Extraction of Chloroquine (CQ) and Hydroxychloroquine (HCQ) from Different Biological Matrices Prior to LC-MS/MS Analysis

Mohamed Youssef, Stephanie J. Marin, Elena Gairloch
Biotage, 10430 Harris Oaks Blvd., Suite C, Charlotte North Carolina 28269, USA

Background/Introduction

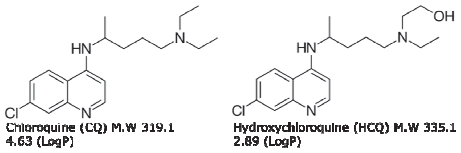

Figure 1 *Chemical structure.*

Hydroxychloroquine (HCQ) and chloroquine (CQ) (Figure 1) are common antimalarial drugs with long half-lives. They are currently being tested as potential treatments for COVID-19. Due to their high publicity in the media, many forensic toxicologists are working on developing methods to extract and detect these compounds in different matrices to understand the adverse effects, potential toxicity, and drug interactions associated with these drugs. These compounds are not typically included in general unknown screens. A simple sample preparation procedure that delivers clean extracts and analyte recovery greater than 90% for CQ and greater than 82% for HCQ with minimum matrix effects and high process efficiency was developed.

Reagents and Materials

Standards

Blank whole blood, urine, and serum were purchased from UTAK laboratories (Valencia, CA). All standards, HPLC grade water and acetonitrile (ACN), ammonium formate (amm formate), and ammonium hydroxide were purchased from Sigma Aldrich (St. Louis, MO) in addition to reagent grade ethyl acetate and formic acid. ISOLUTE® SLE+ 400 µL supported liquid extraction plate (820-0400-P01), and Collection Plate, 2 mL Square (121-5203) were supplied by Biotage (Charlotte, NC).

Instruments

Biotage® PRESSURE+ 96 Positive Pressure Manifold (Pressure +96), Biotage® SPE Dry 96 (SD-9300-DHS-NA), and Biotage® ACT Plate Adapter (414355SP) to prevent cross contamination from evaporation were all supplied by Biotage (Figure 2). Analysis was conducted using an Agilent 1260 HPLC System (Agilent Technologies, Santa Clara, CA) coupled with Sciex 4000 triple quadrupole mass spectrometer (Sciex, Framingham MA). A Ultra AQ C18 3.0 µm 100 X 2.1 mm (9178312) analytical column was supplied by Restek (Bellefonte, PA).

Figure 2. *PRESSURE+ 96, SPE Dry 96, and ACT Plate Adaptor.*

Sample Preparation

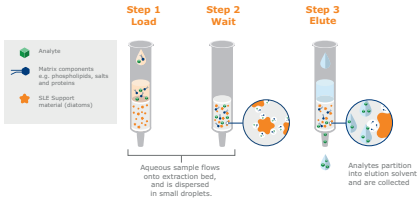

Figure 3. *Schematic of ISOLUTE® SLE+ Supported Liquid Extraction procedure.*

ISOLUTE® SLE+ Optimized Procedure (Figure 3) 96-well Plates: ISOLUTE® SLE+ 400 µL capacity; P/N: 820-0400-P01.

Sample pre-treatment: In a 2 mL 96-well collection plate, 100 µL aliquots of blank biological fluids (whole blood, serum and urine) was spiked with equal concentrations of 10 ng/mL of both analytes, diluted with 300 µL of 0.5 M ammonium hydroxide, and mixed well.

Sample Application: 375 µL was taken from the collection plate and pipetted onto the SLE+ plate. A pulse of positive pressure was applied to initiate flow and the samples were left to absorb for 5 minutes.

Analyte Extraction: 750 µL of ethyl acetate was applied to the plate and allowed to flow under gravity for 5 minutes. A second aliquot of ethyl acetate was applied to the plate and allowed to flow under gravity for 5 minutes. A 5 second application of positive pressure was applied to push remaining solvent through the plate.

Post Extraction: The eluate was evaporated to dryness and reconstituted in 100 µL of mobile phase (A) and analyzed via LC-MS/MS.

Chromatography and Mass Spec Methods

HPLC Parameters and Gradient

HPLC	Parameter
Column	Ultra AQ C18 3.0 um 100 X 2.1 mm
MPA	ACN:20 mM Amm. formate (15:85) + 0.2% formic acid
MPB	0.1% Formic Acid in Acetonitrile
Flow Rate	0.4 mL/min
Column Temp.	25 °C
Sample Temp.	20 °C
Injection Volume	5 µL

Table 1*. HPLC conditions.*

The LC gradient started with 80% aqueous and gradually decreased to 2% aqueous over 2 minutes with a total run time of 4 minutes. This allowed for full separation of both compounds. Figure 4 show the extracted chromatograms for both HCQ and CQ.

Mass Spectrometry Parameters

Instrument: A SCIEX 4000 triple quadrupole Mass Spectrometer with Turbo Ionspray® Ion interface was used. MRM transitions and optimized source parameters are shown in tables 2&3.

