

Investigation of Reserpine Oxidation Using On-Line Electrochemistry/Electrospray Mass Spectrometry

Vilmos Kertesz; Gary J Van Berkel;

Oak Ridge National Laboratory, Oak Ridge, TN

Keywords: Electrospray Ionization (ESI); On-line Analysis; Oxidation; Reaction Monitoring;

Novel Aspect: This is the first comprehensive study of reserpine oxidation using direct coupling an electrospray mass spectrometer with an electrochemical cell.

Introduction:

Electrochemical oxidation products of reserpine formed in acetonitrile and acetonitrile/water solutions were identified using electrochemistry coupled on-line with electrospray mass spectrometry. The main reserpine oxidation product observed was 3,4-dehydroreserpine formed by a two-electron, one-proton oxidation process, while further oxidation products (e.g. lumireserpine) were also identified. Multiple hydrolysis products were also detected during the oxidation. Based on the results a reaction scheme is proposed describing the oxidation/hydrolysis reactions of reserpine.

Methods:

The outlet of the thin-layer, three-electrode electrochemical cell –where the oxidation of reserpine took place- was directly coupled to the inlet of the mass spectrometer ion source. This way the oxidized sample may reach the mass spectrometer in < 10 s based on the flowrate used making it possible to analyze the products that formed in the EC cell on-line. The electrochemical experiments was accomplished using a CH Instruments model 660 electrochemical workstation, while the MS experiments were performed on a PE Sciex API 165 single quadrupole mass spectrometer (MDS Sciex, Concord, Ontario, Canada).

Preliminary results:

Cyclic voltammogram of reserpine reveals a non-reversible (in the examined 10-1000 mV/s scan rate range) oxidation peak and indicates a relatively easy oxidation of the drug. In the potential region where no oxidation occurs, the ES mass spectrum reveals only one peak at m/z 609 that corresponds to the protonated molecule of reserpine. Oxidation of reserpine starts around $E=0.8$ V vs Ag/AgCl and the first oxidation products appear up at m/z 607 and m/z 625. The peak at m/z 607 corresponds to 3,4-dehydroreserpine that is formed in a two-electron, one-proton oxidation process, while the peak at m/z 625 indicates hydroxylated products formed by chemical followup reactions. Increasing the working electrode potential a new peak at m/z 605 arises indicating formation of 3,4,5,6-tetrahydroreserpine. At the same time peaks at m/z 639 and 655 appears indicating the appearance of multiply-hydroxylated products as well. When the working electrode potential is increased even further, a new peak at m/z 415 shows up in the spectrum. This peak corresponds to methylreserpate that forms by hydrolysis and cleavage of a trimethoxy-benzoic acid molecule from the reserpine. Experiments are in progress to identify the structure of all oxidation/hydrolysis products by using MS/MS technique coupled on-line with the electrochemical cell and to investigate the oxidation of other important drugs using the EC/ES-MS technique.

Research sponsored by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences, U.S. Department of Energy, under contract No. DE-AC05-00OR22725 with Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC.