

2020/5/21 (Thu)

Simultaneous analysis for Remdesivir and its metabolite [with CRAM-2030]

Shimadzu Corporation
Global Application Development Center
Eishi IMOTO

Method

System : CLAM-2030, Nexera X2, LCMS-8060

- HPLC Conditions :
 - Analytical Column : Shim-pack Scepter C18-120 (50 mm x 2.1 mm I.D., 1.9 μm)
 - MP A : 0.05% Formic Acid - Water
 - MP B : 0.05% Formic Acid - Acetonitrile
 - Flow Rate : 0.4 mL/min
 - Column Temp : 40 °C
 - Injection Volume : 2.0 μL (Co-injection with 20 μL water)
 - Rinse : MeOH : IPA = 1 : 1 (v/v) External only
- MS Conditions : ESI (Positive)
 - Interface Temp : 300°C
 - Heating Gas : 10 L/min
 - Nebulizer Gas : 3 L/min
 - Drying Gas : 10 L/min
 - DL Temp : 200 °C
 - Heat Block Temp : 400 °C

Gradient Program

Time (min)	B. conc. %
0	5
0.30	5
0.35	30
1.50	70
1.80	90
2.80	90
2.90	5
4.50	STOP

Co-injection program

STEP	Event
1	Rinse needle
2	Aspirate from target vial
3	Rinse needle
4	Aspirate 20 μL water from control rack
5	Rinse needle
6	Injection



CLAM and LC-MS/MS system

Structural formula of Remdesivir and GS-441524

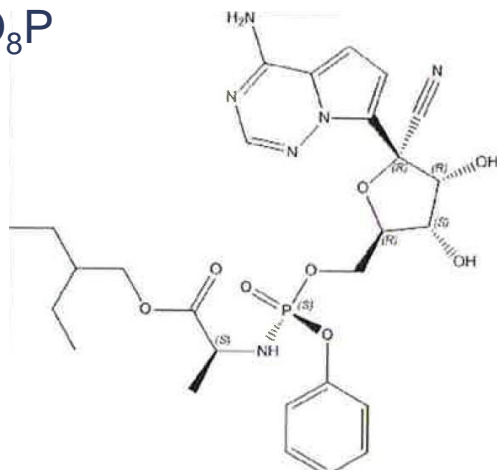
These compounds were purchased from Alsachim

Remdesivir

Formula : $C_{27}H_{35}N_6O_8P$

MW : 602.2254 g/mol

Diluted in MeOH

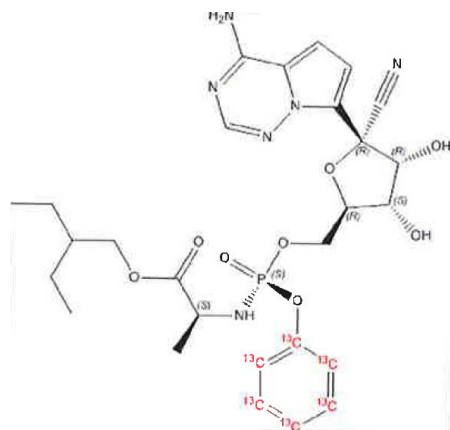


[U-Ring- $^{13}C_6$]-Remdesivir

Formula : $C_{21}^{13}C_6H_{35}N_6O_8P$

MW : 608.2455 g/mol

Diluted in MeOH

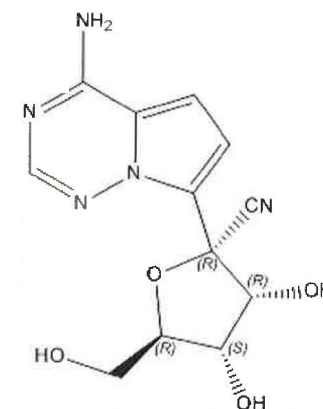


GS-441524

Formula : $C_{12}H_{13}N_5O_4$

MW : 291.0968 g/mol

Diluted in MeOH : DMSO= 1:1 (v/v)

