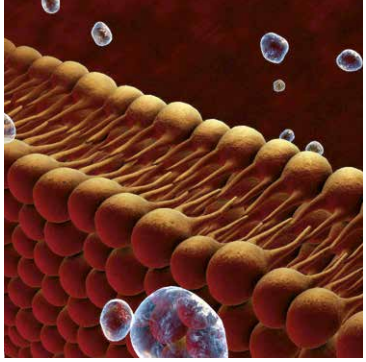
Application Note
Lipidomics / Cholesterol



Electrochemical Reactions upfront MS – EC/MS

Proteomics & Protein Chemistry
S-S bond reduction
HDX
Peptide bond cleavage
Na+, K+ removal
Drug-protein binding

Lipidomics & Fatty Acids
Cholesterol
Oxysterol
FAME Biodiesel

Drug Metabolism
Mimicking CYP 450
Phase I & II
Biotransformation

Synthesis (mg)
Metabolites & Degradants

Pharmaceutical Stability
Purposeful degradation
API testing
Antioxidants

Environmental
Degradation & persistence
Transformation products
Surface & drinking water

Food & Beverages
Oxidative stability
Antioxidants

Forensic Toxicology
Designer drugs
Illicit drugs

Healthcare & Cosmetics
Skin sensitizers

Genomics
DNA Damage
Adduct formation
Nucleic acid oxidation

Generation of Multiple Oxysterols by Oxidation in an Electrochemical Flow-Through Cell

- **Cholesterol oxidation**
- **Easy and fast generation of oxysterols**
- **Mimicking free radical and enzymatic oxidation**

Introduction

Electrochemical (EC) oxidation using EC flow-through cells becomes a popular technique for fast simulation of biological and technologically relevant redox reactions. Combined with mass spectrometry (MS), EC oxidation allows characterization of diverse oxidation products and intermediates formed during an oxidation process, and thus provides deeper understanding of free radical oxidation mechanism and indications for potential products generated in vivo (Jahn & Karst 2012, Faber et al. 2014). EC-MS, often in combination with liquid chromatography (LC), was successfully applied for simulation of oxidation processes in the environment (Hoffmann et al. 2010), elucidation of xenobiotics degradation (Chen et al. 2012), mimicking cytochrome P450 enzyme activities (Jurva et al. 2003), fast prediction of phase I and II drug metabolism and detoxification (Lohmann & Karst 2006, Baumann et al. 2009a), as well as for studying nucleic acid oxidation (Baumann et al. 2009b), disulfide bond arrangements of peptides/proteins (Zhang et al. 2011, 2012), and other protein post-translational modifications (Lohmann et al. 2008, Jahn et al. 2012).

Oxysterol species are formed in vivo by enzymatic and non-enzymatic oxidation of cholesterol. Oxysterols are intermediates in the biosynthesis of bile acids and steroid hormones, but also possess per se versatile bioactivities, such as controlling gene expression, affecting calcium-signaling and immune or inflammatory responses. Many functions of oxysterols are not fully understood and others may not have been discovered yet, especially those of non-enzymatic origin. The lim-

ited number of commercially available standards challenges both analyses and functional studies.

Here we report the generation of numerous cholesterol oxidation products in short reaction times by using an amperometric flow-through cell (ROXY EC system, Antec, NL) and characterization of obtained species by normal phase thin layer chromatography (NP-TLC) and reverse phase (RP)-HPLC-MS.

