

APPLICATIONS

Determination of Chloroquine, Hydroxychloroquine and its Metabolite Desethyl Hydroxychloroquine in Plasma Samples by LC-MS/MS on a Kinetex® 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.

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® 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

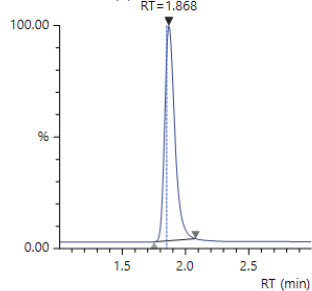
Table 2. Identification of Analytes

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

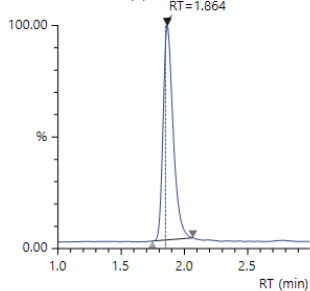
Hydroxychloroquine

Conc 0.537
Area 12787421
Q 336.10>179.20 (+)



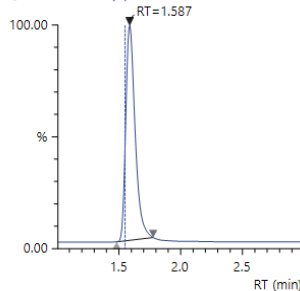
Hydroxychloroquine D5

Conc 1.000
Area 972790
ISTD 341.10>179.10 (+)



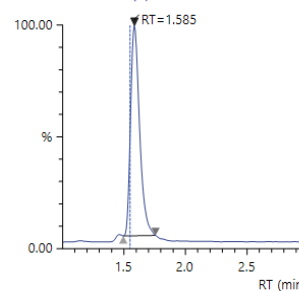
Desethyl hydroxychloroquine

Conc 0.524
Area 15380465
Q 308.10>179.15 (+)



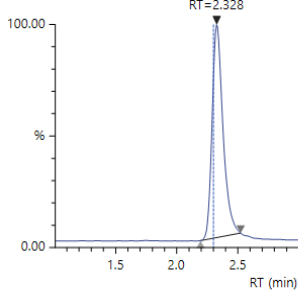
Desethyl hydroxychloroquine D4

Conc 1.000
Area 1237106
ISTD 312.10>179.15 (+)



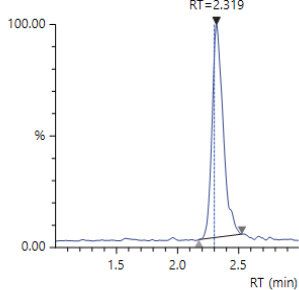
Chloroquine

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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