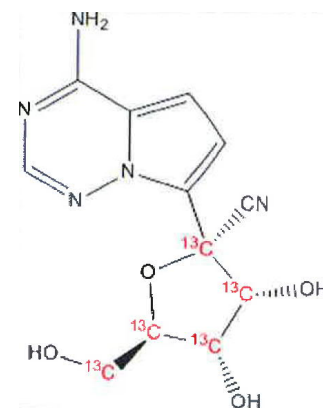


[$^{13}C_5$]-GS-441524

Formula : $C_7^{13}C_5H_{13}N_5O_4$

MW : 296.1136 g/mol

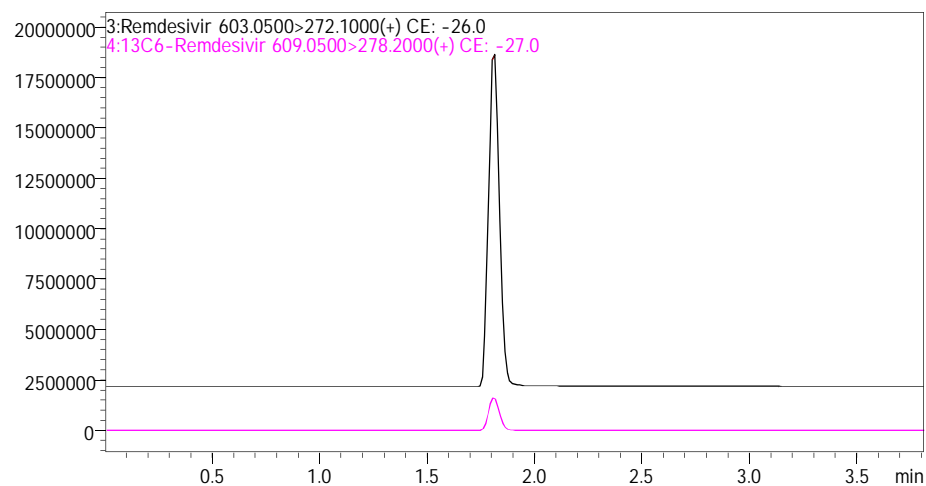
Diluted in MeOH : DMSO= 1:1 (v/v)



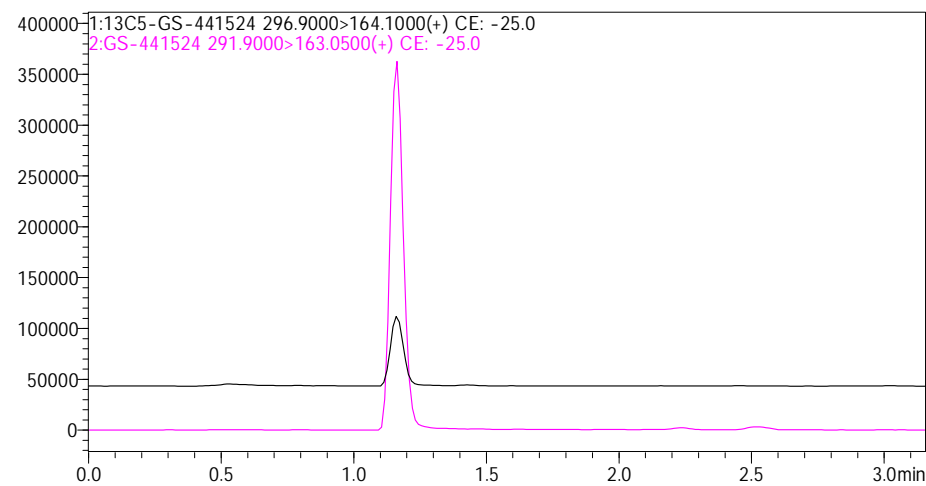
MRM Transitions

Compounds	Ion	Precursor ion (m/z)	Product ion (m/z)	Q1 PreBias (V)	Collision Energy (V)	Q3 PreBias (V)
Remdesivir	MRM Quant	603.05	272.10	-28	-26	-14
	MRM Qual	603.05	229.00	-30	-23	-25
[¹³ C ₆]-Remdesivir	MRM Quant	609.05	278.20	-24	-27	-14
	MRM Qual	609.05	229.15	-24	-21	-26
GS-441524	MRM Quant	291.90	163.05	-14	-25	-17
	MRM Qual	291.90	173.05	-15	-25	-19
[¹³ C ₅]-GS-441524	MRM Quant	296.90	164.10	-14	-25	-18
	MRM Qual	296.90	174.10	-11	-25	-18

