

# The New Panacea in Metabolomics, Proteomics and Genomics – Electrochemistry/Mass Spectrometry

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## 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:

- electrochemical synthesis of metabolites in larger amounts (micro preparative)
- the rapid risk assessment of covalent protein binding of new drugs
- signal enhancement in MS
- oxidative damage of DNA

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 typically 50µM solutions in ammonium formate/acetonitrile (50/50,v/v) are pumped through the electrochemical cell at 1 to 10µL/min. For higher amounts (yield) of oxidation product(s) the use of low flow rate, e.g., 1µL/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 2V (Glassy Carbon) or 3V (Magic Diamond<sup>™</sup>; MD). For LC/EC/MS the working electrode was kept at constant potential and the effluent from the EC cell was collected directly in a 10µL loop. 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 and EC/LC systems is shown in Figure 1.

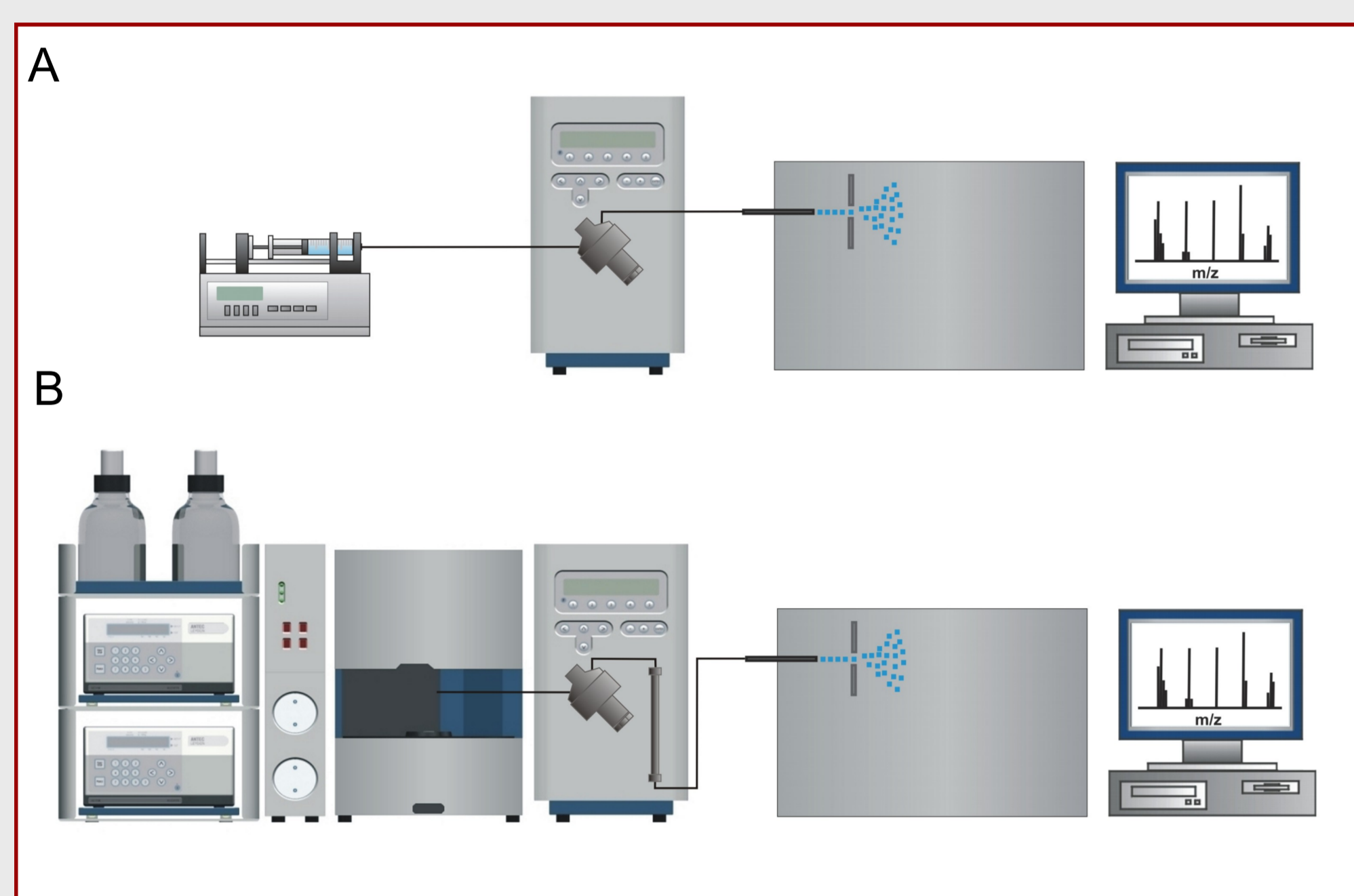


Figure 1: Schematics Antec hardware  
A) ROXY EC system for single compound metabolism studies and/or high yield metabolite synthesis. B) ROXY EC/LC system for automated screening.



