

# Simulating nucleic acid oxidation using electrochemistry/liquid chromatography/mass spectrometry

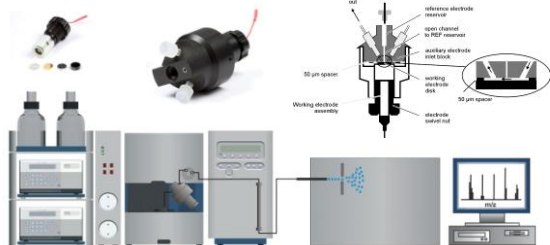
## Background

Nucleic acids present within living systems are continuously exposed to reactive chemicals. Reactive oxygen species represent one class of reactive chemicals that give rise to nucleic acids modification. The formation of covalent adducts between nucleic acids and small molecules represents another mechanism involved in nucleic acids alteration. 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.

Electrochemistry (EC) can be used as biomimetic system to study oxidation processes. Particularly, LC/ESI-MS(/MS) is applied to monitor the obtained reaction products. EC/LC/MS has been applied to study oxidative modification of a number of different compounds including drugs, peptides and proteins, but not of nucleic acids so far.

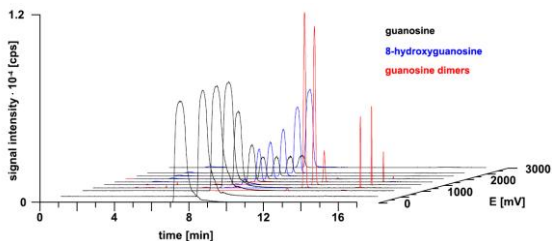
## Experimental Setup

For the electrochemical conversion of nucleic acids commercially available instrumentation was used (ROXY, Antec, The Netherlands). EC was performed in an electrochemical thin-layer cell. A conductive diamond electrode was used as working electrode material. LC was performed on a column (200x0.2 mm) packed with Eurospher 100-5 C18 particles (Knauer, Berlin, Germany). Chromatographic separations were accomplished using gradients of acetonitrile in 10 mM ammonium formate (pH 7.3). The flow rate was set to 3.0 µl/min. The column outlet was directly coupled to the mass spectrometer (Qstar XL, Applied Biosystems, Foster City, CA USA). MS and MS/MS experiments were used to elucidate the structures of the oxidator products.

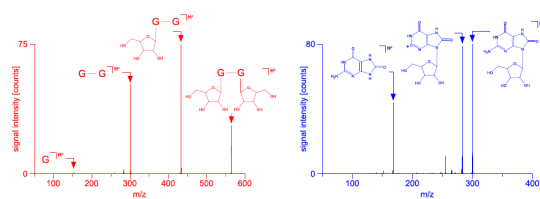


## EC/LC/MS(/MS) of Guanosine

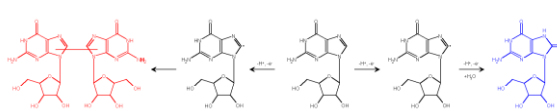
(a) Extracted ion voltammograms



(b) Tandem mass spectra of a guanosine dimer and of 8-hydroxyguanosine

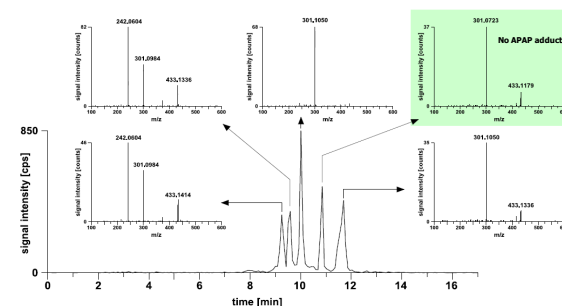


(c) Proposed reaction mechanisms

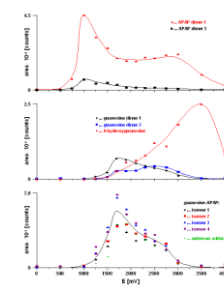


## Formation of Drug-DNA Adducts

(a) EC/LC/MS/MS of a mixture of guanosine and acetaminophen (APAP)



(b) Extracted ion voltammograms



(c) Proposed reaction mechanisms

