Application Note

Therapeutic Drug Monitoring of COVID-19 Drugs with Components of a Commercial Assay

Introduction

Researchers are working furiously to test a variety of potential drugs to find a suitable treatment for COVID-19, the disease caused by SARS-CoV-2. Dose tolerance and dose response exploration studies are usually required that demands for a great analysis. Pharmacokinetic and pharmacodynamic studies are also often involved in these phases. But also later, should one of the drugs be approved, TDM may be a requirement.

To help laboratories with this challenge, we describe a protocol* that allows the determination of 9 COVID-19 drug candidates and metabolites in a short run time on the basis of ***MassTox***® TDM Series A, a modular system that allows the determination of more than 200 drugs.

Materials and Methods

Sample Prep

The sample preparation was performed in line with the MassTox Series A protocol (Chromsystems). In brief: 50 µl of sample, reconstituted 3PLUS1® calibrator (order no. 92055) or ***MassCheck***® control (order no. 0268, 0269), containing 9 drugs and metabolites (see table 1), was pipetted into a 1.5 ml reaction vial. 25 µl of Extraction Buffer (order no. 92005) was added and briefly mixed. 250 µl of a mixture (Internal Standard Mix Anti-HIV drugs, order no. 92844, and Precipitation Reagent, order no. 92012, at a 1:16 ratio), was added and vortexed for 30 s and centrifuged for 5 min at 15000 g. The supernatant was then diluted, depending on sensitivity of the LC-MS/MS system, with Dilution Buffer I (92007; ratio 1:5) or Dilution Buffer II (92008; ratio 1:20). The substances were separated on MasterColumn® A (order no. 92110) according to the protocol below and detected with electrospray ionisation in positive ion mode with a Sciex 6500 Citrine mass spectrometer. MRM transitions and internal standard assignments are found in table 3.

LC-MS/MS-Parameter

Injection volume: *10-20 µl*
Column and autosampler temperature: *ambient*
Flow rate: *0.6 ml/min*
Gradient: *binary*
Column: *MasterColumn A® (order. no 92110)*
Analysis time: *3.5 min*.

Table 1: The Drugs of COVID-19 Calibrator

GS 5734/Remdesivir	Ritonavir	Hydroxychloroquine
GS 441524 *(active metabolite of Remdesivir)*	Favipiravir	Nafamostat
Lopinavir	Chloroquine	Azithromycin

Table 2: Binary gradient conditions

Time	Mobile Phase A order no. 92001	Mobile Phase B order no. 92002
0.00 min	100%	0
0.50 min	100%	0
0.51 min	50%	50%
1.50 min	50%	50%
1.51 min	0	100%
2.50 min	0	100%
2.51 min	100%	0
3.50 min	100%	0

Table 3: MRMs and corresponding ISTD

Analyte	Corresponding ISTD MRM	MRM
Remdesivir	ISTD 2 (520/117)	603 → 402
GS 441524	ISTD 2 (520/117)	292 → 147
Lopinavir	ISTD 2 (520/117)	629 → 155
Ritonavir	ISTD 16 (637/163)	721 → 140
Favipiravir	ISTD 2 (520/117)	158 → 85
Chloroquine	ISTD 2 (520/117)	320 → 247
Hydroxychloroquine	ISTD 2 (520/117)	336 → 158
Nafamostat	ISTD 2 (520/117)	348 → 162
Azithromycin	ISTD 12 (727/146)	749 → 158

**For research-use only and not for diagnostic purposes.*

Results

The lower limits of quantification for COVID-19 drugs were between 1.7 and 418 µg/l, upper limits of quantification were in a range of 400 to 40000 µg/l (see table 5). Interassay and intraassay precision were determined between 1 and 7 % (see table 4). All drugs could be analysed with a short run time of 3.5 min (fig.1).

Table 4: Inter- and intra assay data at different concentrations of COVID-19 drugs

	Level I, 0268			Level II, 0269		
Analyte	**Conc [µg/l]**	**CV [%] Interassay**	**CV [%] Intraassay**	**Conc [µg/l]**	**CV [%] Interassay**	**CV [%] Intraassay**
Remdesivir	699	2%	3%	1987	3%	4%
GS 441524	35,5	2%	3%	70	4%	5%
Lopinavir	1083	1%	2%	4928	2%	3%
Ritonavir	779	1%	1%	4887	3%	3%
Favipiravir	2612	2%	4%	7872	4%	5%
Chloroquine	113	5%	7%	340	6%	7%
Hydroxychloroquine	464	5%	5%	922	5%	6%
Nafamostat	46.6	5%	5%	159	3%	4%
Azithromycin	158	3%	3%	361	2%	2%

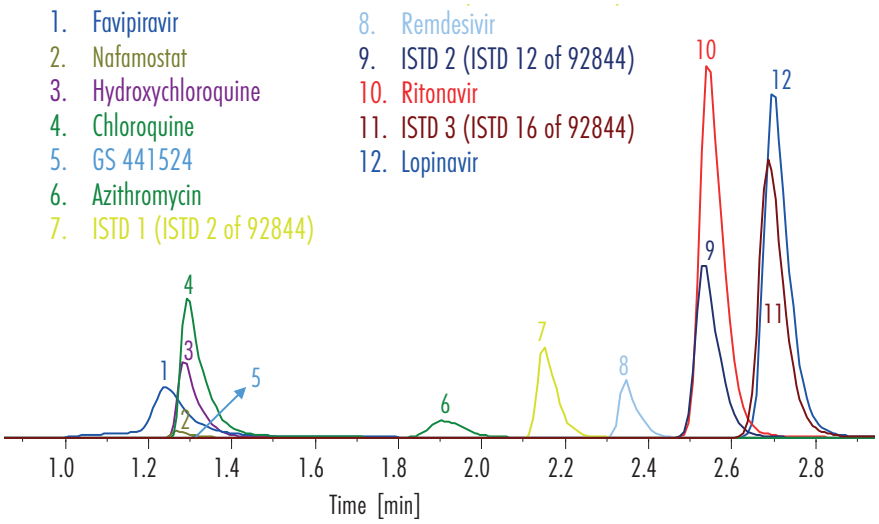

Figure 1: Chromatogram of COVID-19 drugs

Table 5: Upper and lower limit of quantification

Analyte	**LLOQ [µg/l]**	**ULOQ [µg/l]**
Remdesivir	20,8	12000
GS 441524	11,8	400
Lopinavir	104	24000
Ritonavir	12.5	24000
Favipiravir	418	40000
Chloroquine	4.97	2000
Hydroxychloroquine	251	4500
Nafamostat	1.72	1000
Azithromycin	11,20	2000

Ordering Information

Product	**Order No.**
3PLUS1® Multilevel Calibrator Set COVID-19 Drugs	92055
MassCheck® controls COVID-19 Drugs	0268, 0269
Extraction Buffer	92005
Internal Standard Mix Anti-HIV Drugs	92844
Precipitation Reagent	92012
Dilution Buffer I/II	92007/92008
MasterColumn® A	92110
Mobile Phase A	92001
Mobile Phase B	92001

Conclusion

We demonstrate with this protocol that ***MassTox***® TDM Series A can be employed for the determination of 9 COVID-19 drug candidates/metabolites in serum/plasma. Laboratories that have already ***MassTox***® TDM Series A in operation can simply use the same chromatographic setup, sample prep and MasterColumn® A. All you need are a few additional components from ***MassTox***® TDM Series A.

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