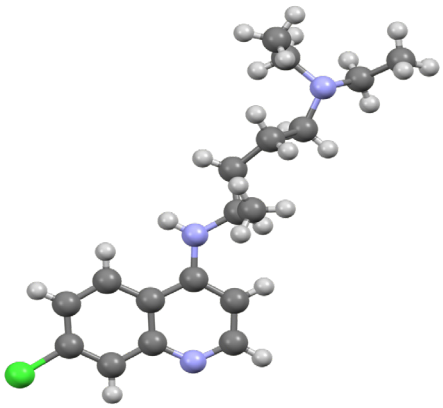
Analysis of Chloroquine, Hydroxychloroquine, and Desethylchloroquine in Urine Using SPE and LC-MS/MS



UCT Part Numbers

SSDBX063
Styre Screen® DBX
60 mg, 3 mL Column

SLDA100ID21-3UM
Selectra® DA HPLC Column
100 X 2.1 mm, 3 µm

SPHPHO6001-10
Select PH Buffer Pouches
100 mM Phosphate pH 6.0

SLDAGDC21-3UM
Selectra® DA Guard Column
10 X 2.1 mm, 3 µm

SLGRDHLDR-HPOPT
Guard Column Holder

Summary:

Since the outbreak of the Novel Coronavirus (COVID-19) triggered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), controversy over the use of the antimalarial drugs Hydroxychloroquine and Chloroquine to treat the virus has surfaced as the side effects and multiple risks associated with these medications have not been fully evaluated [1]. In addition, the US Food and Drug Administration (FDA) is concerned that Hydroxychloroquine and Chloroquine are being used inappropriately to treat non-hospitalized patients who are positive with the coronavirus or as a prophylactic to prevent the disease in the first place [1]. This situation makes the extraction and determination of Chloroquine and Hydroxychloroquine in urine an important unaddressed need in the clinical and forensic markets.

This application note describes a simple and robust solid-phase extraction (SPE) procedure for Chloroquine, Hydroxychloroquine, and the primary metabolite, Desethylchloroquine, in urine. The use of a high capacity polymeric cation-exchange SPE sorbent ensures efficient extraction of the drug residues while removing undesired matrix components and yielding purified results. HPLC separation was carried out using a Selectra® DA column which resulted in excellent retention and baseline separation (<6 minutes) of the three polar drugs.

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SPE Procedure

1. Sample Preparation

a) To 1 mL of urine add 1 mL of pH 6 phosphate buffer (0.1M) and internal standard(s).

b) Mix/Vortex briefly.

Note: A hydrolysis protocol may be required if conjugated compounds are to be included into the above drug panel.

2. Condition Cartridge

a) 1 x 1 mL MeOH

b) 1 x 1 mL DI H_2O.

3. Apply Sample

a) Load sample at 1- 2 mL/minute.

4. Wash Cartridge

a) 1 x 1 mL PH 6 phosphate buffer (0.1M).

b) 1 x 1 mL MeOH.

c) Dry cartridges under full vacuum or pressure for 2 minutes.

5. Elute Analytes

a) 1 x 2 mL MeOH: NH_4OH (98:2)

b) Collect at 1-2 mL/ minute.

6. Dry Eluate

a) Evaporate to dryness at < 40°C.

7. Reconstitute

a) Reconstitute sample in 100 µL of mobile phase or other appropriate organic solvent.

LC-MS/MS Parameters	
System	Shimadzu LCMS-8050
UHPLC Column	Selectra® DA (100 X 2.1 mm, 3 µm)
Guard Column	Selectra® DA (10 X 2.1 mm, 3 µm)
Column Temperature	40°C
Flow Rate	0.4 mL/min
Injection Volume	5 µL

Gradient Program		
Time (min)	**Mobile Phase A (%) (0.1% Formic Acid in Water)**	**Mobile Phase B (%) (0.1% Formic Acid in Water)**
0	100	0
0.5	90	10
5.5	65	35
6.5	0	100
7.5	0	100
11	100	0

Chromatogram:

