Jean-Claude Alvarez*, Pierre Moine, Isabelle Etting, Djillali Annane and Islam Amine Larabi

Quantification of plasma remdesivir and its metabolite GS-441524 using liquid chromatography coupled to tandem mass spectrometry. Application to a Covid-19 treated patient

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Abstract

Objectives: A method based on liquid chromatography coupled to triple quadrupole mass spectrometry detection using 50 µL of plasma was developed and fully validated for quantification of remdesivir and its active metabolites GS-441524.

Methods: A simple protein precipitation was carried out using 75 µL of methanol containing the internal standard (IS) remdesivir-$^{13}C^6$ and 5 µL ZnSO4 1 M. After separation on Kinetex® 2.6 µm Polar C18 100A LC column (100 × 2.1 mm i.d.), both compounds were detected by a mass spectrometer with electrospray ionization in positive mode. The ion transitions used were m/z 603.3 → m/z 200.0 and m/z 229.0 for remdesivir, m/z 292.2 → m/z 173.1 and m/z 147.1 for GS-441524 and m/z 609.3 → m/z 206.0 for remdesivir-$^{13}C^6$.

Results: Calibration curves were linear in the 1–5000 µg/L range for remdesivir and 5–2500 for GS-441524, with limit of detection set at 0.5 and 2 µg/L and limit of quantification at 1 and 5 µg/L, respectively. Precisions evaluated at 2.5, 400 and 4000 µg/L for remdesivir and 12.5, 125, 2000 µg/L for GS-441524 were lower than 14.7% and accuracy was in the [89.6–110.2%] range. A slight matrix effect was observed, compensated by IS. Higher stability of remdesivir and metabolite was observed on NaF-plasma. After 200 mg IV single administration, remdesivir concentration decrease rapidly with a half-life less than 1 h while GS-441524 appeared rapidly and decreased slowly until H_{24} with a half-life around 12 h.

Conclusions: This method would be useful for therapeutic drug monitoring of these compounds in Covid-19 pandemic.

Keywords: Covid-19; GS-441524; LC–MS/MS; remdesivir.

Introduction

The current pandemic coronavirus disease 2019 (Covid-19) has developed interest in developing treatment in order to decrease impact on human life [1]. Many pharmacologic treatments have been proposed against the severe acute respiratory syndrome coronarovirus 2 (SARS-CoV-2) in cause in this pandemic [2]. There are currently no approved effective therapeutic agents available for the treatment of Covid-19. The most promising therapy appeared to be remdesivir [2].

Remdesivir (GS-5734) is a broad-spectrum small molecule antiviral drug that has demonstrated activity against RNA viruses in different families. Its therapeutic efficacy has been primary found against Ebola virus in rhesus monkey [3]. It has been found to have superior antiviral activity to lopinavir and ritonavir *in vitro* against Middle East respiratory coronavirus (MERS-CoV), a precedent coronarovirus disease [4]. In vitro, it inhibits SARS-CoV-2 virus replication in Vero E6 cells with an estimated 50% effective concentration (EC_{50}) around 13.8 mg/L. This concentration is decreased to less than 3.5 mg/L in association with emetine, another antiviral compound [5]. Wang et al. (2020) has shown EC_{50} much lower on the same type of cell (Vero cells) with values around 0.4 mg/L [6]. However, remdesivir is a prodrug of a parent adenosine analog, called GS-441524, both of which being metabolized in human cell into an active nucleoside triphosphate [7].

***Corresponding author: Professor Jean Claude Alvarez**, Laboratoire de Pharmacologie-Toxicologie, Université de Versailles Saint-Quentin en Yvelines, Inserm U-1173, Hôpital Raymond Poincaré, AP-HP, 104, Boulevard R. Poincaré, 92380, Garches, France, Tel: +33 1 47 10 79 46; Fax: 33 1 47 10 79 23, E-mail: jean-claude.alvarez@aphp.fr
Pierre Moine and Djillali Annane: Intensive Care Unit, Paris-Saclay University (Versailles Saint-Quentin-en-Yvelines), Inserm U-1173, Raymond Poincaré Hospital, AP-HP, Garches, France
Isabelle Etting and Islam Amine Larabi: Department of Pharmacology and Toxicology, Paris-Saclay University (Versailles Saint-Quentin-en-Yvelines), Inserm U-1173, Raymond Poincaré Hospital, AP-HP, Garches, France

GS-441524 is the major circulating metabolite of remdesivir. No EC_{50} has been published for GS-441524 on SARS-CoV-2 virus, but this metabolite appeared to be more potent than the parent drug. When activated, both seem to inhibit RNA-dependent RNA polymerase from coronavirus, targeting the viral genome replication process [8, 9].

