The Application of Electrochemistry/MS to Pharmaceutical Stability Testing and Degradant Synthesis

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Introduction

Electrochemistry (EC) was used to generate oxidative degradation products of a model pharmaceutical compound. The compound was oxidized at different potentials using a µ-PrepCell fitted with a Glassy Carbon working electrode, a Pd/H_2 reference electrode and a Titanium auxiliary electrode. The oxidation products were identified and characterized by LC-ESI–MS/MS using a high resolution Q-TOF MS. Results from electrochemical oxidation using different pH were compared to those from chemical oxidation and from accelerated stability studies. Additionally, oxidative degradation products using an in silico software were compared to those obtained from the various experimental methods [1].

Structure Pharmaceutical Compound

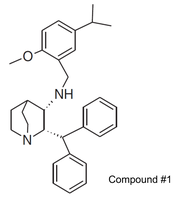

Figure 1: Chemical structure of the model compound ((2S,3S)-2-(diphenylmethyl)-N-[2-methoxy-5-(propan-2-yl) benzyl]-1-azabicyclo[2.2.2]octan-3-amine) used in this study.

Figure 2: Electrochemical flow cell: µ-PrepCell™ (Antec)

Instrumentation

Off-Line Electrochemistry

The electrochemical oxidation was performed using a 3-electrode flow cell (µ-PrepCell™), fitted with a glassy carbon (GC) working electrode, a Pd/H_2 reference electrode and a titanium auxiliary electrode connected to a ROXY potentiostat controlled by Dialogue software (Antec, Zoeterwoude, The Netherlands).

LC-UV-MS Analysis

A HPLC–MS (1100 Series) with 6120 single quadrupole MS controlled by OpenLAB software (Agilent, Waldbronn, Germany) was used. A reversed-phase superficially porous particle column (Phenomenex, Kinetex 2.6 µm XB-C18, 100 Å, 150 × 4.60 mm) was used. Separation was achieved by gradient elution, using aqueous trifluoroacetic acid (0.05%) and acetonitrile as eluting solvents. The flow rate was 1.0 mL/min. The temperature of the column was 40°C. Compounds were detected by UV (λ= 225 nm) or by MS. The UV detector and MS were connected in parallel using a flow splitter.

LC–MS/MS Experiments

A 1290 Infinity HPLC system consisting of a variable wavelength UV detector and 6550-Q-TOF ion funnel MS fitted with an electrospray source controlled by Masshunter software (Agilent, Waldbronn, Germany) was used for high resolution mass and tandem mass spectrometry experiments. The oxidation products were detected in the positive ion mode. A reversed-phase UHPLC column (ACQUITY UPLC®HSS 1.8µm, 2.1 × 100 mm) was used. Aqueous formic acid (0.1%) and acetonitrile were used as eluting solvents. Compounds were detected by UV at λ= 225 nm and by high resolution MS. For tandem mass spectrometry (MS/MS experiments), the pre-cursor ion of interest was selected using the quadrupole analyzer and the product ions were analyzed using a TOF analyzer. All the spectra were recorded under identical experimental conditions using collision energies of 20 eV or 40 eV.

Methods

Electrochemical Oxidation

0.086 mg/mL solutions of the Compound #1 (Fig. 1) dissolved in10 mM aqueous ammonium acetate solution at three pH values (3.9, 7.1, and 8.8) were pumped through the EC cell. Samples analyzed at basic pH were dissolved in a mixture 10 mM ammonium acetate (pH = 8.8)/ acetonitrile (1:1) to ensure solubility of the substrate. The applied potential was increased from 0 to +2 V in steps of 100 mV, and a sample of the cell effluent was collected into separate HPLC vials at each potential. The collected samples were analyzed offline by HPLC-UV-MS.

Oxidative Forced Degradation

Two oxidative forced degradation studies, using **peroxide** and a **radical-initiated oxidation,** were performed on Compound #1. The concentration of each sample was 0.86 mg/mL and all samples were protected from light. For each reaction 3 different samples were prepared (drug substance exposed to the challenge condition, H_2O_2 for peroxide oxidation or AIBN for radical-initiated oxidation). Prior to the analysis by HPLC–UV–MS the samples were diluted by 50:50 (v/v) acetonitrile:water.

In Silico Predictions

Predictions were performed using the Zeneth 6.0 application with Knowledge Base Z2014.2.0.mdb (Lhasa Limited, Leeds, UK). This application is an expert decision support system which predicts the forced degradation pathways of organic compounds.

Degradation Pathways Drug Compound #1

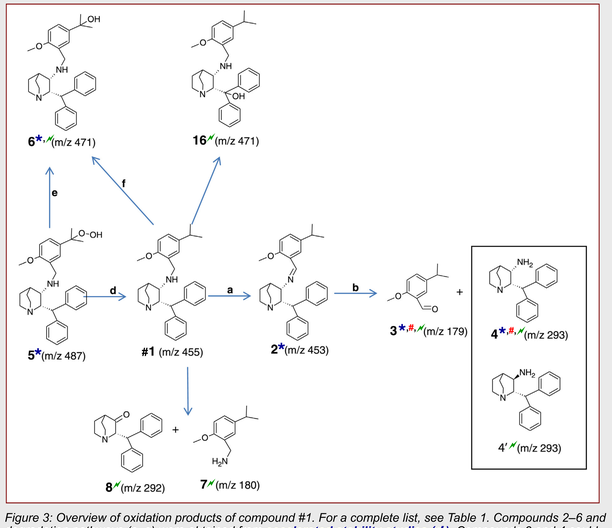

*Figure 3: Overview of oxidation products of compound #1. For a complete list, see Table 1. Compounds 2–6 and degradation pathways (a–e) were obtained from **accelerated stability studies (✱)**. Compounds 3 and 4 could be generated both by: **forced degradation (#)** and **electrochemically (✓)**. Compound 6 and 16 were only electrochemically generated. Chemical structures for compounds 3, 4, 4', 6, 7, 8 and 16 were derived on the basis of exact masses, and MS/MS fragmentation patterns.*

