

## Electrochemically Assisted Ionization for LC/ESI-MS and LC/APCI-MS of Small Molecules: Quantitation and Differentiation of Isobaric Species.

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### **Introduction or Invited Speaker Abstract:**

Several studies have shown that solution-phase electrochemical (EC) conversion of certain redox active compounds can lead to the formation of more readily ionized species thus facilitating their study with LC-MS. Porous carbon working electrodes with high electrolytic conversion efficiencies have been used to extend the capabilities of both APCI and ESI sources when used for quantitative LC-MS analysis of neutral and non-polar molecules. This work involved the use of a 3-electrode, controlled potential EC cell placed upstream and electrically isolated from the MS ion source. The applicability of EC-assisted ionization across a structurally diverse range of low molecular weight molecules was examined in the context of LC conditions that are commonly used in drug discovery and development.

### **Methods:**

Flow injection analysis was used to assess ion abundance for 45 structurally diverse compounds with the following ionization conditions: pH7 (20mM ammonium acetate) ESI and APCI negative ion; pH3.9 (10mM ammonium formate, 50mM formic acid) ESI and APCI positive ion. Solvent was 50% aqueous methanol. Each compound solution was injected with the EC cell (Model 5021, ESA Inc.) potential at 0, 500 and 1000mV vs. Pd reference. Based on these results, selected compounds were analyzed by LC using reversed phase acetonitrile: water gradient conditions with a Q TRAP<sup>TM</sup> LC/MS/MS System (Applied Biosystems/MDS Sciex) to further assess the capability of EC oxidation to improve quantitation of otherwise poorly ionized species and for increasing selectivity for isobaric species.

### **Preliminary Data:**

Compounds examined included: tertiary (n=8), secondary (n=6) and primary (n=2) aliphatic amines; aryl amines (n=7); phenolic acids (n=4); neutral phenols (n=11); phenols with basic side chain (n=1); thiol and thioether (n=2); and thiophosphate (n=3). With pH 7 solvent conditions, base peak negative ion abundance was high for 16 (36%) and 20 (44%) of the compounds when using only ESI and APCI, respectively. With pH3.9 conditions, base peak positive ion abundance was high for 20 (44%) and 21 (47%) of the compounds when using only ESI and APCI, respectively. EC reactions were observed according to the following general rank order (by relative ease of oxidation) *o*, *p*-quinol and *o*, *p*-aminophenol > tertiary amine > *m*-quinol ≈ phenol ≈ arylamine > secondary amine ≈ thiol > thioether. No EC oxidation was observed for primary amines and aliphatic alcohols and oxidation of secondary and tertiary amines was less facile at pH3.9 than at pH 7.0. These observations provide a means of distinguishing isobaric species (e.g. phenol vs. benzyl alcohol). In general, EC oxidation of solution-phase ionic species (e.g. amines, pH 3.9) resulted in decreased base peak ion abundance. EC oxidation resulted in increased (up to ≈120-fold) base peak ion abundance for solution-phase neutral and nonpolar species including phenols and arylamines (pH 3.9 and 7), amines at pH 7 and thiophosphates at pH 3.9. When combined with reversed phase LC separations, EC oxidation extended the range of MS applicability when optimal chromatographic conditions resulted in sub-optimal MS ionization conditions (e.g. low pH for phenolic acids).

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