Sample Preparation Method for Determination of GS-441524 (Remdesivir Metabolite) in Plasma Using ISOLUTE® ENV+ SPE Columns

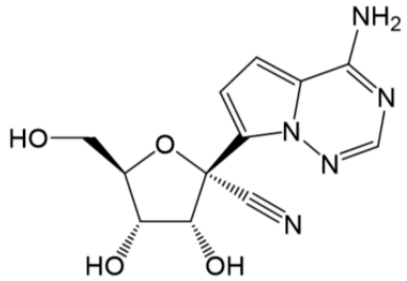

Figure 1. Structural Formula for GS-441524.

Introduction

GS-441524 (Figure 1) is the main plasma metabolite of the antiviral drug remdesivir. Remdesivir is a nucleoside analog antiviral drug developed by Gilead Sciences for the treatment of Ebola, and both have been found to exhibit antiviral activity against several coronaviruses, including SARS-CoV-2 (COVID-19). GS-441524 is also a promising and inexpensive drug candidate in the treatment of COVID-19 and future emerging coronaviruses. Therapeutic Drug Monitoring (TDM), which measures blood levels of the antibacterial drugs and helps designs dosages for individual patient administration, is critical for effective and safe use of the drugs.

In this application note, ISOLUTE® ENV+ based on a hydroxylated polystyrene divinylbenzene copolymer (Figure 2) was used as a sample preparation column to clean up samples prior to LC-MS/MS analysis. It is suitable for retention of highly polar compounds that are typically not retained by silica-based C18 columns. In this application, GS-441524 could be extracted from plasma samples with high recovery.

Analytes

GS-441524 (Remdesivir metabolite, CAS: 1191237-69-0)
Doripenem (Doripenem, CAS: 148016-81-3) used as an Internal Standard (IS).

Sample Preparation Procedure Format

Format

ISOLUTE® ENV+ 50 mg/ 1 mL columns,
(p/n: 915-0005-A)

Sample Pre-treatment

To 200 µL of plasma, add doripenem as an internal standard at a concentration of 0.1 µg/mL. To this, add 200 µL of 10 mmol/L ammonium formate solution containing 0.1% formic acid and vortex mix for 30 seconds.

Conditioning

Condition the column with methanol (1 mL).

Equilibration

Pass through 1 mL of 10 mmol/L ammonium formate solution containing 0.1% formic acid.

Sample Loading

Load 400 µL of the pre-treated sample.

Wash

Elute interferences with 1 mL of a 10 mmol/L ammonium formate solution containing 0.1% formic acid.

Elution

First, elute with 0.4 mL of water:methanol = 8:2 (v/v)
Next, elute with 0.4 mL of methanol.
Mix the two eluates to obtain sample for LC/MS/MS.

Dilution

Depending on the range of calibration concentration in LC/MS/MS to be used, dilute the sample solution further*.

* In this application note, samples were diluted 2.5-fold using purified water.

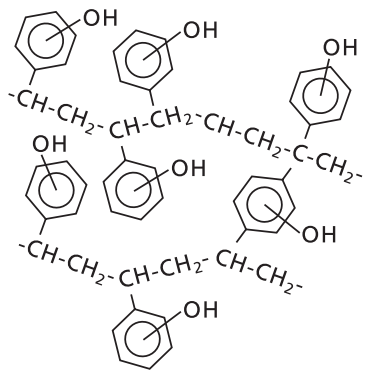

Figure 2. Schematic Representation of ISOLUTE® ENV+.

UHPLC Conditions

Instrument
Shimadzu Nexera LC-30AD

Column
Waters ACQUITY UPLC® BEH C18 1.7 µm (2.1 mm × 50 mm column)

Mobile Phase
A: 10 mmol/L ammonium formate solution containing 0.1% formic acid

B: Methanol

Flow Rate
0.4 mL/min.

Gradient Conditions
Time/% of B: 0/5 ⟶ 1/5 ⟶ 3.5/90 ⟶ 4/90 ⟶ 4.1/5 ⟶ 5/5

Column Temperature
50 °C

Injection volume
1 µL

Mass Spectrometry Conditions

Instrument
Shimadzu LCMS-8060

Ionization Mode
ESI positive

Nebulizer Gas Flow Rate
2.80 L/min.

Drying Gas Flow Rate
10.00 L/min.

Heating Gas Flow Rate
10.00 L/min.

Interface Temperature
350 °C

DL Temperature
200 °C

Heat Block Temperature
350 °C

CID Gas
270 kPa

SRM Transitions

GS-441524:
m/z 292.20 › 202.20, Rt 1.92 min,
Collision Energy; -10

Doripenem:
m/z 420.90 › 274.00, Rt 1.55 min, Collision Energy; -18

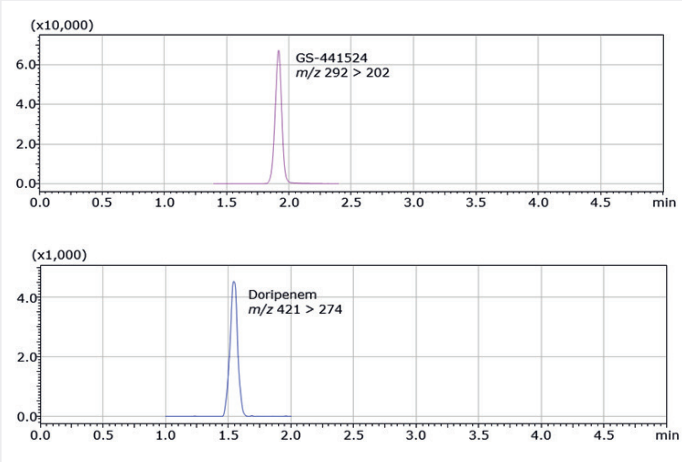

Figure 3. SRM Chromatograms of GS-441524 (top) and Doripenem (bottom).

