

## Hyphenated Analytical techniques in Pharmaceutical Chemistry

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Metabolism is generally regarded as a process contributing to the detoxification of xenobiotics. During this process, xenobiotics such as drugs are converted into more polar compounds that are easily eliminated from the body. In many cases, the resulting metabolites are less toxic than the corresponding parent drug. However, some drugs undergo metabolic reactions that lead to the formation of reactive species. These reactive intermediates may modify cell proteins and DNA by covalent binding to the macromolecules and thus cause drug-induced toxicity and cell damage.

The detection of reactive metabolites using conventional *in vivo* and *in vitro* techniques is hampered because the intermediately formed reactive species are prone to covalent binding to cellular macromolecules. Therefore, the application of improved methods is required. The on-line coupling of an electrochemical reactor with liquid chromatography/mass spectrometry (EC/LC/MS) allows the direct detection of reactive metabolites of pharmaceuticals, which are all known for readily binding to cellular macromolecules after metabolization by cytochrome P450 enzymes.

EC/LC/MS experiments were compared to rat liver microsome incubations and proved to be valuable complementary methods since reactive quinone, quinone imine and quinone diimine species could be detected directly and not only after trapping with glutathione. Furthermore, *N*-dealkylation and *N*-oxidation of the target compounds were successfully simulated by electrochemical oxidation reactions. A comparison with liver cell microsomes shows that the reactive metabolites cannot be observed directly and that only their reaction products with nucleophiles such as glutathione are observed when using microsomes. The simulation of the adduct formation between the electrochemically generated reactive metabolites and proteins such as beta-lactoglobulin A was achieved in an on-line arrangement. While the native protein (one free cysteine residue) was modified only once, the reduced protein (five cysteine residues) was modified on three to five positions. The modification sites were identified by tryptic digestion and subsequent tandem mass spectrometry. These experiments prove the adduct formation of the reactive metabolites at free thiol groups in the protein. Therefore, EC/LC/MS is a promising tool for the identification of both reactive and stable metabolites as well as for the simulation of protein adduct formation in drug development.