Electrochemistry/MS – a Powerful Tool in Drug Metabolism

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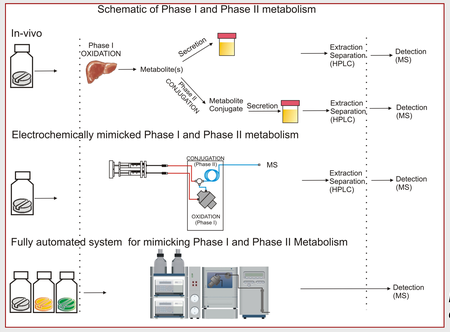

Figure 1: Mimicking liver drug metabolism.

Introduction

Electrochemistry (EC) in combination with mass spectrometry creates a powerful platform to simulate various oxidation and reduction processes in life sciences. Electrochemistry is a complementary technique to traditional in vivo or in vitro metabolism studies, and delivers the oxidative metabolic fingerprint of a (drug) molecule in a very short time. Mass spectrometry delivers selective and sensitive detection and allows for unambiguous identification of all products generated in the electrochemical cell. Additionally, automated data analysis by use of data bases (proteomics) or mass spectral trees (metabolomics) can shorten considerably the total time needed for the experiment. Furthermore, the electrochemical cell can be used for preparative synthesis of reactive metabolites in a short period of time. The cell can be hyphenated to MS or LC/MS to perform separation and identification of the created oxidative compounds i.e., intermediates, (reactive) metabolites. Alternatively, the cell can be used off-line and the generated metabolites can then be collected for supplementary research such as NMR.

Methods

A preparative electrochemical cell (µ-PrepCell, Antec) equipped with a Glassy Carbon (GC) or Magic Diamond™ (BDD) working electrode was used for synthesis of metabolites. Typically, 2-3µM concentration of the drug were used in experiments with MS detection. 250 µM solution of the Verapamil in 20mM ammonium formate, pH 7.4 in acetonitrile (50/50, v/v) was used as a model drug. The electrochemically synthesized metabolites of Verapamil were collected off-line, followed by MS analysis of the collected fractions (Figure 2C). The flow rate used in the synthesis experiments was 50 µL/min. For the formation of GSH adducts, 50 µM GSH solution was added after the EC cell using a mixing coil. Optimization of the metabolite synthesis was performed based on scanning voltammetry. An LTQ-FT (Thermo, USA) or HCT ion trap (Bruker Daltonics, Germany) mass spectrometer equipped with electrospray (ESI) source was used to monitor the oxidation or reductive products.

Figure 2: ROXY EC system (Antec). Syringe pump, ROXY potentiostat and µ-PrepCell with different electrodes.

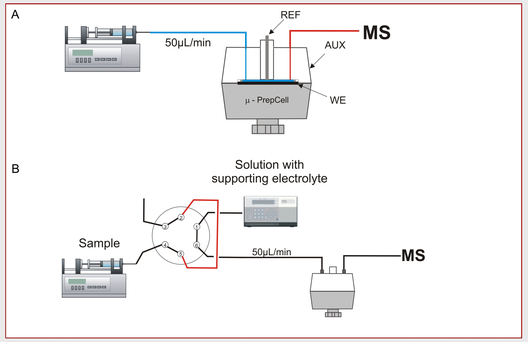

*Figure 3: Instrumental set-up of used for mimicking drug metabolism. **A:** direct infusion approach. **B:** Flow injection approach.*

Results

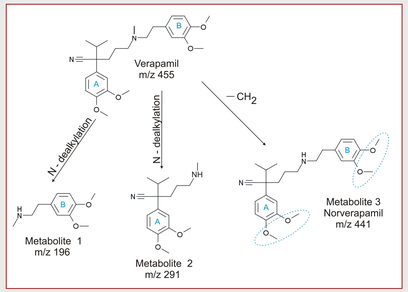

Figure 4: An excerpt of the Verapamil metabolic pathway. Blue dotted ellipses are indicating other places of possible loss of CH_2.

