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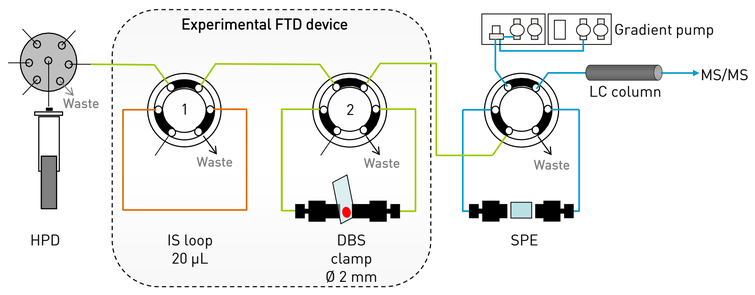
# Analysis of antimalarial drugs in DBS by flow-through desorption coupled online to SPE-LC-MS/MS

## Introduction

Malaria is one of the most abundant infectious diseases in tropical countries. Nowadays, much effort is directed towards investigations and studies with respect to malaria-related therapeutics and pharmacokinetics. In this regard, the development and validation of methods for the accurate measurement of antimalarial drugs in blood is a pre-requisite for reasonable medication. However, sampling, sample storage, preparation and shipment of liquid samples is challenging in tropical remote areas due to extreme temperatures and humidities, and a lack of adequate local conditions as for instance sample freezing possibilities. Dried blood spot (DBS) methods are the most suitable alternative here enabling low volume, high throughput, and field applicable studies.

The drugs Chloroquine (CQ) and Amodiaquine (AQ) find widespread application in the treatment of malaria. For determination of these drugs together with their most abundant metabolites Desethylchloroquine (CQm) and Monodesethylamodiaquine (AQm), respectively, flow-through desorption of DBS coupled online to SPE-LC-MS/MS was evaluated. Preliminary validation data applying optimized desorption and SPE conditions are presented.

## Online FTD-SPE-LC (Spark Holland)

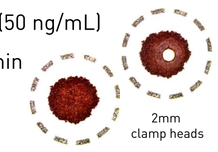



The experimental Flow-Through Desorption (FTD) device for DBS cards comprises a card clamp, a sliding holder for positioning the card in the clamp and two switching valves. The latter are used for switching the DBS clamp and the loop, respectively, online or offline with the SPE flow path. A High Pressure Dispenser (HPD) delivers conditioning and equilibration solvent to the SPE cartridge (DBS clamp offline) and desorption solvent through the DBS card (DBS clamp online). The loop which is filled with internal standard is switched into the desorption flow stream simultaneously with starting the desorption. The HPD delivers wash solvent to remove matrix components from the SPE cartridge after trapping (DBS clamp offline). An automated SPE cartridge exchange system (ACE) is used to provide a new SPE cartridge for every analysis and to switch the cartridge between trapping and elution position. The entire system is controlled via SparkLink version 4.10 software.

## Experimental conditions

### DBS

| | |
|---|---|
| Filter card: | Whatman DMPK-C cards |
| Sample: | 25 µL blood (dried over night at RT) |
| Internal Standard: | Chloroquine-d4 and Amodiaquine-d4 in desorption solution (50 ng/mL) |
| Desorption: | 1 mL 40/60 $(NH_4)_2CO_3$ buffer 20 mM (pH 7 )/ACN at 1.5 mL/min |
| DBS Clamp flush: | 2 mL 70/30 ACN/water 1% HAc at 5 mL/min |
| | 2 mL 75/25 MeOH/ACN at 5 mL/min |
| | 1 mL 40/60 $(NH_4)_2CO_3$ buffer 20 mM (pH 7)/ACN at 5 mL/min |




### SPE

| | |
|---|---|
| Cartridge: | HySphere Mixed Mode weak cation,10 µm, 2 x 10 mm |
| Conditioning: | 1 mL 75/25 MeOH/ACN at 3 mL/min |
| Equilibration: | 1 mL 40/60 $(NH_4)_2CO_3$ buffer 20 mM (pH7)/ACN at 2 mL/min |
| Sample transfer: | see DBS Desorption |
| Cartridge wash: | 1 mL 40/60 $(NH_4)_2CO_3$ buffer 20 mM (pH7)/ACN at 2.5 mL/min |
| | 1 mL 75/25 MeOH/ACN at 2.5 mL/min |
| Elution: | LC gradient |
| SPE Clamp flush: | see DBS Clamp flush |

