These methods provide a powerful analytical tool for clinical therapeutic monitoring of remdesivir. However, MS apparatuses are usually quite expensive, and this cost may be prohibitive to clinical laboratories. As a result, despite many advantages of MS detection, the application of separation methods based on MS can be problematic in clinical practice.

Unlike complicated analytical techniques, miniaturized analytical systems offer a low-cost, fast, easy-to-use, and on-site analysis method to explore the full potential of TDM. Also, on-site TDM has the potential to improve patient outcomes and extremely reduce health-care costs. Therefore, it is recommended that future trends should focus on the design and development of a highly sensitive, portable and miniaturized biosensor for monitoring of remdesivir. At last, it is hoped that this study provides new ideas and prospective for researchers involved in the development of new analytical methods, formulations, and quality and medical control of remdesivir.

Conflict of Interest: The author has no conflicts of interest to disclose.

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