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

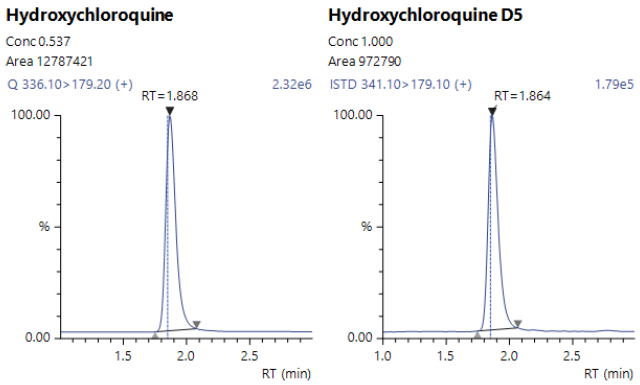
Table 1. MS Parameters

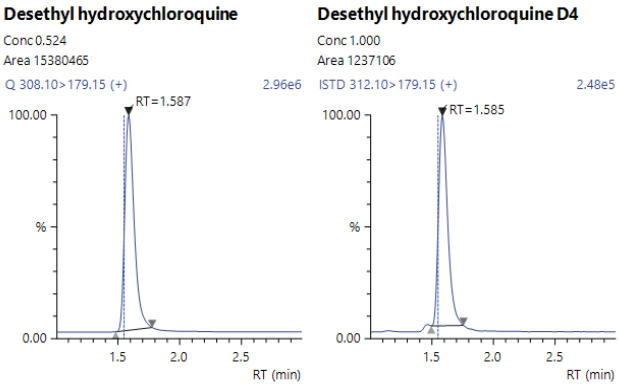
Source Parameter	Setting
Nebulizing Gas	3 L/min
Heating Gas	10 L/min
Interface Temperature	300 °C
DL Temperature	250 °C
Heating Block Temperature	400 °C
Drying Gas Flow	10 L/min
Event Time	0.099 s

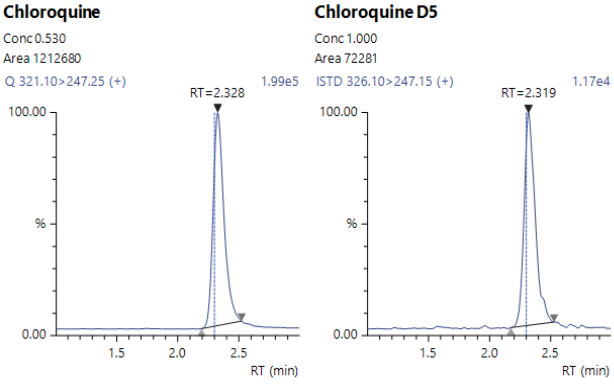
Table 2. Identification of Analytes

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







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.

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