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

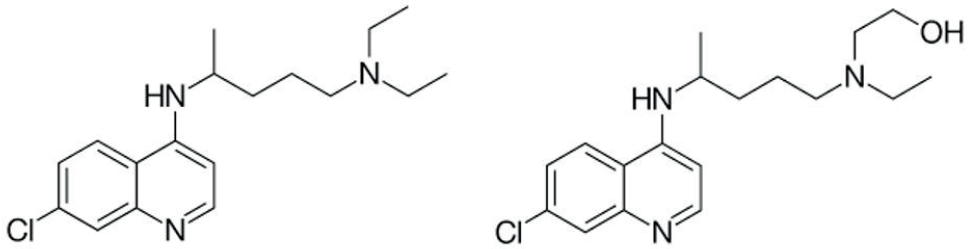

Figure 1. Structures of Chloroquine (CQ) and Hydroxychloroquine (HCQ).

Introduction

The objective of this study was to develop a fast and reliable extraction for Chloroquine (CQ) and Hydroxychloroquine (HCQ) from different human matrices (whole blood, serum and urine) using ISOLUTE® SLE+ Supported Liquid Extraction plates prior to LC-MS/MS analysis.

The simple sample preparation procedure delivers clean extracts and analyte recovery greater than 90% for CQ and greater than 80% for HCQ with minimal matrix effects and high process efficiency.

Analytes

Chloroquine (CQ), and Hydroxychloroquine (HCQ)

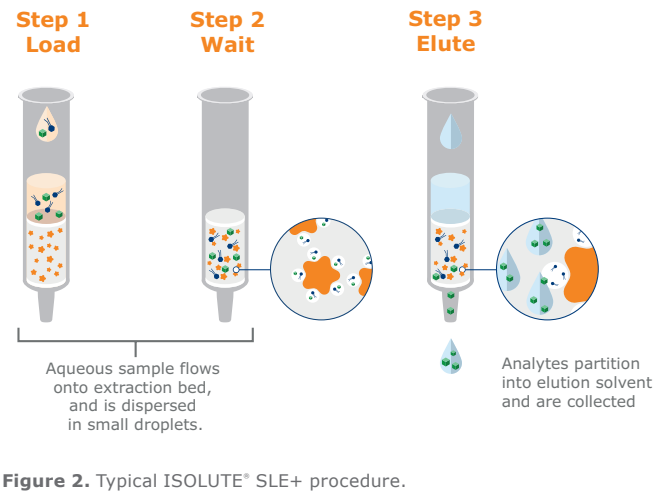

Figure 2. Typical ISOLUTE® SLE+ procedure.

Sample Preparation Procedure[1]

Format

ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Plate (P/N 820-0400-P01)

Sample Pre-Treatment

Dilute the biological sample (100 µL) with 0.5M ammonium hydroxide (300 µL). Vortex briefly and let stand for 5 mins.

Sample Loading

Load pre-treated samples (375 µL) onto the ISOLUTE® SLE+ 400 µL plate. Using a Biotage® PRESSURE+96 Positive Pressure Manifold (P/N PPM-96), apply a pulse of pressure to load samples onto the sorbent. Wait 5 minutes for the sample to equilibrate on the sorbent.

Elution

Apply ethyl acetate (750 µL) to each well, and allow to flow by gravity for 5 minutes. Apply a second aliquot of ethyl acetate (750 µL). Wait 5 minutes. For complete solvent recovery apply a pulse of positive pressure at 10 psi (10–20 seconds).

Evaporation

Dry the extract under a stream of nitrogen using a Biotage® SPE Dry at 40 °C, 20 to 40 L/min for approximately 25 minutes.

Reconstitution

Reconstitute with mobile phase A (100 µL) and mix thoroughly.

UHPLC Conditions

Instrument
Agilent 1260 Infinity II LC System

Column
Restek Ultra AQ C18 3.0 um 100 X 2.1 mm. Cat # 9178312

Flow Rate
0.4 mL/min

Column Temperature
25 °C

Injection Volume
5 µL

HPLC Gradient

Table 1. HPLC Gradient.