Until now, remdesivir treatment is not European Medicines Agency (EMA) approved, but has been recently approved by US Food and Drug Administration (FDA). It is still being tested in ongoing randomized trials or in compassionate use for patients with severe Covid-19 [10, 11].

Few data are available on pharmacokinetics (PK) of remdesivir and its metabolite in humans. Only those from the report on "Summary on compassionate use" released by Gilead, the pharmaceutic company which markets this product were found [12]. Due to an intensive hepatic metabolism, remdesivir being a substrate for cytochrome P450 2C8 (CYP2C8), CYP2D6, and CYP 3A4, which would likely result in almost complete first-pass clearance, this drug is considered as not suitable for oral administration. Consequently, only IV route is actually available for remdesivir. Despite remdesivir is a substrate for different CYP, its main metabolism in humans after IV administration is likely to be predominantly mediated by hydrolase activity [12]. Elimination is predominantly in urine (74%), the predominant form being GS-441524 (49%) followed by remdesivir (10%) and other metabolites not currently clearly identified.

After 3 to 225 mg single-dose IV infusion over 2 h in humans, it has been shown that remdesivir and GS-441524 presented a linear PK profile. After a 150 and 225 mg 2 h IV infusion in eight healthy human subjects, remdesivir peak plasma concentration (C_{max}) observed at the end of infusion were 2280 µg/L (CV 30.1%) and 4421.3 µg/L (CV 16.0%), respectively. Half-life was approximately 1 h, this short value leading to a non-accumulation following multiple once-daily administrations [12]. After this kind of chronic administration, GS-441524 reached steady-state by day four, and accumulated around 2-fold compared to single administration, in accordance with its longer half-life, around 24 h [12]. C_{max} for the metabolite was measured at 152 µg/L on day one following 30 min IV infusion of 200 mg loading-doses, and 142 µg/L on day five following a 200 mg loading-dose on day 1 and 100 mg daily for four days [12].

For the treatment of Covid-19, in order to target exposures of the virus in plasma and in cells, the selected regimen proposed by Gilead® is 200 mg loading IV dose on day one followed by nine days of 100 mg once-daily [12]. Studies of the pharmacokinetic/pharmacodynamics relationship of remdesivir and its metabolite appeared necessary in the context of Covid-19. Moreover, there is no data available on the pharmacokinetics of remdesivir in patients with renal or hepatic impairment, which are frequent in Covid-19. As remdesivir appeared to be rapidly cleaved by hydrolases, the effect of hepatic impairment on its plasma levels is probably low, but no data are available for the metabolism of its active metabolite. In patients with impaired renal function, even if remdesivir is not cleared unchanged in urine to any substantial extent, its metabolite GS-441524 is found in great amount in urine and its plasma concentration may theoretically increase.

Despite these study needs, to our knowledge, no method of quantification of remdesivir and its metabolite in plasma is available. The purpose of this study was to develop and validate a liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method, and to applied it to a Covid-19 treated patient after the first loading-dose administration. Since esterases are present in great quantity in whole blood, stability of remdesivir and that of its metabolite were also investigated in two different sampling tubes and in different storages conditions.

Materials and methods

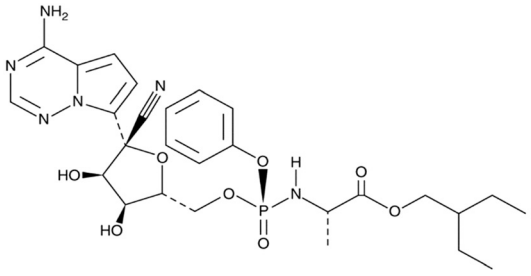
Chemicals and reagents

The reference standards of remdesivir (Figure 1A, $C_{27}H_{35}N_6O_8P$, MW: 602.6 g/mol, purity 99.6%), GS-441524 (Figure 1B, $C_{12}H_{13}N_5O_4$, MW: 291.3 g/mol, purity 98.6%) and remdesivir-$^{13}C_6$, used as internal standard (IS) were purchased from Alsachim (Illkirch, France). Formic acid and methanol were high-performance liquid chromatography (HPLC) grade while acetonitrile was mass spectrometry (LC/MS) grade and were obtained from Merck (Saint-Quentin Fallavier, France). Zinc sulfate ($ZnSO_4$) was obtained from Prolabo (Paris, France) and prepared at a concentration of 1 M in ultra-pure water (18 MΩ) obtained by ultrafiltration with a Q-Pod (Millipore Corp., Molsheim, France). Formate buffer containing 10 mM ammonium formate in 0.1% formic acid was prepared in ultra-pure water and stored after each analysis at +4 °C for a maximum of one week. Blank plasma was obtained from the Etablissement Français du Sang (EFS, La Plaine Saint-Denis, France).