Figure 2: µ-PrepCell<sup>™</sup> with inlet, outlet and reference electrode (top) and working electrode contact (bottom).

## 1. Fast synthesis of metabolites (µ-preparative mode)

The novel micro preparative cell (µ-PrepCell<sup>™</sup>) was designed for highly efficient metabolite generation (Fig. 2). The µ-PrepCell<sup>™</sup> can handle much higher flow rates than the ReactorCell<sup>™</sup> (200 µL/min), resulting in higher metabolite yield. The ReactorCell and µ-PrepCell equipped with a working electrode and HyREF<sup>™</sup> reference electrode were used for the generation of amidarone metabolites (Fig. 3). In particular the yield under different flow rates and working electrode materials (Magic Diamond<sup>™</sup> and Glassy Carbon) was investigated.

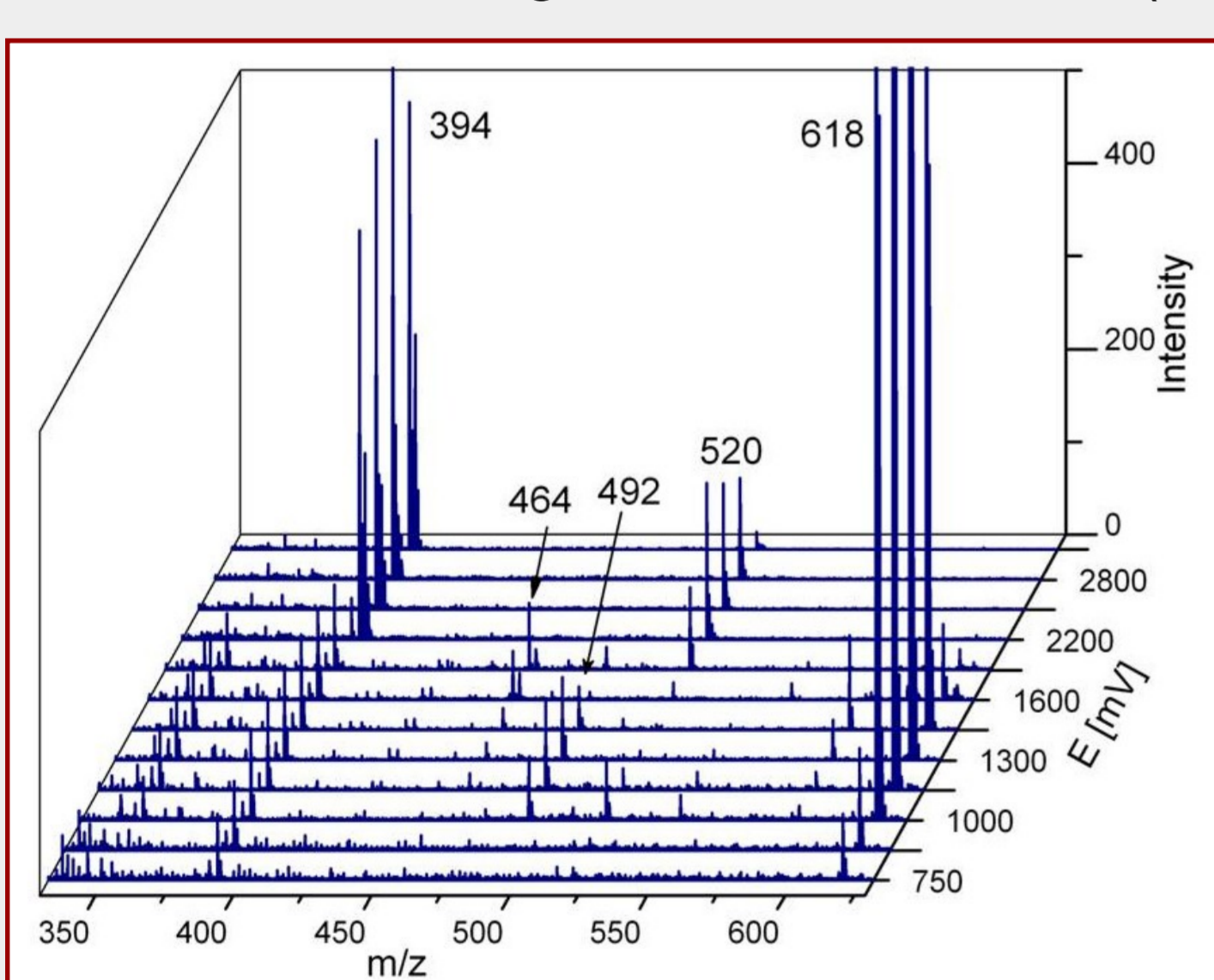


Figure 3: Mass voltammogram of amidarone obtained with µ-PrepCell (Flow rate was 50µL/min). Ion abundance versus m/z as a function of EC potential.

### µ-PrepCell vs. ReactorCell

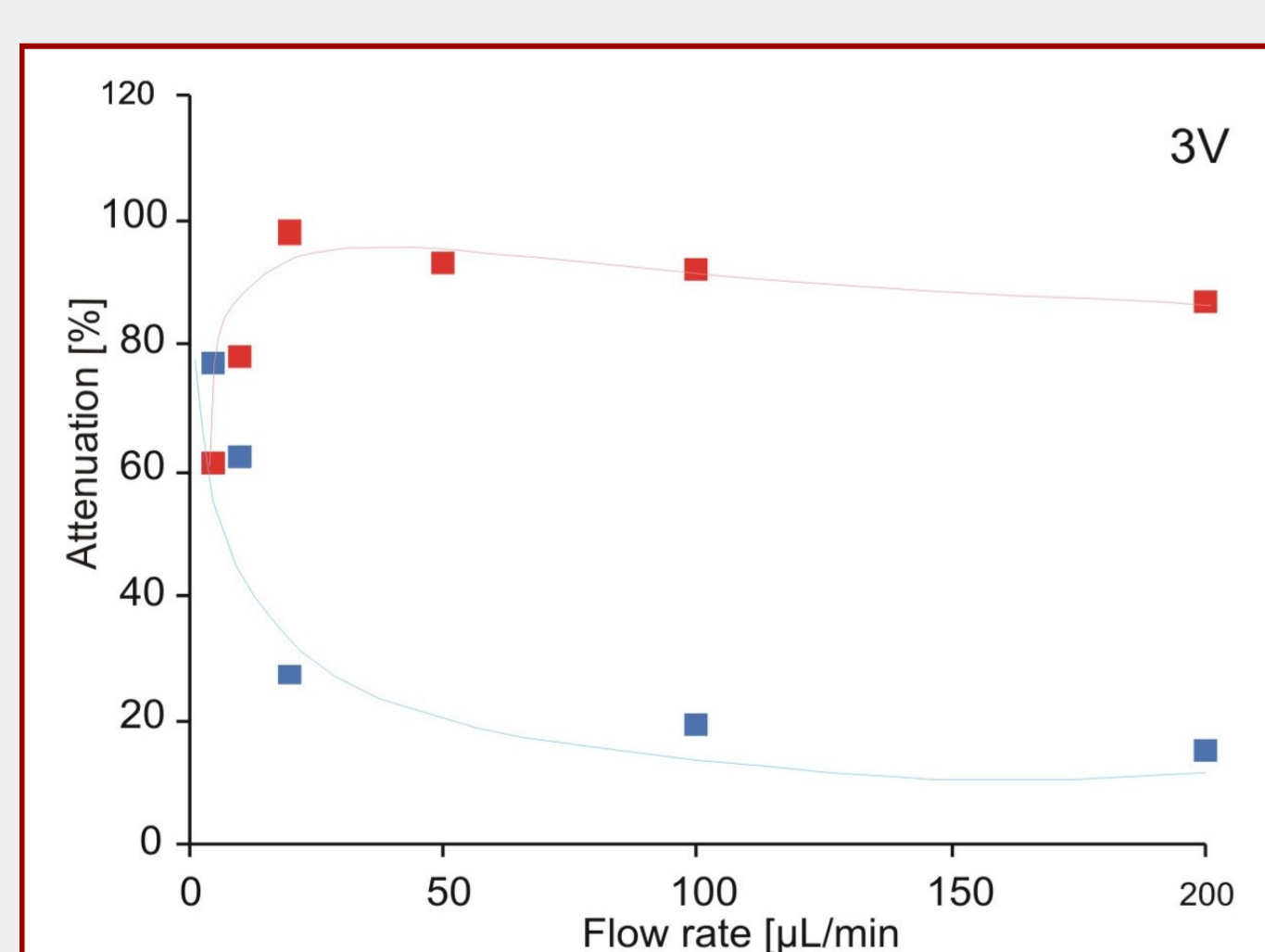


Figure 4: Comparison of amidarone signal attenuation for different flow rates. MD electrode; E=3V.

