On-line Electrochemistry/MS - A Powerful Tool for Fast Prediction of Phase I and II Drug Metabolism

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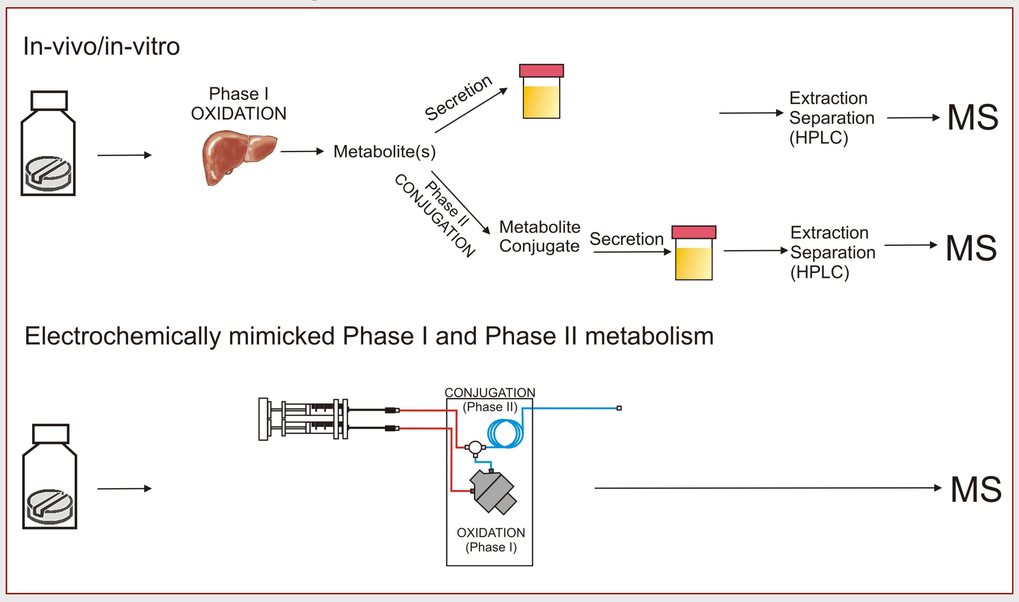
Electrochemistry vs. in-vitro:



- ☺ seconds vs. weeks
- ☺ no isolation/clean-up
- ☺ phase I and II metabolism
- ☺ complementary to in vivo
- ☺ saving rodents (rats, mice)

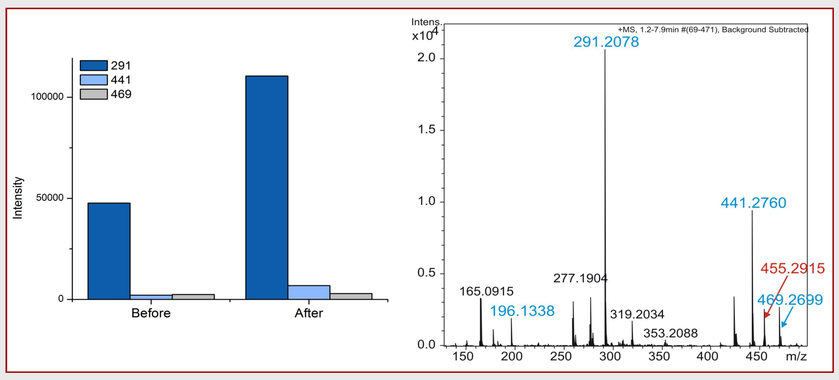
Methods / Instrumentation

An analytical and preparative electrochemical cell (Antec) equipped with a glassy carbon (GC) or conductive diamond (MD) working electrode were used for the oxidation of drug compounds. The cell potential was ramped from 0 to 2V (GC) or to 3.5 V (MD) during the experiments. The outlet of the electrochemical cell was connected directly to the electrospray source of a MicrOTOF-Q (Bruker Daltonics, Germany). Typically 10 µM solutions in ammonium formate (acetate)/acetonitrile are pumped through the electrochemical cell at a flow rate of 10—50 µL/min. For the formation of GSH adducts, 50 – 100 µM GSH was added after the EC cell.

