Electrochemical Simulation of Redox Reactions in Drug Metabolism

Agnieszka Kraj, Hendrik-Jan Brouwer, Nico Reinhoud, Jean-Pierre Chervet
Antec, Zoeterwoude, The Netherlands

Introduction

For almost two decades electrochemistry (EC) has been successfully coupled to mass spectrometry. The electrochemical cell is used as a reactor in which a controlled oxidation or reduction takes place prior to MS detection. The oxidation products show excellent agreement with cytochrome P450 reaction products in nature (e.g., liver), mimicking the enzymatic Phase I biotransformation (biomimetic oxidation). This purely instrumental approach is making the use of costly enzymes and the risk of non-specific reactions, obsolete. The reaction products are formed instantaneously in the EC cell allowing for direct coupling with MS and the measurement of short-lived compounds. Significant time and cost savings result using EC/MS compared to current in vitro (microsomes) or in vivo (rodents) approaches.

Methods / Instrumentation

All experiments were performed on ROXY EC system (Antec, The Netherlands) consisting of a Potentiostat, equipped with an electrochemical reactor cell and an infusion pump (Harvard Apparatus, USA). A preparative electrochemical cell (µ-PrepCell, Antec, The Netherlands) equipped with a Glassy Carbon (GC) or Magic Diamond (MD) working electrode were used in the experiments. For the oxidative fingerprint of the selected drug compounds, typically 10 µM solutions in 20mM ammonium formate/acetonitrile (50/50, v/v) were pumped through the electrochemical cell at a flow rate of 50 µL/min. Automated user defined programs were used to find the optimal potential. A Bruker HCT plus (Bruker Daltonics, Germany) mass spectrometer equipped with electrospray (ESI) source was used to monitor the oxidation products during the optimization steps.

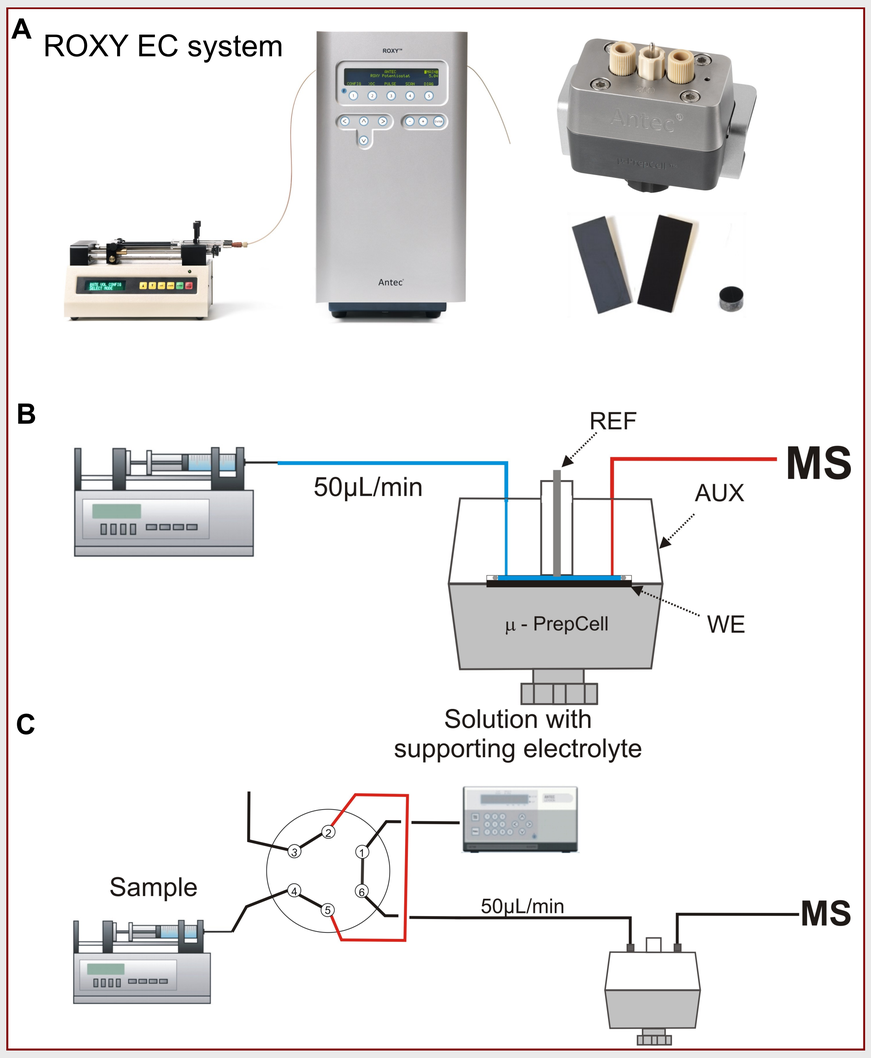

*Figure 1: **A:** ROXY™ EC system. µ-PrepCell and comparison of working electrodes (µ-PrepCell vs. Reactor-Cell). **B:** Set up used for direct infusion experiments. C. Set up used in flow injection experiments.*