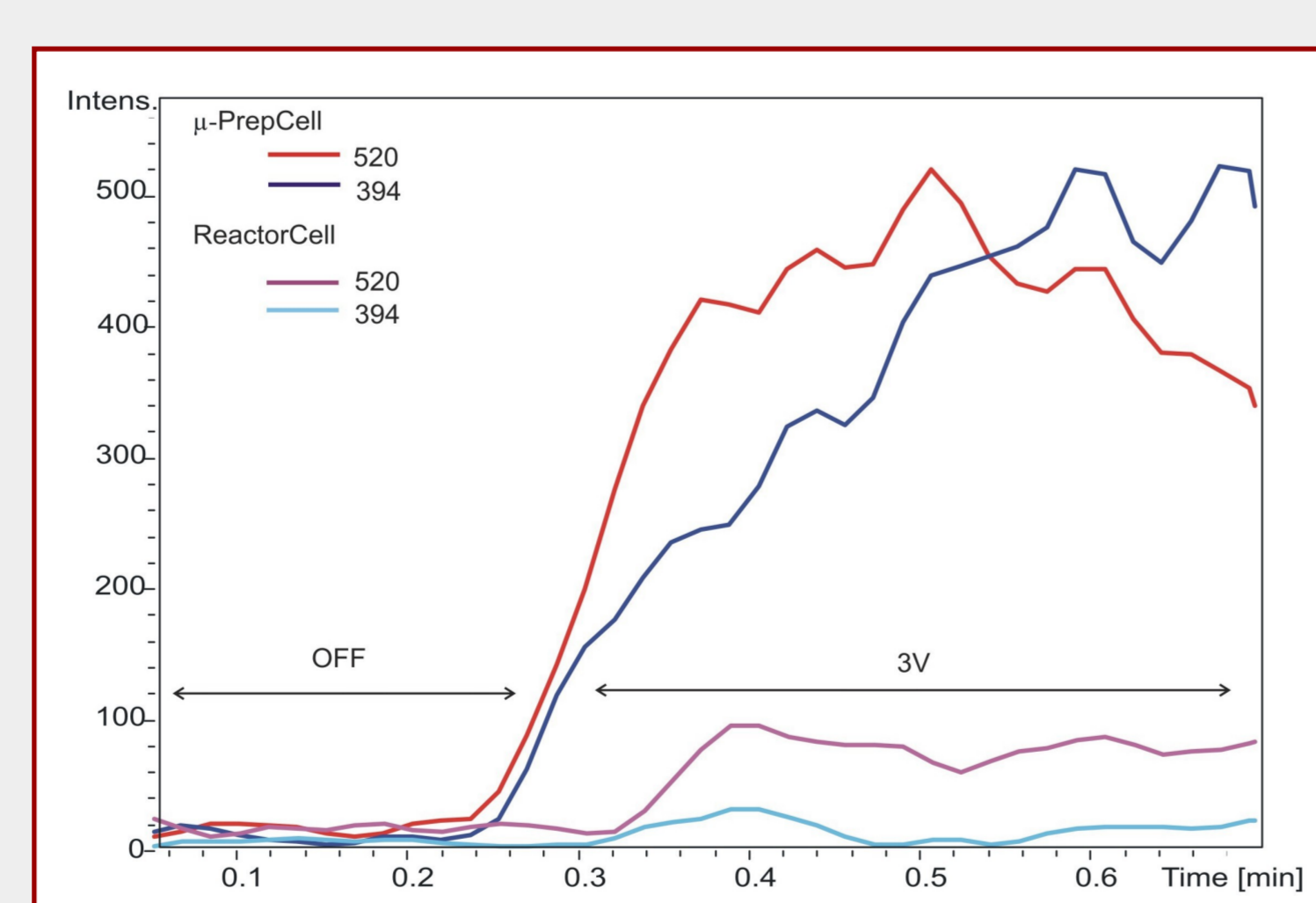


Figure 5: Metabolite generation with flow rate of 200µL/min. EIC for m/z of 520 and 394, MD electrode; E=3V.

