

Overview

On-line electrochemistry (EC) mass spectrometry (MS) has become a powerful tool for the simulation and the prediction of metabolic processes such as oxidative metabolism of xenobiotics. Using EC/LC/MS results in:

- Fast, synthesis of metabolites (seconds vs. days/weeks using in-vivo methods)
- Zero matrix effect, no isolation steps
- Identification of oxidative labile sites in drug molecule
- Zero risk of adduct formation with cell material

Introduction

The use of on-line EC coupled to MS for studying redox reactions of various substances including biomolecules date back to the late 1980 [1,2]. Soon it became clear that this technique offers interesting features for the simulation of oxidative processes. Recently in several studies the usefulness of EC to mimic metabolic reactions of drugs has been demonstrated. EC/MS was applied to mimic the oxidative metabolism of N,N-dimethylaniline [1], of dopamine [3], of the dopamine agonist 2-(N-propyl-N-2-thienylethylamino)5-hydroxytetralin [4], of paracetamol [5,6], of zotepine [7], of clozapine [6], of troglitazone [8], diclofenac [9], tetrazepam [10], and other compounds. These efforts have been reviewed recently [11,12]. Especially the combination of on-line EC/LC/MS has been shown to be a very promising tool to study oxidative metabolism in drug discovery. In this poster we present the appropriate EC/LC hardware to conduct such studies.

Instrumentation

Antec (Leyden) has developed a dedicated Analyzer for Oxidative Metabolism (ALEXYS OxMet™ Analyzer) that is based on two HPLC pumps for gradient elution, an autosampler for automatic sample handling and the Decade II Potentiostat equipped with a reactor cell for EC conversion of target compounds into the appropriate metabolites. Figure 1. shows a picture of the ALEXYS OxMet™ Analyzer used as front-end introduction system in MS.



Figure 1: ALEXYS OxMet™ Analyzer, Antec (Leyden)

Instrumentation

In Figure 2 the typical hardware configurations for phase I metabolism are shown using EC/MS and EC/LC/MS. Figure 2A is the schematics of choice for high yield oxidation of target compounds using a syringe pump for sample introduction (infusion) into the reactor cell and direct detection of the oxidation products by MS or MS/MS. This EC/MS approach is ideally suited to generate larger amounts of metabolites (micro preparative work) and for exhaustive MS/MS investigations. Figure 2B represents the schematics for on-line EC/LC/MS and automated metabolism screening. With a specially modified autosampler the drug compounds are introduced into the reactor cell and the oxidation products are sequentially separated by LC prior to MS detection. For conjugation reactions (phase II metabolism) an additional infusion pump with mixing coil for the addition of glutathione (GSH) or other reagents is used (schematics not shown).

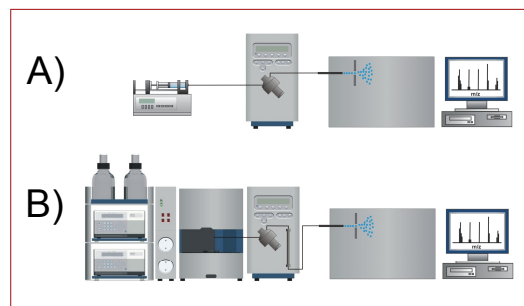


Figure 2: Hardware schematics for oxidative metabolism
A) EC/MS for single compound metabolism studies using the Decade II Potentiostat (Antec)
B) EC/LC/MS for automated multiple compound metabolism screening ALEXYS OxMet™ Analyzer (Antec)

System Features

- Compatibility with any MS
Compatibility with MS via contact closure and the use of Clarity software (Data Apex) for system control.
- Automatic screening
By using a specially modified autosampler easy and automatic screening of xenobiotics is possible.
- System flexibility
Different system configurations are possible, i.e., for higher yield sample conversion, fast screening of multiple samples, Phase I and/or II metabolism, etc.
- Decade II Potentiostat
Large working potential range, +/- 5 volt
From 1 up to 4 reactor cells (simultaneous use of different working electrodes and/or potentials)
Pulse mode for optimal reactor cell activation and cleaning (working electrode)
Scan mode for rapid measurements of the optimal conversion potential
- Reactor cell
Proprietary thin layer reactor cell to assure minimal sample adsorption
Easy and quick working electrode replacement
Large variety of working electrodes e.g., GC, Au, Pt, Ag, MD (Magic Diamond™)
Micro preparative cells, using different spacer geometries



Figure 2: Reactor cell with inlet and outlet (left) and electrode holder with different working electrodes (right).

