Enhanced Pharmaceutical Stability Testing using online Electrochemical Reactions up-front MS

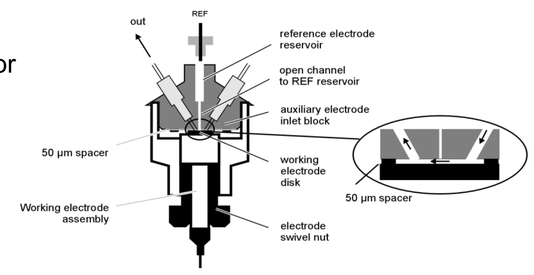
Jean-Pierre Chervet[1], Agnieszka Kraj[1], Nico Reinhoud[1], Mark R. Taylor[2]
[1]Antec, Zoeterwoude, The Netherlands; [2]Pfizer Inc., Sandwich, Kent, U.K.

1. Introduction

Understanding pharmaceutical stability is of fundamental importance to the industry. Stability studies at varied temperature and humidity and purposeful degradation experiments using chemical and thermal methods are widely applied to aid understanding of the stability and degradation of active pharmaceutical ingredients and formulated drug products during development. Many pharmaceutical degradation reactions occur by REDOX mechanisms and the recent advent of commercial flow-through electrochemical (EC) reaction cells has provided a new and convenient method of studying these reactions, with on-line high-resolution mass spectrometry (HR-MS) providing the means to identify and quantify degradation product profiles under varied experimental conditions. EC-HR-MS can be scaled up to synthesize mg quantities of degradation products. After isolation and purification by e.g., liquid chromatography these degradation products can be used or further study by MS and/or NMR. This poster shows results from some preliminary experiments applying EC-HR-MS and EC-LC-HRMS-MS to study the electrochemical degradation of active pharmaceutical compounds and the fast electrochemical synthesis thereof.

2. Instrumentation for Electrochemical Reactions & Synthesis

Solutions were typically prepared in MeCN:Water containing 5 mM NHOAc at concentrations of 5 µg/mL for direct MS infusion and 50 µg/mL for off-line collection ahead of LC-MS-MS



ReactorCell™ (analytical)

µ-PrepCell™ (micro preparative)

Easy interchangeable electrodes, BDD, Au, Pt, etc. (selectivity)

Pump in API solution (or API + soluble excipients, excipient Fragments, etc.)

Ramp the voltage and profile the products by LCMS

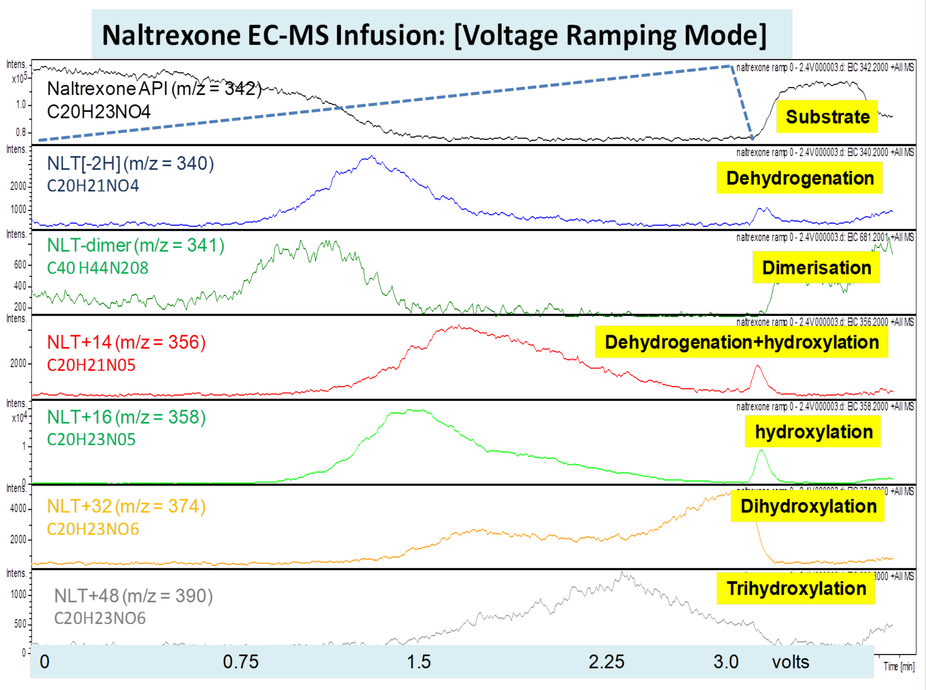
Or hold the voltage to isolate specific species for further Characterization (synthesis)

