Mimicking Drug Metabolism by EC/MS

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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 biotransformation (biomimetic oxidation). This purely instrumental aproach 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. A preparative electrochemical cell (µ-PrepCell, Antec, The Netherlands) equipped with a Glassy Carbon (GC) or Magic Diamond (BDD) 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 50 µL/min. Automated user defined prgrams 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.

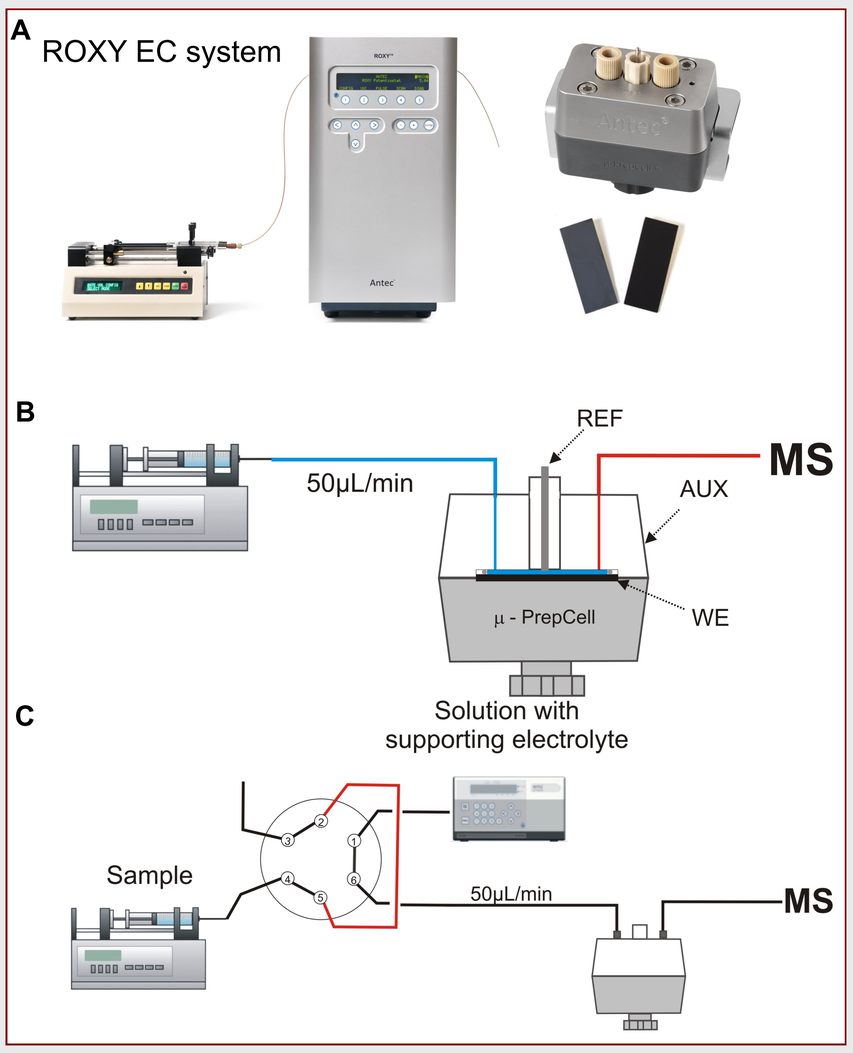

Figure 1:
***A:** ROXY™ EC system. µ-PrepCell and comparison of working electrodes, GC vs. BDD*
***B:** Set-up used for direct infusion experiments.*
***C**: Set up used in flow injection experiments, EC/MS and EC/LC/MS.*