Results

On-line EC/LC/MS was used to simulate the oxidation (phase I metabolism) and the detoxification mechanism (phase II metabolism) of paracetamol in the human body. Paracetamol (PC), also brand named as Panadol, Tylenol, etc., was oxidized in the reactor cell on a GC working electrode at a potential of 600 mV with formation of a quinoneimine intermediate. The quinoneimine further reacted with glutathione (GSH) and/or N-acetylcysteine (NAC) to form isomeric adducts via the thiol function. The adducts were characterized by LC/MS. These reactions are similar to those occurring between paracetamol and glutathione under catalysis by cytochrome P450 enzymes in the human body.

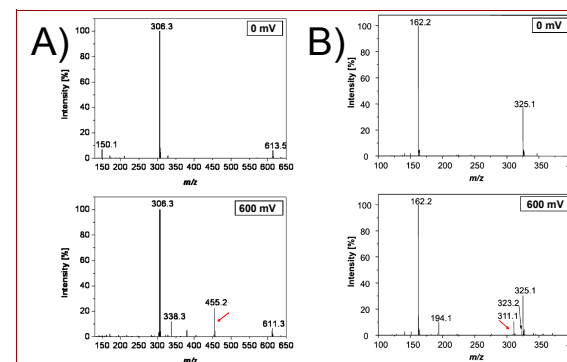


Figure 4: MS spectra prior and after oxidation of paracetamol (PC)
A) in presence of 5 fold excess GSH (5% MeOH, 95% 20 mM aq. NH₄Ac buffer, pH 7) at 0 and 600 mV
B) in presence of 5 fold excess NAC (5% MeOH, 95% 20 mM aq. NH₄Ac buffer, pH 7) at 0 and 600 mV

Results

The electrochemical reactions that take place in the reactor cell are summarized in Figure 6. The oxidation of PC is induced by the loss of one electron and one proton resulting in an intermediate radical. In a follow-up reaction the radical undergoes a second electron and proton transfer leading to the formation of the toxic metabolite N-acetyl-p-benzoquinoneimine (NAPQI). In the presence of thiol containing molecules like GSH or NAC, NAPQI is quenched by adduct formation. This adducts could be easily reproduced in the EC/LC/MS approach and are known as the common detoxification pathway of NAPQI in the human liver (phase II metabolism).

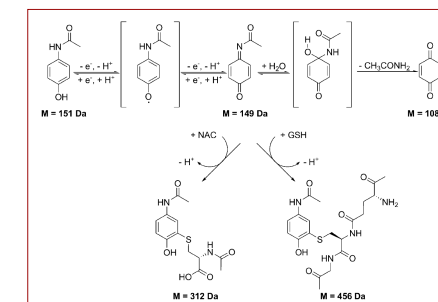


Figure 6: Reaction scheme for electrochemical oxidation of paracetamol (PC) in the presence of GSH and NAC

In Figure 5 the EC/LC/MS chromatograms of paracetamol (PC) are shown. With reactor cell switched off, i.e., 0 mV, only 3 peaks could be detected (Figure 5A). For comparison the chromatograms resulting from the oxidation at 600 mV are shown in Figure 5B. The m/z 150 peak for PC was much smaller at 600 mV, indicating that PC was converted almost completely into different reaction products. The m/z 311 and m/z 455 mass traces represent the GSH and NAC adducts of PC, respectively.

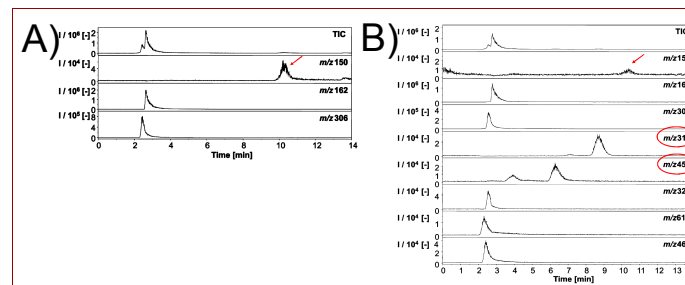


Figure 5: EC/LC/MS chromatograms of a mixture containing PC (10⁻⁴ M), GSH (10⁻⁴ M), and NAC (10⁻⁴ M).
A) EC off, 0 mV: with combined TIC, and mass traces of PC (m/z 150), GSH (m/z 306) and NAC (m/z 162).
B) EC on, 600 mV: with combined TIC, and mass traces of PC (m/z 150), NAC (m/z 162) and GSH (m/z 306), NAC adduct of PC (m/z 311), GSH adduct of PC (m/z 455), NAC dimer (m/z 323) GSH dimer (m/z 611) and a conjugate of NAC and GSH (m/z 467)

Conclusions

Paracetamol (PC) was successfully used as model compound to mimic the oxidative metabolic detoxification pathway in the human liver by EC/MS and EC/LC/MS experiments. 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 LC/MS using either PC alone or in the presence of glutathione and/or N-acetylcysteine. These results clearly illustrate the potential of EC/LC/MS as a powerful tool for predicting metabolic processes.

Acknowledgements

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