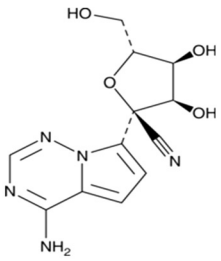
Li-heparin and NaF-containing tubes used in the stability study were obtained from Becton Dickinson (Rungis, France). Drug-free human blood for this study was obtained from five healthy volunteers (one man and four women) from the laboratory who are not medically treated.

Working solutions, calibration standards and quality controls

Stock solutions of remdesivir, remdesivir-$^{13}C_6$ and GS-441524 were prepared in methanol at a concentration of 1 mg/mL. Working solutions for calibration standards (CS) were obtained by dilution of the stock solution of remdesivir and GS-441524 in methanol to obtain solutions at 10, 1, 0.1, and 0.01 µg/mL for remdesivir and five times more concentrated



(A) Remdesivir



(B) GS-441524

Figure 1: Remdesivir and GS-441524 chemical structures.

for the metabolite. Working solution for quality control (QC) preparation was also prepared at the same concentrations by dilution in methanol of another 1 mg/mL stock solution prepared separately. IS was diluted in methanol to obtain a working solution at 0.2 µg/mL.

CS used for calibration curve were prepared each day by spiking with appropriate volumes of the previously mentioned working solutions in 50 µL blank plasma in order to obtain the following concentrations: 1, 5, 10, 25, 50, 100, 250, 500, 1000, and 5000 µg/L for remdesivir, and 5, 25, 50, 125, 250, 500, 1250, and 2500 µg/L for GS-441524, with a zero CS.

QC samples were prepared in bulk in blank plasma at concentrations of 1.0 (lower limit of quantification, (LLOQ)), 2.5, 400 and 4000 µg/L for remdesivir and 5.0 (LLOQ), 12.5, 125, 2000 µg/L. Stock and working solutions and plasma QCs were stored at −20 °C.

Sample preparation

Fifty µL of plasma were precipitated with 75 µL methanol containing IS and 5 µL of $ZnSO_4$ 1 M. After vortex mix for 15 s, samples were left at +4 °C for 10 min, and then centrifuged (10 min, 18000 *g*). Supernatant was transferred into injection vials for analysis (injection volume 20 µL).

LC-MS/MS system and conditions

Chromatography was performed on a Dionex Ultimate 3000 pump (ThermoFisher, Les Ulis, France) using a Kinetex® 2.6 µm Polar C18 100A LC column (100 × 2.1 mm i.d.) (Phenomenex, Le Pecq, France) preheated at 30 °C. 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, and hold for 1 min to 100%. The equilibration time between two consecutive runs was set at 2 min. The total run time was 5 min. The mobile phase was used at a constant flow rate of 0.5 mL/min. Compounds were detected by a TSQ Endura triple-quadrupole mass spectrometer (ThermoFisher) equipped with an electrospray ionization (ESI) source set in a positive mode with ion spray potential at +3.5 kV. Capillary temperature was set at 350 °C. Nitrogen (Nitrox UHPLCMS 18, nitrogen generator; Domnick Hunter, Villefranche sur Saone, France) was employed as sheath gas at 35 arbitrary pressure unit. The argon gas collision-induced dissociation was used with a pressure of 1.5 mTorr. Data were collected in multiple reactions monitoring (MRM) mode. The ion transitions (and corresponding collision energies) used to monitor compounds were m/z 603.3 → m/z 200.0 (35%) and m/z 229.0 (23%) for remdesivir, m/z 292.2 → m/z 173.1 (24%) and m/z 147.1 (29%) for GS-441524 and m/z 609.3 → m/z 206.0 (33%) for remdesivir-$^{13}C_6$. Data acquisition was performed using Xcalibur and LC-Quan softwares (both ThermoFisher).

Method validation procedure

This method was validated according the EMA guidelines [13].