Time (min.)	%A	%B
0.0	80	20
1.9	25	75
2.0	2.0	98
3.0	2.0	98
4.0	80	20

MS Sciex 4000 QQQ

Table 2 Mass spec source parameters.

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

Table 3 Transitions and retention times for target analytes in positive mode.

ID	Q1	Q3	DP (Volt)	CE (Volt)	RT	EP (Volt)	CPX(Volt)
CQ 1	320.0	247.0	41,43	27	1.3	14	14
CQ 2	320.0	142.0	53,43	30	1.3	14	10
HCQ 1	336.1	247.0	49	30	1.6	14	12
HCQ 2	336.1	158.1	43,70	33	1.6	14	12

Results and Discussion

Figure 3 shows an extracted ion chromatogram of a 10 ng/mL mixed standard solution of chloroquine and hydroxychloroquine. Two transitions for each analyte were used and total chromatographic separation was achieved.

Figure 4 shows an extracted ion chromatogram of a 10 ng/mL mixed solution of chloroquine and hydroxychloroquine spiked into blank human whole blood and extracted using the method described.

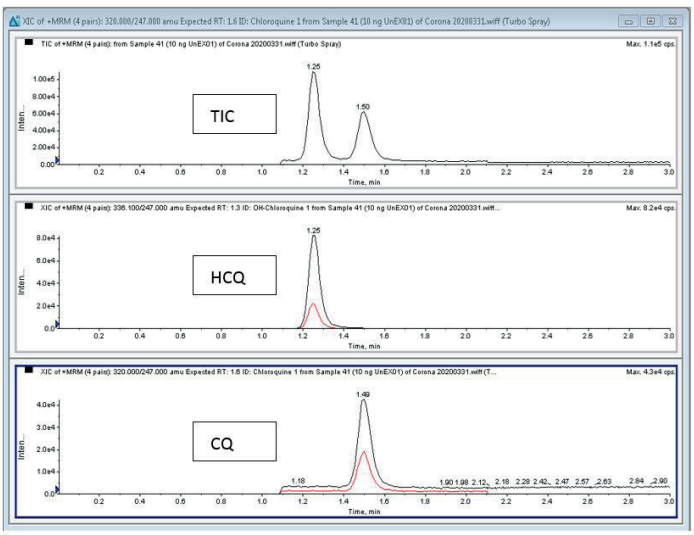

Figure 3. TIC and XIC for unextracted HCQ and CQ @10 ng/mL.

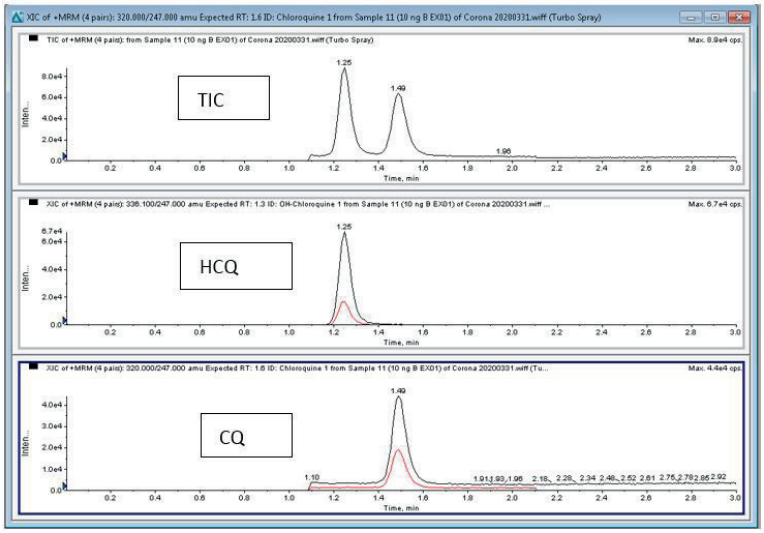

Figure 4. TIC and XIC for HCQ and CQ @10 ng/mL extracted from whole blood.

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Figure 5 shows an extracted ion chromatogram of a 10 ng/mL mixed solution of chloroquine and hydroxychloroquine spiked into blank human serum and extracted using the method described.

