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

## UCT Part Numbers

**SSDBX063**
Styre Screen® DBX
60 mg, 3mL Column

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

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

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

**SLGRDHLDR**
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.



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

## SPE Procedure:

1) **Sample Preparation:**
   - To 1 mL of urine add 1 mL of pH 6 phosphate buffer (0.1M) and internal standard(s)
   - 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**
   - 1 x 1 mL MeOH
   - 1 x 1 mL DI $H_2O$
3) **Apply Sample:**
   - Load sample at 1- 2 mL/minute
4) **Wash Cartridge**
   - 1 x 1 mL PH 6 phosphate buffer (0.1M)
   - 1 x 1 mL MeOH
   - Dry cartridges under full vacuum or pressure for 2 minutes
5) **Elute Analytes**
   - 1 x 2 mL MeOH: $NH_4OH$ (98:2)
   - Collect at 1-2 mL/ minute.
6) **Dry Eluate**
   - Evaporate to dryness at < 40°C.
7) **Reconstitute**
   - Reconstitute sample in 100 µL of mobile phase or other appropriate organic solvent.

## LC-MS/MS PARAMETERS:

| 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: 4**0°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 MeOH) |
| 0 | 100 | 0 |
| 0.5 | 90 | 10 |
| 5.5 | 65 | 35 |
| 6.5 | 0 | 100 |
| 7.5 | 0 | 100 |
| 11 | 100 | 0 |