Prediction of Drug Metabolism (I)

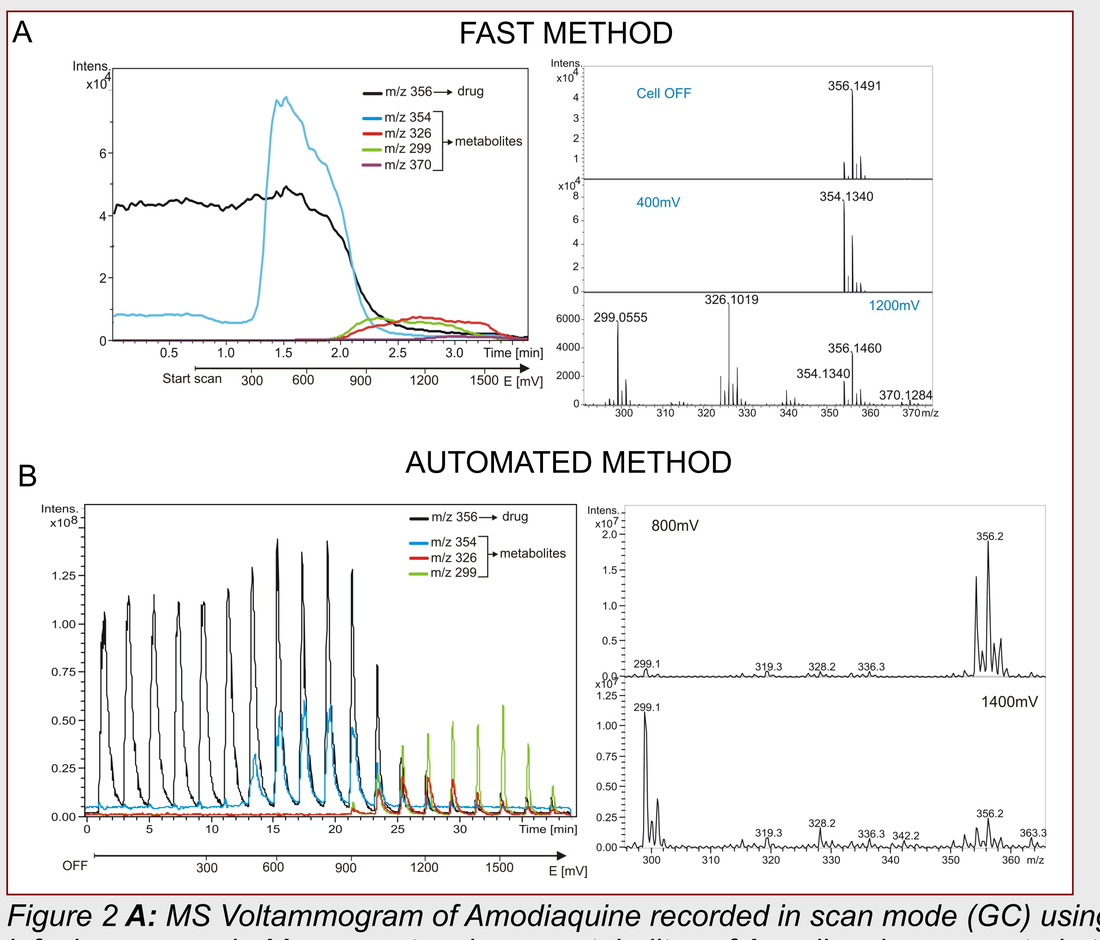

*Figure 2 **A:** MS Voltammogram of Amodiaquine recorded in scan mode (GC) using direct infusion approach. Mass spectra shows metabolites of Amodiaquine generated at different potentials. **B:** MS Voltammogram of Amodiaquine recorded in DC mode (MD). 2µL of Amodiaquine was injected. Potential was ramped from 0 –1700 mV with incremental steps of 100mV every 2 minutes. Mass spectra shows the metabolites of Amodiaquine formed at 800mV and 1400mV. This measurement was performed using FIA approach and was fully automated by using event program in Dialogue.*

