Oxidative Metabolism of Amodiaquine using the ROXY™ EC System

- **Amodiaquine, Camoquin, Flavoquine**
- **Fast mimicking and predicting drug metabolism < 10 min.**
- **Oxidative metabolism (phase I) and adduct formation (phase II)**
- **Ideal for system performance evaluation (reference system)**

**Introduction**

Amodiaquine (AQ) is an antimalarial agent which is used against Plasmodium falciparum, a protzoan parasite which can cause cerebral malaria. Though the drug was withdrawn from the market because of its hepatotoxicity, it is still widely applied for the treatment of Malaria in Africa. Amodiaquine is metabolized to reactive electrophilic metabolites, which are difficult to detect since they are shortlived, and the metabolites can undergo further reactions resulting in stable products.

Amodiaquine (trade names: Camoquin, Flavoquine; IUPAC: 4-((7-chloroquinolin-4-yl)amino)-2-(diethylaminomethyl)phenol) was chosen as a model drug to investigate the nature of the oxidative metabolism using the ROXY EC System.

Electrochemical conversion of the amodiaquine into reactive phase I metabolites and their GSH conjugates were successfully achieved.
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Figure 1: Metabolic pathway of amodiaquine with the 3 most abundant metabolites.

Table 1

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>Monoisotopic mass [u]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amodiaquine (AQ)</td>
<td>C_{20}H_{22}ClN_{3}O</td>
<td>355.14514</td>
</tr>
<tr>
<td>1 (quinoneimine)</td>
<td>C_{20}H_{20}ClN_{3}O</td>
<td>353.12949</td>
</tr>
<tr>
<td>2 (desethyl; quinoneimine)</td>
<td>C_{20}H_{18}ClN_{3}O</td>
<td>325.09819</td>
</tr>
<tr>
<td>3 (bis desethyl; aldehyde)</td>
<td>C_{18}H_{16}ClN_{3}O</td>
<td>298.05091</td>
</tr>
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Method

The ROXY EC System (Figure 2) for compound screening (p/n 210.0070A) includes the ROXY potentiostat equipped with a ReactorCell™, infusion pump and all necessary LC connections. The ROXY EC System is controlled by Antec Dialogue software.

The amodiaquine sample was delivered to the system with a syringe pump equipped with a 1000 μL gas tight syringe. A MicrOTOF-Q (Bruker Daltonik, Germany) with an Apollo II ion funnel electrospray source was used to record mass spectra and MS data were analyzed by Compass software. The relevant mass spectrometer parameters are listed in Table 3. The method was optimized on a 10μM amodiaquine solution. Mass spectrometer calibration was performed using sodium formate clusters at the beginning of the measurements.

Table 2

<table>
<thead>
<tr>
<th>Conditions</th>
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<tbody>
<tr>
<td>EC</td>
<td>ROXY™ EC System (p/n 210.0070)</td>
</tr>
<tr>
<td>Cell</td>
<td>ReactorCell™ with GC WE and HyREF™</td>
</tr>
<tr>
<td>Flow rate</td>
<td>10 μL/min</td>
</tr>
<tr>
<td>Potential</td>
<td>0 – 1500 mV (scan mode)</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>20 mM ammonium formate (pH 7.4 adjusted with ammonium hydroxide) with 50% acetonitrile</td>
</tr>
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The ReactorCell equipped with Glassy Carbon working electrode and HyREF™ reference electrode was used for the generation of amodiaquine metabolites.
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Oxidative metabolism – Phase I
A 10μM amodiaquine solution in 20mM ammonium formate (pH 7.4 adjusted with ammonium hydroxide) with 50% acetonitrile was pumped at a constant flow rate of 10 μL/min through the ReactorCell using an infusion pump. The outlet of the reactor cell was connected directly (online) to the ESI-MS source. The scan mode was used to register the MS Voltammogram with the working electrode potential ramped from 0 – 1500 mV at a scan rate of 10 mV/s in the half cycle. The mass spectra for each change of the cell potential were recorded continuously and saved in one file. The total run time to record the mass voltammogram was approximately 2.5 min. Instrumental set-up of ROXY EC System for oxidative metabolism phase I is shown in Figure 3.

Adduct formation – Phase II
A 10μM amodiaquine solution in 20mM ammonium formate (pH 7.4 adjusted with ammonium hydroxide solution) with 50% acetonitrile was pumped with a constant flow of 10 μL/min through the ReactorCell using an infusion pump. Adduct formation of amodiaquine metabolites and glutathione (GSH) was established using a 100μL reaction coil placed between the ReactorCell and the electrospray source. 100μM glutathione in mobile phase was added at the same flow rate via a T-piece into the coil and the reaction time at the specified flow rate was 5 min. The effluent from the reaction coil was injected directly into the ESI-MS. The instrumental set-up of the ROXY EC System for adduct formation (phase II reactions) is shown in Figure 4. The DC potentials of 400mV and 1200mV were applied to form conjugates with Metabolite 1, and Metabolites 2 and 3 (Fig. 1), respectively.