Selectivity and carry-over

Six different drug-free blank plasma were analyzed to investigate whether endogenous plasma constituents could interfere at retention times and ion channels of remdesivir, metabolite and IS. Absence of interfering components was accepted if the response was less than 20% of the LLOQ for the analytes and 5% for the IS. In order to determine a possible carry-over, a replicate of blank sample was analyzed immediately after the highest CS (upper limit of quantification or ULOQ). Results for blank sample following ULOQ should not be greater than 20% of the LLOQ and 5% for the IS.

Calibration curve

The response of the instrument with regard to the concentration was tested on six different calibration curves analyzed on six different days using the CS previously described. Quantitation was achieved by plotting the peak area ratios of remdesivir and metabolite to the IS versus concentration. The equation showing the lowest and most constant percentage total bias from nominal CS values was considered as the best-fit model. The back calculated concentrations of the CS should be within ±15% of the nominal value, except for the LLOQ for which it should be within ±20%, and at least 75% of the CS must fulfill this criterion. Concentration of analytes in the unknown samples was calculated from their peak area ratios and the calibration curve.

LLOQ and limit of detection (LOD)

The LLOQ was the lowest concentration of remdesivir and GS-441524 which can be measured with accuracy in the range 80–120% and a precision with a coefficient of variation (CV) of less than 20%. It was validated by analysis of a specific QC sample. Moreover, the signal of analytes for the LLOQ sample had to be at least 5 times the signal of blank sample. The limit of detection was evaluated as the lower concentration with a signal/noise ratio > 3.

Accuracy and precision

The accuracy (reported as percent of the nominal value) and precision (CV) of the assay were determined for the three QC samples and at the LLOQ. For the intraday assay, six replicates of each QC were processed the same day. For the inter-day assay, six replicates of each QC level were processed at three different days. The concentrations obtained were analyzed using analysis of variance (ANOVA), which separated the intraday and interday standard deviation and the corresponding coefficients of variation (CV). An accuracy within the range 85–115% of the nominal values and a precision with a CV of ±15% were required.

Matrix effect

Two procedures (A and B) were performed on six different blank plasma at two concentrations (25 and 400 µg/L for remdesivir and 125 and 2000 µg/L for GS-441524) in order to evaluate matrix effect: (A) both compounds and the IS were spiked in 100 µL of methanol and directly injected; (B) both compounds and the IS were spiked afterward in blank matrix samples after the precipitation step and recuperation of 100 µL of supernatant, and then injected. For both compounds and IS, the matrix factor (MF) was calculated for each different lot of plasma by calculating the ratio of the peak area in the presence of matrix (B) to the peak area in absence of matrix (A). The IS-normalized MF was also calculated by dividing the MF of the analyte by the MF of the IS. The CV of the IS-normalized MF calculated from the six lots of matrix should not be greater than 15%.

Influence of blood collection tube (effect of anticoagulant)

In order to evaluate compounds stability in container material and anticoagulant, we drawn blood samples in Lithium-heparin (Li-heparin) and sodium fluoride (NaF) tubes from five volunteers in the laboratory and spiked them with 1000 µg/L of remdesivir and 100 µg/L of GS-441524. An aliquot was immediately analyzed for all tubes. Then, we evaluated the stability in whole blood at room temperature and +4 °C for 24 h.

Stability

Stability of stocks and working solutions were evaluated for two weeks. Stability was carried out in Li-heparin and NaF-plasma at room temperature for 24 h, +4 °C for 24 h, −20 °C for 3 days, and after two freeze/thaw cycles (n=3 samples for each study) at the same concentrations used for matrix effect. Autosampler stability of the processed sample at autosampler temperature (8 °C) was evaluated for 24 h and 48 h. Compounds were considered stable when the differences compared to either freshly prepared samples or concentrations obtained in the first injection for autosampler stability do not exceed 15%.

