

OXIDATIVE METABOLISM OF AMODIAQUINE USING THE ROXY™ EC SYSTEM



PHARMACEUTICAL & BIOTECH ANALYSIS EVER FORMULATED

Aminoglycosides

Amikacin
Framycetin Sulphate
Gentamicin Sulphate
Kanamycin Sulphate
Lincomycin
Neomycin
Spectinomycin
Tobramycin

PET imaging tracer

FDG

Macrolide antibiotics

Azithromycin
Azaerythromycin
Clarithromycin
Erythromycin
Roxithromycin

Bioanalysis of pharmaceuticals

Acetaminophen
Artemisinin
Dihydro-artemisinin
Artemether
Etoposide
8-OH-DPAT
mesna BNP7787
Vincristine

INTRODUCTION

Amodiaquine (AQ) is an anti-malarial agent which was used against Plasmodium falciparum, a protozoan parasite which can cause cerebral malaria. The drug was withdrawn from the market because of its hepatotoxicity. Amodiaquine is metabolized to reactive electrophilic metabolites, which are difficult to detect since they are short-lived, and the metabolites can undergo further reactions resulting in stable products. Amodiaquine (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 dedicated for single component screening.

Electrochemical conversion of the amodiaquine into reactive phase I metabolites and their GSH conjugates were successfully achieved.

- Amodiaquine
- Simulating Cytochrome Oxidation using on-line EC/MS
- Phase I and II Oxidative Metabolism
- Versatile and User-Friendly Platform
- System performance evaluation

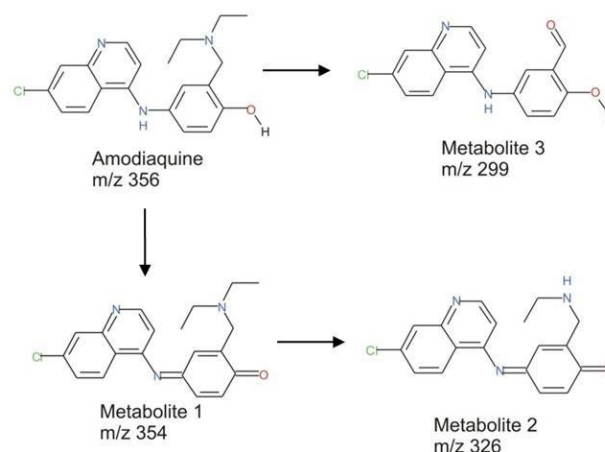


Fig. 1. Fragment of the metabolic pathways of amodiaquine.

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 single compound screening (p/n 210.0070) 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.



Fig. 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.

Conditions	
EC	ROXY™ EC System (p/n 210.0070)
Cell	ReactorCell™ with GC WE and HyREF™
Flow rate	10 μ L/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 μ L 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 10 μ M amodiaquine solution. Mass spectrometer calibration was performed using sodium formate clusters at the beginning of the measurements.

MS settings	
Parameter	Value
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	0 eV
Hexapole	100 Vpp
Ion energy	5 eV

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 Reactor-Cell 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.

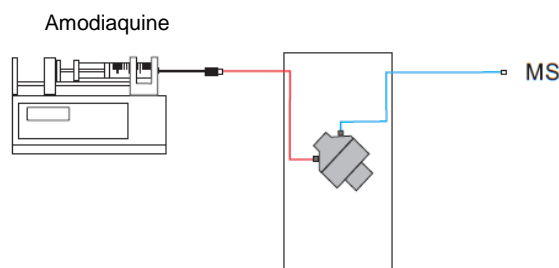


Fig. 3. Instrumental set-up of ROXY EC System for oxidative metabolism phase I.

Oxidative metabolism – 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 oxidative metabolism phase II 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.

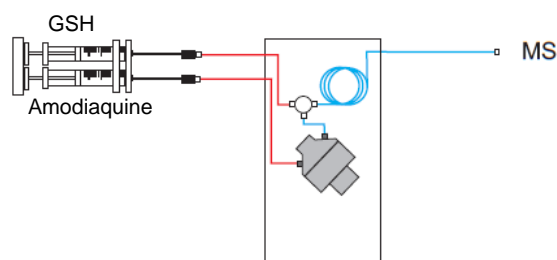


Fig. 4. Instrumental set-up of ROXY EC System for oxidative metabolism phase II.

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).

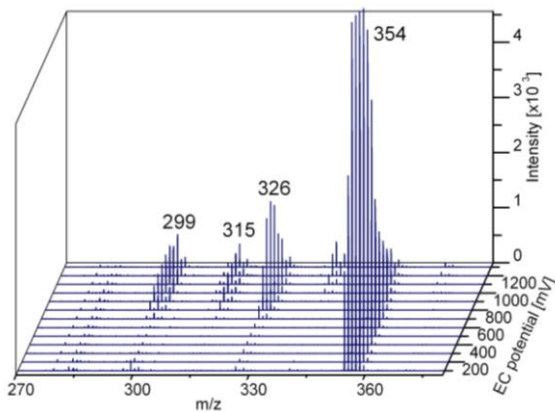


Fig. 5. Mass voltammogram of Amodiaquine. Ion abundance versus m/z as a function of EC potential.