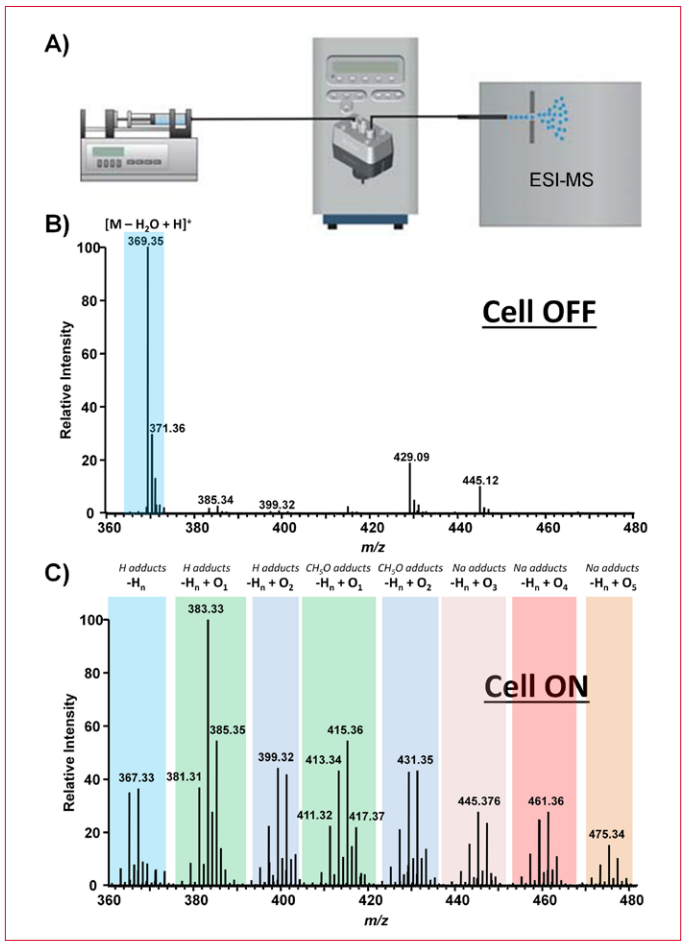


Figure 1: Schematical representation of the ROXY EC system (including the syringe pump and the ROXY potentiostat with the µ-PrepCell) coupled to ESI-MS (A) and the mass spectra aquired by the ESI-LTQ-Orbitrap XL mass spectrometer in positive ion mode for a 100 µmol/L cholesterol solution under the applied EC-cell OFF (B) vs. EC-cell ON (C) conditions. Protonated ion species, sodium- and methanol-adducts are shown.

Material & Methods

Cholesterol (100 µmol/L in 90 % MeOH containing 20 mmol/L ammonium formate) was introduced by syringe pump (50 µL/min) into the µ-PrepCell equipped with a boron doped diamond working electrode and a Pd/H2 reference electrode controlled by the ROXY potentiostat via the Dialogue Software (ROXY EC system, Antec, NL, Fig. 1A). The voltage-dependent oxidation-process was first monitored by coupling the system directly to the ESI-LTQ-Orbitrap XL mass spectrometer (Thermo Scientific, Fig. 1B,C). Later, 2V were constantly applied, the output flow collected and stored at -20°C (with/without 0.05 % BHT) for further analyses by NP-TLC and RP-LC-MS. The electrochemically (EC) generated oxidized products of cholesterol were compared to a set of 19 commercially available oxysterols. The EC-oxidized solution (200 µL) and standards (2 µg) were separated on HPTLC Silica gel 60 F_{254} plates (15 cm x 10 cm, Merck KGaA, Darmstadt, Germany), developed with a mixture of ethylacetate and toluene (50:50, v/v) and dipped into primuline solution (0.02 % in acetone/water, 8:2, v/v) for visualization (Biorad GelDoc EZ Imager, UV Tray). The generated cholesterol oxidation products were additionally analyzed by LC-MS and LC-MS/MS analysis by multiple reaction monitoring (MRM) on a QTRAP 4000 (AB-Sciex) mass spectrometer coupled on-line to C18-column.

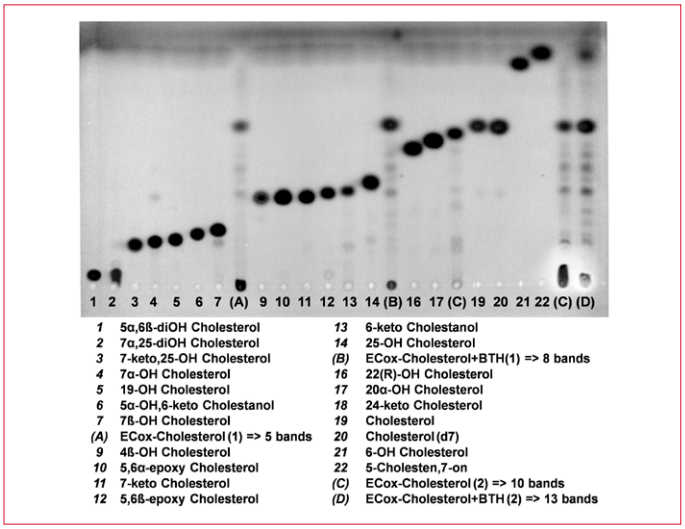


Figure 2: Alalysis of the compounds generated by EC-oxidation of cholesterol by NP-TLC. Lanes 1-22: commercially available oxysterol standards (2µg). A-D: EC-oxidized cholesterol mixture equivalent to 2 pmol of initial cholesterol)