Pharmacokinetic study

The proposed analytical method was applied to a pharmacokinetic study in a treated patient by remdesivir for Covid-19. The patient was a woman, 54 years old, height 165 cm and weight 120 kg (body mass index=44), with onset of symptoms 11 days before presenting headache, epigastralgia treated by non-steroid anti-inflammatory, zolmitriptan and lamaline® in a peripheric hospital. Her polymerase chain reaction screening of Covid-19 was first negative, with pO2 at 65 mm Hg and saturation at 92%, and was treated by ceftriaxone and josamycine. She was then transferred to intensive care unit (ICU) of our universitary hospital 5 days later. She presented bilateral pneumoniae with positive diagnosis of Covid-19 and was intubated and ventilated. Six days later, she was selected for a compassionate use of remdesivir. At that time, she presented Simplified Acute Physiology Score (SAPS) II (measure the severity of disease for patients admitted to ICU) of 33 and Sequential Organ Failure Assessment (SOFA score) of 8. Her treatment was piperacillin/tazobactam, hydroxychloroquine, atracurium, enoxaparin, midazolam, sufentanil, hydroxycortisone, furosemide. She received a loading remdesivir IV dose of 200 mg on day 1 in 60 min. Blood samples were collected in Li-heparin at the following time points, simultaneously to biochemical assessment: before administration (T_0), then 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 15 h and 24 h after the beginning of perfusion. Tubes were kept at +4 °C in the ICU before being brought to the laboratory where they were immediately centrifuged and plasma stored at −20 °C until analysis. She received after the loading dose 100 mg once-daily, but died seven days later.

Plasma log concentration-time profiles and PK parameters were calculated using GraphPad Prism V8.3.0 software: C_0, the initial concentration for T=0, half-life, and volume of distribution (dose/C_0, monoconpartmental model).

Results

LC-MS/MS analysis

The chromatogram obtained for remdesivir-$^{13}C_6$, remdesivir and GS-441524 in a blank sample, the low QC sample and T_{4h} of the pharmacokinetic of the patient were shown in Figure 2. The retention time was 1.69 min for metabolite, 2.41 and 2.42 min for remdesivir and IS.

Method validation

Selectivity, carry over

No interference was observed from the six different blank matrices on the retention times and ion channel of both compounds and IS (Figure 2A). A signal less than 2% of the signal observed at the LLOQ was observed at the retention time of all compounds on the blank reinjected after the highest calibration standard. There was no significant carry-over.

Linearity

A linear regression analysis with weighing factor ($1/x$) was the best fitting model as determined by bias analysis in the 1–5000 µg/L range for remdesivir and 5–2500 µg/L range