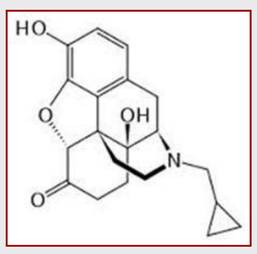
ROXY EC System (Antec)
- Roxy Potentiostat
- Electrochemical cells
- Infusion pump

HR-MS experiments were performed using Bruker Maxis and LC-MS was also performed using Waters Acquity UHPLC with LCT premier TOF-MS

SynthesisCell™
80 mL bulk cell for synthesis of mg quantities of degradants

3. Voltage Ramping EC-HR-MS of Naltrexone API Solutions





Naltrexone

Figure 1: Overlaid EC-HR-MS EICs (Mass Voltammograms) from naltrexone solution infused to Bruker Maxis HRMS. Voltage ramp of 0-3 V in 5 min. Molecular formulae of oxidation products determined rapidly from Acc. Mass ESI-MS using Bruker Compass Smart Formula Chem. Software.

4. EC-UHPLC-UV-ESI-TOF-MS of Naltrexone API Solutions

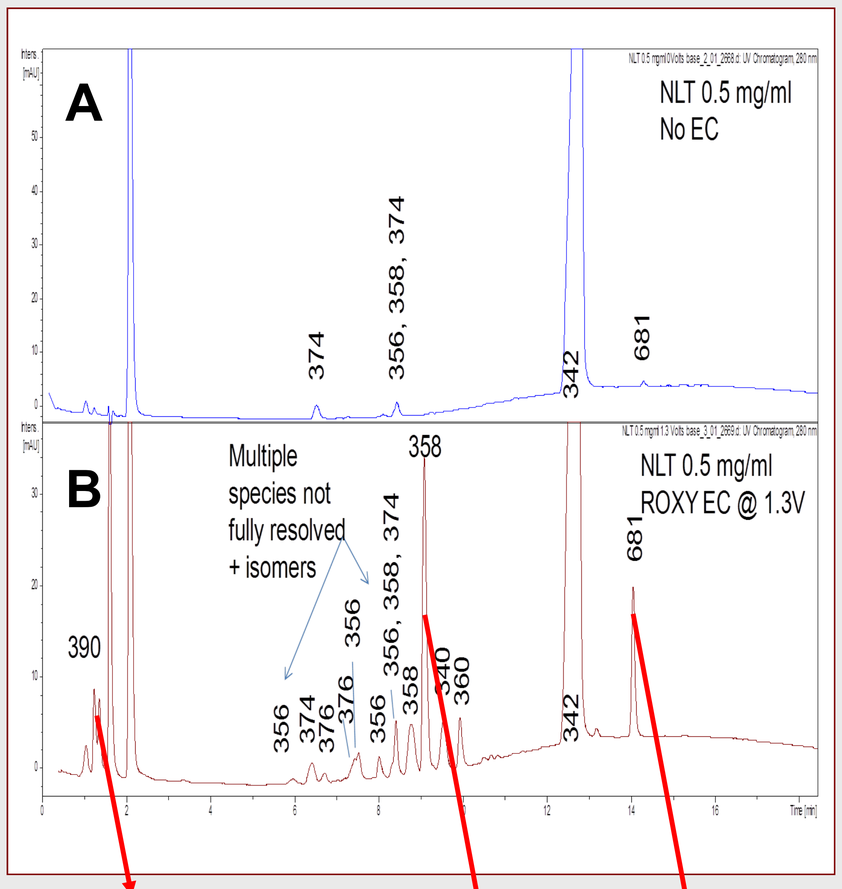
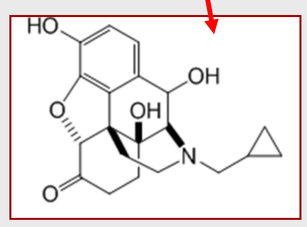


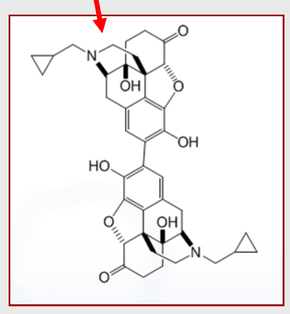
Figure 2: Overlaid UV chromatograms (280 nm) of 500 µg/mL solutions of **A**: aged naltrexone HCl standard and **B**: naltrexone HCl standard oxidised by EC at 1.3V in DC mode showing base-peak mass assignments from TOF-MS. The LC method was not optimised and some co-elution of degradation products occurred.