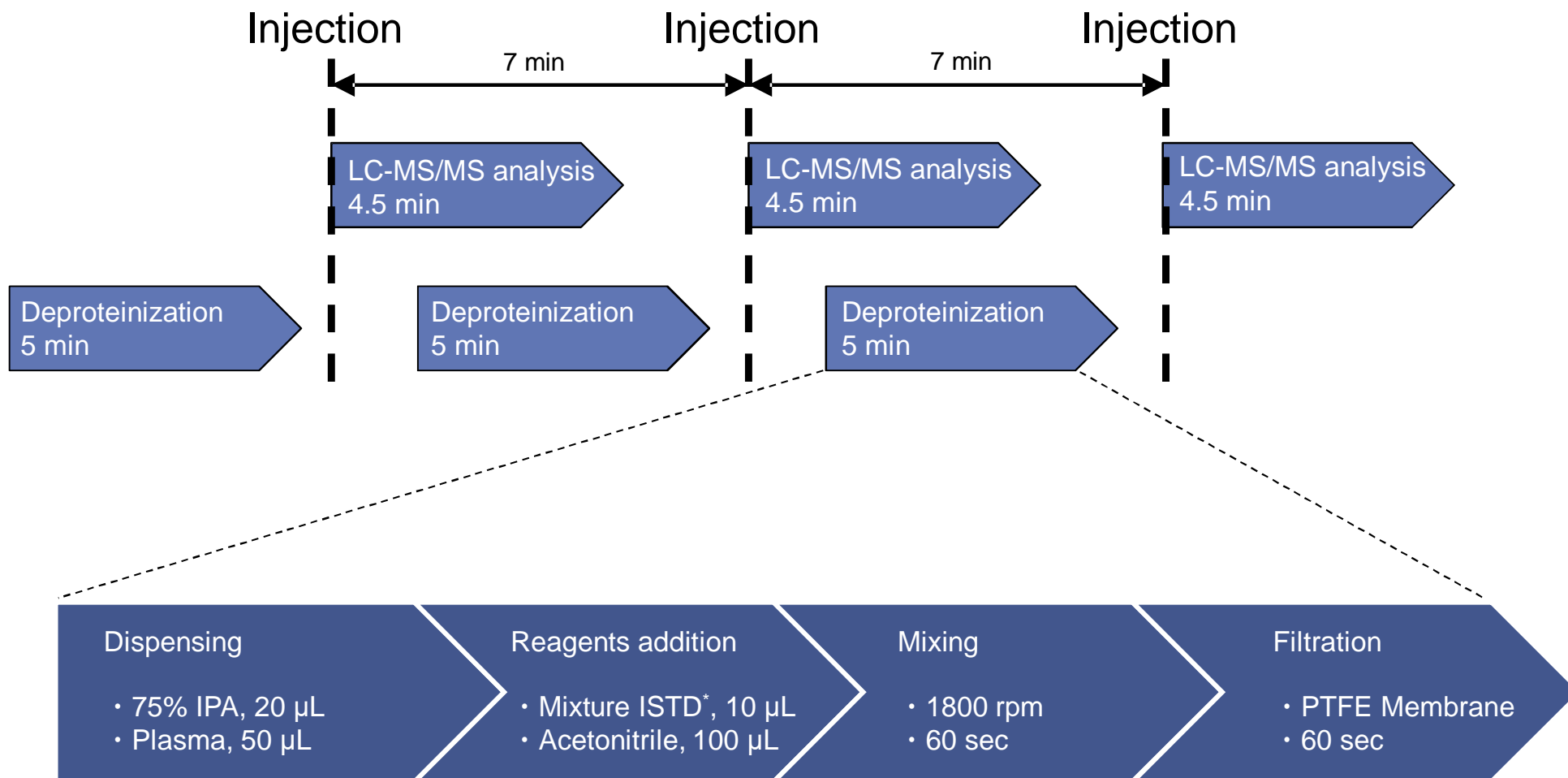
MS Chromatogram Remdesivir and ISTD



MS Chromatogram GS-441524 and ISTD

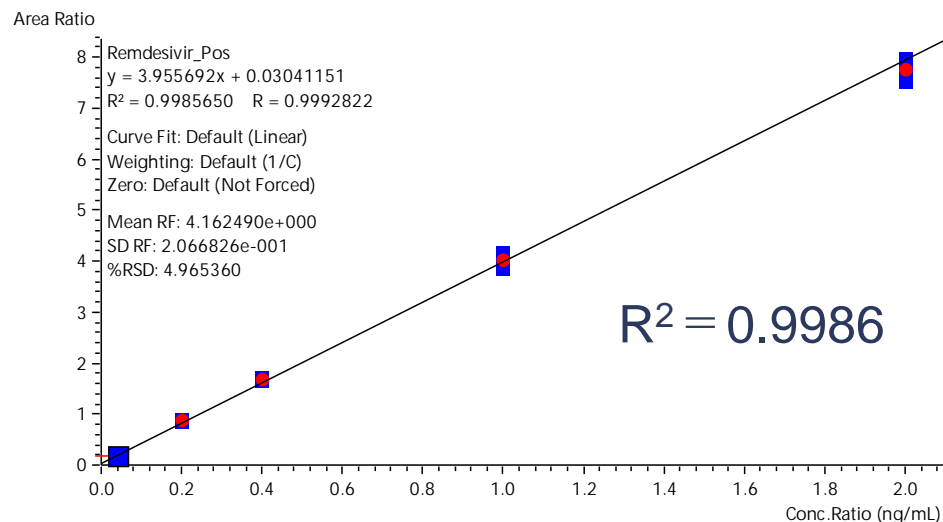


Fully automated sample prep using CLAM-2030



*[¹³C₆]-Remdesivir
 : 2.5 µg/mL in MeOH
¹³C₅]-GS-441524
 : 0.25 µg/mL in MeOH

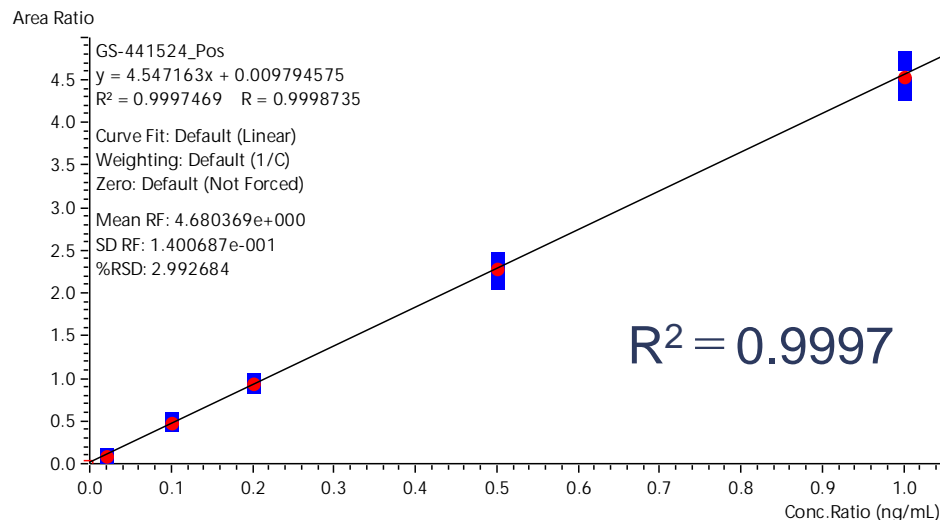
Calibration Curve: Remdesivir using CLAM-2030+LCMS-8060



n=5

ID	Area	Area %RSD	Area Ratio	Area Ratio %RSD	ISTD Area	ISTD Area %RSD	Spiked Conc (ng/mL)	Measured Conc (ng/mL)	Calibration Point	Accuracy %
Blank	---	---	---	---	5,959,187	5.6	---	---	—	---
Level 1	1,043,299	1.2	0.169333	0.5	6,161,122	0.8	100	87.8	○	87.8
Level 2	5,343,514	0.6	0.884055	0.8	6,044,637	1.0	500	539	○	107.9
Level 3	10,499,912	1.4	1.69425	1.2	6,197,352	0.5	1000	1052	○	105.2
Level 4	24,822,342	3.2	4.04333	2.9	6,142,849	4.2	2500	2536	○	101.4
Level 5	48,666,338	2.0	7.75981	2.5	6,272,254	0.7	5000	4885	○	97.7

Calibration Curve: GS-441524 using CLAM-2030+LCMS-8060



n=5

ID	Area	Area %RSD	Area Ratio	Area Ratio %RSD	ISTD Area	ISTD Area %RSD	Spiked Conc (ng/mL)	Measured Conc (ng/mL)	Calibration Point	Accuracy %
Blank	---	---	---	---	232,222	3.4	---	---	—	---