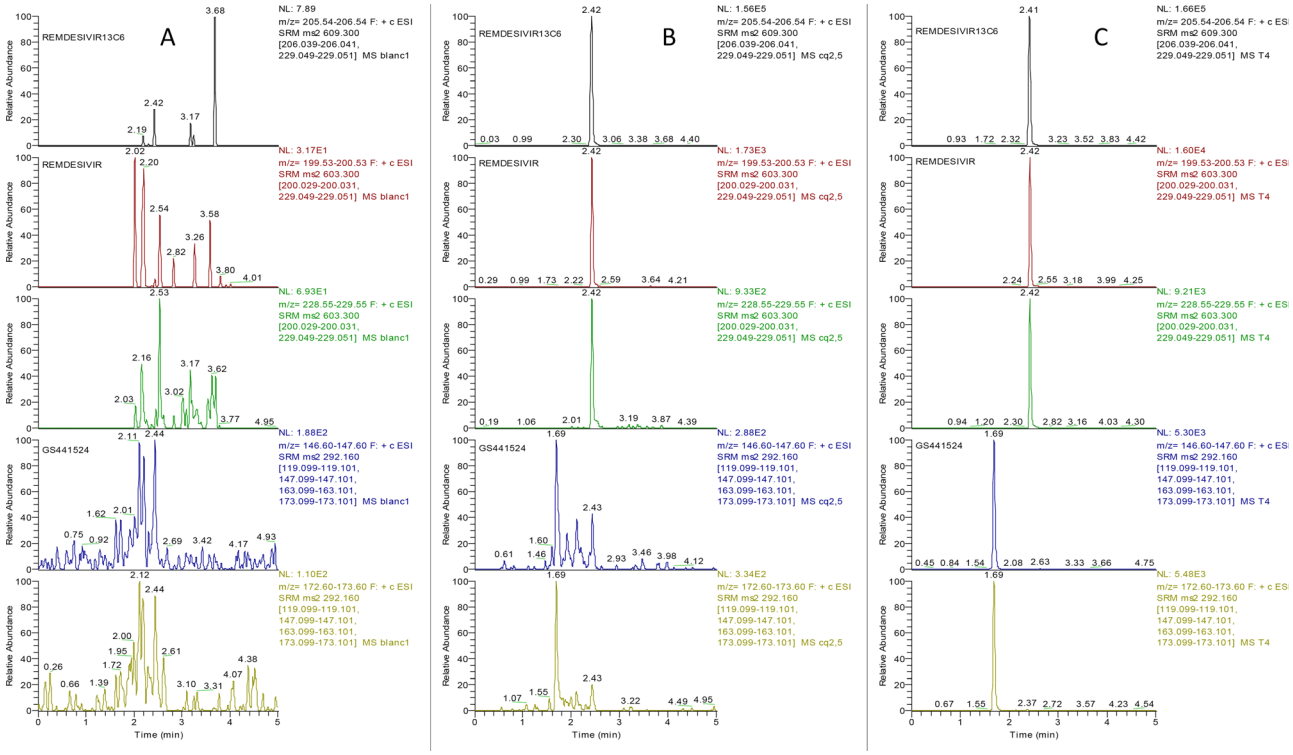


Figure 2: Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) chromatograms of internal standard (IS) (upper graph), remdesivir (medium graphs), and GS-441524 (lower graphs) of a blank plasma sample (A), low quality control (QC) sample at 2.5 µg/L of remdesivir and 12.5 µg/L of GS-441524 (B) and plasma of the treated patient (H_4 after beginning of infusion) with 29 µg/L of remdesivir and 265 µg/L of GS-441524 (C).

for GS-441524. The equations for the curves (n=6) were: $y=(0.00301/x) - 0.0000643$ for remdesivir and $y=(0.00015/x) - 0.0000448$ for GS-441524. Coefficient of determination r^2 were=0.998 (± 0.001) and 0.997 (± 0.001), respectively. Interday CV ranged from 1.7 to 13.4% and bias from –3.4 to 3.7% for the back-calculated concentrations of the CS for remdesivir, and 2.7 to 10.2 % and –5.7 to 6.4% for GS-441524, respectively. 100% of the CS fulfilled the criterion for validation for remdesivir and 93% for GS-441524.

Limit of quantification (LLOQ) and detection (LOD)

The LLOQ was set at 1 µg/L for remdesivir and 5 µg/L for GS-441524, with their corresponding accuracy and precision shown in Table 1. The signal-to-noise ratio was greater than five in both cases. The LODs were estimated at concentration of 0.3 µg/L and 2 µg/L, respectively.

Accuracy and precision

Intra-day and inter-day precision and accuracy of QC samples were summarized in Table 1. All values were in the [89.6–110.2%] range for accuracy and lower than 14.7% for precision.

Matrix effect

Matrix effect values were reported in Table 2. For remdesivir, GS-441524 and IS, MF was similar, ranging from 72 to 84%. The IS-normalized MF was in the [93–107%] range, with CV obtained in the six lots of plasma <9% for remdesivir and its metabolite.

Influence of blood collection tube (effect of anticoagulant)

The influence of blood collection tubes between Li-heparin and NaF on whole blood stability was presented in Table 3. Remdesivir and GS-441524 were stable in whole blood stored at +4 °C for 24 h but not at ambient temperature.

Stability

Stocks solutions of the three compounds and working solutions were stable for at least two weeks. Stability in plasma in different storage conditions were shown in Table 3. Stability for remdesivir was adequate in NaF-plasma when frozen at –20 °C. On Li-heparin-plasma, a 50–60%

Table 1: Validation settings of remdesivir and GS-441524.

			Remdesivir	GS-441524
Accuracy, %	Intra-assay	LLOQ	97.7	101.0
		Low	100.3	94.8
		Medium	108.7	99.7
		High	88.9	103.5
	Inter-assay	LLOQ	100.4	98.4
		Low	98.6	99.1
		Medium	110.2	101.7
		High	89.6	104.1
Precision (CV, %)	Intra-assay	LLOQ	7.3	8.2
		Low	4.6	3.9
		Medium	2.8	4.1
		High	2.5	5.3
	Inter-assay	LLOQ	5.6	12.7
		Low	11.9	14.7
		Medium	5.2	4.2
		High	2.6	3.2

CV, coefficient of variation; QC levels, remdesivir: LLOQ, 1 µg/L, Low, 2.5 µg/L, medium, 400 µg/L, high, 4000 µg/L; GS-441524, LLOQ, 5 µg/L, low, 12.5 µg/L, medium, 125 µg/L, high, 2000 µg/L.

Table 2: Accuracies and precisions of lower limit of quantification (LLOQ) and quality control (QC) samples.