ID	Q1 Mass	Q3 Mass	DP Volt	EP (Volt)	CE (Volt)	CXP (Volt)
CQ 1	320.0	247.0	100	14	27	14
CQ 2	320.0	142.0	100	14	30	10
HCQ 1	336.1	247.0	100	14	30	12
HCQ 2	336.1	158.1	100	14	33	12

Table 2. *MRM transitions.*

Curtain Gas	35
Collision Gas	Medium
IonSpary Voltage(IS)	5500
Temperature	650
Ion Source Gas (GS1)	60
Ion Source Gas (GS2)	60
Interface Heater	On

Table 3. *Mass Spec Parameter.*

Results

The initial conditions of the LC gradient allowed for improved chromatographic separation (figure 4) and minimized matrix effects from coeluting peaks from the matrices. Recoveries, matrix effects, and process efficiencies for both analytes were calculated for all three matrices. For all three matrices, calculated matrix effects for chloroquine showed slight enhancement (between 2-12%), while hydroxychloroquine showed slight suppression (between 4-13%) (figure 5). Both analytes had calculated recoveries of 82-97% when using ISOLUTE® SLE+ for all three matrices (figure 6). Process efficiencies when using this extraction technique were 66-99% for all three matrices (figure 7).

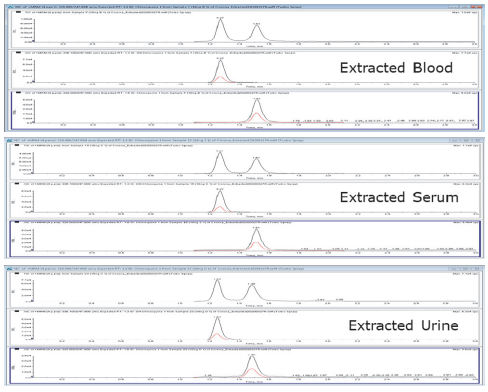

Figure 4. *Extracted Ion Chromatogram for analytes from whole blood, serum, and urine at 10 ng/mL.*

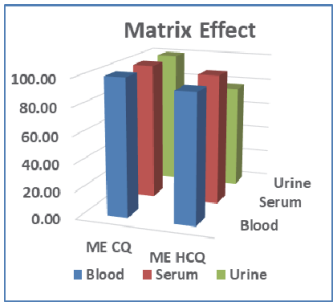

Figure 5. *Matrix Effect.*

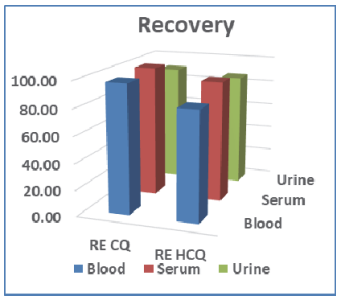

Figure 6. *Recovery.*

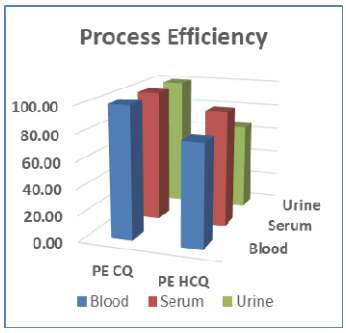

Figure 7. *Process Efficiency.*

Conclusions

ISOLUTE® SLE+ provides the selectivity and sensitivity required to quantitate Chloroquine (CQ) and Hydroxychloroquine (HCQ). The combination of a simple unified extraction for different matrices and the short run time maximizes sample throughput and allows for implementation of the method in high-throughput laboratories.

Other Considerations

- It is recommended to keep the column temperature at 25 ˚C with a 400 µL/min flow rate to allow the analytes to come out later in the gradient.
- Carryover can be observed at higher concentrations:
  1. It is recommended to make mobile phase A as a mixture of aqueous and organic
  2. It is recommended to use a needle wash mix of acetonitrile, methanol, isopropanol, and water (25:25:25:25) and smaller injection volumes of 2-5 µL.
  3. Extending the column rinse step with 98% organic for 1 min and a re-equilibration time of 1 min will also help reduce carryover.
  4. When using deuterated internal standard, it can be prepared in 0.5 M ammonium hydroxide diluent
- It is recommended to use the ACT plate adaptor to reduce evaporative crosstalk in extracted biological samples
- The assay can be automated using the Biotage® Extrahera™

References

1-Chhonker, Y. S., Sleightholm, R. L., Li, J., Oupický, D., & Murry, D. J. (2018). Simultaneous quantitation of hydroxychloroquine and its metabolites in mouse blood and tissues using LC–ESI–MS/MS: An application for pharmacokinetic studies. Journal of Chromatography B, 1072, 320-327. doi:10.1016/j.jchromb.2017.11.026

This Poster was presented at SOFTember, September 2020online
Part Number: P224.V.1