Level 1	22,799	3.4	0.0957136	2.4	238,162	1.5	5	4.72	○	94.5
Level 2	114,124	2.7	0.485467	4.8	235,285	2.6	25	26.2	○	104.6
Level 3	226,889	3.3	0.934008	4.4	243,036	1.9	50	50.8	○	101.6
Level 4	555,288	1.1	2.28184	4.9	243,735	4.1	125	125	○	99.9
Level 5	1,131,711	1.8	4.52778	4.1	250,168	2.9	250	248	○	99.4

Intra-day analysis using CLAM-2030+LCMS-8060

The result of Intra-day accuracy and precision of Remdesivir and GS-441524 spiked in plasma

Compounds	QC Sample	Conc. Added (ng/mL)	Intra-day analysis (<i>n</i> =6)		
			Conc. Found (ng/mL)	%RSD	Accuracy
Remdesivir	LLOQ	100	90.5	2.0	90.5
	Low	750	797	1.7	106
	Medium	1,000	1045	0.9	105
	High	3,750	3393	2.0	90.5
GS-441524	LLOQ	5	4.51	3.1	90.2
	Low	37.5	33.2	2.5	88.5
	Medium	50	45.2	2.3	90.4
	High	187.5	171.7	3.6	91.6

For Remdesivir and GS-441524, the intra-day precisions were 0.9 – 2.0% and 2.3 - 3.6%, and accuracies ranged 90.5 - 106% and 88.5 – 91.6%, respectively

The LLOQ reproducibility was less than 20% and accuracy 80-120% in the concentration range

Inter-day analysis using CLAM-2030+LCMS-8060

The result of Inter-day accuracy and precision of Remdesivir and GS-441524 spiked in plasma

