Application of high resolution mass spectral trees for identification of electrochemically synthesized metabolites


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Introduction

Combining electrochemistry with MS creates a powerful platform for oxidative metabolic studies and helps to overcome many of the tedious tasks by traditional metabolites formed in vivo (i.e., plasma, etc.) or in vitro (microsomes) [1]. Furthermore, the electrochemical cell can be used for preparative synthesis of reactive metabolites in a short period of time. The calls can be hypothesized to MS or LC/MS to perform separation and identification of the oxidized metabolites or native metabolites formed in vitro. Alternatively, the call can be used off-line and the generated metabolites can be collected for supplementary research such as FAB/MALDI. Here, highly efficient metabolites by applying square-wave potential pulses is presented. Metabolites are generated continuously for more than 2 hours using a highly conserved electrochemical detection system (i.e., and the fragment less peak representation (b) [2]).

Methods/Instrumentation

A preparative electrochemical cell (Antec) equipped with a Glassy Carbon (GC) working electrode was used for synthesis of metabolites. The GC worked at 300 μA solution of the Verapamil was used in a new electrolyte (500 μM) containing 0.1 M acetate buffer (pH 4.5). The current density was 1.5 μA/cm². The flow rate was 1 mL/min. A single (full) cycle to MS. The control samples were collected in triplicate. The reference compounds were processed with the so-called NIST library.

Results

We propose 4 steps protocol for an optimized, electrochemical synthesis of drug/xenobiotic metabolites:

Step 1: Scan Voltammetry with electrochemical detection

Step 2: Scan Voltammetry with mass spectrometric detection

Step 3: Optimization of square wave pulses parameters

Step 4: Metabolic synthesis with off-line samples collection

In Step 1, a working potential range and maximum potential are established (Figure 3A). It is not possible to identify the metabolites based on a single profile acquired in Step 1. Therefore, an semi-automated process with on-line MS detection to verify hystone fragments in the mass spectra of the synthesized metabolites (Step 2). Figure 2B shows the multistage mass spectral trees of VERPAILIN. The mass spectrometer allows direct monitoring of metabolic generation over long periods of time. Optimization of the metabolite synthesis was performed based on scanning voltammetry. An LTQ Orbitrap (Thermo, USA) was used to record the mass spectra of the control samples. The control samples were collected in triplicate. The reference compounds were processed with the so-called NIST library.

Conclusions

A long-lasting, stable and efficient electrochemical synthesis of metabolites is possible by applying a square-wave pulse. Selective time points can be selected based on the multistage mass spectral trees of the control samples. The control samples were collected in triplicate. The reference compounds were processed with the so-called NIST library.

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References


Figure 1: An excerpt of the Verapamil metabolic pathway.

Figure 2: A: RIDSY ECG system (Antec). B: PreCell (Antec). C: Instrumental set-up for metabolite synthesis. D: Preparation of the control samples (a) and the fragment less peak representation (b) [2].

Figure 3: Application of a square wave pulse is beneficial for metabolite synthesis. The main advantage of square-wave potential pulses is preconditioning. The electrode surface is continuously reactivated during the run, reducing adsorption/fouling, and helps to overcome many of the laborious tasks by isolating the metabolites formed in vivo (i.e., plasma, etc.) or in vitro (microsomes) [1].

Figure 4: A: MS Voltammetry of Verapamil. B: Mass spectrum corresponding to primary metabolites (background was subtracted). C: Different potential pulses were tested (I, II, III, IV) with experiment performed in Step 1.

Figure 5: Pulse mode optimization

A: The potential was chosen for a regime where oxidation was occurring (STEP 1). B: Different potential pulses were tested (I, II, III, IV). C: Ion abundances correspond to the mass spectra (m/z 475 and its 2 metabolites) measured with I, II, III (pulse settings).

Figure 6: A: MS Voltammetry of Verapamil. B: Mass spectrum corresponding to primary metabolites (background was subtracted). C: Different potential pulses were tested (I, II, III, IV). C: Ion abundances correspond to the mass spectra (m/z 475 and its 2 metabolites) measured with I, II, III (pulse settings).

Figure 7: Fragment tree representation of Norverapamil in comparison to the parent drug (Verapamil). Combined with the fragment tree (Fragment 1) of the synthesized metabolite, this is very useful for identifying unknown metabolites or confirming the origin of the known ones.

Figure 8: MS fragment spectroscopic data of Norverapamil (m/z 310) and Verapamil (m/z 330) (Verapamil, not shown here) results in the same fragment pattern (peaks 16 and 17).

Figure 9: The fragmentation tree representation of Norverapamil in comparison to the parent drug (Verapamil). Combined with the fragment tree (Fragment 1) of the synthesized metabolite, this is very useful for identifying unknown metabolites or confirming the origin of the known ones.