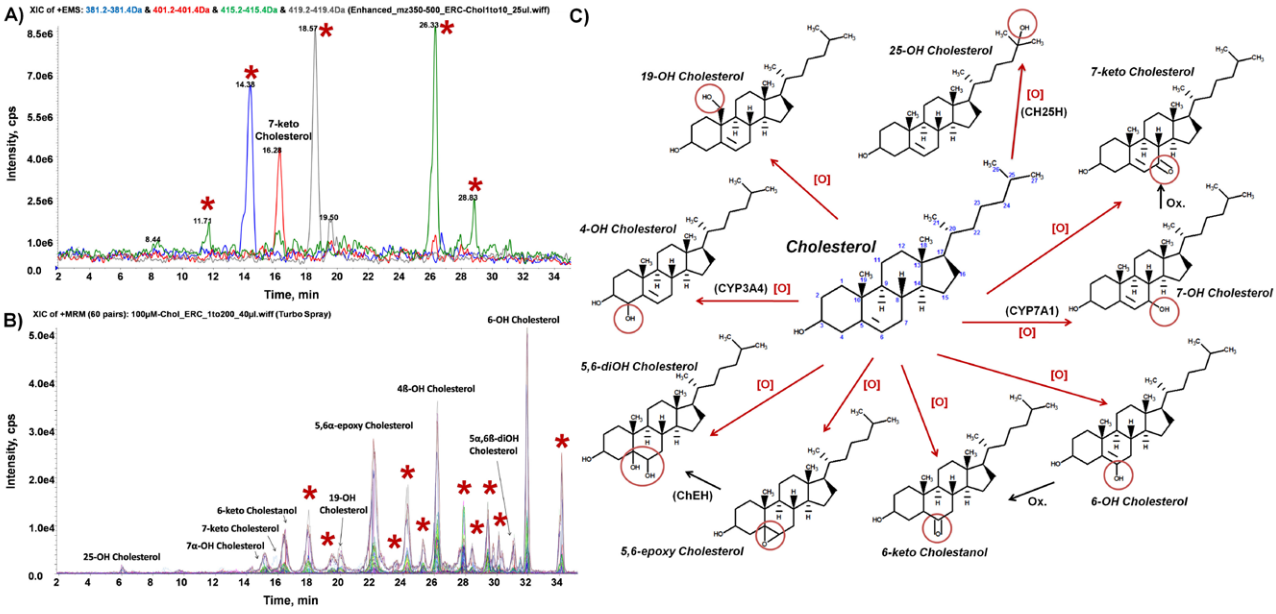


Figure 3: Analysis of the products generated by EC-oxidation of cholesterol by RP-HPLC-MS (extracted ion chromatograms from full scan MS (A) and MRM (B) on the QTRAP 4000). Unknown compounds are marked by asterisks. C: Cholesterol and the generated oxysterols identified by RP-HPLC-MRM. The carbon numbering at the cholesterol backbone is shown in blue. The sites of oxidation are highlighted by red circles. Indicated are the free radical driven (red arrows) and possible consecutive reactions (black arrows) leading to the formation of the identified compounds, as well as enzymes (in brackets) involved in the generation of oxysterols *in vivo*.

Results

The ROXY™ EC system (Antec, NL) equipped with the u-Prep-Cell™ allowed us to oxidize cholesterol yielding numerous oxidation products within short reaction times (Fig. 1C), which were analyzed by NP-TLC (Fig. 2) and RP-HPLC-MS (Fig. 3A, B) relative to 19 standard compounds. Besides the six oxysterols identified by both techniques, more than ten additional electrochemically generated compounds were detected. The identified products were mostly oxidized near the double-bound at the B-ring (and to a lower extent at the tertiary carbon in position 25), which is in agreement with susceptibility to free radical driven oxidation (Fig. 3C). Interestingly, some of the new electrochemically generated oxysterols were also present in lipid extracts obtained from cell culture models of nitrosative stress. Further investigation of electrochemically generated compounds (e.g. using SynthesisCell™ for higher production yields) will allow identification and characterization of new oxysterols in vivo.

Acknowledgment

All data of this application note were kindly provided by Dr. Maria Fedorova[1, 2] et al., and will be presented at the 4th European Lipidomics Meeting, September 22-24, Graz, Austria and as poster at the SFRR 2014, September 5-7, 2014, Paris, France

1 Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy,
2 Center for Biotechnology and Biomedicine (BBZ), University of Leipzig, Leipzig, Germany

Conclusion

By using an electrochemical flow-through cell, cholesterol can be easily oxidized to different oxysterols. The obtained oxysterols show excellent agreement with the known enzymatic biotransformation reactions and with some of the radical driven reactions.

Electrochemistry in combination with LC/MS and/or TLC/MS has great potential for the identification and discovery of oxysterols, thereby mimicking enzymatic and free radical reactions including nitrosative stress.

References

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Figure 4: ROXY EC System consisting of ROXY Potentiostat, dual syringe pump and µ-PrepCell

Ordering information

210.0074A	ROXY EC system, incl. dual syringe pump, µ-PrepCell and electrodes. All parts included for described Electrochemical (EC) application.

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.

Antec Scientific (USA)
info@AntecScientific.com
www.AntecScientific.com
T 888 572 0012

Antec Scientific (worldwide)
info@AntecScientific.com
www.AntecScientific.com
T +31 71 5813333

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