Compounds	QC Sample	Conc. Added (ng/mL)	Day 1st (n=3)			Day 2nd (n=3)			Day 3rd (n=3)		
			Conc. Found (ng/mL)	%RSD	Accuracy	Conc. Found (ng/mL)	%RSD	Accuracy	Conc. Found (ng/mL)	%RSD	Accuracy
Remdesivir	LLOQ	100	91.6	1.1	91.6	82.2	4.9	82.2	85.1	1.9	85.1
	Low	750	788	1.8	105	734	1.4	97.9	770	0.1	103
	Medium	1,000	1037	0.7	104	999	0.7	99.9	1018	0.6	102
	High	3,750	3765	1.3	100	3441	1.3	91.8	3994	7.2	107
GS-441524	LLOQ	5	4.54	4.3	90.8	4.54	7.7	90.8	4.50	7.8	90.0
	Low	37.5	33.1	1.8	88.3	34.1	2.9	90.9	32.5	3.2	86.7
	Medium	50	44.8	3.2	89.6	44.5	2.7	89.0	43.8	0.4	87.6
	High	187.5	174.0	3.7	92.8	172.6	3.0	92.1	167.5	0.7	89.3

For Remdesivir and GS-441524, the inter-day precisions were 0.1-7.2% and 0.4-7.8%, and accuracies ranged 82.2-107% and 86.7-92.8%, respectively.

Recovery Test using manual sample prep + LCMS-8060

Sample preparation for evaluate matrix effect and recovery rate

Standard	Post-Spike	Pre-Spike
75% IPA: 20 µL		
Internal Standard: 10 µL		
ACN: 100 µL	Deproteinized ACN: 100 µL	ACN: 100 µL
Water: 45 µL		Plasma: 45 µL
Remdesivir (1,000 ng/mL) + GS-441524 (500 ng/mL): 5 µL		
Vortex and centrifugation		

Calculation of matrix effect and recovery rate of each samples

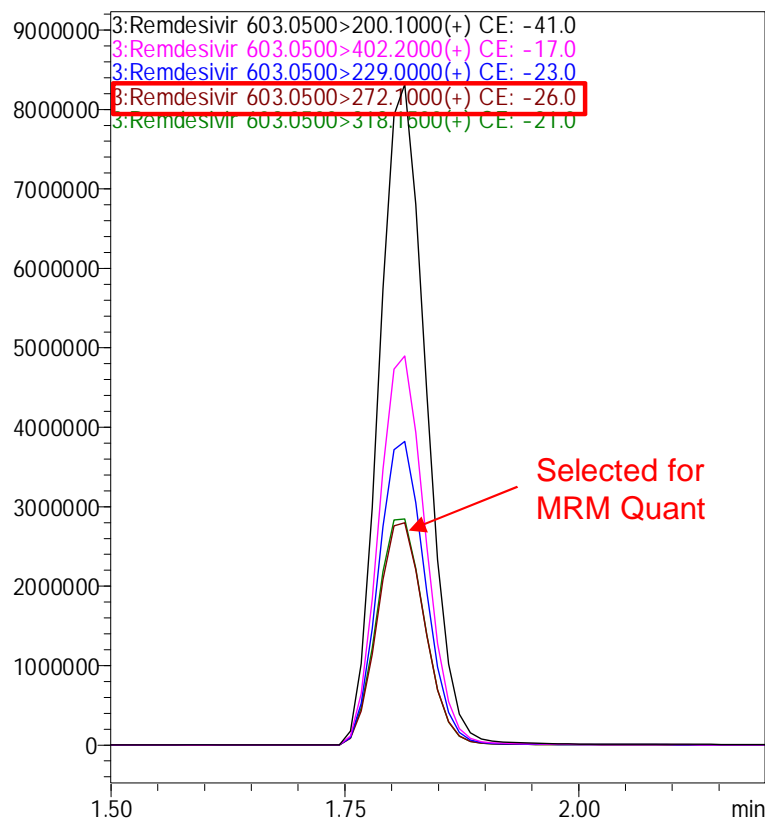
Compounds	Standard Peak Area [-]	Post-Spike Peak Area [-]	Pre-Spike Peak Area [-]	<i>n=3</i>	
				Matrix effect*1 %	Recovery Rate*2 %
Remdesivir	1,032,259	1,029,720	1,016,067	<u>99.8</u>	<u>98.7</u>
GS-441524	250,236	210,700	191,396	<u>84.2</u>	<u>90.8</u>

*1 [Matrix effect] = [Post-Spike Peak Area] / [Standard Peak Area] x 100
 *2 [Recovery Rate] = [Pre-Spike Peak Area] / [Post-Spike Peak Area] x 100