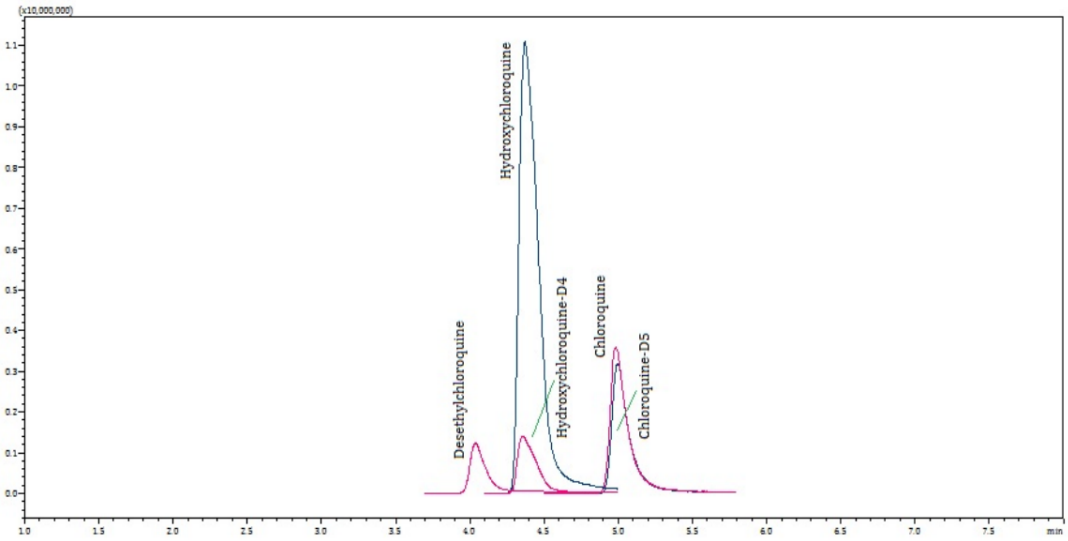

Figure 1. Chromatogram of a 50 ng/mL extracted sample.

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MRM Table:

MRM's							
Analyte	**Parent Ion**	**Product Ion 1**	**CE**	**Product Ion 2**	**CE**	**Product Ion 3**	**CE**
Chloroquine	319.95	247.1	21	142.15	22	179	37
Hydroxychloroquine	335.95	247	16	179	38	158.1	24
Desethylchloroquine	291.95	179	22	247.05	20	114.1	21
Hydroxychloroquine-D4	340.45	247.1	22	179.05	39	162.2	25
Chloroquine-D5	325.45	247.1	20	147.2	22	179	37

Results:

Recovery (n=5)				
Analyte	**2.5 ng/mL**	**RSD (%)**	**25 ng/mL**	**RSD (%)**
Chloroquine	95%	5.6	100%	3.3
Hydroxychloroquine	94%	3.0	99%	4.2
Desethylchloroquin	80%	5.8	88%	5.9

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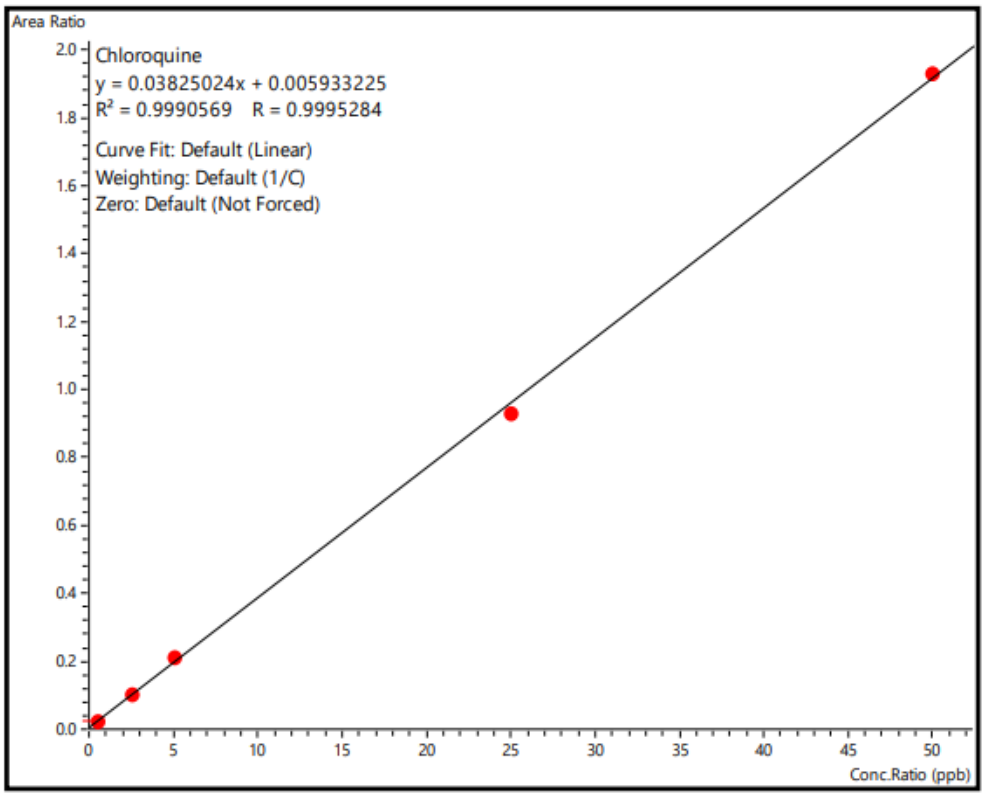
Calibration Curves:



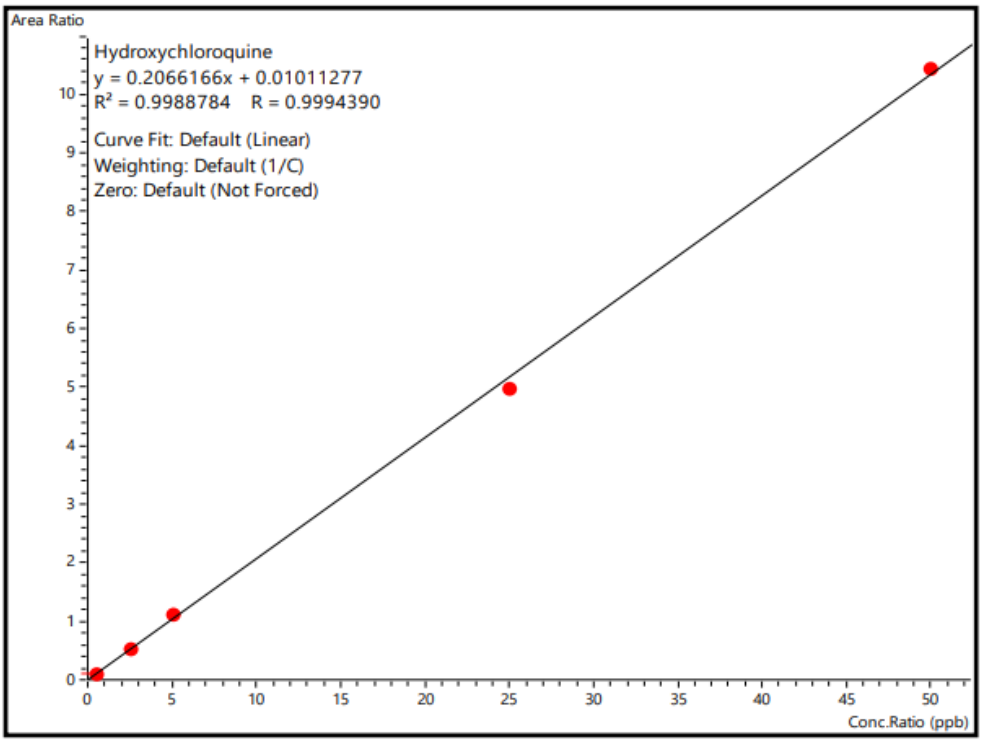


Figure 2. Calibration curves of Chloroquine & Hydroxychloroquine (1, 5, 10, 50, 100 ng/mL), Avg R^2 0.9985.

