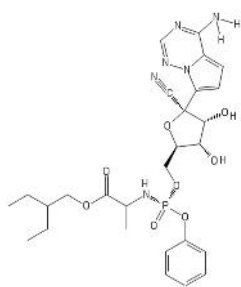


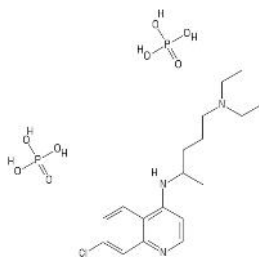
# Rapid LC-MS-MS Method for the Simultaneous Determination of Potential Medicines for COVID-19 with Endeavosil<sup>®</sup> C18-PFP Column

## Introduction

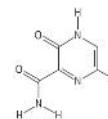
The emergence of SARS-CoV-2 at the end of 2019 and its rapid spread throughout world has created need for drugs to treat COVID-19. This is of particular importance given the high transmissibility of the virus as well as current unavailability of any effective vaccine. Recently, Remdesivir, Chloroquine Phosphate and Favipiravir have been proposed to demonstrate potential activity against this novel coronavirus. Therefore, in this article, Dikma technicians developed a rapid LC-MS-MS method to detect several potential drugs by using an UHPLC column packed with novel stationary phase C18-PFP. Six related drugs were assayed simultaneously and the total run time was 4 min. This method is simple and rapid which will make the detection of these potential drugs convenient and efficient and may contribute to additional studies for treatment of COVID-19.



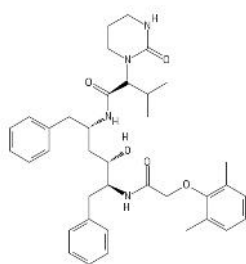
Remdesivir



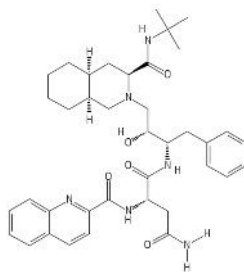
Chloroquine Phosphate



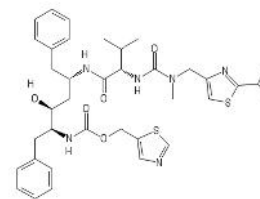
Favipiravir



Lopinavir



Saquinavir



Ritonavir

Fig.1 Molecular structures of 6 compounds

## Experimental

Column: Endeavorsil® 1.8  $\mu\text{m}$  C18-PFP, 100  $\times$  2.1 mm

LC System: Agilent 1290 Infinity UPLC and AB SCIEX 4000+ MS

Mobile Phase: A: 0.1% Formic acid in water

B: 0.1% Formic acid in acetonitrile

Gradient: 10% B to 80% B in 1.5 min, 80 to 90% B in 4.5 min, hold 0.5 min, 90 to 10% B in 0.5 min, equilibrate at 10% B for 3 min

Scan mode: ESI+; Flow Rate: 0.2 mL/min; Injection: 1  $\mu\text{L}$ ; Temperature: 30  $^{\circ}\text{C}$

Samples: Except Chloroquine Phosphate which was dissolved in mixed solution of methanol and water (1:1 v/v), other standard stock solutions were made by methanol, then a standard mixture of 6 analytes was prepared with each standard stock solution of 1 mg/mL. The concentration of each analyte in the mixed standard solution is listed in Table 1.