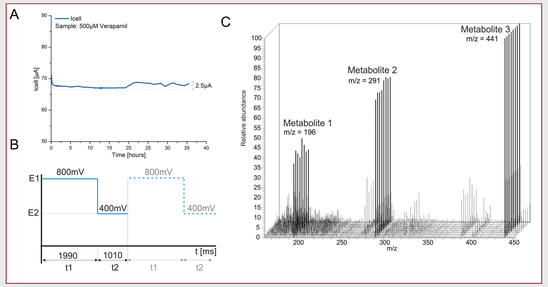

*Figure 5: **A:** Long term current stability experiment. **B:** Optimized pulse settings. **C:** An overlay of 8 mass spectra of the 100x diluted control samples. GC electrode.*

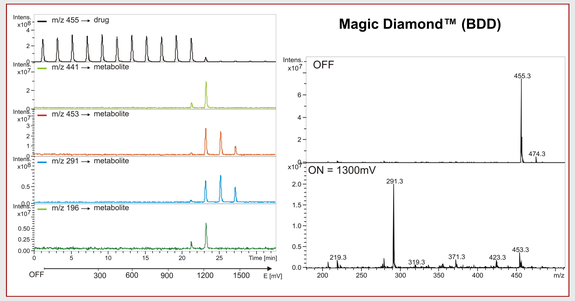

Figure 6: MS Voltammogram of Verapamil and its metabolites. Mass spectrum is corresponding to OFF and 1300mV. The experiment was automated. BDD electrode was used.

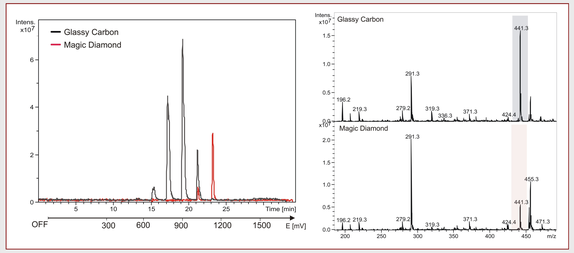

Figure 7: MS Voltammogram of Norverapamil (m/z = 441) registered on BDD & GC electrodes. Mass spectrum corresponds to optimal potential for formation of this metabolite on both electrodes, respectively.

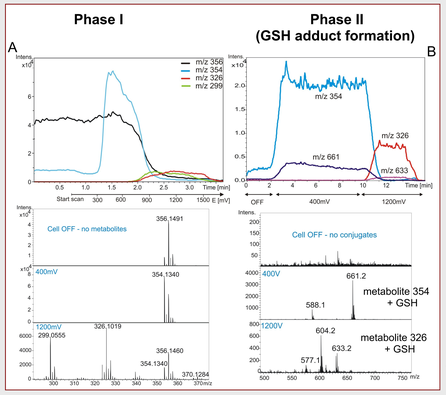

*Figure 8**A:** MS Voltammogram of Amodiaquine (Scan mode) and mass spectra of metabolites of Amodiaquine. **B:** Example of phase II reactions (adduct formation of metabolite with GSH). EICs of Metabolite 1 (m/z 354) and its adduct with glutatione (m/z 661) and Metabolite 2 (m/z 326) and its adduct with glutathione (m/z 633). Reaction was performed in DC mode. The spectrum with cell OFF confirms that the adducts (phase II reaction) are formed ONLY if potential is applied. GC electrode was used.*

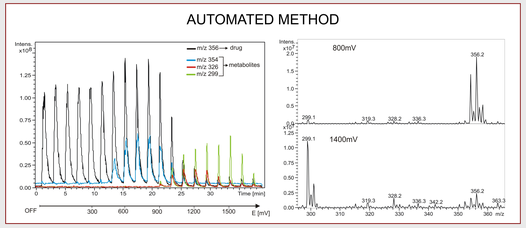

Figure 9: MS Voltammogram of Amodiaquine recorded in DC mode (BDD). 2µL of Amodiaquine was injected. Potential was ramped from 0 –1700 mV with incremental steps of 100mV every 2 minutes. Mass spectra shows the metabolites of Amodiaquine formed between 800mV and 1400mV. This measurement was performed using FIA approach and was fully automated by using event program in Dialogue software (Antec).

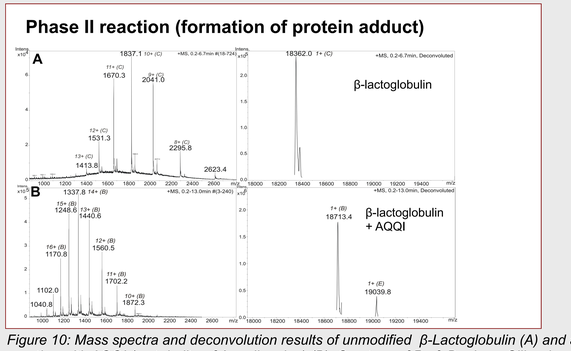

Figure 10: Mass spectra and deconvolution results of unmodified β-Lactoglobulin (A) and after reaction with AQQI (metabolite of Amodiaquine) (B). Courtesy of Prof. Dr. Jerzy Silberring (AGH University of Science and Technology, Kraków, Poland).

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 were 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.

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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