CLINICAL

FORENSICS

## Chromatogram:

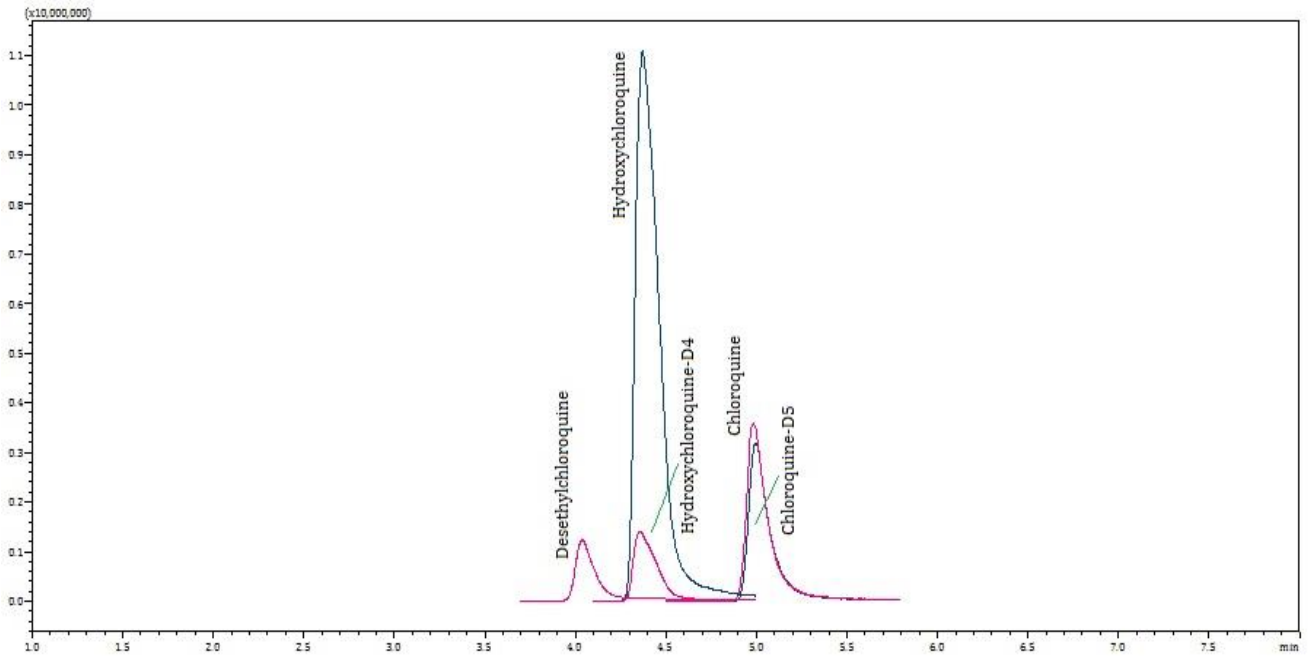



**Figure 1:** Chromatogram of a 50 ng/mL extracted sample.

## MRM Table:

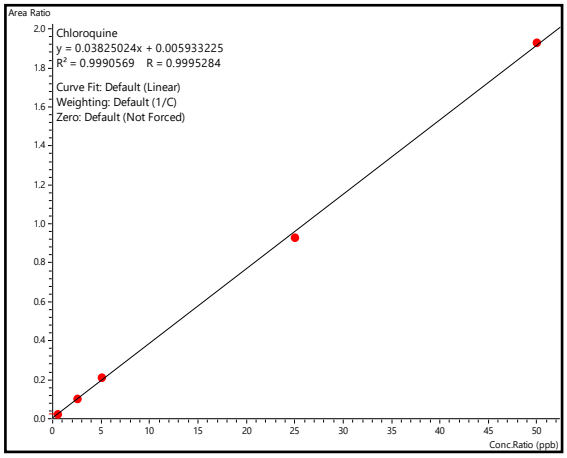
| Analyte | MRM's | | | | | | |
|---|---|---|---|---|---|---|---|
| | Parent Ion | Product Ion 1 | CE | Product Ion 2 | CE | Product Ion 3 | CE |
| Chloroquine | 319.95 | 247.1 | 21 | 142.15 | 22 | 179 | 37 |
| Hydroxychloroquine | 335.95 | 247 | 16 | 179 | 38 | 158.1 | 24 |
| Desethylchloroquine | 291.95 | 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:

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

CLINICAL FORENSICS

## Calibration Curves:



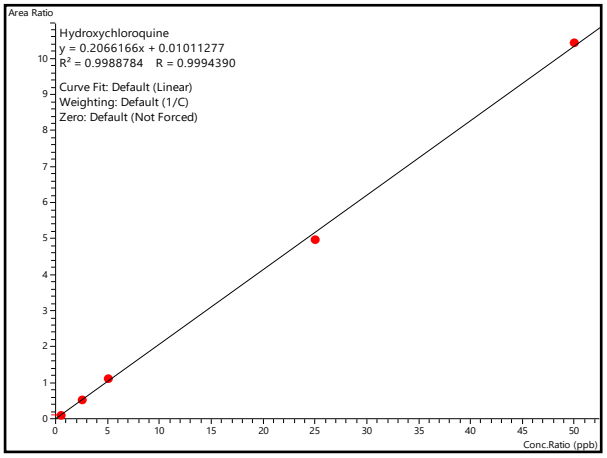




**Figure 2:** Calibration curves of Chloroquine & Hydroxychloroquine (1, 5, 10, 50, 100 ng/mL), Avg $R^2$ 0.9985.

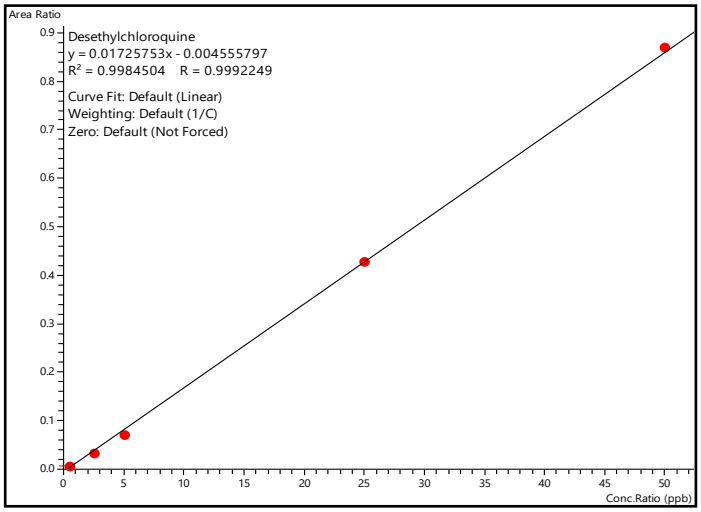



**Figure 3:** Calibration curve of Desethylchloroquine (1, 5, 10, 50, 100 ng/mL), $R^2$ 0.9984.

## 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 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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