Figure 1: ROXY™ EC system (left). ReactorCell™ and different WE (right).

Figure 2: µ-PrepCell™ and different working electrodes (µ-PrepCell vs. ReactorCell).

Electrochemical Activation of the Electrodes



- Simple and automated procedure
- Quick (6 minutes)
- No need for additional solvents only mobile phase
- No need for cell disassembly
- Applicable for MD and GC electrodes

Figure 3: Verapamil metabolites before and after MD activation procedure (left). Mass spectrum of Verapamil after oxidation in the µ-PrepCell (right). Blue — known metabolites; Red — parent ion.

Scan Mode for Efficient Metabolite Synthesis

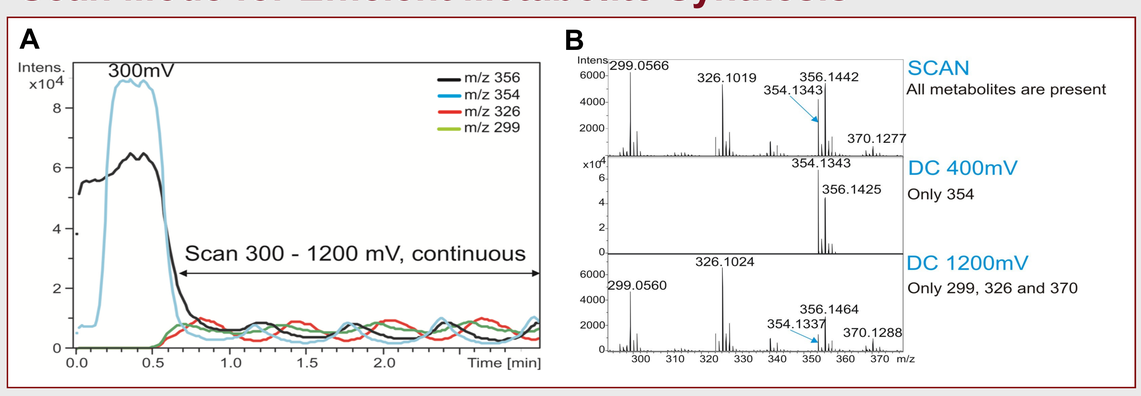


*Figure 4**(A):** Principle of using scan mode for stable oxidation. Amodiaquine metabolites (µ-PrepCell; 50µL/min). **(B):** Mass spectra corresponding to scan and DC modes for metabolites synthesis.*

- All metabolites are synthesized in one run
- Stable oxidation conditions for a prolonged period of time
- No need for electrode maintenance during oxidation of highly concentrated samples

Results

Amodiaquine was chosen as model drug to investigate 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.