Prediction of Drug Metabolism (II)

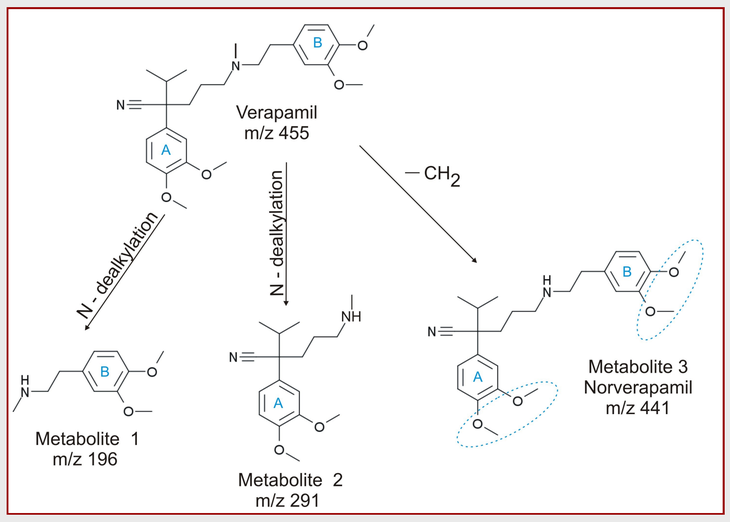

Figure 3: An excerpt of the Verapamil metabolic pathway. Blue dotted ellipses are indicating other places of possible loss of CH_2.

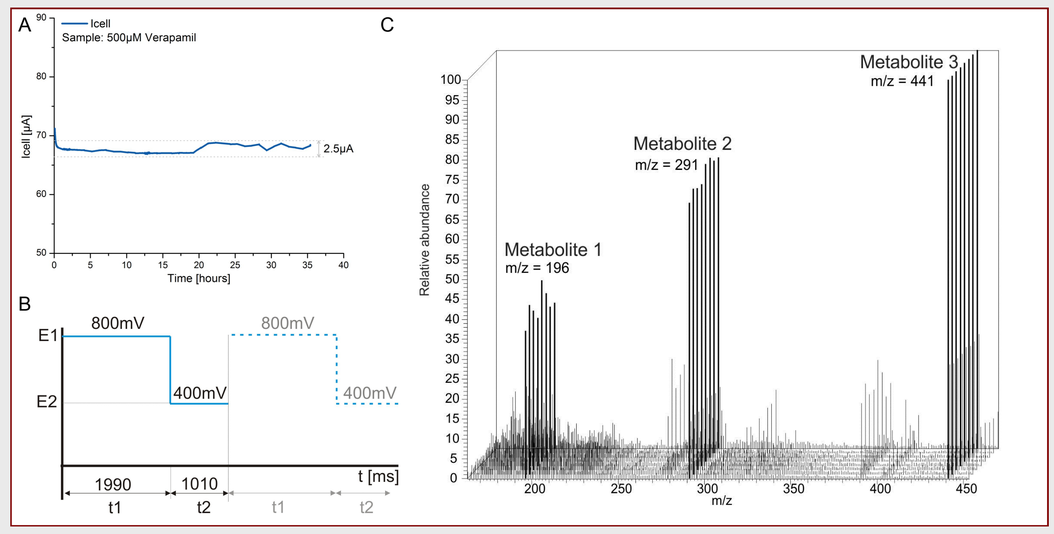

*Figure 4: **A:** Long term current stability experiment. **B:** Optimized pulse settings. **C:** An overlay of 8 mass spectra of the 100x diluted control samples. GC electrode.*

