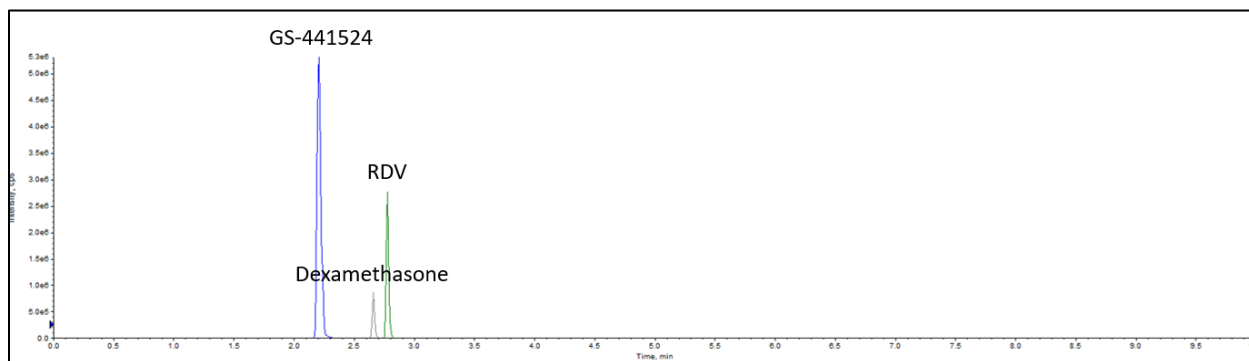


## Remdesivir and dexamethasone method validation brief

We developed a 10-minute method to quantify Remdesivir (RDV, GS-5734), its primary metabolite GS-441524, and dexamethasone in serum using LC-MS/MS, using Dapivirine-d11 as the internal standard. We validated the method using a 13-point calibration curve and three quality control (QC) levels spanning the low, mid, and high ends of the linear range.



### 1. Range and linearity (Passing criteria for linearity: $R^2 \geq 0.95$ )

The method uses 50 $\mu$ L of serum which is diluted 15-fold during the extraction. This means that there is a 15-fold difference between the instrument range and the method range. Both are displayed below for clarity (all concentrations in ng/mL).

Instrument range:

QC	RDV	GS-441524	Dexamethasone
LOD	0.0025	0.025	0.25
LLOQ	0.0025	0.025	0.25
ULOQ	9	90	90

Method range:

QC	RDV	GS-441524	Dexamethasone
LOD	0.0375	0.375	3.75
LLOQ	0.0375	0.375	3.75
ULOQ	135	1350	1350

The mean  $R^2$  for RDV, GS-441524, and dexamethasone across three runs was 0.997, 0.998, and 0.997 respectively.

### 2. Precision and accuracy (Passing Criteria: Precision $\leq 15\%$ CV; Accuracy $\leq 15\%$ Rel. Error)

Precision of the three QC levels was less than 5% CV for all three analytes when analyzing precision between three runs and within each individual run. Relative error was less than 8% for all three analytes between three runs and within each individual run.

		Within-run			Between-run		
		Low	Mid	High	Low	Mid	High
RDV	Precision (CV)	5.57%	3.51%	2.29%	4.11%	2.92%	1.86%
	Accuracy(RE)	4.40%	5.22%	7.29%	4.51%	6.99%	5.32%
GS-441524	Precision (CV)	5.95%	3.62%	2.87%	3.89%	2.14%	1.82%
	Accuracy(RE)	7.12%	6.59%	7.17%	3.02%	4.62%	7.72%
DXM	Precision (CV)	4.84%	2.06%	1.42%	4.09%	2.14%	2.42%
	Accuracy(RE)	1.96%	0.07%	6.94%	3.51%	4.53%	7.95%

### 3. Matrix Effect (Passing Criteria: Precision of Matrix Effect $\leq 15\%CV$ )

The matrix strongly enhanced RDV signal. For GS-441524 and dexamethasone, the matrix weakly suppressed signal. The precision of matrix effect for all QC levels of the three analytes are within 12% CV.

	RDV	CV	GS-441524	CV	DXM	CV
Matrix effect LQC	601.69%	11.28%	2.06%	3.38%	14.74%	11.73%
Matrix effect MQC	787.48%	9.46%	-2.23%	2.49%	-10.60%	3.29%
Matrix effect HQC	634.46%	8.14%	-2.41%	2.44%	-15.78%	3.92%
Matrix effect mean	674.54%	9.63%	-0.86%	2.77%	-3.88%	6.31%

### 4. Recovery (Passing Criteria: Precision of Recovery $\leq 15\%CV$ )

Mean recovery of all three analytes was greater than 85%. The precision of recovery for all QC levels of the three analytes are within 8% CV.

	RDV	CV	GS-441524	CV	DXM	CV
Recovery LQC	86.69%	5.95%	85.32%	4.73%	88.38%	7.53%
Recovery MQC	87.06%	4.72%	92.72%	2.88%	86.38%	2.16%
Recovery HQC	85.64%	1.90%	88.91%	2.01%	82.11%	2.77%
Recovery Mean	86.46%	4.19%	88.98%	3.21%	85.62%	4.15%

### 5. Injection Repeatability (Passing Criteria: Repeatability $\leq 15\%CV$ )

The CV for five injections at each QC level was  $< 4\%$  for all three analytes. The table below shows the CV at each QC level.

QC	RDV	GS-441524	Dexamethasone
LQC	1.4%	2.5%	3.7%
MQC	1.8%	2.0%	1.0%
HQC	2.0%	3.0%	2.6%

### 6. Carryover

No signal above the threshold signal-to-noise ratio of three was detected in any of the matrix blank samples for GS-441524 or dexamethasone following injection of the high QC. RDV signal increased following injection of the high QC but returned to baseline after one matrix blank injection.

### 7. Specificity

No interfering signal for RDV, GS-441524, nor dexamethasone was detected after running 20 other common cold, flu, and antiviral drugs.

Group	Drug	Low (ng/mL)	High (ng/mL)
<b>A</b>	Dextromethorphan	10	50
	Tenofovir	10	50
	Codeine	10	50
	Diphenhydramine	10	50
	Dolutegravir	10	50
	Ibuprofen	10000	50000
	Aspirin	10000	50000
<b>B</b>	Cortisone	100	500
	Rilpivirine	100	500
	Prednisone	100	500
	Emtricitabine	100	500
	Prednisolone	100	500
	Pseudoephedrine	100	500
<b>C</b>	Cortisol	1000	5000
	HCQ	1000	5000
	Cabotegravir	1000	5000
	Darunavir	1000	5000
	Efavirenz	1000	5000
	Dapivirine	1000	5000
	Acetaminophen	1000	5000