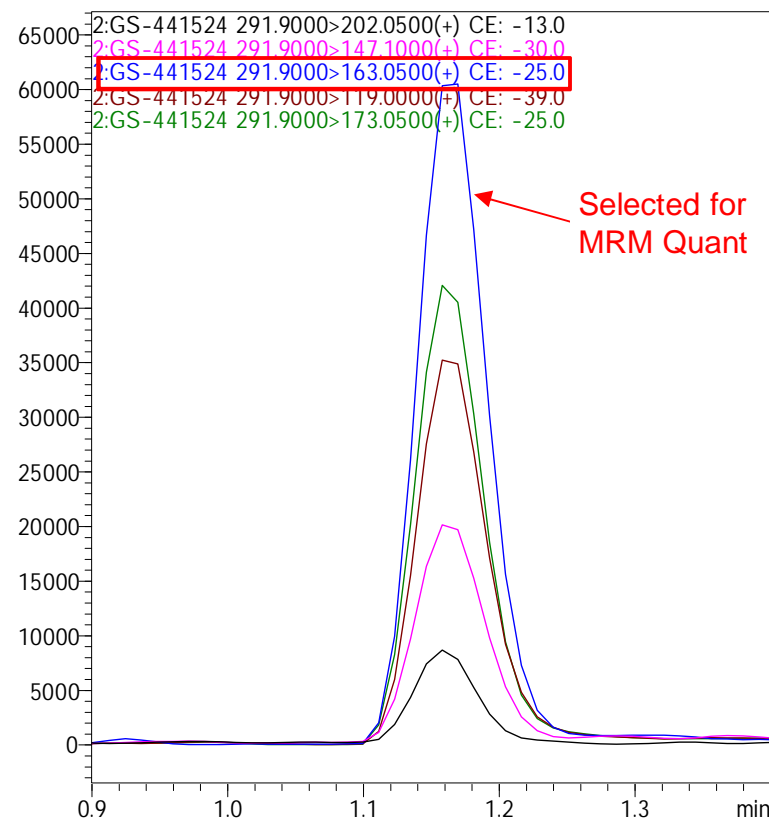
Technical Point ① Selection of MRM Transition

<Result of optimization>

The top 5 MRM transitions from ion intensities of Remdesivir



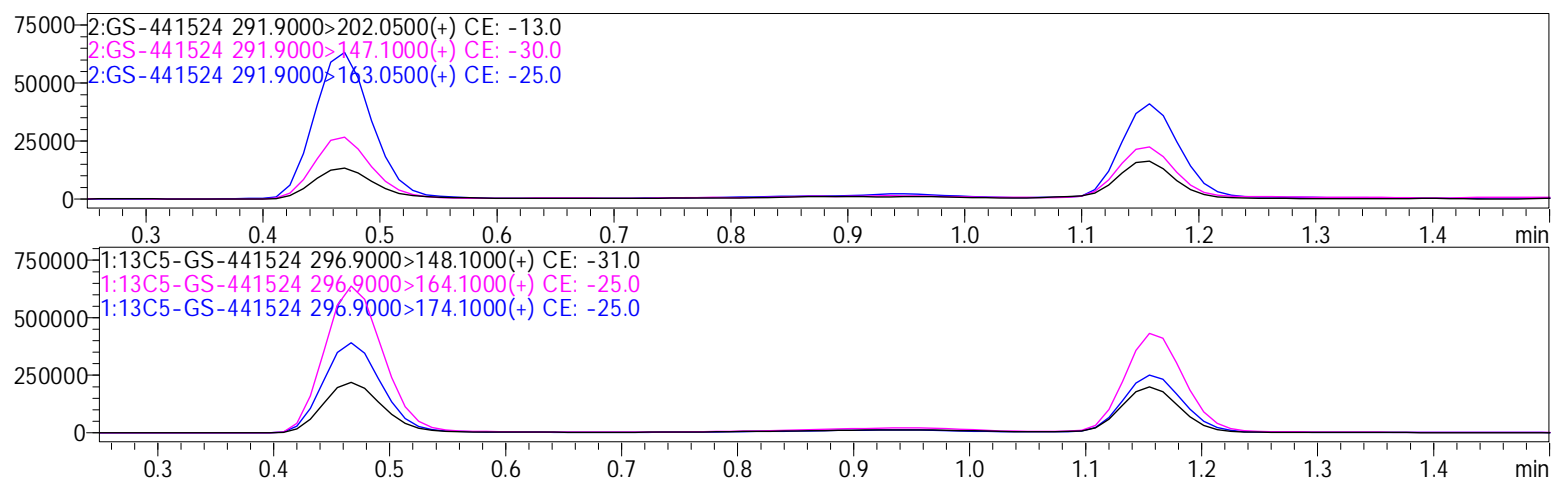
The top 5 MRM transitions from ion intensities of GS-441524



