

Application News

No. C217

LC-MS

Simultaneous Analysis of Remdesivir and Metabolites in Human Plasma Using Fully Automated Sample Preparation LC/MS/MS System

■ Introduction

Remdesivir (brand name: Veklury®), which was developed by Gilead Sciences (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 Application News C218, we introduced an robust, highly-sensitive simultaneous measurement method using LC/MS/MS with manual pretreatment. Meanwhile, manual pretreatment of plasma samples requires a certain level of workload. This report introduces a method of simultaneously analyzing remdesivir and its metabolite using the automated sample preparation LC/MS/MS system that can reduce variation between procedures, mix-ups of the samples, and risk of exposure to the samples (Fig. 1).

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Fig. 1 Fully Automated Sample Preparation LC/MS/MS System (CLAM™+LC/MS/MS)

■ Analysis of Remdesivir in plasma with Fully Automated Pretreatment

For analysis of low-molecular compounds in plasma using LCMSTM, it is common to use supernatant collected following deproteinization by adding an organic solvent. With the fully automated sample preparation LC/MS/MS system, these preparatory steps are done automatically just by placing a blood collection tube in the system after plasma separation (Fig. 2). Pretreatment of the next sample can also be performed in parallel with LC/MS/MS analysis, which can greatly reduce the time required to analyze each sample.

This analysis was performed in a per-sample cycle time of seven minutes from plasma pretreatment to the simultaneous analysis of remdesivir and its metabolite using LC/MS/MS.

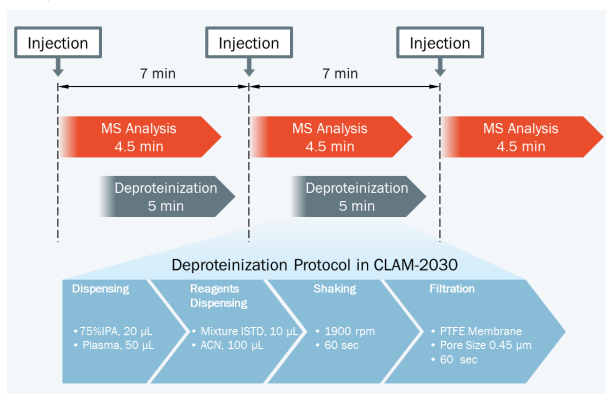


Fig. 2 Workflow of Fully Automated Sample Pretreatment

■ Analysis Conditions and Pretreatment of Samples

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. To commercially available human plasma treated with EDTA 2K, remdesivir and GS-441524 were added. Following this, the calibration curves and QC samples 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). Samples are automatically prepared through a series of steps. These comprise mixing 20 µL of 75%IPA, 50 µL of plasma, 10 µL of ISTD and 100 µL of acetonitrile, shaking the mixture, and then filtration of the mixture using a PTFE membrane filter, as shown in Fig. 2. Finally, the prepared sample is used for LC/MS/MS analysis.

*Alsachim's product numbers

Table 1 LC and MS Analytical Conditions

<LC Analysis Conditions>		<MS Analysis Conditions>	
UHPLC	Nexera™ X2	LC/MS/MS system	LCMS-8060
Analysis 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 [C ₂₇ H ₃₅ N ₆ O ₈ P]	Quantitation ion	603.05	272.10
	Qualification ion	603.05	229.00
[¹³ C ₆]-Remdesivir [C ₂₁ ¹³ C ₆ H ₃₅ N ₆ O ₈ P]	Quantitation ion	609.05	278.20
	Qualification ion	609.05	229.15
GS-441524 [C ₁₂ H ₁₃ N ₅ O ₄]	Quantitation ion	291.90	163.05
	Qualification ion	291.90	173.05
[¹³ C ₅]-GS-441524 [C ₇ ¹³ C ₅ H ₁₃ N ₅ O ₄]	Quantitation ion	296.90	164.10
	Qualification ion	296.90	174.10

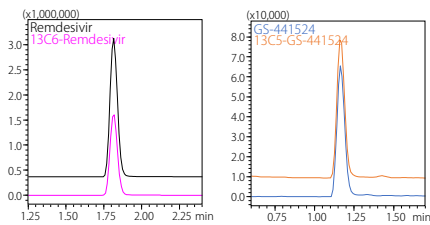


Fig. 3 MS Chromatograms of Remdesivir, [U-Ring-¹³C₆]-Remdesivir (Left) and GS-441524, [¹³C₅]-GS-441524 (Right)

Table 3 Linearity, Accuracy and Precision of Remdesivir and GS-441524 in plasma Obtained from Analysis Using Fully Automated LC/MS/MS

