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Hydroxychloroquine and Chloroquine Therapeutic Drug Monitoring in Blood at 8 Seconds per Sample Using Luxon-MS/MS

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Introduction

Chloroquine and hydroxychloroquine have displayed the ability to inhibit the replication of multiple coronaviruses *in vitro*. Publications support the hypothesis that both chloroquine and hydroxychloroquine could improve the clinical outcome of patients diagnosed with coronavirus disease 2019 (COVID-19). Hydroxychloroquine appears to be safer and more potent in inhibiting the causative virus, severe acute respiratory syndrome coronavirus 2 (SARSCoV-2), *in vitro*. The numerous molecular mechanisms by which they can achieve such results remain to be further explored. Early reports of its use *in vivo* have been promising. However, no randomized clinical trials have been conducted to inform clinical guidance on the use, dosing, and duration of chloroquine/hydroxychloroquine for prophylaxis or treatment of COVID-19. The potential unprescribed use of chloroquine and hydroxychloroquine is alarming, since it may lead to irreversible vision loss. Although it may appear safe at low doses, chloroquine and hydroxychloroquine retinopathy is most influenced by daily dose, length of use, and cumulative dose.
The kinetics of chloroquine metabolism are complex, as its half-life increases with increasing intake.

Factors associated with hydroxychloroquine toxicity include the following:

- Maintenance dose ≥ 5 mg/kg/day based on real weight (most critical risk)
- Daily dose ≥ 400 mg
- Cumulative ≥ 1,000 mg
- Age ≥ 60 years

Factors associated with chloroquine toxicity include the following:

- Maintenance dose ≥ 2.3 mg/kg/day
- Obesity

Factors associated with toxicity in both drugs include the following:

- Duration of treatment ≥ 5 years (critical factor)
- Use of Tamoxifen (5-fold risk)
- Evidence of renal insufficiency
- Underlying retinal disease or maculopathy (macular degeneration)
- Evidence of liver disease
- P450 polymorphisms leading to higher drug concentration in blood

Our goal for this application note is to present a Therapeutic Drug Monitoring method that allows the quantitation of Chloroquine and Hydroxychloroquine in blood in less than 8 seconds per sample. This high-throughput method enables numerous patients to be continuously monitored, thus preventing irreversible vision loss or impairing effects. Sample preparation is reduced to the minimum to keep up with the analysis throughput.

Luxon Ionization Source

The Luxon Ion Source® (**Figure 1**) is the second-generation sample introduction and ionization source based on the LDTD® technology for mass spectrometry. Luxon Ion Source® uses Fiber-Coupled Laser Diode (**Figure 2**) to obtain unmatchable thermal uniformity giving more precision, accuracy and speed. The process begins with dry samples which are rapidly evaporated using indirect heat. The thermally desorbed neutral molecules are carried into a corona discharge region. High-efficiency protonation and strong resistance to ionic suppression characterize this type of ionization and it is the result of the absence of solvent and mobile phase. This thermal desorption process yields high-intensity molecular ion signal in less than 1 second sample-to-sample and allows working with very small volumes.

Figure 1 - Luxon Ion Source®

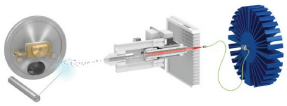

Figure 2 - Schematic of the Luxon ionization source

Sample Preparation Method

50 µL whole blood sample
60 µL internal standard Hydroxychloroquine-d4 at350 ng/mL in Water-Methanol (95:5)

- Vortex

500 µL Acetonitrile

- Vortex
- Centrifuge 14,000 rpm / 2min

Spot 3 µL upper-layer phase on a LazWell™96 plate

- Dry 2 minutes at room temperature with air flow

Table 1 shows the calibration range used for the quantification of both drugs.

Table 1 – Calibration range

Drug	Calibration range
Hydroxycholoroquine	20-2000 ng/mL
Chloroquine	20-2000 ng/mL

Luxon-MS/MS Parameters

Luxon

Model: Luxon SH-960, Phytronix
Carrier gas: 6 L/min (air)
Laser pattern:

- 6-second ramp to 65% power
- 2-second hold at 65% power

MS/MS

MS model: Shimadzu: LC-8060
Scan Time: 25 msec
Total run time: 8 seconds per sample
Ionization: APCI (positive)
Analysis Method: MRM mode

Table 2 – MRM transitions for Luxon-MS/MS

Drug	Transition	CE
Chloroquine, quantitative	320.2 → 247.1	-20
Chloroquine, confirmation	322.2 → 249.1	-20
HydroxyChloroquine, quantitative	336.2 → 247.1	-20
HydroxyChloroquine, confirmation	336.2 → 158.1	-20
HydroxyChloroquine-d4, IS	340.3 → 251.2	-20

Results and Discussion

Linearity

Blood is spiked with drugs to prepare QC (L-low, M-medium and H-high) and standards within the calibration range defined in **Table 1**. Standards are extracted and used to generate a calibration curve. Correlation values greater than 0.99 are obtained for all drugs. **Figure 3** shows calibration curve results for Chloroquine (a) and Hydroxychloroquine (b).