Results
Phase I
Table 1 provides a list of compounds related to amodiaquine metabolism and their monoisotopic masses used for mass spectra interpretation. The 3-D MS Voltammogram shown for amodiaquine (Fig. 5) is a graphical representation of oxidative pattern of the analyte. The data for the MS Voltammogram were recorded using a scan mode with a potential range between 0 and 1500mV, scanned at a 10mV/s rate in the half cycle (Fig. 6).

The background information about MS Voltammogram acquisition using Dialogue are given in the “Dialogue for ROXY user guide” (P/N 210.7017) and in the application note 210_001A “Event Programming for Automated Recording of MS Voltammograms” for details, see our web.
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The extracted ion chromatograms for the mass-to-charge ratio (m/z) of amodiaquine (m/z of 356) and its metabolites (m/z of 354; 326; 299 and 370) are shown in Figure 6 as a 2-D MS Voltammogram. Based on the 2-D MS Voltammogram (Fig. 6), the optimum potential for the formation of the particular metabolites was estimated as 400mV for amodiaquine dehydrogenation (metabolite 1), and 1200mV for formation of metabolites 2, 3 and 4.

Furthermore if the potential is higher than 1400mV, hydroxylation of Amodiaquine (m/z of 370) was observed. Fig. 7 shows the mass spectra corresponding to ReactorCell OFF (control measurement) with applied voltages of 400mV and 1200mV.

Figure 5: Mass voltammogram of Amodiaquine. Ion abundance versus m/z as a function of EC potential.

Figure 6: Amodiaquine abundance vs. EC potential. The 2-D MS Voltammogram was acquired using scan mode.

Figure 7: Mass spectra of phase I metabolites of Amodiaquine.
Phase II
To confirm the presence of the conjugation products of Amodiaquine metabolites and GSH, mass spectra were acquired with the ReactorCell off and at Ec = 400 mV and 1200 mV. EIC traces of Amodiaquine metabolites (1 and 2) are presented in Fig. 8. Mass spectra obtained with different potentials and a control experiment with ReactorCell OFF are shown in Fig. 9.

![Figure 8: Result of conjugation of phase I metabolites of Amodiaquine with GSH. Example of EICs of Metabolite 1 (m/z 354) and its conjugate (m/z 661) and Metabolite 2 (m/z 326) and its conjugate (m/z 633)](image)

![Figure 9: Mass Spectra of GSH-Metabolite adducts formed at 400 and 1200 mV with m/z 661.2 and 663.2, respectively. The spectrum with ReactorCell OFF confirms that the conjugates are formed only if potential is applied.](image)

Conclusion
The on-line coupling of the ROXY™ EC System with MS (EC/MS) provides a versatile and user-friendly platform for fast screening of target compounds (drugs, pharmaceuticals, pollutants, etc.) for oxidative metabolism (phase 1 reactions), thereby mimicking the metabolic pathway of CYP450 reactions.

MS voltammograms can be recorded automatically to obtain a metabolic fingerprint of the compound of interest in less than 10 min.

In addition, rapid and easy studies of adduct formations can be performed simply by adding GSH after the ReactorCell (phase II reactions).
Oxidative Metabolism of Amodiaquine using the ROXY™ EC System

References

Figure 10: ROXY™ EC System consisting of ROXY Potentiostat, dual syringe pump and ReactorCell.

Ordering number
210.0070A ROXY™ EC system, incl. dual syringe pump, ReactorCell, electrodes and LC connection kit for phase I and II reactions. All parts included for described Electrochemical (EC) application.

Figure 8: Mass spectra of GSH-Metabolite adducts formed at 400 and 1200 mV. EIC results of conjugation of phase I metabolites of Amodiaquine with the ReactorCell (phase II reactions).

Figure 10: ROXY™ EC System consisting of ROXY Potentiostat, dual syringe pump and ReactorCell.
mV with m/z 661.2 and 663.2, respectively. The spectrum with ReactorCell
Figure 9: Mass Spectra of GSH-Metabolite adducts formed at 400 and 1200
corresponding to Amodiaquine metabolites (1 and 2) are presented in
Phase II and Metabolite 2 (m/z 326) and its conjugate (m/z 633).

Figure 9: Mass Spectra of GSH-Metabolite adducts formed at 400 mV and 1200 mV. EIC
diaquine metabolites and GSH, mass spectra were acquired
using the ROXY™ EC System.

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