



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 protozoan 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 Though the drug was withdrawn from the market because of its hepatotoxicity, it is still widely applied for the treatment of Malaria in Africa.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.

Electrochemical Reactions upfront MS – EC/MS

Proteomics & Protein Chemistry

S-S bond reduction
HDX
Peptide bond cleavage
Na+, K+ removal
Drug-protein binding

Lipidomics & Fatty Acids

Cholesterol
Oxysterol
FAME Biodiesel

Drug Metabolism

Mimicking CYP 450
Phase I & II
Biotransformation

Synthesis (mg)

Metabolites &
Degradants

Pharmaceutical Stability

Purposeful degradation
API testing
Antioxidants

Environmental

Degradation &
persistence
Transformation products
Surface & drinking water

Food & Beverages

Oxidative stability
Antioxidants

Forensic Toxicology

Designer drugs
Illicit drugs

Healthcare & Cosmetics

Skin sensitizers

Genomics

DNA Damage
Adduct formation
Nucleic acid oxidation

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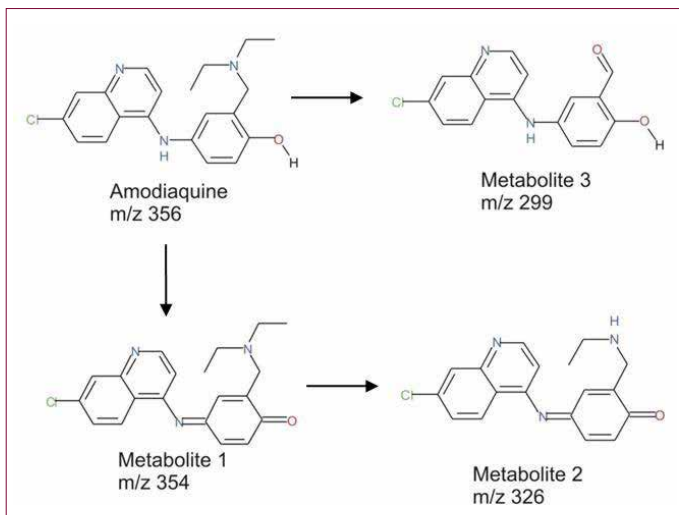


Figure 1: Metabolic pathway of amodiaquine with the 3 most abundant metabolites.

Table 1

Amodiaquine and its (selected) metabolites		
Name	Formula	Monoisotopic mass [u]
Amodiaquine (AQ)	C ₂₀ H ₂₂ ClN ₃ O	355.14514
1 (quinoneimine)	C ₂₀ H ₂₀ ClN ₃ O	353.12949
2 (desethyl; quinoneimine)	C ₁₈ H ₁₆ ClN ₃ O	325.09819
3 (bis desethyl; aldehyde)	C ₁₆ H ₁₁ ClN ₂ O ₂	298.05091

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.

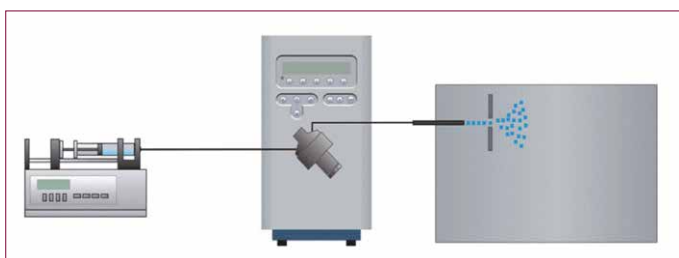


Figure 2: Instrumental set-up of ROXY EC System for oxidative metabolism phase I.

The ReactorCell equipped with Glassy Carbon working electrode and HyREF™ reference electrode was used for the generation of amodiaquine metabolites.

Table 2

Conditions	
EC	ROXY™ EC System (p/n 210.0070)
Cell	ReactorCell™ with GC WE and HyREF™
Flow rate	10 µL/min
Potential	0 – 1500 mV (scan mode)
Mobile phase	20 mM ammonium formate (pH 7.4 adjusted with ammonium hydroxide) with 50% acetonitrile

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

Conditions	
Name	Formula
Mass range	50 – 1000 m/z
Ion polarity	Positive
Capillary voltage	-4500 V
Nebulizer	1.6 Bar
Dry gas	8 L/min
Temperature	200 °C
ISCID energy 100 Vpp	0 eV
Hexapole	100 Vpp
Ion energy	5 eV

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Oxidative metabolism – Phase I

A 10 μ M 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 μ L/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.