Results

Figure 3 shows the SRM (Selected Reaction Monitoring) chromatograms of LC-MS/MS analyses of GS-441524 and doripenem (IS). The calibration curve generated is shown in Figure 4. The required blood concentration range for TDM (Therapeutic Drug Monitoring) of GS-441524 is 10 to 1000 ng/mL. In this method, the concentration of GS-441524 was measured at 10-fold dilution from 1 to 100 ng/mL. As a result, a wide dynamic range and good linearity with a multiple correlation coefficient (r^2) of 0.999 or higher was obtained.

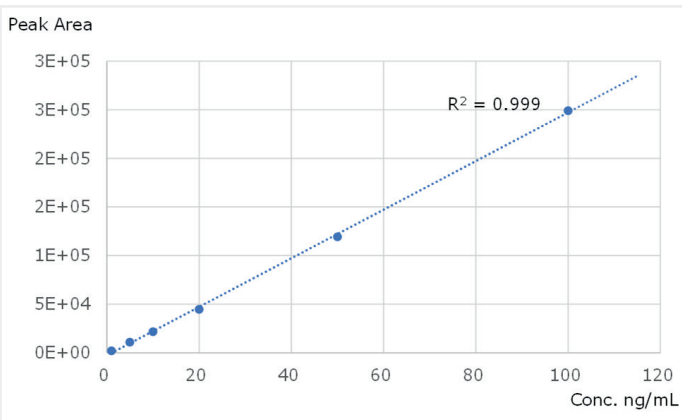

Figure 4. Calibration Curve for GS-441524 (1.0 ~ 100 ng/mL).

Confirmation of Analyte Recovery and Matrix Factors

Sample pre-treatment is very important in samples of biological origin. ISOLUTE® ENV+ solid phase extraction columns were used to eliminate the effects of matrix components like protein, phospholipids and salts, and to allow for improved quantitative analyses. The sample used was control plasma with 10 ng/mL GS-441524. The SRM chromatogram obtained by LC/MS/MS measurement after pretreatment is shown in Figure 5). No interference by contaminants was observed.

Next, we evaluated matrix effects using ISOLUTE® ENV+ for cleanup. Three samples of plasma containing 10, 100, and 1000 ng/mL of GS-441524 were prepared using ISOLUTE® ENV+ columns. The recovery rates and matrix factors are shown in Table 1. The recoveries were calculated by comparing the peak area values in plasma samples spiked with GS-441524 before extraction (A) and the peak area value of plasma samples spiked with GS-441524 after extraction (B). Matrix factors were calculated by comparing the peak area values (B) and the standard solution (S). As a result, a high recovery rate of 86.7% to 98.9% was obtained, and the matrix factor was sufficiently small, ranging from 0.5% to 9.3%, confirming that pretreatment with ISOLUTE® ENV+ can efficiently remove matrix effects.

Table 1. Recovery Rate and Matrix Factors (n=2) in the Spike Recovery Test.

Blood Concentrations (ng/mL)	Recovery Rate (%)	Matrix Factor (%)
10	98.9	9.3
100	98.6	0.5
1000	86.7	5.0

* Recovery rate = [A]/[B] × 100; Matrix Factor = 1-[B]/[S] × 100.

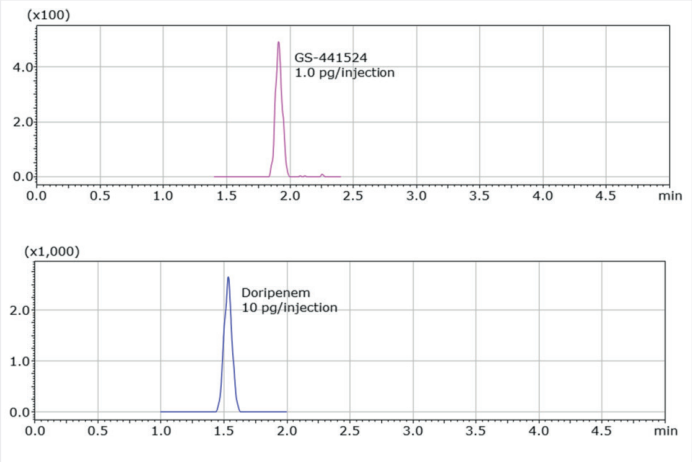

Figure 5. SRM Chromatograms after SPE with ISOLUTE® ENV+ for GS-441524 10 ng/mL in plasma.

Ordering Information

Part Number	Description	Quantity
915-0005-A	ISOLUTE® ENV+ 50 mg/1 mL	100/pk
PPM-48	Biotage® PRESSURE+ 48 Positive Pressure Manifold	1
121-2016	Biotage® VacMaster™ 20 Sample Processing Manifold	1

Acknowledgement

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