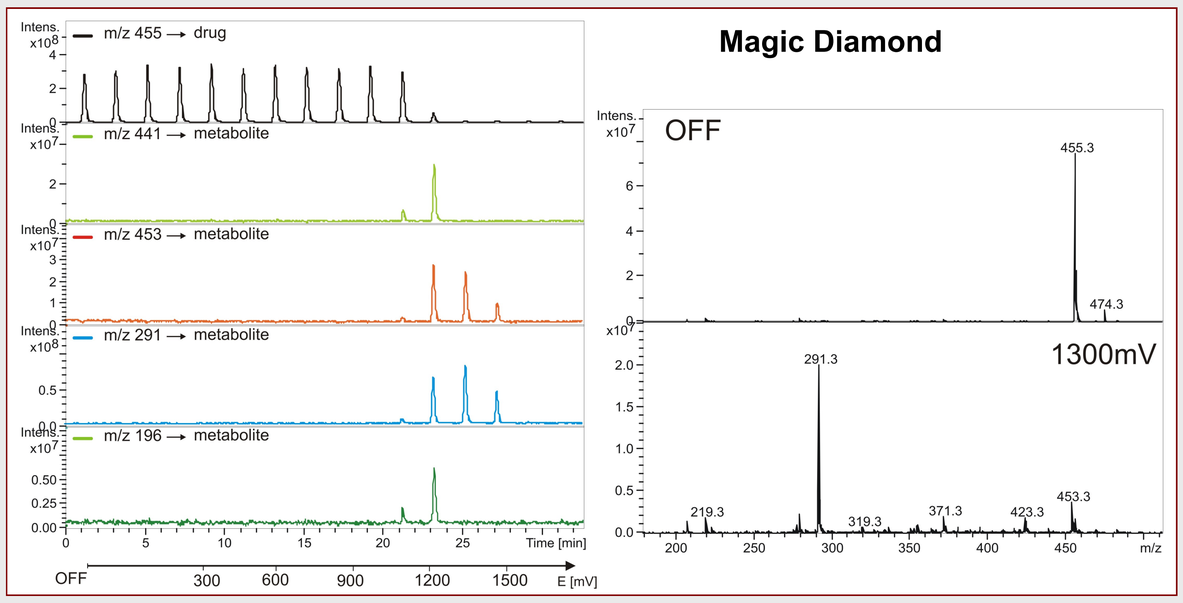

Figure 5: MS Voltammogram of Verapamil and its metabolites. Mass spectrum is corresponding to OFF and 1300mV. The experiment was automated. MD electrode was used.

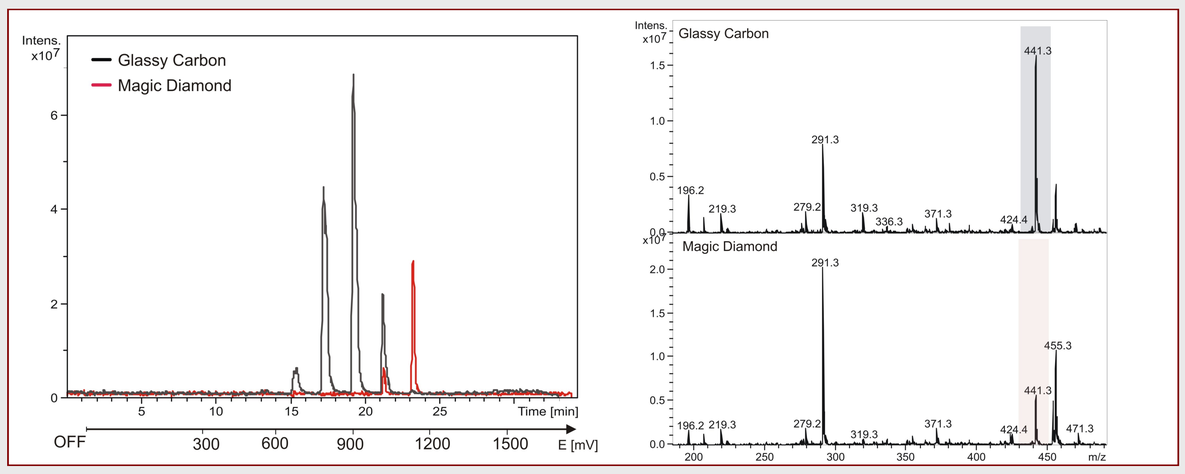

Figure 6: MS Voltammogram of Norverapamil (m/z = 441) registered on MD & GC electrodes. Mass spectrum corresponds to optimal potential for formation of this metabolite on both electrodes, respectively.

Conclusions

Using the ROXY™ EC system on-line with MS results in fast generation of metabolites (seconds vs. days or weeks using in-vitro and/or in-vivo methods), access to phase II reactions as well as reactive metabolites. Amiodaquine and Verapamil was successfully used as model drug to mimic the oxidative metabolic 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.
The data demonstrate that hyphenation of electrochemistry with electrospray mass spectrometry provides a versatile and user-friendly platform for rapid and cost efficient screening of target compounds (drugs, xenobiotics, etc.) in phase I and phase II metabolomics studies.

References

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[2] Lohmann W., et al., *LC-GC Europe.* January (2010) 1
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