### LC

Mobile phase: A) 20mM Ammonium Formiate (with 1.5% FA):ACN 90:10 v/v ; B) ACN

| Gradient | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Time [mm:ss]** | 0.00 | 0.20 | 0.25 | 2.00 | 2.60 | 3.60 | 3.90 | 5.00 |
| **Flow [mL/min]** | 0.7 | 0.7 | 0.7 | 0.8 | 1.0 | 1.0 | 0.8 | 0.8 |
| **B [%]** | 0 | 0 | 10 | 20 | 100 | 100 | 0 | 0 |

Column: Zorbax SB CN, 50 mm x 4.6 mm, 3.5 µm (Zorbax Inc., Wilmington, USA)

### ESI-MS

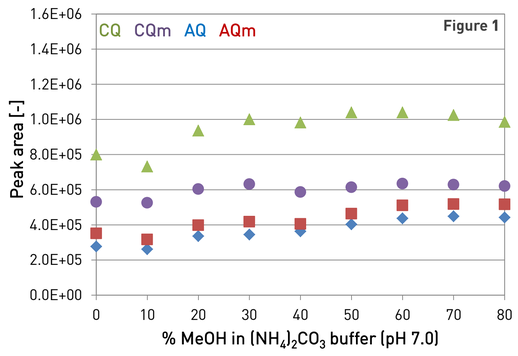
MS: 3200 Triple Quadrupole (AB Sciex, Concord, Canada)

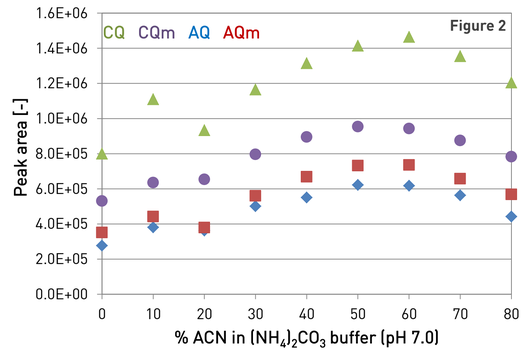
| ESI-MS/MS conditions (positive mode) | | | | | | |
|---|---|---|---|---|---|---|
| **Compound** | **Q1 [m/z]** | **Q3 [m/z]** | **General Settings** | | | |
| Chloroquine (CQ) | 320.2 | 247.2 | IS [V] | 5500 | GS1 (psi) | 60 |
| Desethylchloroquine (CQm) | 292.2 | 197.1 | TEM [°C ] | 600 | GS2 (psi) | 60 |
| Amodiaquine (AQ) | 356.2 | 283.2 | CAD | Medium | Dwell time (msec) | 100 |
| Desethylamodiaquine (AQm) | 328.1 | 283.1 | CUR [psi] | 25 | | |

## Results and Discussion

### Optimization of DBS desorption and SPE conditions

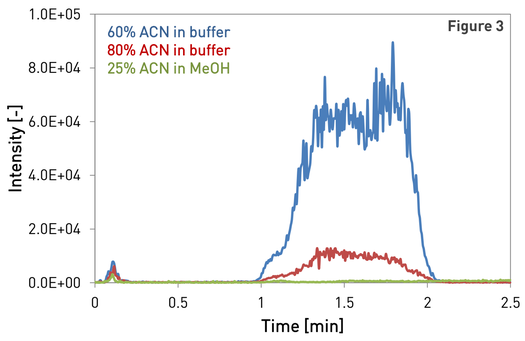
DBS desorption and SPE sample clean-up have been optimized with respect to desorption and wash solvent compositions. In a first experiment desorption and wash were carried out using mixtures of $(NH_4)_2CO_3$ buffer (pH 7) with MeOH or ACN in different ratios. The profiles for 0% to 80% organic content of the desorption and wash solvents are shown in Figures 1 and 2.




Higher peak areas are detected for all compounds when desorption and wash are carried out utilizing ACN; an optimum is observed at 60% ACN.

In order to remove as much matrix as possible the SPE wash step has further been optimized. Additional washing has been carried using either 60% ACN in buffer, 80% ACN in buffer or 25% ACN in methanol. All compounds trapped were directly eluted towards the MS and the phospholipid signature trace m/z 184 was monitored.




The mass traces of the three measurements are overlayed in Figure 3. Best clean-up is obtained when using 25% ACN in methanol in the second SPE catridge wash step as no phospholipids are detected any more .