Generated Degradation (Oxidation) Products

Oxidation products	Molecular formula	Observed m/z ([M+H]$^+$)	Error (ppm)	EC	ST	*in silico*	Accelerated Stability
1* (Drug substance)	$C_{31}H_{38}N_2O$	455.3073	0.29	–	–	–	–
2*	$C_{31}H_{36}N_2O$	–	–	×	×	√	√
3*	$C_{13}H_{18}O_2$	179.1065	1.85	√	√	√	√
4*	$C_{20}H_{28}N_2$	293.2011	0.12	√	√	√	√
4'*	$C_{20}H_{28}N_2$	293.2013	-0.22	√	×	×	×
5*	$C_{31}H_{38}N_2O_3$	–	–	×	×	√	√
6*	$C_{31}H_{38}N_2O_2$	471.2998	0.26	√	×	√	√
7*	$C_{13}H_{21}NO$	180.1378	2.28	√	×	×	×
8*	$C_{20}H_{21}NO$	292.1693	0.67	√	×	×	×
9	–	183.2	–	√	×	×	×
10	–	208.2	–	√	×	×	×
11	–	167.2	–	√	×	×	×
12	$C_{20}H_{18}N_2O$	303.1490	0.50	√	×	×	×
13	$C_{31}H_{32}N_2O$	449.2586	0.45	√	×	×	×
14	$C_{18}H_{24}N_2O$	285.1962	0.08	√	×	×	×
15	–	167.2	–	√	×	×	×
16*	$C_{31}H_{38}N_2O_2$	471.3004	0.77	√	×	√	×

Table 1: Overview of oxidation products generated via pharmaceutically relevant stress testing (ST), electrochemically (EC) and predicted by in silico (computational). Oxidation products for which a chemical structure was assigned are marked with *, with ✓ for generated, and with × for not generated.

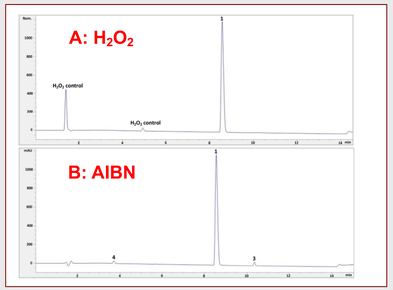

*Figure 4: LC–UV chromatogram at 225 nm of **forced degradation.** Samples after 3 days of challenge condition:*
(A) 0.3% H_2O_2,
(B) 5 mM AIBN; 2,2'-Azobis(2-methy-lpropionitrile), radical initiator

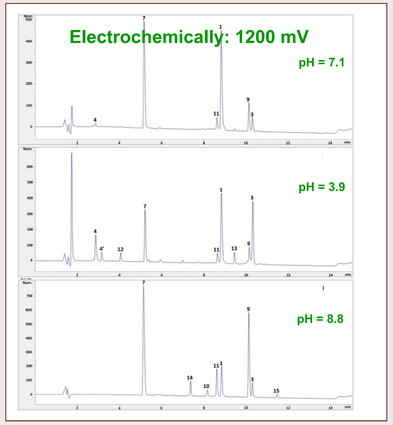

*Figure 5: LC–UV UV chromatograms at λ = 225 nm for **electrochemically** oxidized samples at 1200 mV at three different pH values (pH = 3.9, 7.1, 8.8). Peak 1 corresponds to the drug sub stance under study. For proposed structures see Figure 3. No chemical structures were obtained for oxidation products 9–15.*

Synthesis of mg Quantities

For the fast synthesis of mg quantities of oxidative degradants and metabolites in related studies a bulk synthesis cell was used for the generation of the appropriate products mixtures. The oxidation products were isolated and purified by reverse phase preparative high-performance liquid chromatography and subsequently fully characterized by NMR [2].

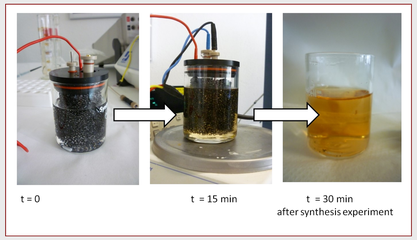

Figure 6: 80 mL bulk SynthesisCell (Antec) for fast synthesis of degradants and other REDOX products.
Up to 100 mg (+) of pure degradant marker solutions in 1 day resulting in tremendous savings in synthesis resources.
Antec's ROXY EC can do it faster, cleaner and greener – we are very excited about the results, Dr. M. Taylor (Pfizer, UK)

Conclusions

The electrochemical (EC) approach proved to be useful as an oxidative stress test.

- All final degradation products observed under accelerated stability studies could be generated
- From the 3 stable degradants generated by EC only 2 were generated using the chemical stress testing with the radical initiator AIBN
- EC is much faster and **"greener"**, it does not require strong oxidizing agents
- EC is selective, it enables the study of different operating parameters and the optimization of the reaction conditions, i.e., pH, applied voltage, to generate different oxidative products
- Very useful as a rapid stress test to generate oxidative degradation products and to study drug stability
- Fast synthesis of mg quantities of degradants for structural elucidation by MS and NMR and for toxicity, bioactivity studies

References

[1] S. Torres et.al., *Journal of Pharmaceutical and Biomedical Analysis*, **115**, (2015) 487-501
[2] S. Torres et al., *Org. Process Res.Dev.*,(2014), http://dx.doi.org/10.1021/op500312e