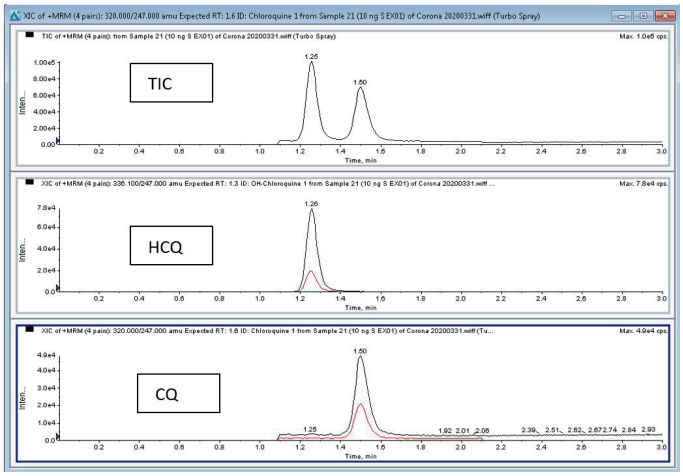

Figure 5. TIC and XIC for HCQ and CQ @10 ng/mL extracted from serum.

Figure 6 shows an extracted ion chromatogram of a 10 ng/mL mixed solution of chloroquine and hydroxychloroquine spiked into blank human urine and extracted using the method described.

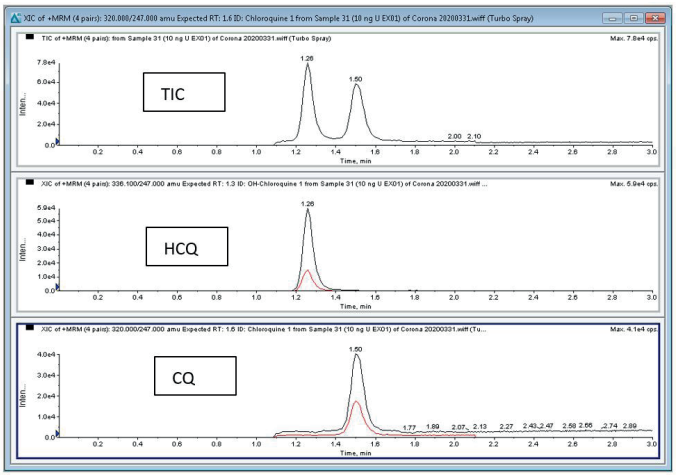

Figure 6. TIC and XIC for HCQ and CQ @10 ng/mL extracted from urine.

Matrix effects, analyte recovery and process efficiency were determined. Figures 7, 8, and 9 display matrix effects, recovery, and process efficiency respectively, obtained for choloroquine and hydroxychloquine using the ISOLUTE® SLE+ 400 µL extraction plates. The study represents 3 sets of samples.[2]

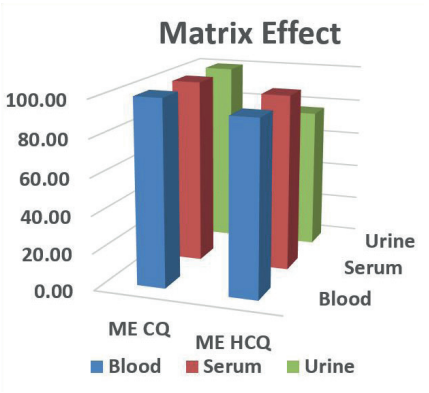

Figure 7. Matrix Effect.

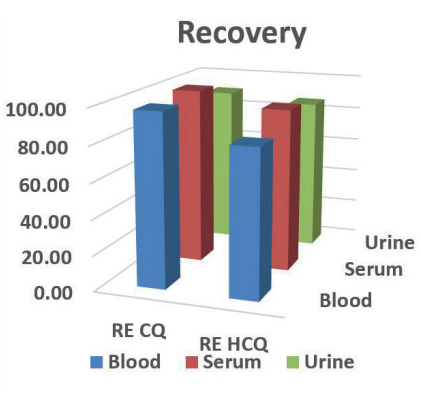

Figure 8. Recovery.