### Linearity

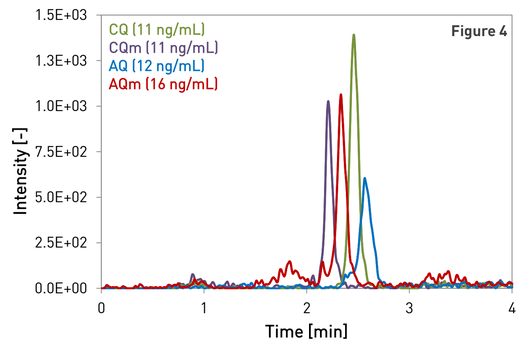
Linearity was determined for all analytes spiked to blood using a 1/x-weighting for calculation.

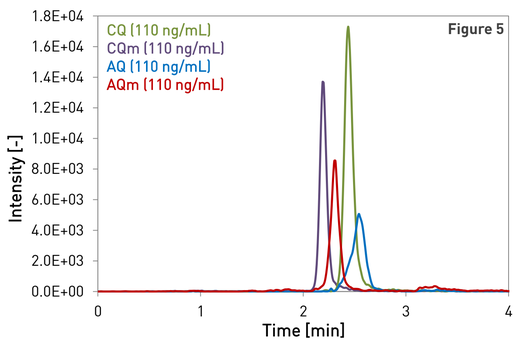
| Linearity (1/x- weighting) | | |
|---|---|---|
| **Compound** | **Concentration range [ng/mL]** | **R** |
| CQ | 3-1500 | 0.9996 |
| CQm | 3-1500 | 0.9998 |
| AQ | 4 - 940 | 0.9989 |
| AQm | 5-940 | 0.9956 |

The Table above shows that the method is linear for the drugs CQ and AQ as well as for their metabolites over more than 3 orders of magnitude.

### Sensitivity

Sensitivities are estimated based on the signal-to-noise ratio. Figure 4 below shows a chromatogram of the analytes at LLOQ (11 ng/mL for CQ and CQm, respectively, 12 ng/mL for AQ and 16 ng/mL for AQm). For comparison a chromatogram at 110 ng/mL is shown in Figure 5.




Sensitivities of the assay would allow for therapeutic drug monitoring of the four compounds in DBS. [1,2]

### Precision and Accuracy

Precision and accuracy of the analyte measurement are determined for spiked DBS. Quality controls low (LLOQ), medium and high are analyzed in threefold.

| Precision and Accuracy | | | |
|---|---|---|---|
| **Compound** | **Concentration [ng/mL]** | **Precision [% CV, n = 3]** | **Accuracy [%]** |
| CQ | 11 | 2.5 | 93.6 |
| | 131 | 0 | 91.6 |
| | 1180 | 4.1 | 96.0 |
| CQm | 11 | 2 | 95.1 |
| | 131 | 0 | 90.8 |
| | 1180 | 1.3 | 97.7 |
| AQ | 12 | 3.2 | 94.1 |
| | 101 | 8.4 | 96.2 |
| | 860 | 4.6 | 98.9 |
| AQm | 16 | 3.1 | 96.4 |
| | 116 | 3.9 | 94.8 |
| | 860 | 2.6 | 91.0 |

Good precisions and accuracies are obtained for all concentration levels of the four analytes. Similar results were obtained when working without IS (data not shown).

## Conclusion

- DBS sampling is the technique of choice for anti-malarial investigations as it can overcome blood sampling challenges based on difficult climate and local conditions in malaria-afflicted remote areas.
- A FTD-SPE-LC-MS/MS method for the determination of Chloroquine (CQ), Amodiquine (AQ) and their metabolites Desethylchloroquine (CQm) and Monodesethylamodiaquine (AQm) in DBS has been developed.
- Preliminary validation results with respect to linearity, sensitivity, precision and accuracy are very promising. Further validation measurements regarding recovery, carry-over and method comparison are in progress.
- The combination of flow-through DBS desorption and online SPE-LC-MS/MS results in an efficient automated workflow for therapeutic drug monitoring.

Literature: **[1]** D Blessborn, S Romsing, Y Bergqvist, N Lindegardh. Bioanalysis 2010;2(11),1839–47; **[2]** M Ntale, C Obua, J Mukonzo, M Mahindi, L Gustafsson, O Beck, JW Ogwal-Okeng. Malaria Journal 2009;8(52)