Hydroxylated, dehydrated and dimerised degradant product peaks were identified and concentrations of the “real world” degradants observed API in the aged standard were increased significantly facilitating structure elucidation by HR-MS-MS. A number of isobaric species were also observed resulting from anomerisation / hydroxylation in different ring positions.

Trihydroxy-NLT isomers



10-hydroxynaltrexone
C20H23NO5
MW = 357



2,2-Bis(naltrexone)
C40H44N2O8
MW = 680

5. Study of Antioxidant Performance / Capacity

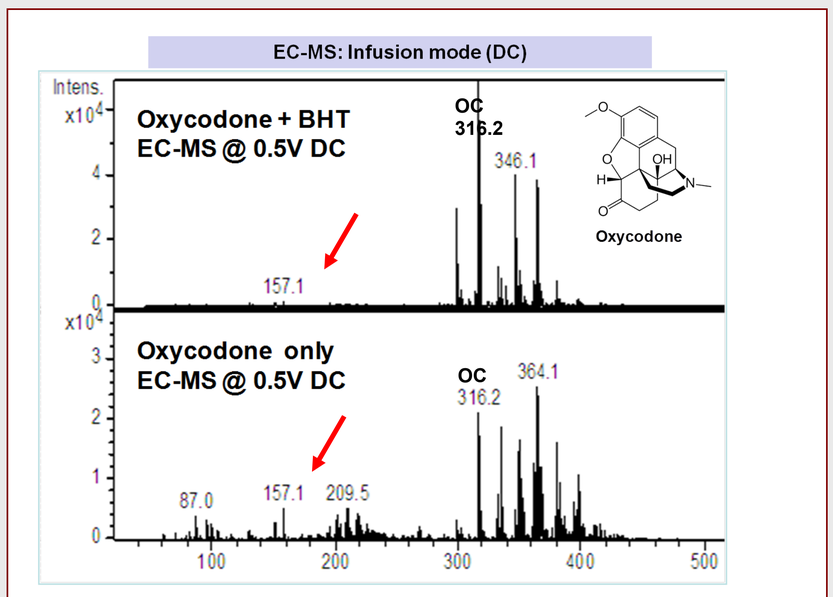
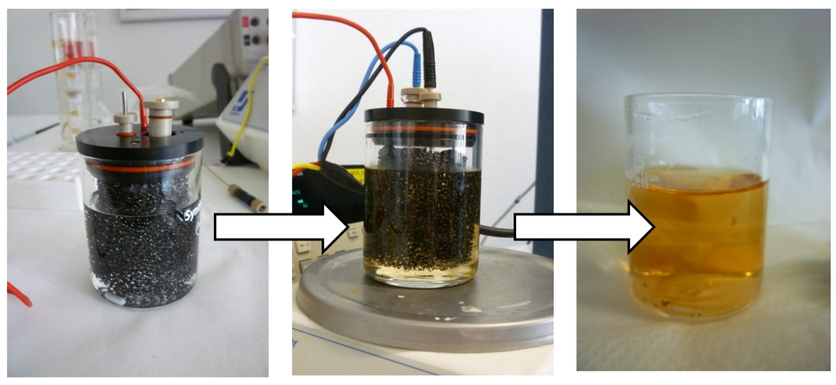


Figure 3: Effectiveness of butylated hydroxytoluene (BHT) as an antioxidant to stabilise oxycodone (OC) in solution was studied by EC-MS and EC-LC-UV-MS.

Addition of BHT signifcantly increased the OC molecular ion base peak intensity and reduced the yield of oxycodone degradant peaks produced when voltage was applied.
The fine voltage / reaction control offered by the EC-MS system allowed reactions to be studied rapidly in real time.

6. Fast Synthesis of mg Quantities of Degradants



t = 0 | t = 15 min | t = 30 min after synthesis experiment

Figure 4: 80 mL bulk SyntehsisCell for fast synthesis of degradants and other REDOX products.
Up to 100 mg (+) of pure degradant marker solutions in 1 day resulting in tremendous savings in synthesis resources. ***ROXY can do it faster, cleaner and greener – we are very excited about the results, Dr. M. Taylor***

Conclusions

EC-HR-MS has given us a new way of studying pharmaceutical stability and a convenient & controllable method of performing purposeful degradation without need for oxidizing agents and with rapid, real-time results.
All degradation products know form Naltrexone were found and re-confirmed by EC. In addition **4 new proprietary compounds** were found in less than 2 days work!
EC has untapped potential for a host of other pharma-related studies including formulation design, standard synthesis and accelerated structure elucidation and we will continue to research these over the next few years.