### Metabolite yield in µ-PrepCell and ReactorCell

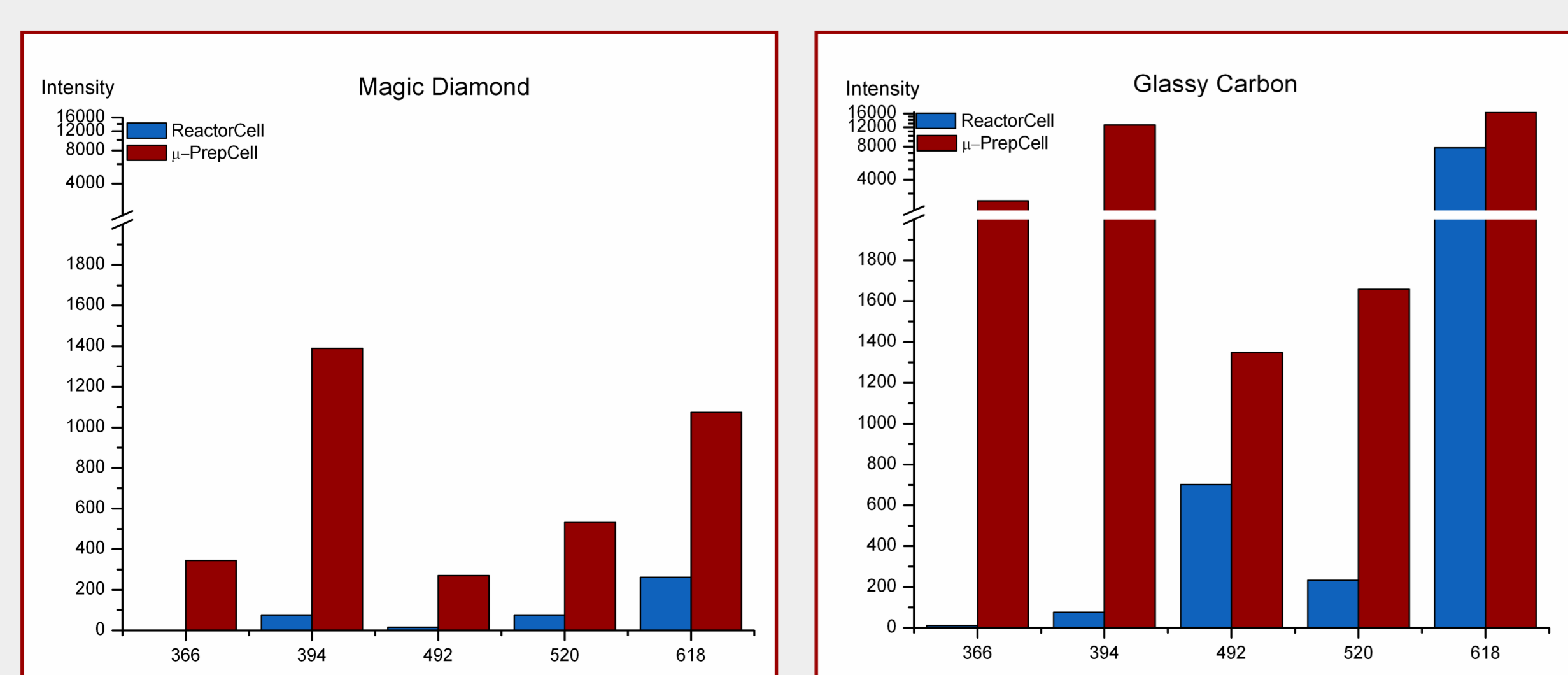


Figure 6: Amidarone was oxidized with potential 1V and 3V for Magic Diamond (left) and 1V and 1.5V for Glassy Carbon (right). Flow rate 50µL/min.

## 2. Rapid risk assessment of drug–protein binding

New chemical entities, e.g., drugs, are easily oxidized in an EC cell and the generated oxidative metabolites (phase I) are exposed to proteins such as β-lactoglobulin A (LGA) to form drug-protein adducts. 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 (Fig.7).