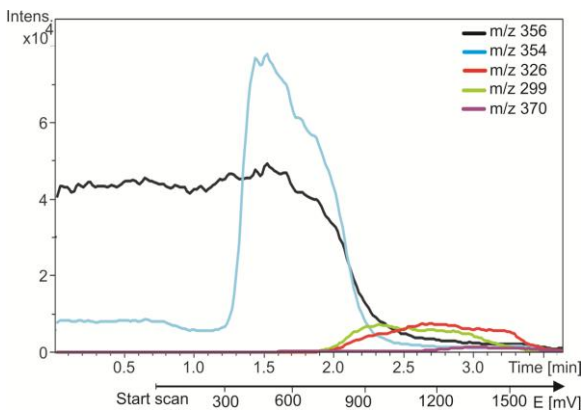


Fig. 6. Amodiaquine abundance vs. EC potential. The 2-D MS Voltammogram was acquired using scan mode.

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.

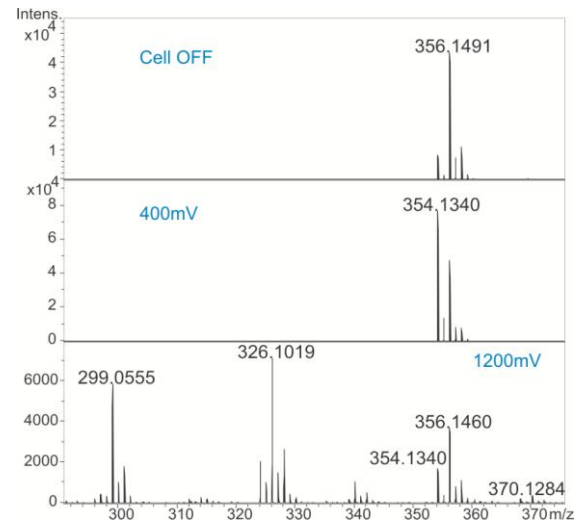


Fig. 7. Mass spectra of phase I metabolites of Amodiaquine.

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 $E_c = 400$ 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.

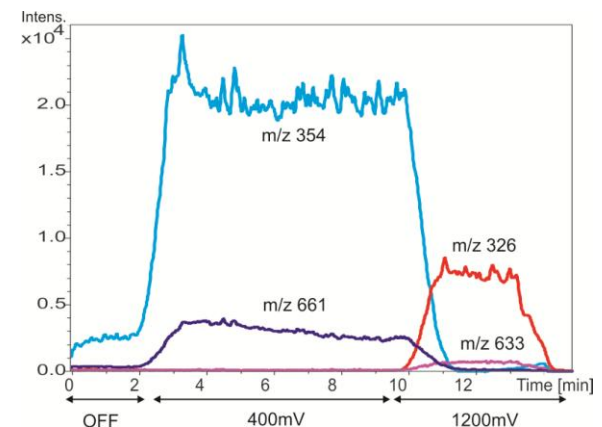


Fig. 8. Result of conjugation of phase I metabolite of Amodiaquine and GSH. Example of EICs of Metabolite 1 (m/z 354) and its conjugate (m/z 661) and Metabolite 2 (m/z 326) and its conjugate (m/z 633)

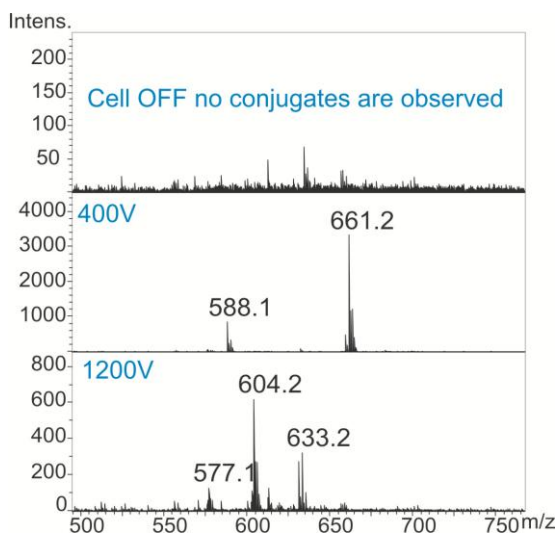


Fig. 9. The mass spectra of conjugation the products formed with different potential. The spectrum with ReactorCell OFF confirms that the conjugates are formed ONLY if potential is applied.

CONCLUSION

The ROXY™ EC System provides a versatile and user-friendly platform for screening of single target compounds (drugs, pharmaceuticals, herbicides, etc.) in phase I and II metabolism studies. MS Voltammograms can be obtained in very short time frame (less than 3 min) and provide a metabolic fingerprint of the compound of interest.

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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Fig. 10. ROXY™ EC System.

PART NUMBERS

210.0070	ROXY™ EC System
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