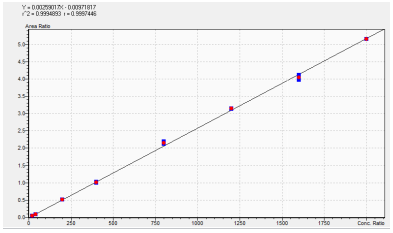

Figure 3a - Standard curve for Chloroquine

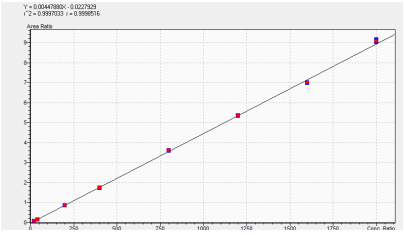

Figure 4b - Standard curve for Hydroxychloroquine

Accuracy and Precision

Calibration curves and QC are extracted and analyzed. For the intra-run precision and accuracy experiments, each fortified sample set is analyzed in six replicates. **Table 3** shows the intra-run results for Chloroquine and **Table 4** shows Hydroxychloroquine results. Each concentration does exceed 15% CV and the mean concentration are within ±15% of expected value. Similar results are obtained for the confirmation transition.

Table 3 - Intra-run precision and accuracy for Chloroquine

Chloroquine	LLOQ	QC-L	QC-M	QC-H	ULOQ
Exp. conc.(ng/mL)	20	60	600	1400	2000
Calc. conc (ng/mL)	19.5	56.6	578.5	1346.3	1987.6
N	6	6	6	6	6
%CV	4.3	3.7	1.1	3.2	1.8
%Nom	97.5	94.3	96.4	96.2	99.4

Table 4 - Intra-run precision and accuracy for Hydroxychloroquine

Hydroxychloroquine	LLOQ	QC-L	QC-M	QC-H	ULOQ
Exp. conc.(ng/mL)	20	60	600	1400	2000
Calc. conc (ng/mL)	21.5	56.9	587.5	1354.9	2022.3
N	6	6	6	6	6
%CV	1.6	1.3	1.0	3.1	0.8
%Nom	107.5	94.8	97.9	96.8	101.1

For the inter-run precision and accuracy experiment, each fortified sample set is analyzed in three different runs. **Table 5** shows the inter-run results for both Chloroquine and Hydroxychloroquine. Each concentration does not exceed 15% CV and the mean concentration is within ±15% of expected value. Similar results are obtained for the confirmation transition.

Table 5 - Inter-run precision and accuracy

	Chloroquine			Hydroxychloroquine		
	QC-L	QC-M	QC-H	QC-L	QC-M	QC-H
Exp. conc.(ng/mL)	60	600	1400	60	600	1400
Calc. conc (ng/mL)	57.4	589.8	1355.5	57.2	590.7	1353.8
N	18	18	18	18	18	18
%CV	2.8	3.2	3.5	1.5	1.6	3.2
%Nom	95.6	98.3	96.8	95.4	98.4	96.7

Evaluation of Matrix effect

Drugs are spiked in six (6) different whole blood matrices at QC-L level. After extraction, concentrations are evaluated against a calibration curve. Replicate extractions are deposited onto a LazWell™ plate and dried before analysis. The precision and accuracy criteria are used.
Results for quantification transitions are shown in **Table 6** and **Table 7** for Chloroquine and Hydroxychloroquine, respectively. Each concentration does not exceed 15% CV and the mean concentration is within ±15% of expected value**.** Similar results are obtained for the confirmation transition.

Table 6 – Matrix effect evaluation for Chloroquine

Chloroquine	M 1	M 2	M 3	M 4	M 5	M 6
Exp. conc.(ng/mL)	60	60	60	60	60	60
Calc. conc (ng/mL)	56.6	55.3	53.9	57.4	53.0	56.6
N	6	6	6	6	6	6
%CV	2.6	2.3	2.6	1.4	1.6	1.9
%Nom	94.4	92.2	89.9	95.7	88.3	94.3

Table 7 – Matrix effect evaluation for Hydroxychloroquine

Hydroxychloroquine	M 1	M 2	M 3	M 4	M 5	M 6
Exp. conc.(ng/mL)	60	60	60	60	60	60
Calc. conc (ng/mL)	57.0	56.7	54.9	60.4	54.0	56.5
N	6	6	6	6	6	6
%CV	0.4	1.9	3.3	1.0	0.9	0.8
%Nom	95.0	94.5	91.4	100.6	90.0	94.1

Conclusion

A system combining Luxon Ion Source® with a Shimadzu 8060 spectrometer system allows ultra-fast (**8 seconds per sample**) quantification of Chloroquine and Hydroxychloroquine in whole blood samples using a simple protein precipitation sample preparation method.

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