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Application News

No. C218

LC-MS

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 vivo[1]. 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-$^{13}C_6$]-remdesivir (P/N: C8845*) and [$^{13}C_5$]-GS-441524 (P/N: C8855*), as their stable isotopes, were purchased from Alsachim, one of the companies of the Shimadzu Group. [U-Ring-$^{13}C_6$]-remdesivir and [$^{13}C_5$]-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-$^{13}C_6$]-remdesivir (2.5 µg/mL) and [$^{13}C_5$]-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

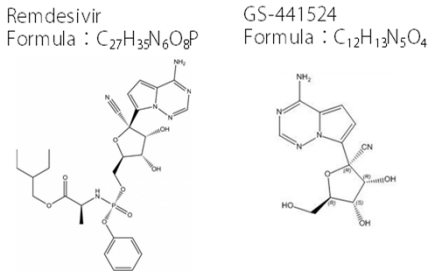

Fig. 2 Structural Formulas of Remdesivir and GS-441524

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

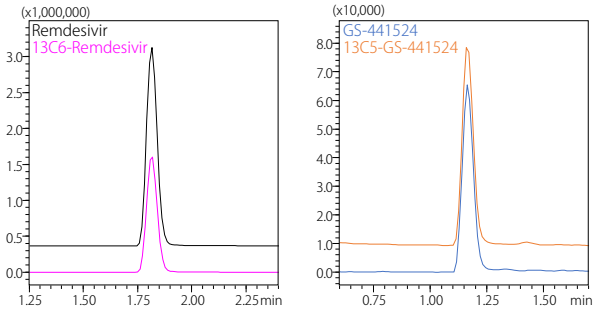

Fig. 3 MS Chromatograms of Remdesivir, [U-Ring-$^{13}C_6$]-Remdesivir (Left) and GS-441524, [$^{13}C_5$]-GS-441524 (Right)

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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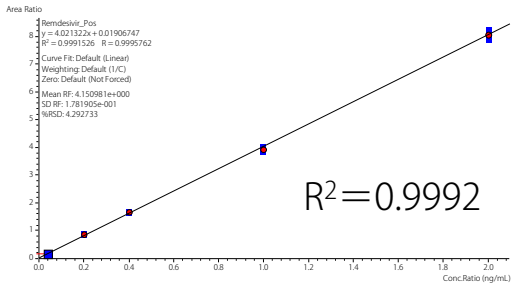
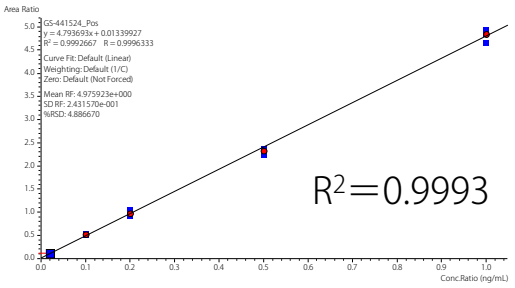
Level 1	100	92.0	1.2	92	5	4.74	3.8	95
Level 2	500	535	0.8	107	25	26.6	2.8	106
Level 3	1000	1036	0.9	104	50	50.7	5.0	101
Level 4	2500	2433	1.8	97	125	121	2.2	97
Level 5	5000	5004	1.8	100	250	252	2.3	101
Calibration Curve								

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.

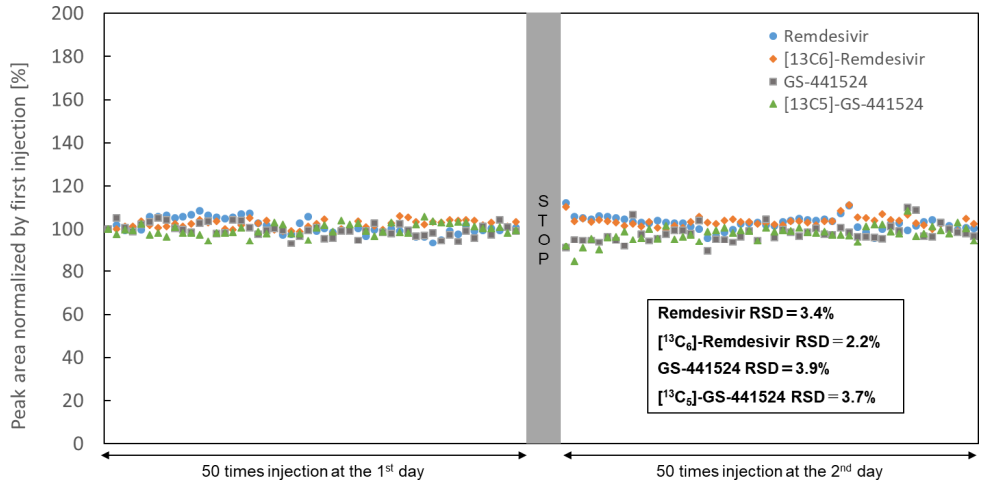


Fig. 4 Measurement Results of 100 Injections of Remdesivir, [U-Ring-$^{13}C_6$]-Remdesivir, GS-441524 and [$^{13}C_5$]-GS-441524
The areas obtained from individual injections were normalized by the area from the first injection and then plotted.

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>
1) 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.

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