Mimicking Metabolism of Amodiaquine

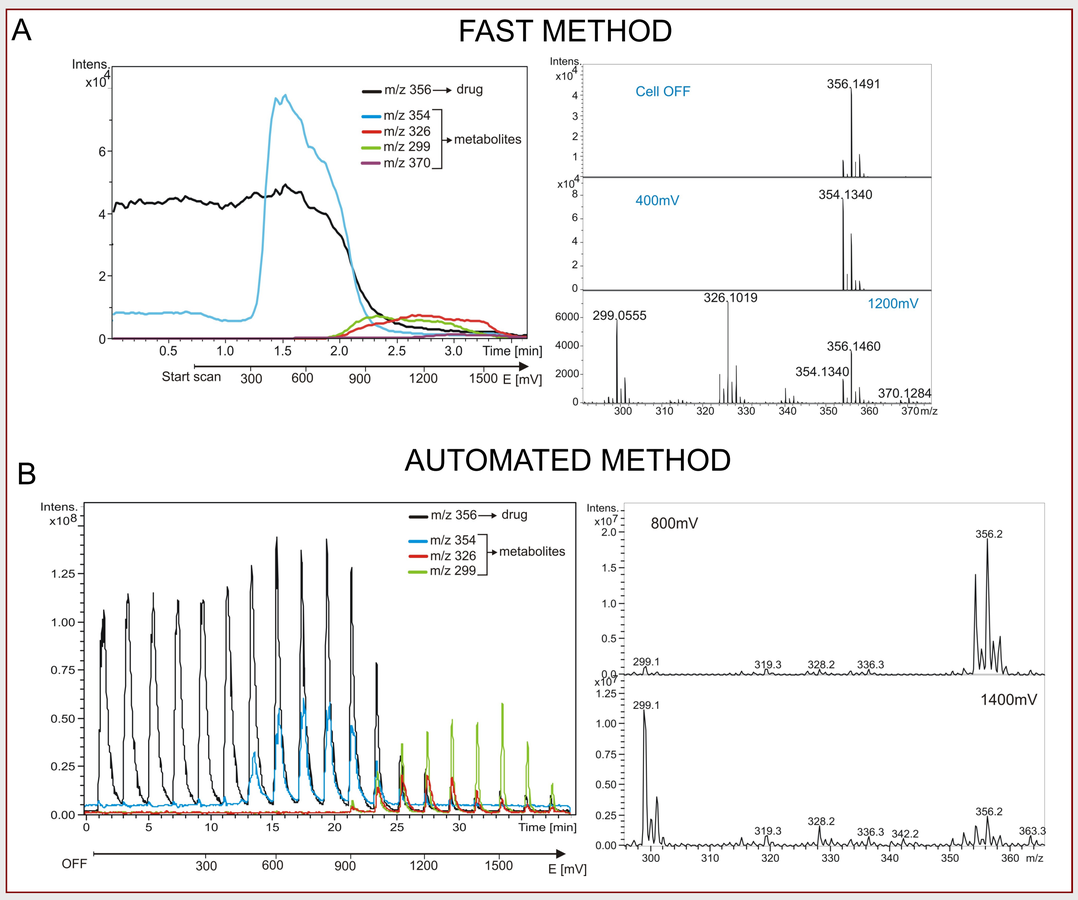

*Figure 2: **A:** MS Voltammogram of Amodiaquine recorded in fast scan mode (GC) using direct infusion approach. Mass spectra shows metabolites of Amodiaquine generated at different potentials. **B:** MS Voltammogram of Amodiaquine recorded in DC mode (MD). 2µL of Amodiaquine was injected. Potential 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.*

Mimicking Metabolism of Verapamil & Norverapamil

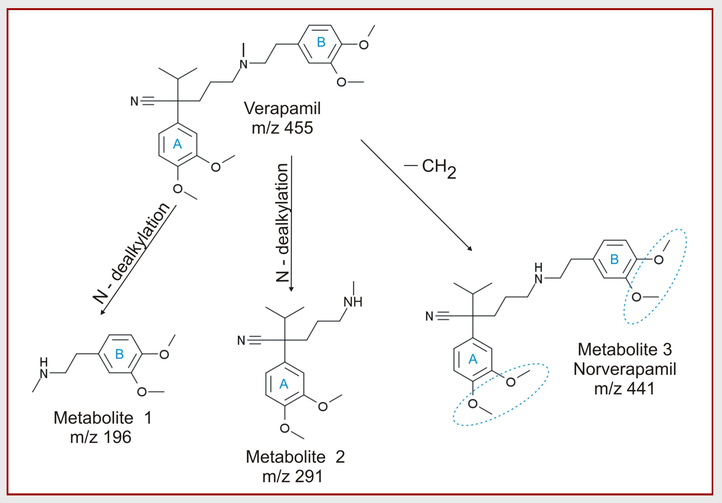

Figure 3: Excerpt of Verapamil metabolic pathway with 3 major metabolites: m/z 196, m/z 291 and m/z 441
Blue dotted ellipses are indicating other places of possible loss of CH_2.

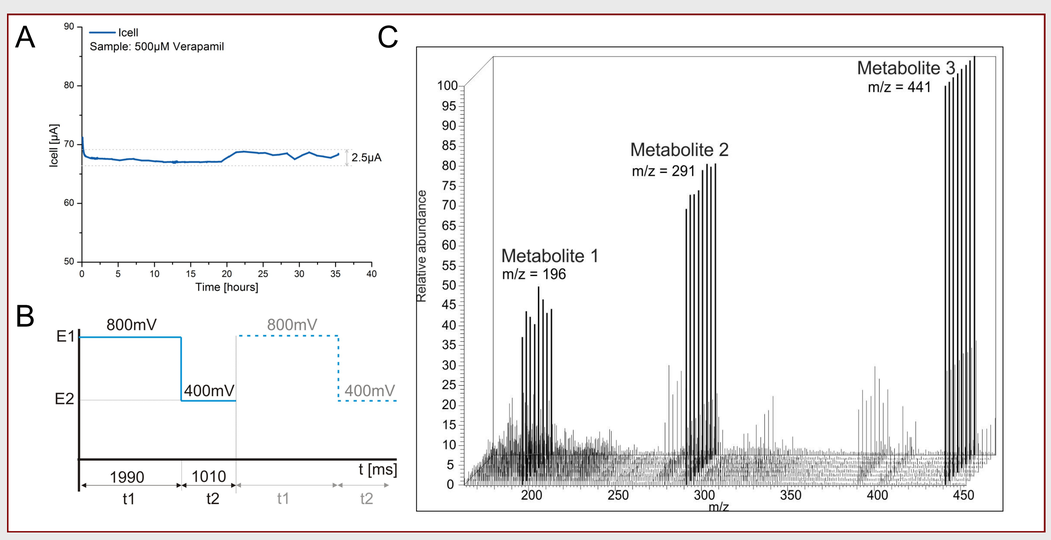

*Figure 4: **A:** Long term current stability experiment. **B:** Optimized pulse settings. **C:** An overlay of 8 mass spectra of the 100x diluted control samples. GC electrode.*