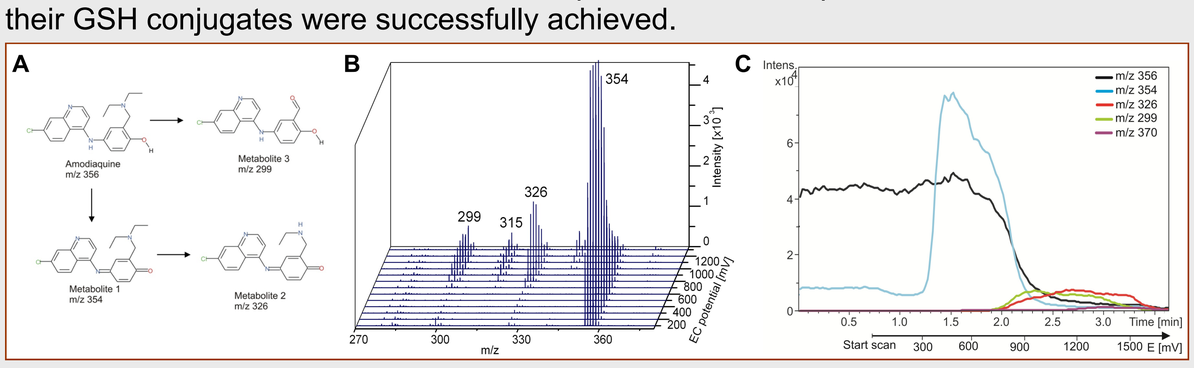


*Figure 5 **(A):** Metabolic pathway of Amodiaquine (selected metabolites). **(B):** 3-D MS Voltammogram of Amodiaquine. The MS Voltammogram represents the metabolic fingerprint of the molecule. The voltammogram was recorded using an event table in Dialogue™ software (Antec). **(C):** 2-D MS Voltammogram of Amodiaquine (Scan mode).*

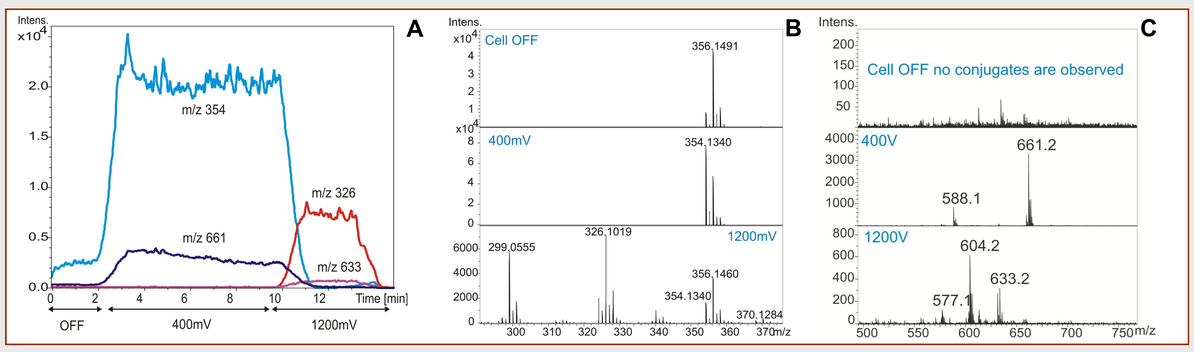


*Figure 6 **(A):** Result of conjugation of phase I metabolites of Amodiaquine and GSH. Example of EICs of Metabolite 1 (m/z 354) and its conjugate (m/z 661) and Metabolite 2 (m/z 326) and its conjugate (m/z 633). Conjugation was performed in DC mode. **(B):** Mass spectra of phase I metabolites of Amodiaquine. **(C):** The mass spectra of the conjugation products formed with different potential. The spectrum with cell OFF confirms that the conjugates are formed ONLY if potential is applied.*

