APPLICATION NOTE

Measurement of Remdesivir & Its Metabolite GS-441524 in Plasma Using the QSight® 200 Series LC-MS/MS

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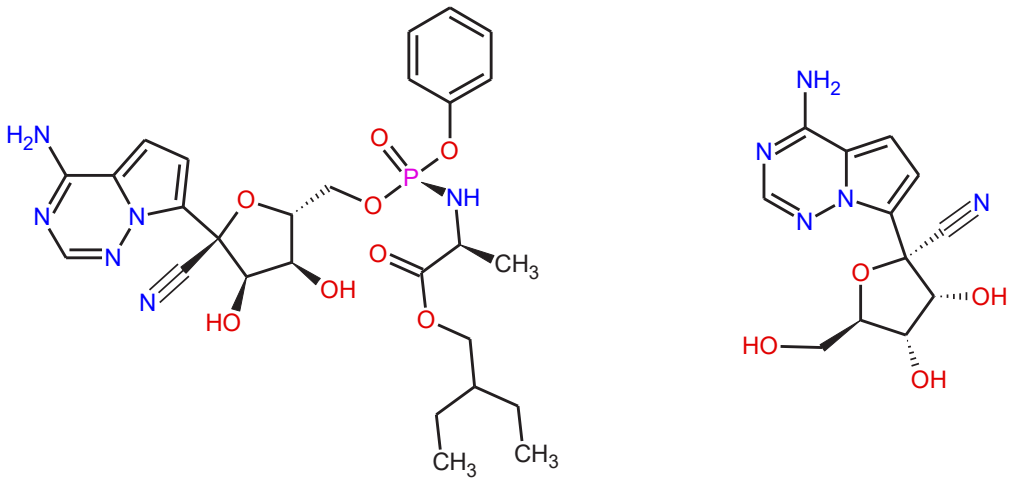
Introduction

Remdesivir (RDV) is a broad-spectrum antiviral medication that can be potentially used for the treatment on several viral diseases[1-2], [5-7] and is a prodrug of an adenine nucleotide analogue. Its core contains the active metabolite GS-441524, which interferes with RNA-dependent polymerases and inhibits viral RNA synthesis[4]. In non-humans primates, following IV administration, remdesivir is rapidly distributed into peripheral blood mononuclear cells (PBMCs) and converted within 2h to the active form, while GS-441524 is detectable in plasma for up to 24h[4]. In order to study their pharmacokinetic and pharmacodynamic profiles, a fast and precise analytical method based on LC-MS/MS is needed.

We report here an ultra-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method validated according to FDA and EMA guidelines [8, 9], for both remdesivir and GS-441524 determination, using the QSight® 200 series mass spectrometer.



(a) (b)

Figure 1. *Chemical structure of a) remdesivir and b) its active form GS-441524*

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Experimental

Chemicals & Materials

HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from VWR Chemicals (Radnor, PA, USA); MS-grade H2O (MilliQ) was produced with a Milli-DI system coupled with a Synergy 185 system by Millipore (Milan, Italy); DMSO and 6,7-dimethyl-2,3-di(2-pyridyl) quinoxaline [QX; purity 98.5%, used as internal standard (IS)] were purchased from Sigma–Aldrich Corporation (Milan, Italy). Blank plasma from healthy donors was supplied by the Blood Bank of Citta` della Salute e della Scienza of Turin (Italy). Remdesivir (purity 98.3%) and its metabolite GS-441524 (purity 98%) were kindly donated by CoQua Lab (Turin, Italy). All powders were stored at -20° C in the dark, in order to prevent any possible degradation.

Sample Preparation

The extraction procedure consisted of a rapid protein precipitation. 100 uL of internal standard (IS) QX solution (100 ng/mL) in H2O:MeOH (70:30) and 600 uL of a precipitation solution consisting in MeOH:ACN (50:50) were added to 50 uL of a plasma sample. After being vortexed for 30s, samples underwent centrifugation (21,000 g for 10 min at 4°C). 300 uL of the supernatant was diluted with 600 uL of water, mixed, and 8 uL were injected into the LC-MS/MS system.

Mass Spectrometry Conditions

The LC-MS/MS analysis was performed using a QSight® 220 mass spectrometer coupled with LX-50 UHPLC, with analysis done in ESI positive ion mode. Table 1 outlines the MS instrumental source parameter settings. The optimized MRM transition parameters for analytes involved in this assay are shown in Table 2.