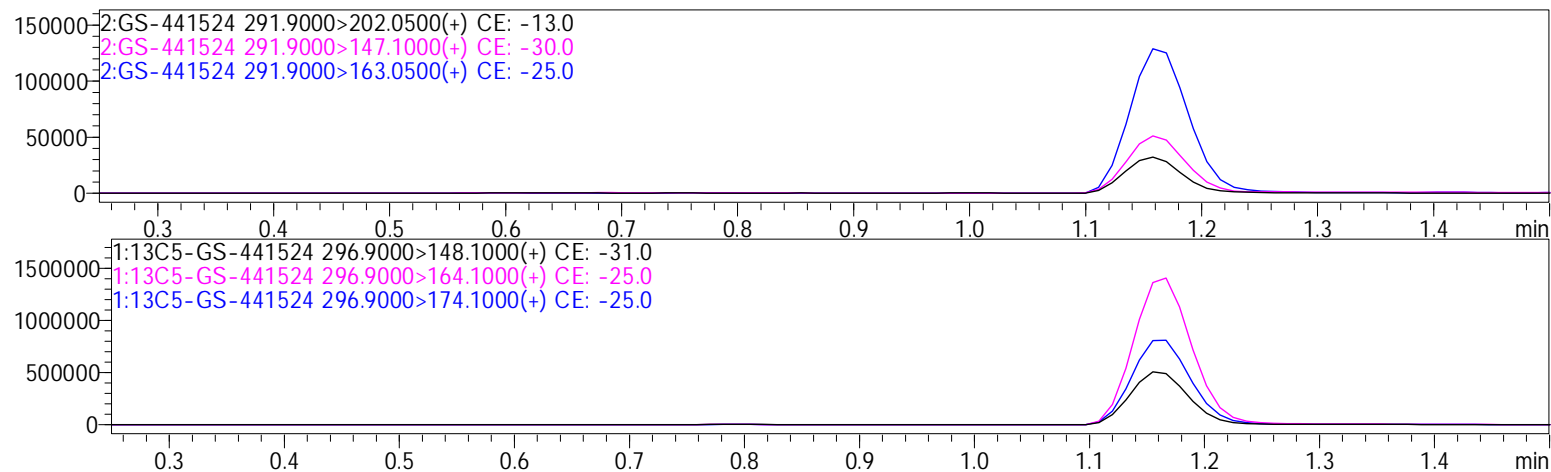
4th MRM was selected as quantification for Remdesivir and, 1st MRM was selected for GS-441524
 The reason why is that conc range in plasma are different between Remdesivir and GS-441524

Technical Point ② Improvement of peak shape

< conventional injection >



< Co-injection with 20 μ L water >

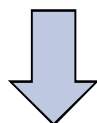
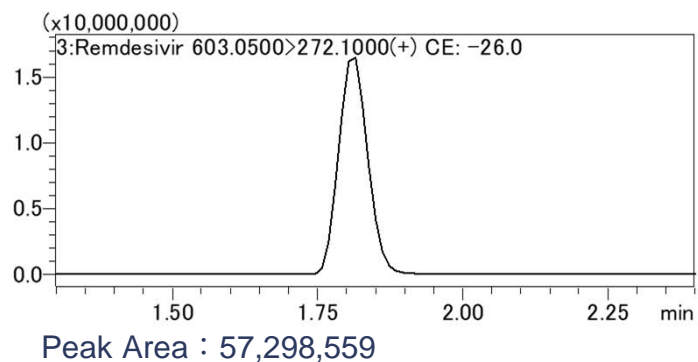


After sample prep, ACN was used as a solvent.
Peak shape was improved using co-injection with 20 μ L water

Carryover test

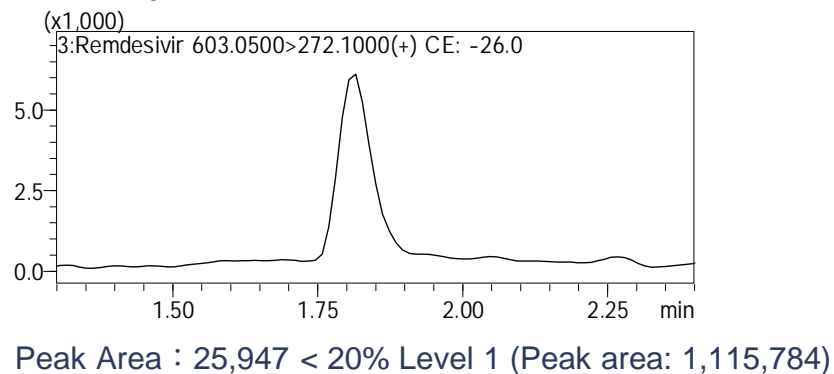
<Remdesivir>

Level 5: 5,000 ng/mL in plasma



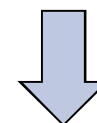
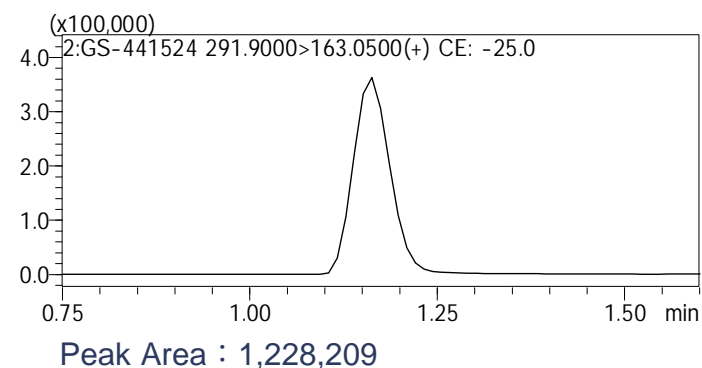
Next Injection

