Rapid Assessment of Metabolism by Electrochemistry–MS

(Drugs, Xenobiotics, Plants)

Jean-Pierre Chervet[1]; Hendrik-Jan Brouwer[1]; Martin Eysberg[2]
[1]Antec Scientific, Alphen a/d Rijn, The Netherlands;
[2]Antec Scientific, Boston, MA, USA

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Introduction

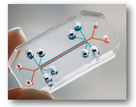
The metabolic pathways and the biotransformation of drugs including xenobiotics or plants are crucial for elucidation of degradation routes of the active compounds. *In vitro* studies are based on incubating drug candidates with, e.g., liver cells (microsomes) and isolating and detecting the metabolic products. With the availability of the ROXY™ Electrochemistry (EC) system oxidative metabolism, which usually occurs in the liver (Cytochrome P450), can be mimicked successfully within seconds and detected by MS. Combining EC with MS creates a powerful platform for oxidative metabolism and overcomes some of the laborious tasks such as isolating the metabolites form *in vivo*, e.g., urine, plasma, or *in vitro* studies, e.g., microsomes. This poster highlights the use of Electrochemistry—MS as a rapid, easy and complementary technique to current approaches.

Techniques used to study metabolism

In vivo
Test animals (rodents), final stage human.
Ethical concerns, time consuming, expensive.
Well established and accepted.

In vitro
Liver microsomes (human, rat, mouse, etc.).
Inexpensive, time required for incubation, no access to short lived metabolism. Established and accepted.

In vitro
Liver-on-a-chip/organ on-a-chip.
Promising new technique, quick screening prior to *in vivo* testing.



In vitro
Electrochemistry–MS (EC-MS)
Rapid prediction and mimicking of metabolism.
Access to short lived and phase I and II metabolism.

Instrumental setup Electrochemistry–MS (EC-MS)

In Figure 1 the schematics of the instrumental setup is shown for direct infusion experiments. When combined with HPLC, the EC system can be positioned either before or after the LC, depending on the sample complexity and the desired outcome.

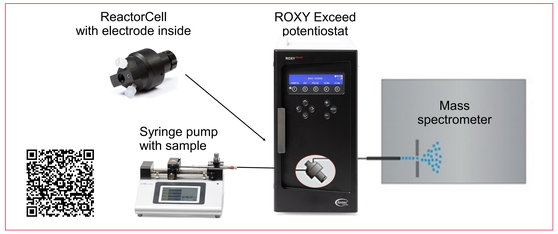


Figure 1: *Schematics instrumental setup for online EC-ESI/MS. From left to right: Infusion pump, ROXY Exceed™ Potentiostat equipped with ReactorCell™ (both Antec Scientific), and MS. Typical working electrodes: Glassy carbon or Boron-Doped Diamond (BDD).*

MS Compatibility ROXY™ Exceed

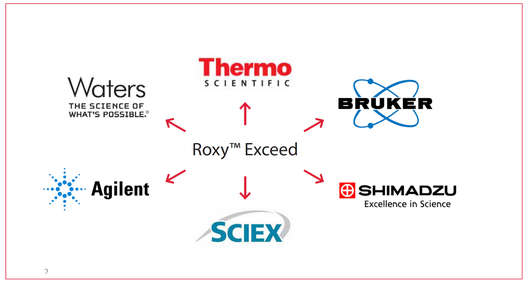


Figure 2: *ROXY Exceed is compatible with all major LC/MS systems. For Thermo Scientific it can be controlled out of their Chromatography Data System (CDS). For all others, it can be controlled out of Dialogue software (Antec Scientific).*

Cytochrome P450 reactions initiated (mimicked) by EC

Typical reactions catalyzed by cytochrome P450	Mimicked by EC
Allylic and aliphatic hydroxylation	Possible at high potentials (only with ROXY)
Benzylic Hydroxylation	Possible
Dealkylation of amines	Possible
Dealkylation of ethers and thioethers	Ethers: Possible Thioethers: not mimicked
Hydroxylation of aromatics	Possible
Epoxide formation	Possible
Oxidation of heteroatoms (N, S)	Possible

Figure 3: *Most of the typically oxidative Cytochrome P450 reactions can be mimicked (initiated) in the electrochemical cell within a few seconds (on the "fly" through the cell) without any biological interactions.*

Phase I and II reactions

Another benefit of electrochemically initiated metabolism is its convenient accessibility for studying phase II conjugation reactions. This can be achieved by introducing Glutathione (GSH) as a scavenger after the electrochemical cell and by directly measuring the formed GSH adducts via the MS. In Figure 4 the schematics of the instrumental setup for phase II reactions is depicted.

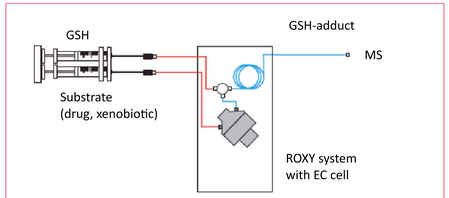


Figure 4: *Schematics instrumental setup for online Phase II conjugation reactions by EC-MS. From left to right: Dual syringe pump containing the substrate and GSH. ROXY Exceed with EC cell, T-piece for GSH addition and reaction coil for adduct formation and direct measurement via MS.*

Results—case study amoidiquine and >100 others examples

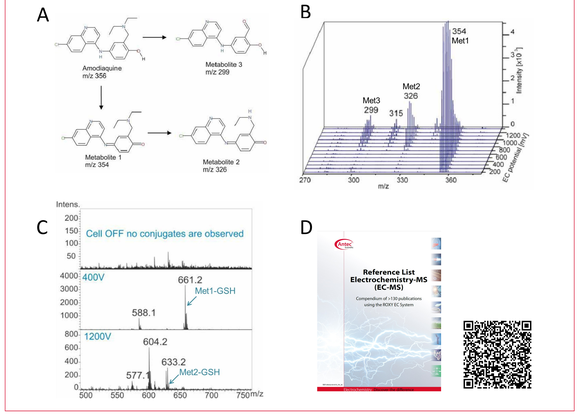


Figure 5: *(A) Structure of amodiaquine and its 3 major metabolites, Met1 m/z 354, Met2 m/z 326 and Met3 m/z 299. (B) MS Voltammogram illustrating the dependency of metabolite formation on the applied potential. (C) Phase II reactions by adduct formation with GSH. Both Met1 and Met2 undergo conjugation. (D) Reference list with >100 publications illustrating the successful use of EC-MS for predicting (mimicking) drug/xenobiotic metabolism.*

Conclusions

EC-MS is the perfect analytical technique for predicting and mimicking metabolism. It complements existing techniques and allows for:

- Rapid generation of (drug)metabolites, intermediates, and degradants
- Excluding matrix interference (e.g. cell membranes, microsomes)
- Eliminating the use of costly enzymes
- Direct identification of reaction products including short lived compounds
- Substantial time and cost savings compared to chemical or enzymatic reactions
- Easy scale-up for synthesis of mg quantities (reference material)

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