

Simultaneous Analysis of Remdesivir and Metabolites in Human Plasma

Introduction

Remdesivir (brand name: Veklury®), which was developed by Gilead Science (U.S.) for treatment of Ebola virus disease, is a prodrug having antiviral activity against single-strand RNA viruses. It is known to be partly metabolized to activated GS-441524, the main metabolite of remdesivir, in vivo¹⁾. In this article, we present the results of research into an analytical system that can analyze remdesivir and its metabolites simultaneously using LC/MS/MS, an analytical method that demonstrates outstanding selectivity (Fig. 1).

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Fig. 1 External Appearance of LCMS™-8060

Analysis Conditions and Sample Pretreatment

Remdesivir (P/N: C8799*) and GS-441524 (P/N: C8847*), as the target compounds, and [U-Ring-¹³C₆]-remdesivir (P/N: C8845*) and [¹³C₅]-GS-441524 (P/N: C8855*), as their stable isotopes, were purchased from Alsachim, one of the companies of the Shimadzu Group. [U-Ring-¹³C₆]-remdesivir and [¹³C₅]-GS-441524 were used as materials of the internal standard. The structural formulas of remdesivir and GS-441524 are shown in Fig. 2. To commercially available human plasma treated with EDTA 2K, remdesivir and GS-441524 were added. Following this, the calibration curves were prepared. Analysis was performed using the LC and MS analysis conditions shown in Table 1 and the multiple reaction monitoring (MRM) data acquisition parameters shown in Table 2. Shim-pack Scepter™ C18-120 (50 mm×2.1 mm I.D., 1.9 μm) was used as the analytical column. Fig. 3 shows the MS chromatograms.

Calibration was performed using 5 calibration points at concentrations of 100, 500, 1000, 2500 and 5000 ng/mL for remdesivir and 5 calibration points at concentrations of 5, 25, 50, 250 and 500 ng/mL for GS-441524 (n = 5 for each calibration point). [U-Ring-¹³C₆]-remdesivir (2.5 μg/mL) and [¹³C₅]-GS-441524 (0.25 μg/mL) were mixed with methanol to be used as the internal standard (ISTD).

The samples are prepared in the following sequence. After 20 μL of 75% IPA, 50 μL of plasma, 10 μL of ISTD and 100 μL of acetonitrile were added and mixed well, the mixture was centrifuged. The supernatant obtained by centrifuging was transferred into an LC vial for analysis.

*Alsachim's product numbers

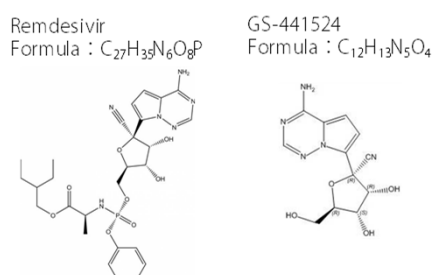


Fig. 2 Structural Formulas of Remdesivir and GS-441524

Table 1 LC and MS Analysis Conditions

<LC Analysis Conditions>		<MS Analysis Conditions>	
UHPLC	Nexera™ X2	LC-MS/MS system	LCMS-8060
Analytical column	Shim-pack Scepter C18-120 (50 mm × 2.1 mm I.D., 1.9 μm)	Interface	Heated ESI
Mobile phase	A: 0.05 % Formic acid–water B: 0.05% Formic acid–acetonitrile	MS analysis mode	MRM (+)
Gradient program (%B)	5 % (0 – 0.30 min) → 30 % (0.35 min) → 70 % (1.50 min) → 90 % (1.80 – 2.80 min) → 5 % (2.90 – 4.50 min)	Heat block temperature	400 °C
Flow rate	0.4 mL/min	DL temperature	200 °C
Column oven temperature	40 °C	Interface temperature	300 °C
Injection volume	2.0 μL (co-injected with 20 μL of water)	Nebulizing gas flow rate	3 L/min
Rinse solution (for external rinse only)	MeOH: IPA = 1:1 (v/v)	Drying gas flow rate	10 L/min
		Heating gas flow rate	10 L/min

Table 2 MRM Transitions of Remdesivir and GS-441524

Compounds	Ion	Precursor ion (m/z)	Product ion (m/z)
Remdesivir	Quantitation ion	603.05	272.10
	Qualification ion	603.05	229.00
[¹³ C ₆]-Remdesivir	Quantitation ion	609.05	278.20
	Qualification ion	609.05	229.15
GS-441524	Quantitation ion	291.90	163.05
	Qualification ion	291.90	173.05
[¹³ C ₅]-GS-441524	Quantitation ion	296.90	164.10
	Qualification ion	296.90	174.10

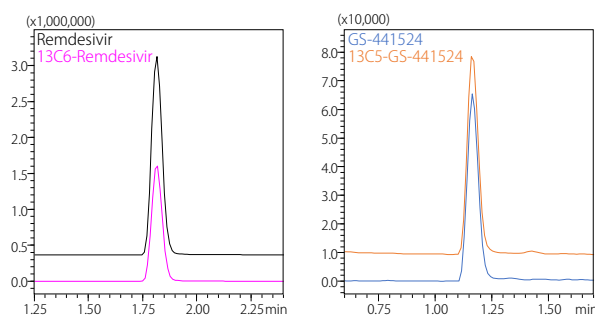


Fig. 3 MS Chromatograms of Remdesivir, [U-Ring-¹³C₆]-Remdesivir (Left) and GS-441524, [¹³C₅]-GS-441524 (Right)

Preparation of Calibration Curves

Calibration curves of plasma-spiked samples are shown in Table 3. For Remdesivir and GS-441524, good linearity was obtained in the set calibration range. The precision (reproducibility) of remdesivir and GS-441524 in the entire concentration range, including the quantitative lower limit was %RSD 0.8 %–1.8 % and %RSD 2.2 %–5.0 %, respectively. Similarly, the accuracy of remdesivir and GS-441524 was 92.0 %–107 % and 94.8 %–106 %, indicating that the accuracy of both was within 100 ± 15 %.

