

Objective

To illustrate the potential of EC/MS and EC/LC/MS as powerful analytical technique beyond classical drug metabolism using a dedicated up-front EC/LC system.

Introduction

Combining electrochemistry (EC) with MS and LC/MS has shown great potential for the investigation of drug metabolism. Recently, the use of on-line EC/LC/MS has been extended towards new applications such as:

- the rapid risk assessment of covalent protein binding of new drugs
- electrochemical synthesis of drug metabolites for reliable quantification by MS
- electrochemical synthesis of metabolites for doping control in sports drug testing
- simulation of pesticide metabolism

All these applications illustrate the tremendous power and broad applicability of this technique and are discussed in more detail in this poster.

Methods/Instrumentation

Depending on the type of compound (drug, pesticide, etc.) typically 50 µM solutions in ammonium formate/acetonitrile (50/50, v/v) are pumped through the electrochemical reactor cell at 1 to 10 µl/min. For higher amounts (yield) of oxidation product(s) the use of low flow rate, e.g., 1 µl/min is recommended. For EC/MS, the effluent of the EC cell is directly introduced into the ESI-MS. The potential at the working electrode was ramped from 0 to 2000 mV. For LC/EC/MS the working electrode was kept at constant potential. In case of oxidation experiments, the effluent from the EC cell was collected directly in a 10 µl loop. For the formation of GSH adducts, 500 µM GSH solution was added after the EC cell. The content of loop is injected into the HPLC. Different types of MS were employed, depending from the type of application. A typical schematics of a dedicated EC/LC Analyzer is shown in Figure 1. For more details about the EC/LC OxMet™ Analyzers, see Corporate Posters ASMS, Antec (USA).

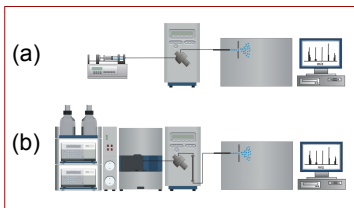


Figure 1: Typical hardware schematics (a) EC/MS for single compound metabolism studies and/or high yield metabolite synthesis using the Decade II Potentiostat (Antec) (b) EC/LC/MS for automated multiple compound metabolism screening using the ALEXIS OxMet™ Analyzer (Antec)

Results

1. Rapid risk assessment of drug—protein binding

New chemical entities, e.g., drugs, are easily oxidised in an EC cell and the generated oxidative metabolites (phase I) are exposed to proteins such as β -lactoglobulin A (LGA) and human serum albumin (HSA) to generate drug-protein adducts. In practice the adduct formation is simply achieved by adding the protein solution via a reaction coil between the EC cell and the HPLC injection valve. Potential adducts of the reactive drug metabolites and the proteins, i.e. covalent drug-protein binding, are identified by MS. This simple set-up allows for easy handling and short analysis times and is therefore ideally suited for the rapid risk assessment of drug-protein binding. In Figure 2 the model compounds used are shown together with the reaction scheme.

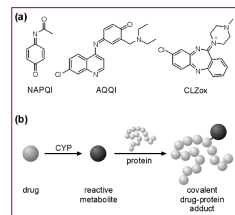


Figure 2: (a) Structures of the reactive metabolites of paracetamol (N-acetyl-p-quinoneimine, NAPQI), amodiaquine (amodiaquine quinoneimine, AQQI), and clozapine (clozapine nitrenium ion, CLZox). (b) Principle of covalent protein binding of drugs after activation by cytochrome P450 (CYP) enzymes to reactive metabolites and as mimicked in EC/LC/MS system

In Figure 3 the TOF/MS spectra are shown for the metabolites of Figure 2(a) after 10 min of reaction with the model protein LGA. For data with the more relevant HSA, see reference [1].

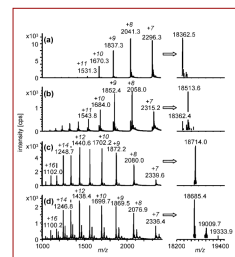


Figure 3: (a) TOF/MS spectra and deconvolution results of (a) unmodified LGA, (b) LGA after reaction with NAPQI, (c) LGA after reaction with AQQI, and (d) LGA after reaction with CLZox. The mass spectra of the drug-protein adducts were obtained after on-line reaction of the protein with the electrochemically generated reactive metabolites

The shift in the m/z values after the reaction with the reactive drug metabolites clearly indicates the occurrence of covalent drug-protein adduct formation. For NAPQI and AQQI the increase in the molecular mass was in good accordance with attachment of one molecule, meanwhile for CLZox a mono-adduct (most abundant) a bis- and tris- adduct were found. For a detailed studies about the modification sites see reference [1].

To sum-up, this simple technique offers two new possibilities [1] rapid risk assessments of covalent drug-protein binding, 2) rapid and clean synthesis of covalently modified proteins.