Table 1. *MS source conditions (AU = arbitrary units)*

ESI Voltage (V)	5000
HSID Temp (°C)	270
Nebulizer Gas Setting (AU)	350
Drying Gas Setting (AU)	130
Source Temp. (°C)	350

Table 2. *Compound-dependent parameters for MRMs monitored (EV = entrance voltage, CC = collision energy, CCL2 = Collision cell lens 2).*

Compound	Precursor (m/z)	Fragment (m/z)	CCL2	CC	EV
Remdesivir quant	603.1	200.0	-116	-53	15
Remdesivir qual	603.1	318.0	-104	-28	12
GS-441524 quant	292.0	163.0	-64	-32	43
GS-441524 qual	292.0	147.0	-80	-50	2
QX (IS) quant	313.2	78.0	-80	-50	30
QX (IS) qual	313.2	246.2	-80	-50	30

LC conditions

The LC separation was performed using a C18 reverse phase (50 x 2.1 mm, 1,8 um) column, in-line with a filter precolumn. The chromatographic separation was achieved with a 4 minute gradient (Table 3) with conditions reported below:

- Mobile Phases: A water with 0.05% formic acid
  B acetonitrile with 0.05% formic acid
- Flow rate: 400 µL/min
- Injection volume: 8 µL
- Column temperature: 40°C

Table 3. *LC gradient*

Time	Flow Rate (µL/min)	%B
0.00	400	5
0.30	400	5
0.35	400	30
1.50	400	70
1.80	400	90
2.80	400	90
2.90	400	5
4.00	400	5

Results & Discussion

Specificity & Selectivity

Specificity and selectivity were evaluated using six individual sources of the blank plasma matrix, individually analysed and evaluated for interference. Mean retention times for the considered analytes were 0.98 min for GS-441524, 1.67 min for remdesivir (RDV) and 1.72 min for QX, the IS (Figure 2). No interferences were observed, as demonstrated in Figure 3, where the lowest calibrator point was compared with a blank plasma. Also, the extent of any interference caused by possible co-administered medications was investigated: briefly, an aliquot of blank plasma was spiked with fourteen antiretroviral drugs currently used for the treatment of HIV (amprenavir, atazanavir, cobicistat, darunavir, dolutegravir, efavirenz, elvitegravir, etravirine, lopinavir, maraviroc, nevirapine, raltegravir, rilpivirine and ritonavir) and analysed [8]. The absence of detectable interfering peaks at the analyte retention times with the monitored MRMs was considered as lack of interference (Figure 4).

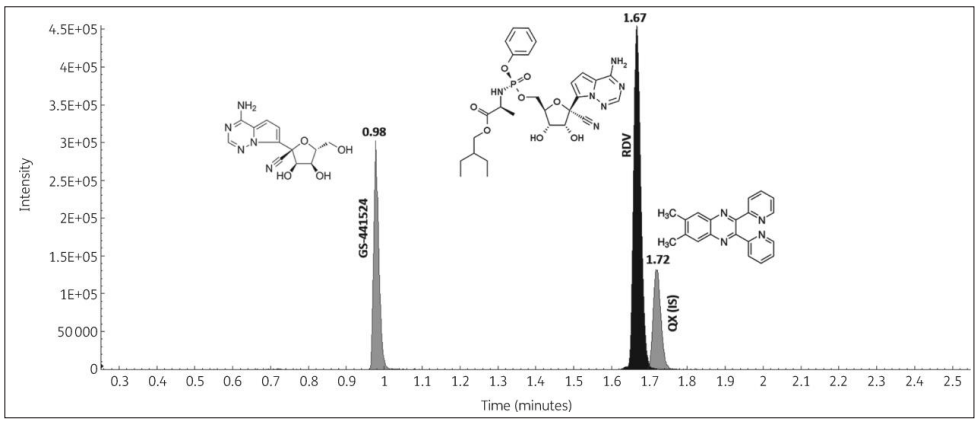

Figure 2. *Spiked plasma sample with RDV, GS-441524 and QX (IS).*

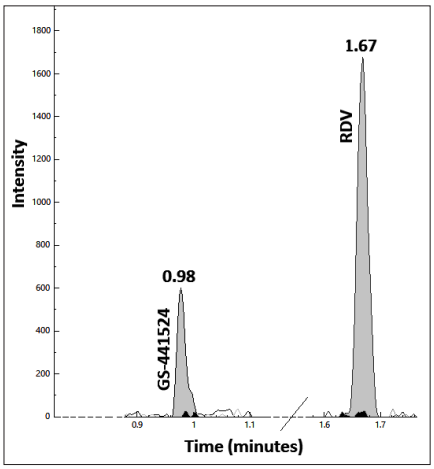

Figure 3. *overlaid chromatograms comparing the signal of lowest calibrator point (in gray) vs the signal of blank plasma sample (in white)*