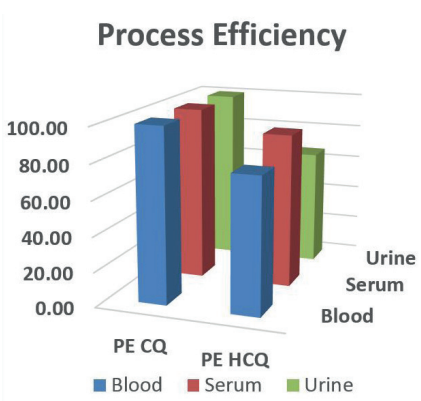

Figure 9. Process Effect.

Conclusion

ISOLUTE® SLE+ provides the selectivity and sensitivity required to extract and quantitate chloroquine (CQ) and hydroxychloroquine (HCQ) in a variety of biological fluid samples. The combination of a simple unified extraction for different matrices and the short run time will maximize the sample throughput and allow implementation of methods in high-throughput laboratories.

Additional information

- It is recommended to maintain column temperature at 25 °C with a 400 µL/min flow rate to allow the analytes to elute later in the gradient.
- Carryover can be observed at higher concentrations. To reduce this:
  - It is recommended to make mobile phase A as a mixture of aqueous and organic
  - It is recommended to use a needle wash mix of acetonitrile, methanol, isopropanol and water (1:1:1:1, v/v) and smaller volume injections 2-5 µL.
  - Extending the column rinse step with 98% organic for 1 min and a re-equilibration time of 1 min. will also help reduce carryover.
- The assay can be automated using the Biotage® Extrahera™
- When using deuterated internal standard, it should be prepared in 0.5 M ammonium hydroxide diluent.

Ordering Information

Part Number	Description	Quantity
820-0400-P01	ISOLUTE® SLE+ 400 µL Supported Liquid Extraction Plate	1
PPM-96	Biotage® PRESSURE+ 96 Positive Pressure Manifold	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 100/120 V	1

Chemicals and Reagents

- Chloroquine diphosphate salt, hydroxychloroquine sulfate, methanol (LC-MS grade), acetonitrile (LC-MS grade), ammonium formate, water (LC-MS grade), formic acid (LC-MS grade), ethyl acetate (anhydrous, 99.8%), ammonium hydroxide were purchased from Sigma Aldrich (St. Louis, MO).
- Blank matrices (human whole blood, serum, and urine were purchased from UTAK labs (Valencia, CA)
- Mobile Phase A: ACN:20 mM Ammonium formate (15:85, v/v) + 0.2% formic was prepared in a 1 L bottle by combining 1.26 g of ammonium formate +150 mL of Acetonitrile + 850 mL of water + 2 mL of formic acid and mixed well, pH approximately 2.6
- Mobile Phase B: Methanol: ACN (75:25, v/v) was prepared in a 1 L bottle by combining 750 mL of methanol and 250 mL of acetonitrile and mixed well.
- 0.5M ammonium hydroxide was prepared by dissolving 1.75 g in 100 mL of water and mixed well.

References

1. Kaewkhao, Karnrawee, et al. “High Sensitivity Methods to Quantify Chloroquine and Its Metabolite in Human Blood Samples Using LC–MS/MS.” Bioanalysis, vol. 11, no. 5, 2019, pp. 333–347., doi:10.4155/bio-2018-0202.
2. Silvestro, Luigi, et al. “Matrix Effects in Mass Spectrometry Combined with Separation Methods - Comparison HPLC, GC and Discussion on Methods to Control These Effects.” IntechOpen, IntechOpen, 29 May 2013, www.intechopen.com/books/tandem-mass-spectrometry-molecular-characterization/matrix-effects-in-mass-spectrometry-combined-with-separation-methods-comparison-hplc-gc-and-discussi.

EUROPE
Main Office: +46 18 565900
Toll Free: +800 18 565710
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 704 654 4900
Order Fax: +1 434 296 8217
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
Outside US: +1 704 654 4900
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 68162810
Fax: +86 21 68162829
cn_order@biotage.com
cn-1-pointsupport@biotage.com

KOREA
Tel: +82 31 706 8500
Fax: +82 31 706 8510
korea_info@biotage.com
kr-1-pointsupport@biotage.com

INDIA
Tel: +91 22 4005 3712
india@biotage.com

Distributors in other regions are listed on www.biotage.com

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