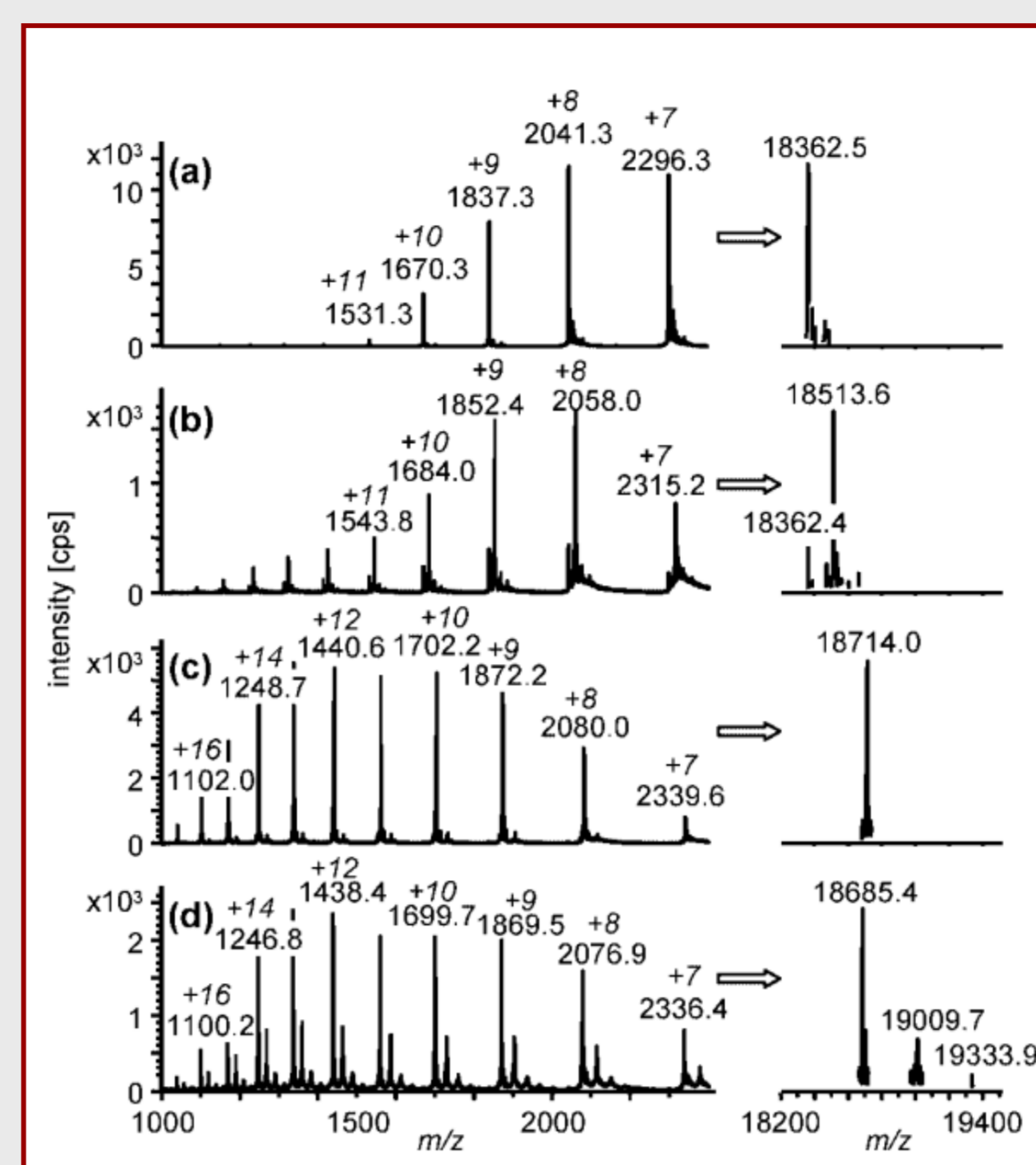


Figure 7: (a) TOF/MS spectra and deconvolution results of (a) unmodified LGA, (b) LGA after reaction with NAPQI, (c) LGA after reaction with AQQI, and (d) LGA after reaction with CLZox. The mass spectra of the drug-protein adducts were obtained after on-line reaction of the protein with the electrochemically generated reactive metabolites [1]. Courtesy of Prof. Dr. Uwe Karst, Westfälische Wilhelms-Universität Münster, Münster, Germany.

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.

## 3. Signal enhancement in MS Proteomics

N-(2-Ferroceneethyl)maleimide (FEM) is introduced as an electroactive derivatizing agent for determining the number of free thiol groups or the total number of free and disulfide-bound thiol groups in proteins. The electrochemical cell provides additional information, because the increase in mass spectrometric response upon electrochemical oxidation of the neutral ferrocene to the charged ferrocenium groups is monitored (Fig. 8).