2. Electrochemical synthesis of drug metabolites

For the risk assessment of drug candidates, the identification and quantification of their metabolites is required by regulatory agencies such as FDA, ICH, etc. The majority of analytical techniques is based on calibration standards for quantification of the metabolites. As these are often not easily available, the use of electrochemical (EC) synthesis followed by MS and LC/MS was investigated and the results were compared with the metabolites found in vivo, i.e., human and/or in vitro experiments, i.e., rat liver microsomes. In Figure 1 the structure of the antiarrhythmic agent Amiodarone (AM), brand named as Pacerone, Cordarone, etc., is shown.

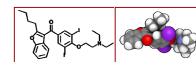


Figure 4: Structure and ball-and-stick model of amiodarone, containing two iodine atoms (purple)

In Figure 5 the mass voltammogram of AM are shown. At 0 mV mainly AM with m/z of 646 could be detected using the EC/MS set-up. In the next step the working electrode potential of the EC cell was ramped between 0 and 1500 mV in steps of 100 mV and the MS spectra were continuously recorded.

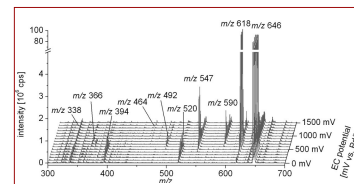


Figure 5: Mass voltammogram of AM (m/z 646) and its metabolites

Main oxidation product (phase I metabolite) can be seen at m/z 618, corresponding to N-deethylamiodarone. All other oxidative metabolites found are in good agreement with the biotransformation in the body and the metabolites found by microsome incubation. For more details see reference [2].

In another example, electrochemical synthesis was used to generate sufficient amounts of a major metabolite of a new steroidal anabolic agent used in sport drug testing to allow NMR and high resolution/high accuracy MS characterization. This metabolite—though commercially yet not available—has been used routinely in more than 5000 doping control urine samples that have been tested for selective androgen receptor modulators (SARMs). For details see reference [3].

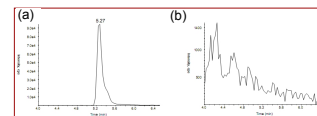


Figure 6: Extracted IC for major SARM metabolite (a) blank urine spiked with ca. 5ng/ml and (b) blank doping control urine sample

3. Simulation of pesticide metabolism

On-line EC/LC/MS was employed to mimic the oxidative metabolism of the fungicide Boscalid. Furthermore a 2nd EC cell in reductive mode was used providing important additional information on the oxidation products. With this set-up, hydroxylation, dehydrogenation, formation of a covalent ammonium adduct and dimerization were detected after initial one-electron oxidation of boscalid to a radical cation.

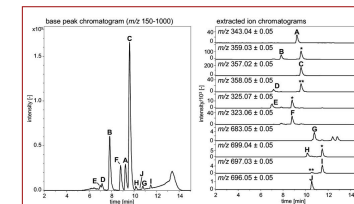


Figure 7: BPC (m/z 150-1000) and EIC of the separation of boscalid oxidation products. The peaks marked with one (*) are the respective ³⁷Cl isotopomers of the compound with a mass of 2 u less, the peak marked with (**) is a ¹³C isotopomer of the compound with a mass of 1 u less

The results of the electrochemical oxidation and the generated metabolites are in good accordance with previously reported in vivo experiments, illustrating that EC/LC/MS is a powerful technique for studying pesticide metabolism. For details see reference [4]

Conclusions

For the risk assessment of covalent protein binding of metabolites (1) the coupling of EC to LC/MS results in a simple and rapid on-line method for the generation and identification of drug-protein adducts. In terms of complexity, EC/LC/MS is superior to the time and labour consuming conventional methods such as microsomal incubations or in vivo experiments, without any need of isolation from complex biological matrices.

For the electrochemical synthesis of iodine containing metabolites of amiodarone and subsequent quantification by ICPMS (2) EC/LC/ICPMS together with EC/LC/ESI-MS allows for qualitative and quantitative analysis of the major metabolites. Similarly the electrochemical synthesis of major metabolite of SARM (3) was generated in amounts sufficient for NMR and MS characterisation. Although in this example the electrochemical approach is not able to fully substitute the in vitro models or the in vivo administration studies, EC/LC/MS enable a rapid insight into possible presence or absence of drugs and their metabolites in doping control. For the simulation of pesticide metabolism (4) the use of EC/LC/MS provides very similar metabolic products than the in-vivo approach with all the advantages thereof, as mentioned above (1). This is also the first time that EC/LC/MS is used in the investigation of pesticide metabolism.

References

- [1] W. Lohmann, H. Hayen and U. Karst, *Anal Chem*, 80 (2008) 9714
- [2] W. Lohmann, B. Meermann, I. Möller, A. Scheffer, U. Karst, *Anal Chem*, 80 (2008) 9769
- [3] M. Thevis et al., *Eur. J. Mass Spectrometry*, 14 (2008) 163
- [4] W. Lohmann et al., *J. Am Soc Mass Spectrometry*, 20 (2009) 138