		MF	IS-normalized MF	
		%	%	CV, %
Remdesivir, µg/L	25	78	101	2.2
	400	84	107	3.2
GS-441524, µg/L	125	72	93	9.0
	2000	84	107	5.8
EI, µg/L	300	76	100	—

MF, matrix factor (ratio of the peak area in the presence of matrix to the peak area in absence of matrix); IS-normalized MF, ratio of MF of the analyte by the MF of the IS; CV calculated from six different lots of plasma.

decrease was observed even if plasma was frozen at −20 °C. GS-441524 is stable in both plasma when frozen at −20 °C. However, after two frozen/thaw cycles, GS-441524 appeared to increase in Li-heparin-plasma for the high concentration (2000 µg/L).

Autosampler stability of the processed sample at autosampler temperature (8 °C) was confirmed for 24 h and 48 h (difference < 5%).

Pharmacokinetic study

The plasma log concentration-time profiles of remdesivir and GS-441524 from the Covid-19 treated patient who received intravenous remdesivir are shown in Figure 3. Plasma concentration of remdesivir was found at 150 µg/L 2 h after the beginning of perfusion, followed by a rapid decrease. After 10 h, concentrations were under the LLOQ. Half-life was evaluated in this patient at 0.8 h. C_0 was evaluated at 800 µg/L, leading to a volume of distribution of 200 L for remdesivir in this patient. GS-441524 was present at H_2 at 300 µg/L and showed a slow concentration decrease during 24 h (concentration of 120 µg/L at H_{24}). Half-life was evaluated at 12 h.

Discussion

We have developed and fully validated according to the EMA guidelines a new method for the measurement of plasma concentrations of remdesivir and its active metabolite, GS-441524. This method was successfully applied to a pharmacokinetic study in a patient suffering from Covid-19.

We used deuterated remdesivir (remdesivir-$^{13}C_6$) as IS. It is always of importance of selecting the most suitable IS when validating a LC-MS/MS method. It is the best practice to use a deuterated analog of the analyte being measured as they have similar chemical properties, ionization response in electrospray ionization mass spectrometry and the same chromatographic retention time. The deuterated IS co-elute with the analyte to be quantified and contain enough mass increase to show a signal outside the natural mass distribution of the analyte (Figure 2). Moreover, deuterated IS allow to compensate matrix effect, which was relatively low for remdesivir (MF=78–84%). For the metabolite, deuterated GS-441524 was not commercially available, but matrix effect of the metabolite (MF=72–84%) was similar to that of remdesivir, and the use of remdesivir-$^{13}C_6$ as IS for GS-441524 allow to compensate this slow matrix effect (IS-normalized MF=93–107%, with CV ≤ 9.0).

According to EMA guidelines [13], the study of dilution integrity should also be evaluated, but it was considered here unnecessary considering the high ULOQ validated in calibration curves (5000 µg/L for remdesivir and 2500 µg/L for GS).

The LLOQs of remdesivir and GS-441524 used in our method are less than 5% of the C_{max} as required [12], and appeared enough when looking at the pharmacokinetics obtained in the treated patient and the EC_{50} of remdesivir on SARS-CoV-2 [5, 6]. The data of accuracies and precisions of QCs samples indicated that the present method is accurate, precise and reproducible for quantification of remdesivir and its active metabolite throughout a wide dynamic range.

Influence of blood collection tube has shown that Li-heparin collection tubes are not suitable if there are not stored at 4 °C rapidly after sampling. When stored at room temperature for 24 h, we observed a 74% decrease in

Table 3: Influence of blood collection tube and stability in plasma of remdesivir and GS-441524.

			Ambient temperature for 24 h	4 °C for 24 h	−20 °C for 3 days	2 frozen/thaw cycles
Remdesivir	Li-heparin	Whole blood	26	92	–	–
		P 25 µg/L	3	40	41	35
		P 400 µg/L	5	54	49	43
	NaF	Whole blood	87	97	–	–
		P 25 µg/L	68	89	100	87
		P 400 µg/L	69	92	101	96
GS-441524	Li-heparin	Whole blood	147	101	–	–
		P 125 µg/L	67	77	91	87
		P 2000 µg/L	87	89	116	147
	NaF	Whole blood	101	103	–	–
		P 125 µg/L	100	83	101	97
		P 2000 µg/L	89	79	98	96

All results in % of initial concentration. P, Li-heparin plasma or NaF-plasma. Concentration in whole blood: remdesivir=1000 µg/L, GS-441524=100 µg/L.