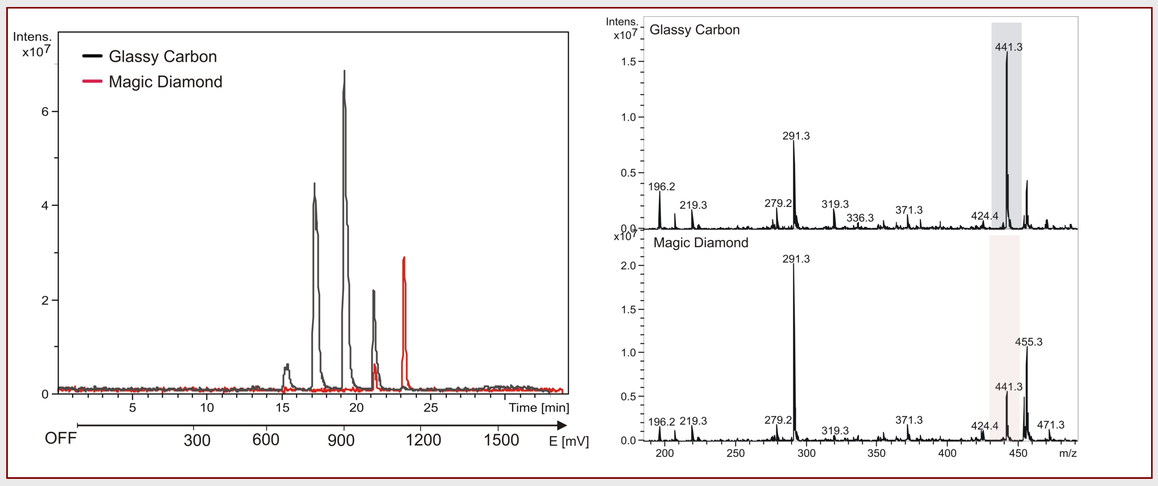

Figure 5: MS Voltammogram of Norverapamil (m/z = 441) registered on BDD & GC electrodes. Mass spectrum corresponds to optimal potential for formation of these metabolites on both electrodes, respectively.

Fast Synthesis of mg Quantities of Metabolites

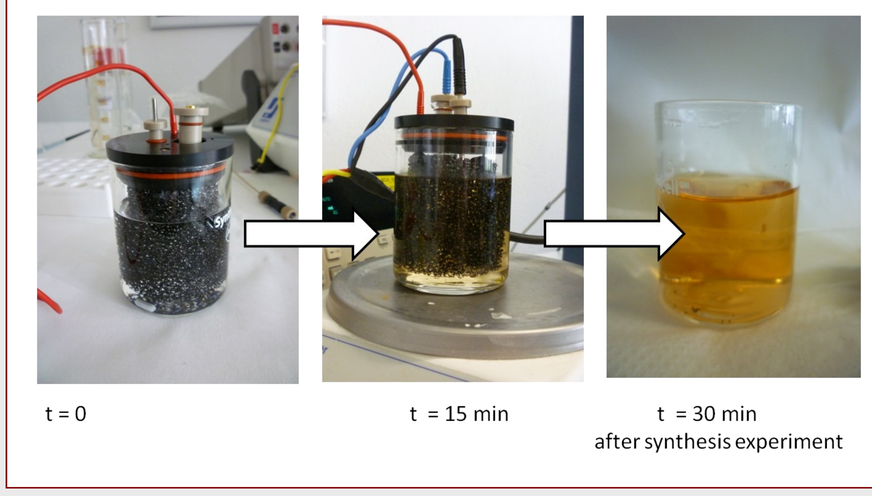

Figure 6: Fast electrochemical synthesis of metabolites using a 80 mL bulk cell.
Up to 100 mg of pure metabolite in less than 1 day! M. Taylor Pfizer U.K.

Conclusions

Using the ROXY™ EC system on-line with MS results in fast generation of metabolites (minutes vs. days or weeks using in-vitro and/or in-vivo methods).
For all drug compounds tested Amiodaquine, Verapamil and Norverapamil all electrochemically generated metabolites showed full agreement with the metabolites known from literature and/or from in-vivo experiments.

The data demonstrate that hyphenation of EC with MS provides a powerful and user-friendly platform for rapid and cost efficient screening of target compounds (drugs, xenobiotics, etc.) on their oxidative metabolism. Furthermore, EC allows for fast synthesis of mg quantities of metabolites and becomes a truly **"Metabolite Synthesizer"**

References

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