Level 0: plasma



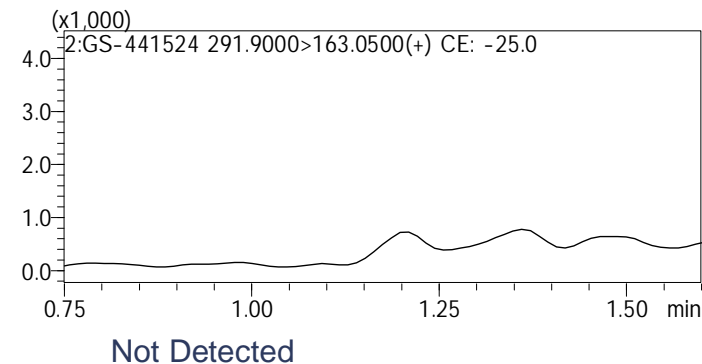
<GS-441524>

Level 5: 250 ng/mL in plasma



Next Injection

Level 0: plasma



Notice

Validation test was conducted 2 days after making plasma samples (LLOQ, Low, Medium, High)

Remdesivir*1

n=6

ID	Area	Area %RSD	Area Ratio	Area Ratio %RSD	ISTD Area	ISTD Area %RSD	Spiked Conc (ng/mL)	Measured Conc (ng/mL)	Accuracy %
LLOQ	657,307	4.4	0.107596	11.2	6,164,397	10.7	100	48.8	48.8
Low	4,405,767	1.6	0.677426	2.5	6,505,409	1.6	750	409	54.5
Medium	5,307,559	0.9	0.816156	1.8	6,504,641	1.8	1000	497	49.7
High	11,405,409	1.9	1.76408	2.0	6,466,304	1.8	3750	1096	29.2

GS-441524*2

n=6

ID	Area	Area %RSD	Area Ratio	Area Ratio %RSD	ISTD Area	ISTD Area %RSD	Spiked Conc (ng/mL)	Measured Conc (ng/mL)	Accuracy %
LLOQ	25,045	11.3	0.0955224	6.8	261,615	6.0	5	4.7	94.3
Low	178,435	1.6	0.665898	2.1	268,008	1.5	37.5	36.1	96.3
Medium	220,653	2.0	0.834107	3.2	264,674	2.5	50	45.3	90.6
High	825,206	1.7	3.22779	2.7	255,721	1.2	187.5	177	94.4

*1: 100 ng/mL for LLOQ, 750 ng/mL for Low, 1000 ng/mL for Medium, 3750 ng/mL for High

*2: 5 ng/mL for LLOQ, 37.5 ng/mL for Low, 50 ng/mL for Medium, 187.5 ng/mL for High

The reason why is that each plasma samples had been stored at 2°C refrigerator
Remdesivir in plasma sample might break down storing at such temperature

Summary

- **Calibration Curve:** *Sensitivity was cleared*

[Manual sample prep + LCMS-8060]

Remdesivir (100 ng/mL – 5000 ng/mL): R2=0.9992 , GS-441524 (5 ng/mL – 250 ng/mL): R2=0.9993

[CLAM-2030 + LCMS-8060]

Remdesivir (100 ng/mL – 5000 ng/mL): R2=0.9986 , GS-441524 (5 ng/mL – 250 ng/mL): R2=0.9997

- **Intra-day Analysis:** *Quantification in a day was cleared*

For Remdesivir and GS-441524, the intra-day precisions were 0.9 – 2.0% and 2.3 - 3.6%, and accuracies ranged 90.5 - 106% and 88.5 – 91.6%, respectively

- **Inter-day Analysis:** *Quantification between 3 days was cleared*

For Remdesivir and GS-441524, the inter-day precisions were 0.1-7.2% and 0.4-7.8%, and accuracies ranged 82.2-107% and 86.7-92.8%, respectively.

- **Stability Test:** *Sufficient robustness*

Medium QC sample was injected 100 times between 2 days

Remdesivir RSD = 3.4%, [¹³C₆]-Remdesivir RSD = 2.2%, GS-441524 RSD = 3.9%, [¹³C₅]-GS-441524 RSD = 3.7%

- **Recovery Test:** *Sufficient recovery rate*

There are no significant matrix effect and recovery rate for Remdesivir and GS-441524

- **Carryover Test:** *Acceptable carryover*

Blank sample was tested after measurement of level 5 calibration point.

Remdesivir: Peak area obtained from blank was under 20% of level 1, GS-441524: N.D. from blank