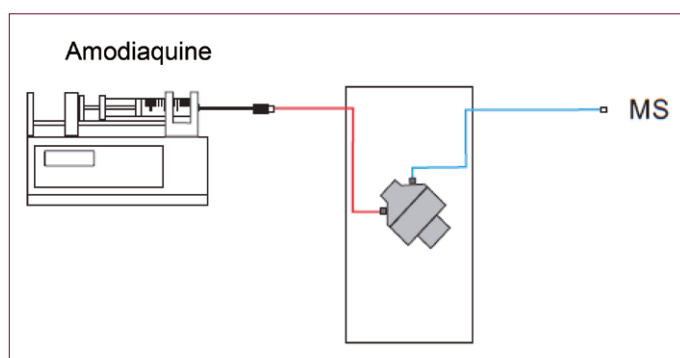


Figure 3: Instrumental set-up of ROXY EC System for oxidative metabolism phase I.

Adduct formation – Phase II

A 10 μ M 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 μ L/min through the ReactorCell using an infusion pump. Adduct formation of amodiaquine metabolites and glutathione (GSH) was established using a 100 μ L reaction coil placed between the ReactorCell and the electrospray source. 100 μ M 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.

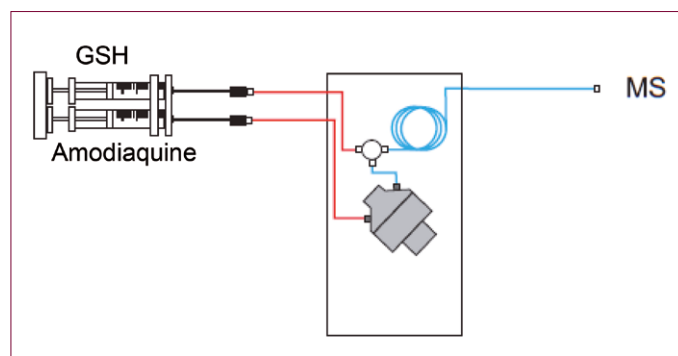


Figure 4: Instrumental set-up of ROXY EC System generating the oxidative metabolites in the ReactorCell (phase I) and subsequent addition of glutathione via a T-piece for GSH-adduct formation (phase II).

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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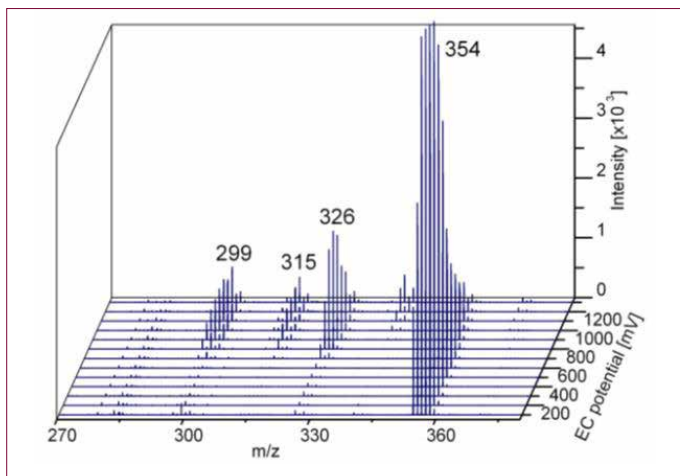


Figure 5: Mass voltammogram of Amodiaquine. Ion abundance versus m/z as a function of EC potential.

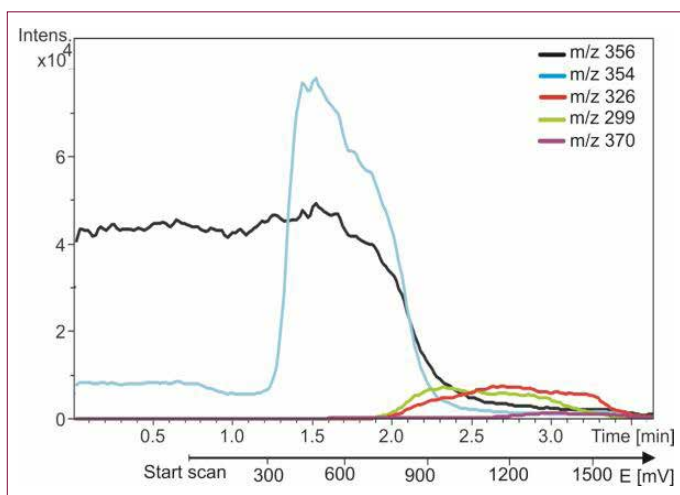


Figure 6: Amodiaquine abundance vs. EC potential. The 2-D MS Voltammogram was acquired using scan mode.

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.