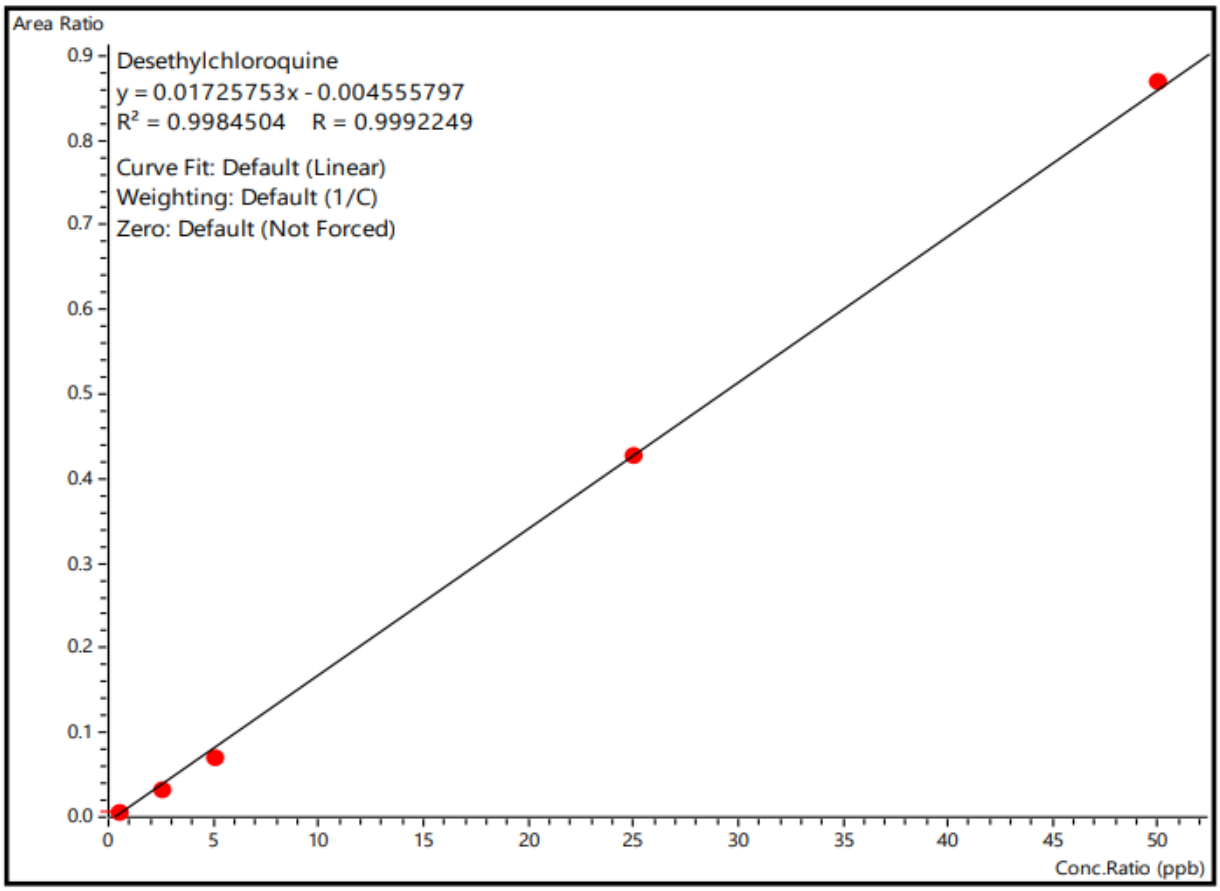


Figure 3. Calibration curve of Desethylchloroquine (1, 5, 10, 50, 100 ng/mL), R^2 0.9984.

Conclusions:

This application note outlines a simple SPE procedure for the analysis of Chloroquine, Hydroxychloroquine, and the primary metabolite Desethylchloroquine in urine using UCT's Styre Screen® DBX polymeric SPE cartridge. Excellent recoveries for all three compounds were obtained using the outlined procedure, namely ≥80% at the 2.5 ng/mL level and ≥95% at the 25ng/mL level. RSD values at both concentration levels were ≤6%. In addition, the chromatographic separation of these analytes was challenging due to the extreme polarity of all analytes. However, the use of a Selectra® DA polyaromatic HPLC column resulted in excellent retention and baseline separation for all the compounds included in the method. This method will be beneficial to any lab looking to implement testing of these controversial drugs.

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References:

[1] https://www.fda.gov/drugs/drug-safety-and-availability/fda-cautions-against-use-hydroxychloroquine-or-chloroquine-covid-19-outside-hospital-setting-or

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