

# APPLICATIONS

## Determination of Chloroquine, Hydroxychloroquine and its Metabolite Desethyl Hydroxychloroquine in Plasma Samples by LC-MS/MS on a Kinetex<sup>®</sup> 2.6 µm F5 100 x 2.1 mm Core-Shell LC Column

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### Introduction

The World Health Organization (WHO) has initiated together with the local Health Ministries of many countries clinical studies to evaluate possible treatments for COVID-19. Two of the drugs which are being tested are Chloroquine (CQ) and Hydroxychloroquine (HCQ).

This application note describes the analysis of these two drugs together with the metabolite Desethyl Hydroxychloroquine (DHCQ) from various biological matrices. The method was developed by Dr. Thomas Lanot from the Institut Fédératif de Biologie – CHU de Toulouse in France. The isocratic method allows for a fast and reliable quantification with a LOQ of 10 µg/L.

### Materials and Method

#### Internal Standard Stock Solutions (ISSSs)

ISSS1: D5-Hydroxychloroquine (HCQ-D5) 1 mg in 654 µL water  
 ISSS2: D4-Desethyl Hydroxychloroquine (DHCQ-D4) 1 mg in 775 µL water  
 ISSS3: D5-Chloroquine (CQ-D5) 1 mg in 781 µL water

#### Internal Working Standard (IWS)

10 µL ISSS1 + 10 µL ISSS2 + 10 µL ISSS3 + 970 µL methanol

#### Precipitation Solvent

400 µL IWS diluted to 200 mL methanol

#### Sample Preparation

1. Add 50 µL Heparin blood to an Eppendorf tube. Add 150 µL precipitation solvent, then vortex for 30 seconds immediately. Settle for 5 minutes at room temperature, then vortex for 15 seconds and then centrifuge at 10,900 rpm.
2. Put 400 µL mobile phase A into a vial and add 100 µL of the supernatant. Vortex for 15 seconds. Inject 10 µL of this solution.

**TIP:** [Impact<sup>™</sup> Protein Precipitation Plates](#) are recommended as a high through-put sample clean-up option.

#### HPLC Parameters

**Column:** Kinetex 2.6 µm F5  
**Dimensions:** 100 x 2.1 mm  
**Part Number:** [00D-4723-AN](#)  
**Mobile Phase:** A: 0.2% Formic acid in water  
 B: 0.1% Formic acid in acetonitrile (A:B, 89:11)  
**Flow Rate:** 0.3 mL/min  
**Temperature:** 40 °C  
**Detection:** Shimadzu<sup>®</sup> LCMS-8060  
**Injection:** 10 µL  
**Run Time:** 3 min

**Table 1. MS Parameters**

Source Parameter	Setting
Nebulizing Gas	3 L/min
Heating Gas	10 L/min
Interface Temperature	300 °C
DL Temperature	250 °C
Heating Block Temperature	400 °C
Drying Gas Flow	10 L/min
Event Time	0.099 s

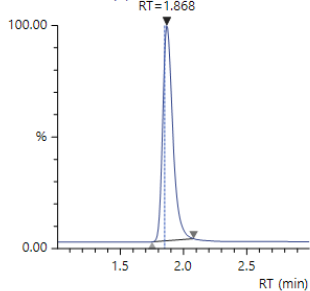
**Table 2. Identification of Analytes**

Analyte	Retention Time (min)	Parent Ion Q1	Fragment Ion Q3	Q1 Pre Bias (V)	CE (V)	Q3 Pre Bias (V)
HCQ	1.86	336.1	179.2	-24	-40	-12
			191.2	-24	-39	-20
HCQ-D5	1.85	341.1	179.1	-24	-41	-12
			191.2	-24	-39	-13
DHCQ	1.58	308.1	179.2	-22	-29	-12
			247.3	-22	-15	-17
DHCQ-D4	1.57	312.1	179.2	-22	-31	-12
			247.3	-23	-27	-12
CQ	2.28	321.1	247.3	-12	-25	-12
			179.2	-23	-39	-18
CQ-D5	2.27	326.1	247.2	-23	-25	-17
			179.1	-23	-40	-18

**Chromatograms**

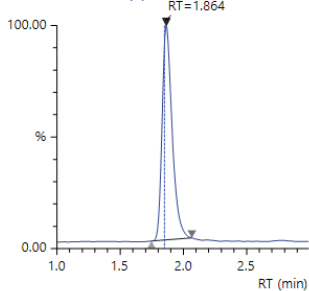
**Hydroxychloroquine**

Conc 0.537  
Area 12787421  
Q 336.10>179.20 (+)



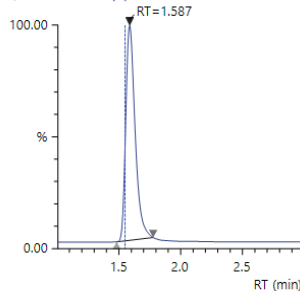
**Hydroxychloroquine D5**

Conc 1.000  
Area 972790  
ISTD 341.10>179.10 (+)



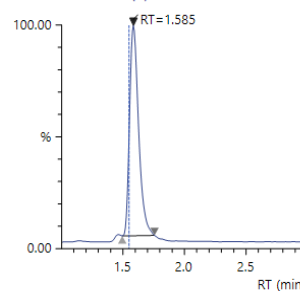
**Desethyl hydroxychloroquine**

Conc 0.524  
Area 15380465  
Q 308.10>179.15 (+)



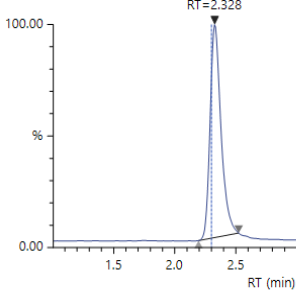
**Desethyl hydroxychloroquine D4**

Conc 1.000  
Area 1237106  
ISTD 312.10>179.15 (+)



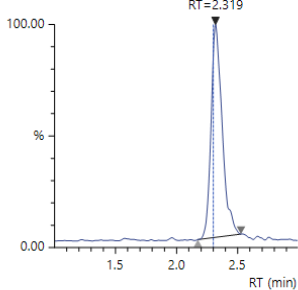
**Chloroquine**

Conc 0.530  
Area 1212680  
Q 321.10>247.25 (+)



**Chloroquine D5**

Conc 1.000  
Area 72281  
ISTD 326.10>247.15 (+)



**Conclusion**

The presented method allows for a fast and reliable quantitation of Chloroquine, Hydroxychloroquine and its metabolite Desethyl Hydroxychloroquine from blood samples.

The Kinetex F5 core-shell LC column used in the method has a very specific selectivity. This allows for the separation of the three aromatic compounds within 3 minutes under isocratic conditions, allowing for high-throughput analysis.

# APPLICATIONS

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