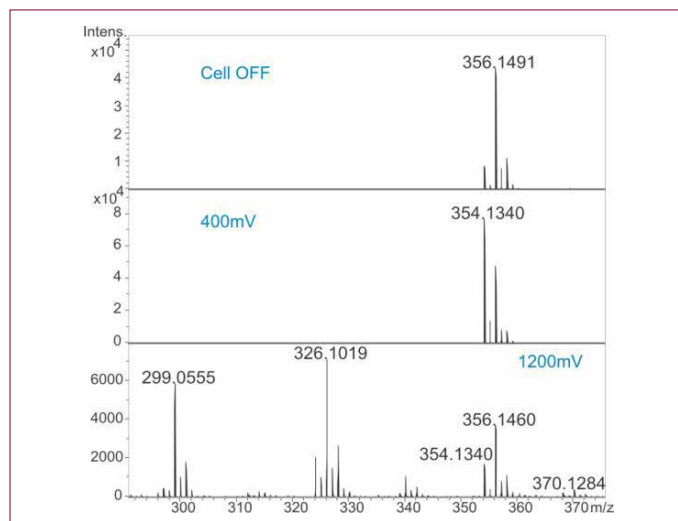


Figure 7: Mass spectra of phase I metabolites of Amodiaquine.

Oxidative Metabolism of Amodiaquine using the ROXY™ EC System



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 $E_c = 400$ 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.

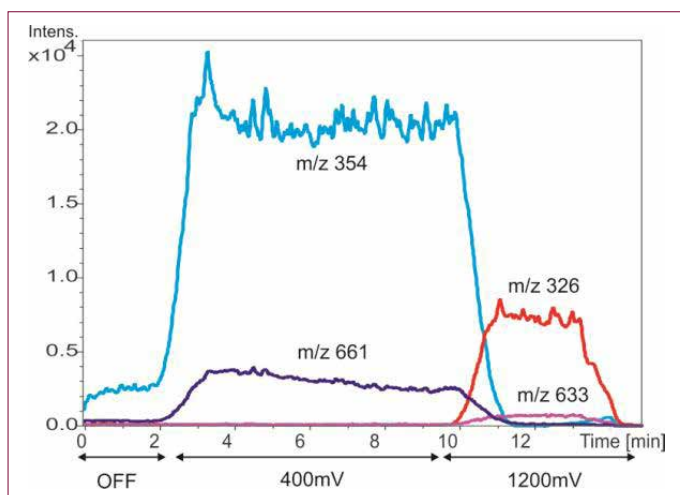


Figure 8: Result of conjugation of phase I metabolites of Amodiaquine with GSH. Example of EICs of Metabolite 1 (m/z 354) and its conjugate (m/z 661) and Metabolite 2 (m/z 326) and its conjugate (m/z 633)

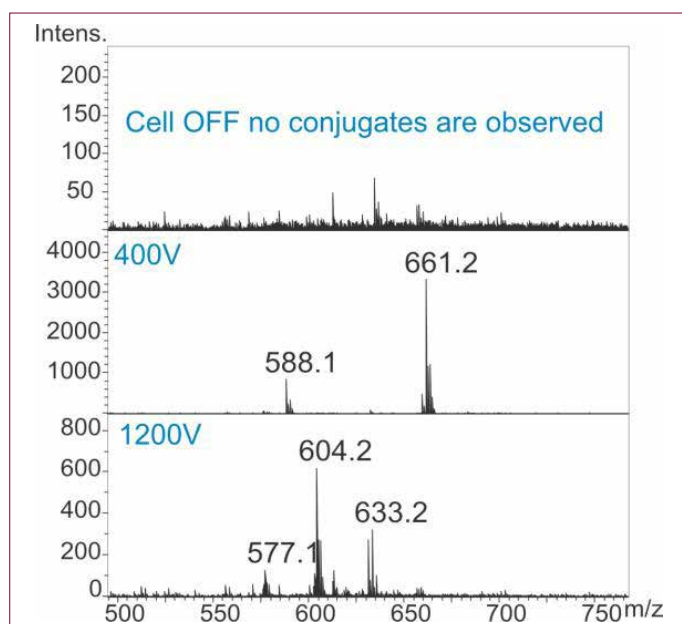


Figure 9: Mass Spectra of GSH-Metabolite adducts formed at 400 and 1200 mV with m/z 661.2 and 663.2, respectively. The spectrum with ReactorCell OFF confirms that the conjugates are formed only if potential is applied.

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).

References

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2. Lohmann W., Hayen H., Karst U., Covalent Protein Modification by Reactive Drug Metabolites Using Online Electrochemistry/Liquid Chromatography/Mass Spectrometry, Anal. Chem.,80, 2008, 9714–9719
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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.
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