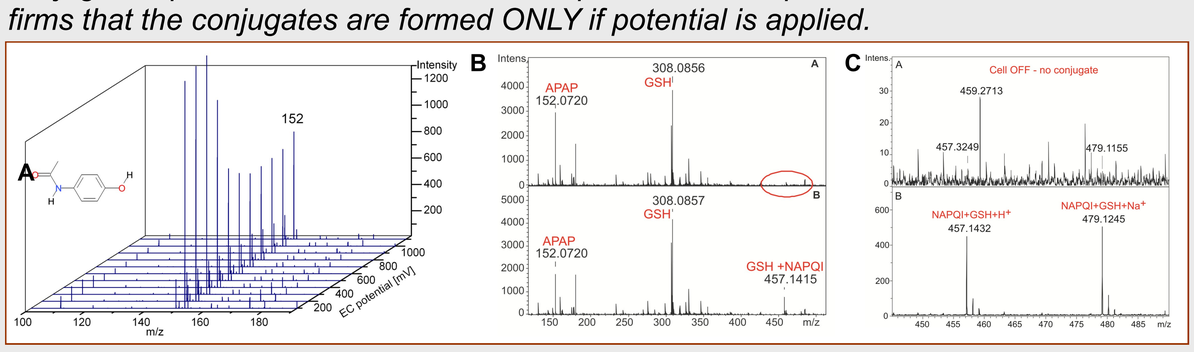


*Figure 7 **(A):** MS Voltammogram of acetaminophen. **(B):** Result of conjugation of phase I metabolite NAPQI and GSH. **(C):** Zoom in of mass range from m/z of 445 to 490. Peak at m/z of 457.1432 corresponds to protonated ion of conjugation product. Peak of m/z of 479.1245 was identified as its Na+ adduct. (A.) Cell OFF, (B.) Cell EC=800mV.*

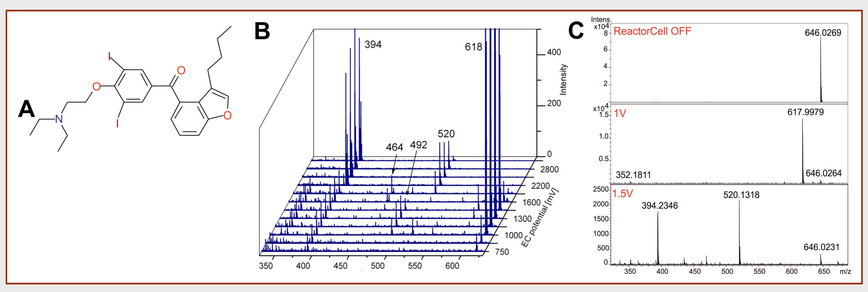


*Figure 8 **(A)**: Structure, **(B)**: MS Voltammogram and **(C):** mass spectra of Amiodarone.*

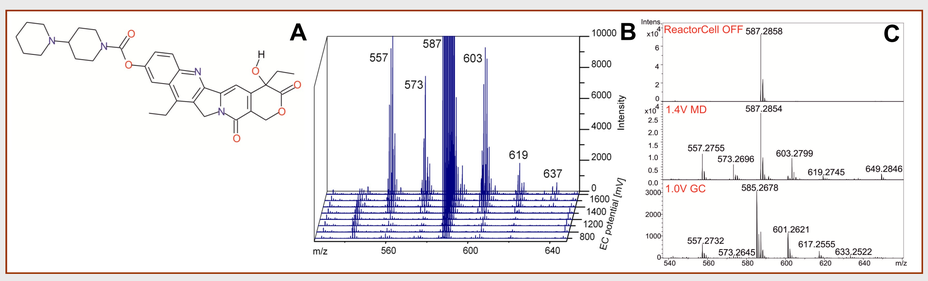


*Figure 9 **(A)**: Structure, **(B)**: MS Voltammogram and **(C):** mass spectra of Irinotecan.*

Conclusions

Amiodaquine was successfully used as model drug to mimic the oxidative metabolic detoxification pathway in the human liver by on-line EC/MS. Phase I and II metabolites, which were already known from the literature as detoxification products *in vivo*, were generated in the EC reactor cell and on-line identified by MS using either amodiaquine alone or in the presence of glutathione. Furthermore, the oxidation pathway was successfully simulated for amiodarone, acetaminophen and irinotecan, and many other compounds, shown in earlier posters. These results clearly illustrate the potential of EC/MS as a powerful tool for predicting metabolic processes. The ROXY™ EC system provides a versatile and user friendly platform for studying phase I and II metabolism of target compounds (drugs, pharmaceuticals, herbicides, *etc.*) including the synthesis thereof by dedicated µ-preparative flow cells.

Acknowledgements

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References

[1] Lohmann W. et al., LC-GC Europe January (2010) 1
[2] Hoffmann T. et al., *Anal Bioanal Chem* 399(5) (2011) 1859
[3] Baumann A. et al., *J Chromatogr A* 22 (2009) 286