## Results

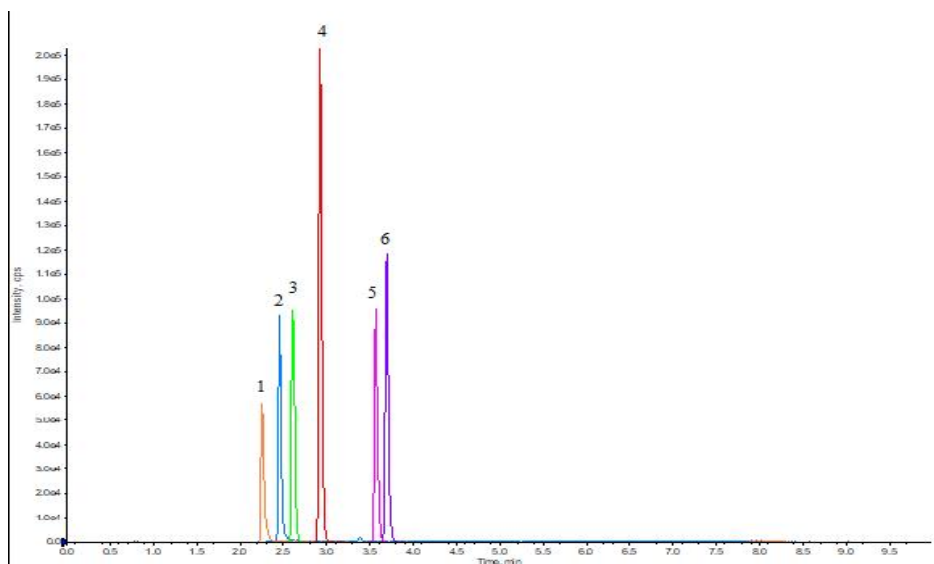


Fig.2 TIC of 6 compound mixture solution

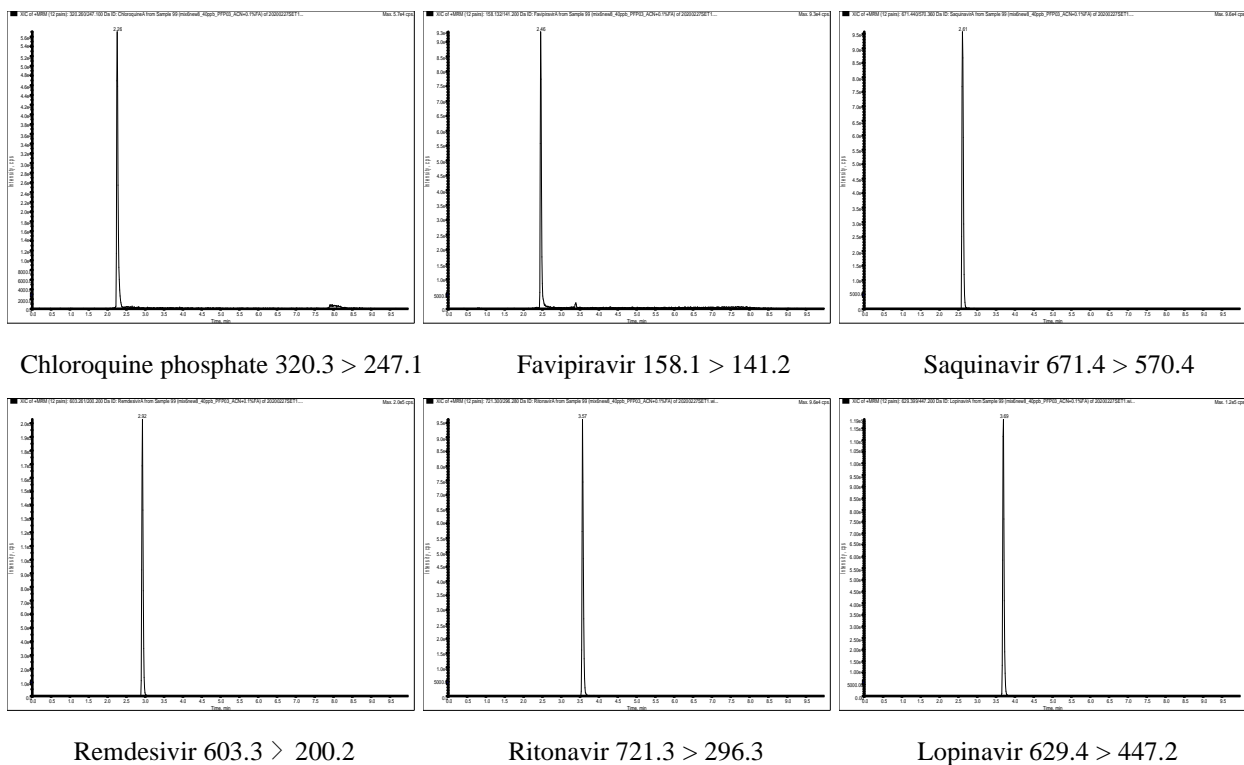


Fig.3 MRM Chromatogram of 6 chemicals standard solution

Table 1 Summary of 6 compound detection parameters and results

No.	Compounds	Concentration (µg/mL)	Retention Time(min)	MS→MS <sub>1</sub> Ions (Precursor→Product 1)	MS→MS <sub>2</sub> Ions (Precursor→Product 2)
1	Chloroquine phosphate	0.008	2.26	320.3 → 247.1	320.3 → 142.3
2	Favipiravir	0.2	2.45	158.1 → 141.2	158.1 → 113.0
3	Saquinavir	0.06	2.63	671.4 → 570.4	671.4 → 128.3
4	Remdesivir	0.02	2.92	603.3 → 200.2	603.3 → 402.2
5	Ritonavir	0.04	3.55	721.3 → 296.3	721.3 → 140.3
6	Lopinavir	0.02	3.69	629.4 → 447.2	629.4 → 155.3

Six drugs were separated on an Endeavorsil<sup>®</sup> C18-PFP column within 4 minutes (Fig. 2). A listing of elution order, concentration, retention time, MS transitions is shown in Table 1.

## Conclusions

The proposed method offers efficient and simultaneous determination of six promising drugs for treatment of COVID-19. Other advantages of this method include a simple mobile phase composition, a relatively short run time of 4 min, good peak shape and high sensitivity. To the best of our knowledge, this is the first reported LC-MS-MS method that can simultaneously separate, verify, and test these six compounds. Hence, we can hope that it can be a useful tool for routine testing in production, research and clinical trial drug development phases of these drugs.