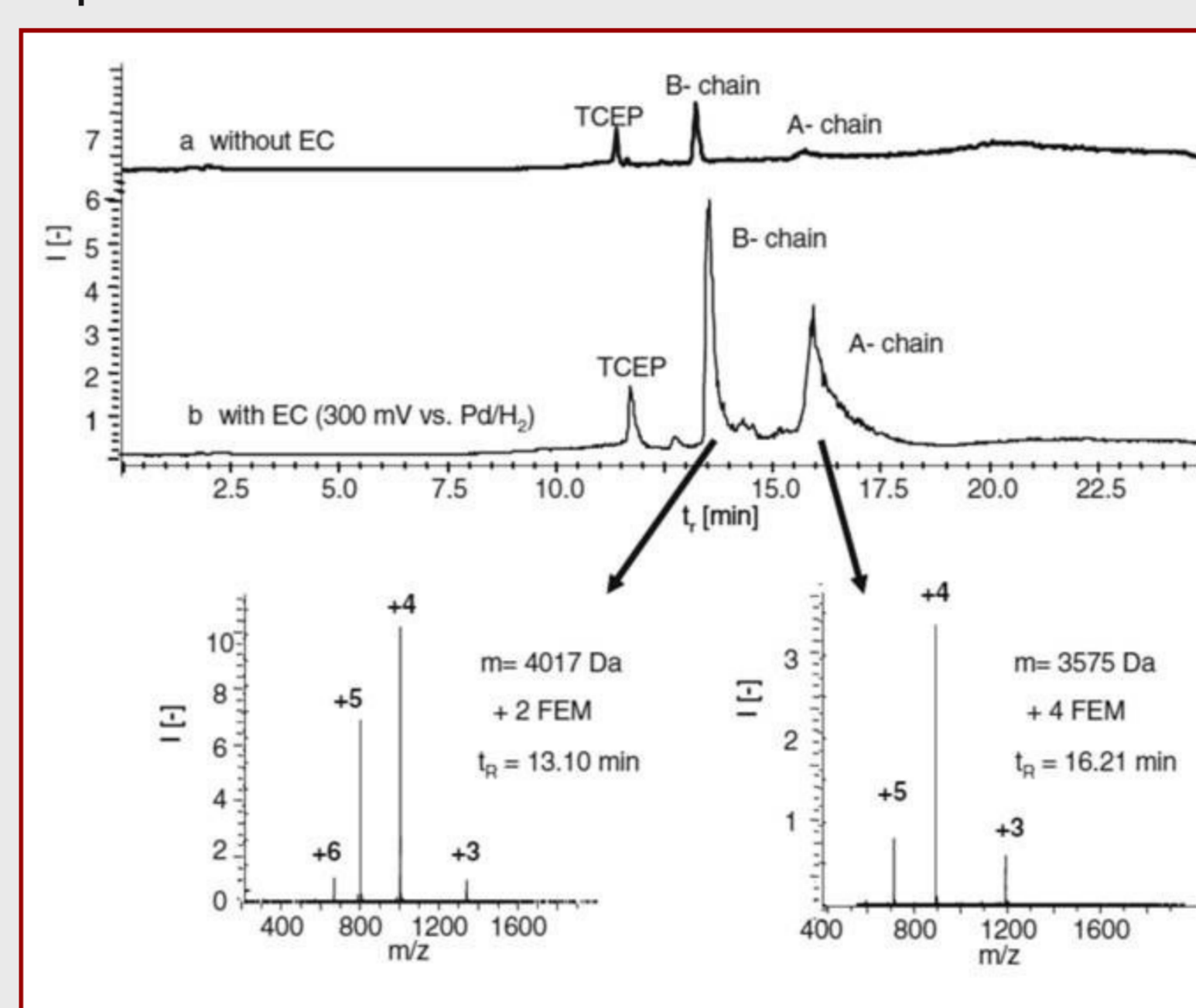


Figure 8: LC/MS (a) and LC/EC/MS (b) chromatograms for insulin, reduced and derivatized, along with the corresponding mass spectra of the A-chain and the B-chain [5]. Courtesy of Prof. Dr. Uwe Karst, Westfälische Wilhelms-Universität Münster, Münster, Germany.

## 4. Oxidative damage of DNA

Nucleic acids present within living systems are continuously exposed to reactive chemicals. Reactive oxygen species (ROS) represent one class of reactive chemicals that give rise to nucleic acids modification. Modified purine and pyrimidine bases are potential substrates for repair enzymes or polymerases, or they can block these activities, triggering biological responses including mutation, cell death, malignancy, and aging.