Table 3 Linearity, Accuracy and Precision of Remdesivir and GS-441524 in Plasma Obtained from Analysis

Compound	Remdesivir				GS-441524				
	ID	Spiked Conc. (ng/mL)	Measured Conc. (ng/mL)	Precision %RSD	Accuracy %	Spiked Conc. (ng/mL)	Measured Conc. (ng/mL)	Precision %RSD	Accuracy %
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Level 1	100	92.0	1.2	92	5	4.74	3.8	95	
Level 2	500	535	0.8	107	25	26.6	2.8	106	
Level 3	1000	1036	0.9	104	50	50.7	5.0	101	
Level 4	2500	2433	1.8	97	125	121	2.2	97	
Level 5	5000	5004	1.8	100	250	252	2.3	101	

Area Ratio

Remdesivir_Pos

y = 4.021322x + 0.01906747

R² = 0.9991326 R = 0.9995762

Curve Fit: Default (Linear)

Weighting: Default (1/C)

Zero: Default (Not Forced)

Mean RF: 4.150981e+000

SD RF: 1.781905e+001

MRSD: 4.292733

R² = 0.9992

Area Ratio

GS-441524_Pos

y = 4.793693x + 0.01339927

R² = 0.9992667 R = 0.9996333

Curve Fit: Default (Linear)

Weighting: Default (1/C)

Zero: Default (Not Forced)

Mean RF: 4.975923e+000

SD RF: 2.411530e+001

MRSD: 4.886670

R² = 0.9993

Stability Test of Analytical System

To evaluate the robustness and reproducibility of the developed analytical system, the human plasma spiked with remdesivir at 1000 ng/mL and with GS-441524 at 50 ng/mL was injected into the system a total of 100 times over two days. Fig. 4 shows the plotted results obtained by normalizing the areas from individual injections by the area from the first injection. In human plasma, the %RSD of each compound was 2.2 %–3.9 %, indicating that excellent injection stability was achieved. This result demonstrates that this analytical system can maintain outstanding sensitivity over a long period thanks to its exceptional robustness.

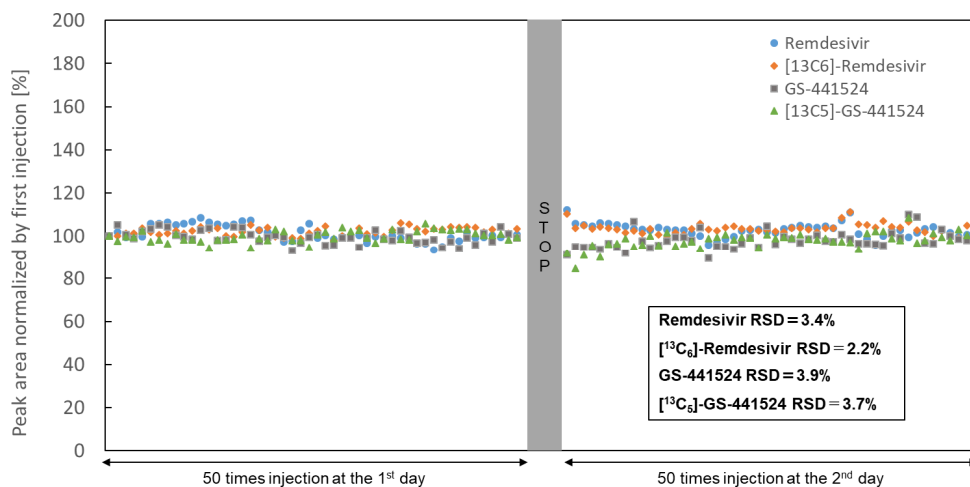


Fig. 4 Measurement Results of 100 Injections of Remdesivir, [U-Ring-¹³C₆]-Remdesivir, GS-441524 and [¹³C₅]-GS-441524
The areas obtained from individual injections were normalized by the area from the first injection and then plotted.

Conclusion

Using plasma samples spiked with remdesivir and GS-441524, its metabolite, an LC-MS/MS analytical system was developed. The prepared calibration curves showed good linearity. Additionally, these samples were injected in the system a total of 100 times to evaluate the robustness of the analytical system. The findings demonstrated that the analytical system can maintain excellent sensitivity over a long period thanks to its exceptional robustness.

<References>

- Richard T et al., "Remdesivir: A Review of Its Discovery and Development Leading to Emergency Use Authorization for Treatment of COVID-19", ACS Cent. Sci.

The product described in this document has not been approved or certified as a medical device under the Pharmaceutical and Medical Device Act of Japan. It cannot be used for the purpose of medical examination and treatment or related procedures.