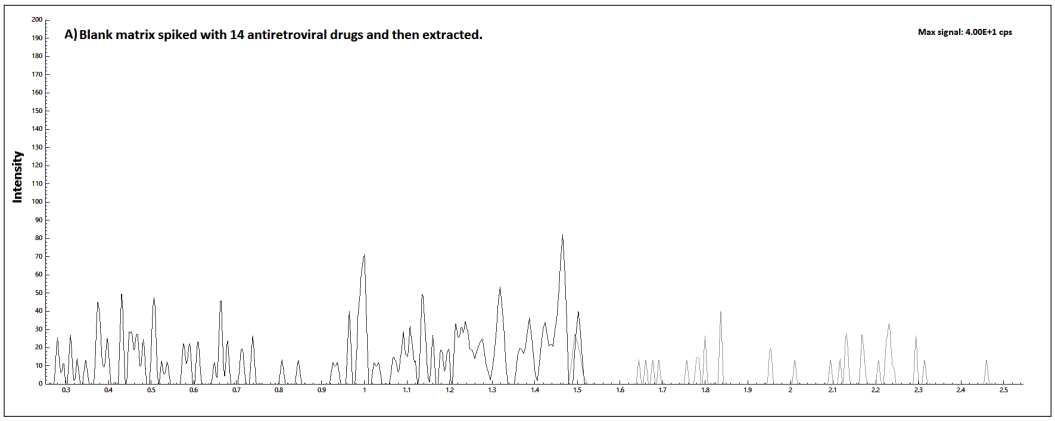

Figure 4. *MRM chromatograms of blank plasma spiked with fourteen antiretroviral drugs*

Accuracy, Precision, ULOQs, LLOQs, LODs & Linearity

Six interday validation sessions were performed, as stipulated by FDA and EMA guidelines[8,9]. Accuracy and interday imprecision were evaluated, performing quantification of the three different QC samples in duplicate during each validation session. Intraday imprecision was evaluated using five replicates. Interday and intraday imprecision were expressed as the relative standard deviation (RSD) at each QC concentration. Integration was performed, considering peak areas for each analyte. Accuracy and imprecision results are summarized in Table 4, and satisfied the FDA and EMA guidelines[8, 9].

The lower limit of quantification (LLOQ) for each analyte was the lowest concentration of analyte in a sample that could be quantified reliably, with a deviation from the nominal concentration (measure of accuracy) and RSD (measure of precision) lower than 20% and with a signal-to-noise ratio higher than five[8]. The limit of detection (LOD) was considered as the lowest dilution of LLOQ that yielded a signal-to-noise ratio higher than three[8, 9]. The ULOQ for both remdesivir and GS-441524 was determined to be 1000 ng/mL. The LLOQ value for both the analytes was 0.98 ng/mL while the LOD values were 0.24 ng/mL for remdesivir and 0.98 ng/mL for GS-441524. For both analytes the 'linear through zero' regression models, with a 1/x weighting factor, showed a good fit with R2 >0.998, in the assayed range.

Recovery (REC), Extraction Efficiency (EE) & Matrix Effect (ME)

REC was evaluated during six validation sessions at high, medium and low concentrations by comparing peak areas from extracted QCs (pre-spiked) with those obtained by the direct injection of a chemical mix containing both the drugs and the IS at the same concentrations as the QCs[9].

The EE was measured by comparing the areas of peaks of pre- and post-spiked samples. Separate plasma samples from six healthy donors were used for the preparation of STDs and for the evaluation of ME. The ME was calculated by comparing the signal from the analysis of post-extraction spiked samples (post-spiked) at high, medium and low QC levels with those from direct injection of the same concentration of analytes without matrix, as described by Taylor 11 and in FDA guidelines (post-extraction addition method)[9]. All these parameters satisfied the FDA and EMA guidelines and are detailed in Table 4.

Table 4. *Overview of method validation parameters for remdesivir and GS-441524 (RSD, relative standard deviation; REC, recovery; EE, extraction efficiency; ME, matrix effect). Low QC level concentration: (10 ng/ml); medium QC level concentration: 100 ng/ml; high QC level concentrations: (800 ng/ml).*

		Accuracy %	Intraday	Interday	Mean ME, % (RSD, %)	Mean REC % (RSD, %)	Mean EE, % (RSD, %)
		Imprecision (RSD, %)					
Remdevisir	High QC Level	104	2	6	2 (2)	67 (6)	66 (7)
	Medium QC Level	100	1	6	-1 (3)	67 (8)	67 (11)
	Low QC Level	87	5	6	16 (7)	78 (4)	67 (9)
	LLOQ	118	10	12			
	Mean (RSD)	102	4.5	7.5	6 (4)	71 (6)	67 (9)
GS-441524	High QC Level	96	2	3	5 (4)	104 (6)	99 (5)
	Medium QC Level	102	6	4	-3 (21)	99 (5)	105 (17)
	Low QC Level	92	9	11	-7 10)	104 (10)	112 (9)
	LLOQ	81	9	14			
	Mean (RSD)	93	6	8	-2 (12)	102 (7)	105 (10)

Conclusions

The QSight® 200 series UHPLC-MS/MS system exhibited excellent sensitivity and accurate determination of remdesivir and GS-441524 in human plasma samples. This method can represent a useful tool for pharmacokinetic and pharmacodynamic studies of remdevisir.

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