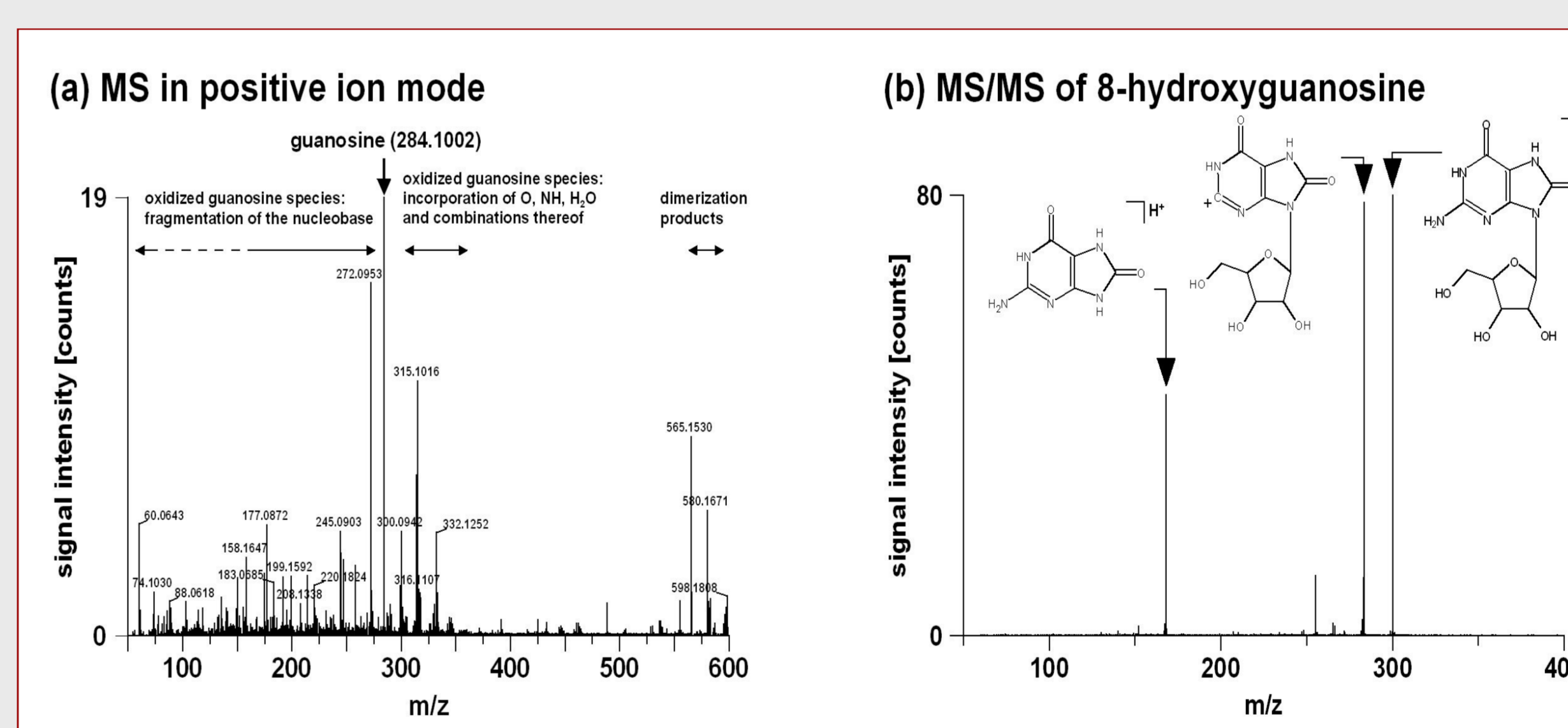


Figure 9: EC/ESI-MS of guanosine. Mass spectra were measured in positive ion mode (E = 2.25 V) (a). Fragmentation of oxidation product of guanosine for positive mode (b). Courtesy of Prof. Dr. Herbert Oberacher, Innsbruck Medical University, Innsbruck, Austria.

## Conclusions

With the availability of the µ-PrepCell (1) a significant increase in the yield of metabolites is achieved. The efficiency of the µ-PrepCell is maintained even at higher flow rates. Glassy Carbon electrodes provide substantial higher yield than conductive Diamond electrodes. For the risk assessment of covalent protein binding of metabolites (2) 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. FEM can be used for the determination of (3) thiol functionalities in the proteins, furthermore ferrocene is converted in the electrochemical cell to ferrocenium resulting in MS signal enhancement. EC/ESI-MS is introduced as efficient and fast method to (4) mimic oxidative modifications of nucleic acids (nucleosides, nucleotides and small oligonucleotides, DNA) occurring in biological systems. EC/ESI-MS represents a valuable tool for studying the fundamental principles of nucleobase oxidation and to find new biomarkers for oxidative stress.

## References

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