

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 a 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.

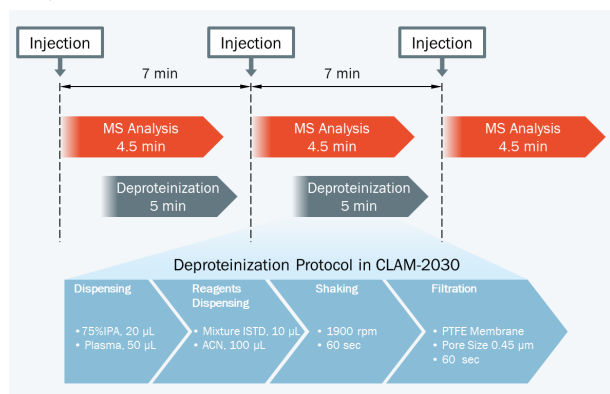


Fig. 2 Workflow of Fully Automated Sample Pretreatment

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) A: 0.05 % Formic acid–water B: 0.05% Formic acid–acetonitrile | Interface | Heated ESI |
| Mobile phase | 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) | MS analysis mode | MRM (+) |
| Gradient program (%B) | 0.4 mL/min | Heat block temperature | 400 °C |
| Flow rate | 40 °C | DL temperature | 200 °C |
| Column oven temperature | 2.0 µL (co-injected with 20 µL of water) | Interface temperature | 300 °C |
| Injection volume | Rinse solution MeOH: IPA = 1:1 (v/v) | Nebulizing gas flow rate | 3 L/min |
| Rinse solution (for external rinse only) | | 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 |

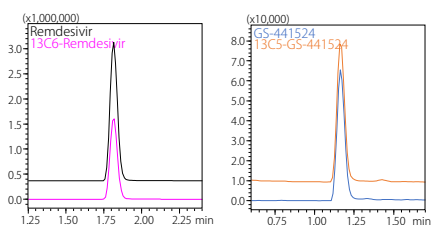


Fig. 3 MS Chromatograms of Remdesivir, [U-Ring-¹³C₆]-Remdesivir (Left) and GS-441524, [¹³C₅]-GS-441524 (Right)

■ Preparation of Calibration Curves

Calibration curves prepared using the fully automated sample preparation LC/MS/MS are shown in Table 3. 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 0.5 %–2.9 % and %RSD 2.4 %–4.9 %, respectively. Similarly, the accuracy of remdesivir and GS-441524 was 87.8 %–108 % and 94.5 %–105 %, respectively, 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 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 % |
| Blank | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 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 | |

| Compound | Remdesivir | | GS-441524 | |
|------------|----------------------|------------------------|----------------------|------------------------|
| | Spiked Conc. (ng/mL) | Measured Conc. (ng/mL) | Spiked Conc. (ng/mL) | Measured Conc. (ng/mL) |
| Remdesivir | 0.0 | 0.0 | 0.0 | 0.0 |
| Remdesivir | 0.1 | 0.1 | 0.1 | 0.1 |
| Remdesivir | 0.2 | 0.2 | 0.2 | 0.2 |
| Remdesivir | 0.5 | 0.5 | 0.5 | 0.5 |
| Remdesivir | 1.0 | 1.0 | 1.0 | 1.0 |
| Remdesivir | 2.5 | 2.5 | 2.5 | 2.5 |
| Remdesivir | 5.0 | 5.0 | 5.0 | 5.0 |
| Remdesivir | 10.0 | 10.0 | 10.0 | 10.0 |
| Remdesivir | 25.0 | 25.0 | 25.0 | 25.0 |
| Remdesivir | 50.0 | 50.0 | 50.0 | 50.0 |
| Remdesivir | 100.0 | 100.0 | 100.0 | 100.0 |
| Remdesivir | 250.0 | 250.0 | 250.0 | 250.0 |
| Remdesivir | 500.0 | 500.0 | 500.0 | 500.0 |
| Remdesivir | 1000.0 | 1000.0 | 1000.0 | 1000.0 |
| Remdesivir | 2500.0 | 2500.0 | 2500.0 | 2500.0 |
| Remdesivir | 5000.0 | 5000.0 | 5000.0 | 5000.0 |
| GS-441524 | 0.0 | 0.0 | 0.0 | 0.0 |
| GS-441524 | 0.1 | 0.1 | 0.1 | 0.1 |
| GS-441524 | 0.2 | 0.2 | 0.2 | 0.2 |
| GS-441524 | 0.5 | 0.5 | 0.5 | 0.5 |
| GS-441524 | 1.0 | 1.0 | 1.0 | 1.0 |
| GS-441524 | 2.5 | 2.5 | 2.5 | 2.5 |
| GS-441524 | 5.0 | 5.0 | 5.0 | 5.0 |
| GS-441524 | 10.0 | 10.0 | 10.0 | 10.0 |
| GS-441524 | 25.0 | 25.0 | 25.0 | 25.0 |
| GS-441524 | 50.0 | 50.0 | 50.0 | 50.0 |
| GS-441524 | 100.0 | 100.0 | 100.0 | 100.0 |
| GS-441524 | 250.0 | 250.0 | 250.0 | 250.0 |
| GS-441524 | 500.0 | 500.0 | 500.0 | 500.0 |
| GS-441524 | 1000.0 | 1000.0 | 1000.0 | 1000.0 |
| GS-441524 | 2500.0 | 2500.0 | 2500.0 | 2500.0 |
| GS-441524 | 5000.0 | 5000.0 | 5000.0 | 5000.0 |

■ 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>

- 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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