Pharmaceutical Stability Testing by On-line Electrochemistry-LC-MS

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1. Objective

Using Electrochemistry-LC-MS (EC-LC-MS) for rapid drug stability testing:

- EC as alternative to oxidative forced degradation and accelerated stability studies
- Eliminating the use of harsh oxidizing chemicals (green)
- Selective and tunable

2. Introduction

Stability testing of drugs and active pharmaceutical ingredients (API) are important to pharmaceutical industry in order to understand and maintain product quality and safety through-out its shelf-life. Oxidative forced degradation studies are among the different types of stability studies performed to understand the intrinsic stability of drug molecules. We have been comparing the use of electrochemistry as an alternative oxidative forced degradation method to traditional forced degradation and accelerated stability studies.

3. Instrumentation

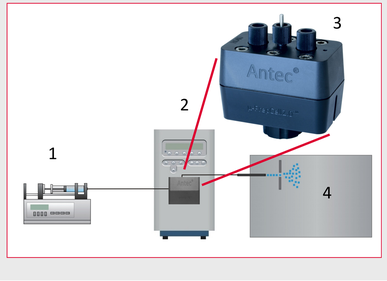

Figure 1: Schematics infusion Electrochemistry/MS system: 1) Infusion pump, 2) ROXY Potentiostat equipped with 3) µ-PrepCell2.0 and Boron Doped Diamond (BDD) or Glassy Carbon (GC) electrode 4) generic MS

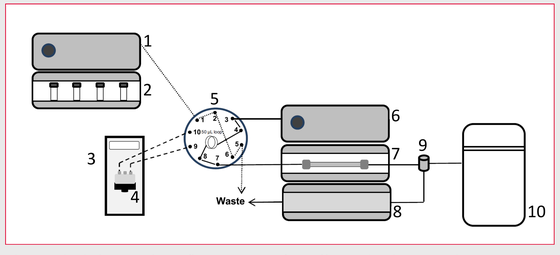

*Figure 2: Schematics of automated on-line EC-LC-UV-MS system used to study the oxidative behavior of **drug compound**. 1) loading/washing pump, 2) autosampler, 3) Roxy potentiostat, 4) electrochemical cell (µ-PrepCell), 5) 10-port switching valve, 6) gradient pump, 7) column compartment, 8) UV detector, 9) flow splitter, 10) MS*

4. Experimental

4.1. Drug Compound

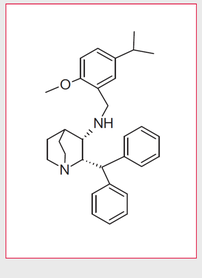

Figure 3: Chemical structure of drug compound ((2S,3S)-2-(diphenylmethyl) -N-[2-methoxy-5-(propan-2-yl) benzyl] -1-azabicyclo[2.2.2]octan-3-amine) used in this study.
Pfizer Worldwide R&D, Groton, USA

4.2. Electrochemical Oxidation

The EC reactions were conducted with the ROXY Potentiostat (Antec Scientific) consisting a flow-through µ-PrepCell 2.0 equipped with a Glassy Carbon (GC) working electrode and a Pd/H_2 reference electrode.

4.3. EC-LC-UV-MS

A 2.6 µm, XB-C18, 150 × 4.60 mm (Phenomenex, Kinetex) was used for the separation. TFA (0.05% in water) and ACN were used for the gradient elution. Total run time 20 min. Flow rate 1.0 mL/min; injection loop 50 µL, column temperature 50°C. The volume of each sample delivered by the loading pump was 100 µL. The UV detector (Agilent 1100 Series) and the mass spectrometer was an Agilent 6120 single quadrupole MS fitted with a ESI and/or APCI. UV and MS were connected in parallel using a flow splitter with 80% of the flow diverted to the UV and 20% to MS. The LC-UV-MS was controlled by the Agilent OpenLAB software (Waldbronn, Germany).

5. Results

5.1. Off-line EC-MS

Previously, off-line EC was applied to study the oxidative behavior of the drug compound [1]. All of the final stable oxidation products could be generated. For these studies the drug was oxidized by pumping the solution through the cell and collecting the effluent into vials for off-line LC–MS analysis. This approach is time consuming especially when large numbers of drug compounds are studied. Furthermore, there is a risk to miss short-lived oxidation products

5.2. On-Line EC-LC-UV-MS

By on-line coupling the EC cell to LC-UV-MS the shortcomings of the off-line system could be overcome [2]. The system allows to study multiple experimental parameters (pH, voltage, substrate concentration) without operator intervention, resulting in significant better reproducibility.

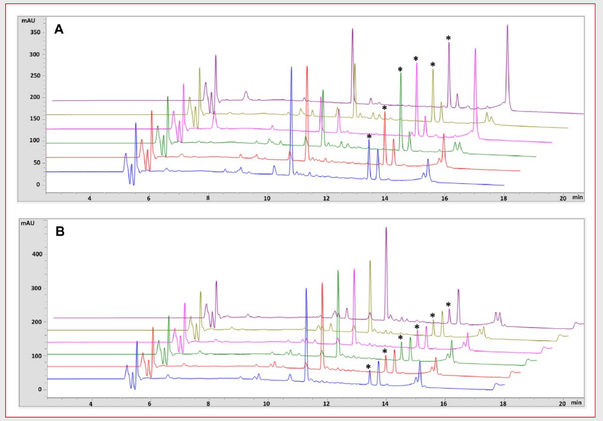

*Figure 4: EC-LC-UV chromatograms (225 nm) showing EC degradation of drug compound oxidized at 1.5 V (6 consecutive runs). **A)** Oxidative profiles without a cleaning cycle. **B)** Oxidative profiles after introduction of a cleaning cycle between each run. Results show increased reproducibility by introduction of a cleaning cycle. Drug compound marked with asterisk.*

6. Conclusions

The on-line coupling of EC-LC-UV-MS allows for:

- Rapid and automated screening of multiple drug compounds on their oxidative stability
- Testing of multiple experimental conditions (electrode material, voltage, pH, organic solvent content)
- Significant reduction of the operator intervention and time in comparison to the off-line approach
- Studying of short-lived oxidation products

7. References

[1] S. Torres et al.; J. Pharm. Biomedical Analysis 115 (2015) 487-501
[2] S. Torres et al.; J. Pharm. Biomedical Analysis 131 (2016) 71-79