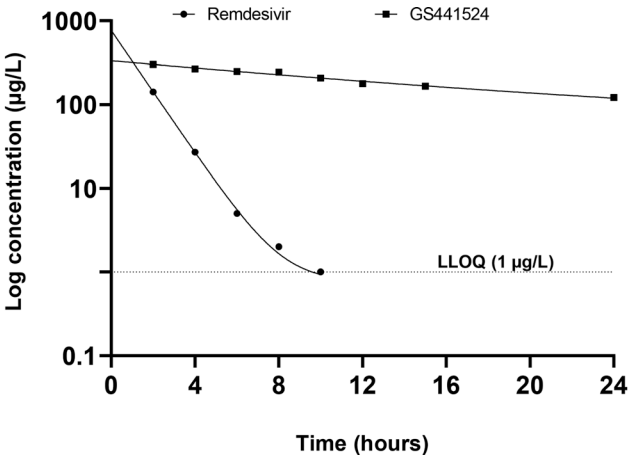


Figure 3: Pharmacokinetics of remdesivir and GS-441524 observed in the treated patient.

remdesivir with a simultaneous increase in the metabolite, probably due to the degradation of remdesivir. This important degradation was also seen in Li-heparin-plasma stored at room temperature (more than 90% of decrease) while it is weaker in the NaF-plasma (around 30% of decrease). The difference is due to the inhibition of enzymes like esterases by fluorure of sodium, leading to a much higher stability in NaF tube, which should be used when sampling patients for remdesivir therapeutic drug monitoring or pharmacokinetic studies.

Unfortunately, the patient treated here was sampled before this stability study, and Li-heparin tubes were used. Whole blood samples were kept at +4 °C before being brought to the laboratory, but the plasma was immediately frozen on arrival at the laboratory and it can be assumed that around 50% of remdesivir was lost according to our stability results, with conservation of the metabolite. As the loss of remdesivir is similar between low (41% at 25 µg/L) and higher concentration (49% at 400 µg/L), the half-life of remdesivir should not be modified (stability of the slope of the concentration curve). Stability in post mortem whole blood has not been tested. However, since esterases cease their activity after death, sampling in NaF tubes at autopsy must allow to determine the post mortem concentrations of the drugs.

Our patient received a loading IV dose of 200 mg in 60 min, and considering the absence of data from Covid-19 patients, resuscitators from ICU asked for a therapeutic drug monitoring of the drug and its active metabolite.

In the literature, the only data available are those of the report of Gilead. C_{max} of remdesivir was found at 2280 µg/L in eight subjects (CV=30.0%) after a single 2 h IV infusion of remdesivir 150 mg, and at 4421.3 µg/L in eight subjects (CV=16.0%) after a dose of 225 mg. C_{max} following 30-minutes IV infusion of remdesivir loading-dose of 200 mg in eight subjects was found at 5400 µg/L (CV=20.3%) on day one, and 2610 µg/L (CV=12.7%) on day 5 in seven subjects after the loading-dose followed by 100 mg daily for four days [12].

C_{max} (C_0) in the patient treated here was evaluated at 800 µg/L. Considering the probable degradation of remdesivir, the concentration should be probably two-timing more elevated. However, Gilead studies were realized in healthy subjects, which are different from Covid-19 patients hospitalized in ICU. Our patient was in a critical state when she was treated, with SAPS II score of 33 and SOFA score of 8. The volume of distribution was found very important (200 L) in our patient suffering from morbid obesity (BMI=44), which is another difference with healthy subject. Half-life was found

at 0.8 h, very closed to that described in healthy subjects (0.89 to 0.98 h) [12]. GS-441524, which is the active metabolite of remdesivir, appeared rapidly in plasma since it was already at its maximal concentration at H_2, consistent with metabolism by hydrolases. Its half-life is longer than remdesivir, around 12 h in our patient and around 24 h in healthy subject [12]. This long half-life of the active metabolite allows the use of remdesivir infusion once-daily despite the short half-life of remdesivir itself.

Conclusions

To our knowledge, this is the first method of quantification of remdesivir and its active metabolite in plasma with application to a treated patient. A small quantity of plasma is required, making the method also suitable for children plasma monitoring of the drugs. Sample preparation requirements are minimal and short analytical time enables high throughput analysis. Stability was evaluated revealing that blood sampling should be drawn in NaF tubes. This method should be useful for the pharmacokinetic/pharmacodynamic relationship studies of these compounds in Covid-19 pandemic.

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