

Simulation of Oxidative Cytochrome P450 Metabolism Reactions of Amodiaquine by Means of Electrochemistry / Liquid Chromatography / Mass Spectrometry (EC/LC/MS)

Sebastian Götz¹, Wiebke Lohmann², Gabriela Zurek¹, Andreas Germanus¹, Uwe Karst²

¹ Bruker Daltonik GmbH, Fahrenheitstr.4, 28359 Bremen, Germany

² University of Muenster, Institute of Inorganic and Analytical Chemistry, Corrensstrasse 30, 48149 Muenster, Germany

Introduction

The complete characterization of all metabolic reaction products plays an important role in the development of a new chemical entity. This process usually involves the use of living cells, liver microsomes, blood serum and/or certain enzyme mixtures.

A fast and easy approach to simulate the oxidative cytochrome P450 metabolism is the electrochemical conversion of drug compounds. For this purpose the respective compound is pumped through an electrochemical flow cell to produce the desired metabolites.

In this work Amodiaquin, an anti-malaria drug known for its toxic metabolic side effects, is electrochemically converted and analyzed by LC/MS.

The results of the electrochemical conversion are compared to natural metabolism reactions in the human body.

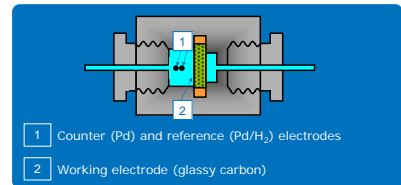


Fig. 1 Electrochemical flow cell

Experimental

An electrochemical flow cell with a glassy carbon working electrode (fig. 1, ESA Biosciences, Chelmsford, MA) is used to mimic the cytochrome P450 metabolism pathways. Prior to separation the Amodiaquine solution (pH 7.4) is pumped through the cell (50 µL/min) at a constant potential of 700 mV. Under these conditions, the very large surface area of the flow cell electrode allows for an almost quantitative conversion of the pharmaceutical compound to its respective oxidized metabolites. All yielded products are separated using a HP 1200 chromatography system (Agilent Technologies, Palo Alto, CA) and subsequently analyzed by a micrOTOF ESI-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) in ESI-positive mode (m/z 90 – 1000) using external calibration. MetaboliteTools_{2.0} was used to compare two full scan LC/MS chromatograms (oxidized sample and reference) using the eXpose™ algorithm to detect all compounds

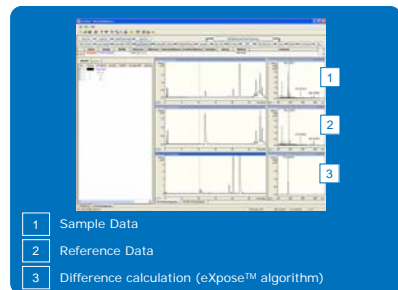


Fig. 2 MetaboliteDetect_{2.0} for the unambiguous detection of metabolites

exclusively present in the sample (fig. 2). Accurate mass spectra combined with true isotopic pattern information are used to unambiguously identify the generated metabolites.

Additionally a second sample containing glutathione (GSH) was electrochemically oxidized to enable the generation of phase II metabolites (GSH-adducts).

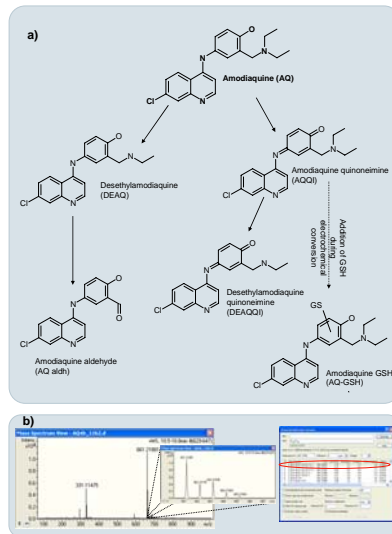


Fig. 3 a) Structures of Amodiaquine and its observed metabolites. b) Exemplary MS-spectrum of AQ-GSH showing isotopic pattern and mass accuracy results

Results

Under given conditions several electrochemical conversion products could be observed (fig. 3a). Amodiaquine is known to form a reactive quinone imine^[1] (AQOI), which is found as the main metabolite. (bis-)Desethylation is a common metabolic conversion seen in rat liver microsome incubations^[2]. While the des-ethylated DEAO (and its quinone imine DEAOOI) could also be observed with electrochemistry, the bis-desethylated form of AQ could not be reproduced.

Instead AQ aldehyde, a possible further oxidation product of the bis-desethylated AQ, could be observed.

Former studies showed that the reactive intermediate AQOI could not be observed *in vivo*, as it was readily reacted with glutathione (GSH), forming the AQ-GSH adduct, which is the major metabolite of AQ in rat bile^[3].

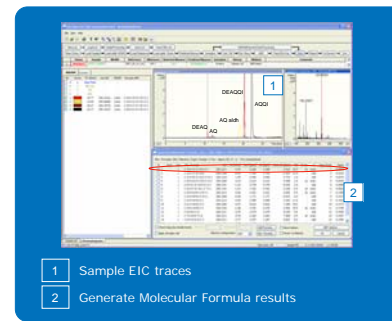


Fig. 4 a) Extracted ion chromatograms of detected metabolites

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To be able to mimic these phase II metabolic conversions, GSH was added to the amodiaquine solution prior to the electrochemical conversion. This way during the *in vitro* metabolization the reactive AQOI could react immediately with the GSH, forming the respective GSH adduct. Figure 3b shows the mass spectrum of the detected AQ-GSH (fig 3b).

[1] Maggs, J. L.; Tingle, M. D.; Kitteringham, N. R.; Park, B. K. *Biochem. Pharmacol.* 1988, 37, 303-311.

[2] Jewell, H.; Maggs, J. L.; Harrison, A. C.; O'Neill, P. M.; Ruscoe, J. E.; Park, B. K. *Xenobiotica* 1995, 25, 199-217

[3] Harrison, A. C.; Kitteringham, N. R.; Clarke, J. B.; Park, B. K. *Biochem. Pharmacol.* 1992, 43, 1421-1430

Conclusions

The oxidative metabolism of amodiaquine could successfully be simulated by means of EC/LC/MS. Several metabolites produced with rat liver microsomes, could also be found using the described electrochemical conversion method.

To further mimic the *in vivo* generation of phase II metabolites, glutathione was added to the amodiaquine solution prior to oxidation, leading to the successful generation of the respective amodiaquine-GSH adduct.