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	87.8	0.5	88	5	4.72	2.4	95
Level 2	500	539	0.8	108	25	26.2	4.8	105
Level 3	1000	1052	1.2	105	50	50.8	4.4	102
Level 4	2500	2536	2.9	101	125	125	4.9	100
Level 5	5000	4885	2.5	98	250	248	4.1	99
Calibration Curve	<div>Area Ratio</div> <div><div>Remdesivir_Phas</div><div>$y = 18.95502x + 0.020411$ $R^2 = 0.998602$ $n = 0.00000002$</div></div> <div><div>Curve Fit (Default: Linear)</div><div>Weighting: Default (1/x)</div><div>Zero: Default (Not forced)</div></div> <div><div>Mean: $W = 0.62969e+000$ $SD(W) = 2.0062e-001$ $NRSD = 4.961360$</div><div>$R^2 = 0.9986$</div></div>				<div>Area Ratio</div> <div><div>GS-441524_Phas</div><div>$y = 4.44716x + 0.000796175$ $R^2 = 0.999704$ $n = 0.00000001$</div></div> <div><div>Curve Fit (Default: Linear)</div><div>Weighting: Default (1/x)</div><div>Zero: Default (Not forced)</div></div> <div><div>Mean: $W = 0.62630e+000$ $SD(W) = 4.0008e-001$ $NRSD = 2.920884$</div><div>$R^2 = 0.9997$</div></div>			

Validation Test of the Analytical System Using QC Samples

Remdesivir and GS-441524 were prepared at the following concentrations as QC samples: for remdesivir, 100, 750, 1000 and 3750 ng/mL; for GS-441524, 5, 37.5, 50 and 187.5 ng/mL to evaluate their repeatability (Table 4) and between-days reproducibility comparing results of three days (Table 5). Based on the repeatability test result, the precision of remdesivir was %RSD 0.9 %–2.0 %, while that of GS-441524 was %RSD 2.3 %–3.6 %. The accuracy of remdesivir was 90.5 %–106 %, while that of GS-441524 was 88.5 %–91.6 %, indicating that their reproducibility was within 100 ± 15 %. Based on the test results for between-days reproducibility, the precision of remdesivir was %RSD 0.1 %–7.2 %, while that of GS-441524 was %RSD 0.4 %–7.8 %. Additionally, the accuracy of remdesivir was 82.2 %–107 %, while that of GS-441524 was 86.7 %–92.8 %, indicating that their accuracy was within 100 ± 20 % at the LLOQ and within 100 ± 15 % in other concentration ranges.

Table 4 Repeatability of Remdesivir and GS-441524 in plasma

Compounds	QC Sample	Spiked Conc. (ng/mL)	Intra-Assay (n=6)		
			Average Conc. (ng/mL)	Precision %RSD	Accuracy %
Remdesivir	LLOQ	100	90.5	2.0	91
	Low	750	797	1.7	106
	Medium	1000	1045	0.9	105
	High	3750	3393	2.0	91
GS-441524	LLOQ	5	4.51	3.1	90
	Low	37.5	33.2	2.5	89
	Medium	50	45.2	2.3	90
	High	187.5	171.7	3.6	92

Table 5 Between-Days Reproducibility of Remdesivir and GS-441524 in plasma

Compounds	QC Sample	Spiked Conc. (ng/mL)	Day 1st (n=3)			Day 2nd (n=3)			Day 3rd (n=3)		
			Average Conc. (ng/mL)	Precision %RSD	Accuracy %	Average Conc. (ng/mL)	Precision %RSD	Accuracy %	Average Conc. (ng/mL)	Precision %RSD	Accuracy %
Remdesivir	LLOQ	100	91.6	1.1	92	82.2	4.9	82	85.1	1.9	85
	Low	750	788	1.8	105	734	1.4	98	770	0.1	103
	Medium	1000	1037	0.7	104	999	0.7	100	1018	0.6	102
	High	3750	3765	1.3	100	3441	1.3	92	3994	7.2	107
GS-441524	LLOQ	5	4.54	4.3	91	4.54	7.7	91	4.50	7.8	90
	Low	37.5	33.1	1.8	88	34.1	2.9	91	32.5	3.2	87
	Medium	50	44.8	3.2	90	44.5	2.7	89	43.8	0.4	88
	High	187.5	174.0	3.7	93	172.6	3.0	92	167.5	0.7	89

Conclusion

A system for analyzing remdesivir and GS-441524, its metabolite, by adding them to plasma was developed using the LC/MS/MS with fully automated sample preparation. The repeatability and between-days reproducibility of remdesivir and GS-441524 were evaluated using QC samples. Good accuracy and reproducibility were obtained.

<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.

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