# A new tool for proteomics: Atmospheric Pressure Electron Capture Dissociation

**Davin Carter**[1]; Jason Rogalski[1]; Damon Robb[2]; Michael Blades[2]; Juergen Kast[1,2]

[1] The Biomedical Research Centre, [2] The Department of Chemistry, The University of British Columbia, Vancouver, Canada

## Introduction

• We are developing new analytical methods that will offer further insights into the relationships between platelets, monocytes, and the endothelium.

• Electron capture dissociation (ECD) and electron transfer dissociation (ETD) have emerged as powerful tools for the analysis of post-translational modifications

• We have developed a novel in-source atmospheric pressure (AP)-ECD method as an alternative to conventional *in vacuo* ECD or ETD, capable of providing ECD functionality to all types of electrospray mass spectrometers, without modifications to the instrument.

• Here, we demonstrate the use of AP-ECD in the LC/MS analysis of model sulfated and glycosylated peptides as well as the reproducibility of AP-ECD

## AP-ECD Method

• The AP-ECD source is comprised of a sprayer, a spray chamber, and a source block with a photoionization detector (PID) lamp.

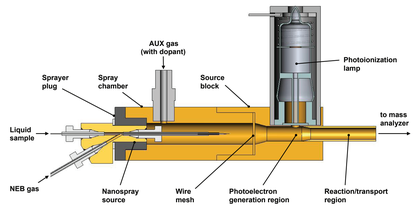



**Figure 1:** *AP-ECD source*

• Multiply-charged peptide ions are created within the heated spray chamber by the enclosed nanospray source. These ions are transported through the spray chamber by a flow of gas to the photoelectron generation region.

• Electrons are produced from interactions between photons, generated from a photoionization lamp, and acetone dopant (D) added to the auxiliary (AUX) gas: $D + h\nu \longrightarrow D^{+} + e^{-}$

• Photogenerated electrons interact with peptide ions to cause in-source ECD preserving labile modifications. The ions are then introduced to the mass spectrometer.

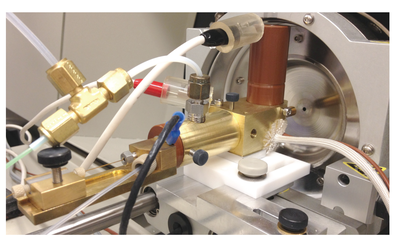


**Figure 2:** *AP-ECD interfaced with a QStar XL Q-ToF*

## Experimental

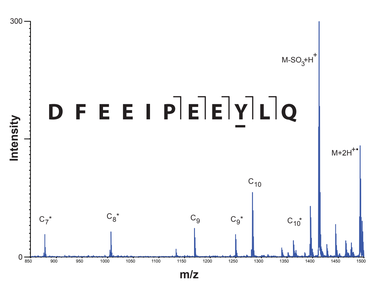
• AP-ECD source: uses nanospray emitters (New Objective) for peptide ionization and a PID lamp (Heraeus Noblelight) for generation of electrons via photoionization of the acetone dopant (0.2 ul/min); the auxiliary gas is high-purity nitrogen (~7 l/min). The source temperature was 100°C. The nanospray voltage was 2.8 kV and the voltage of the ion source block was 1.2 kV. The declustering potential was 80 volts.

• Mass Spectrometer: unmodified QStar XL Q-ToF from AB Sciex

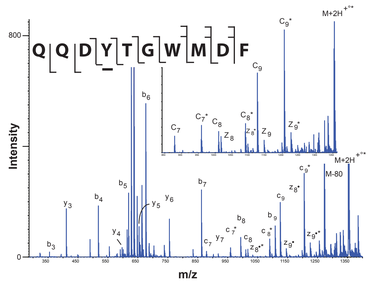
• Chromatography: LC packings Ultimate with Famos autosampler, column 10 cm x 75 um RP C18; flow = 200 nl/min; A standard gradient of: mobile phase: A= 0.1% formic acid in water, B = 0.1% FA in acetonitrile/water.

• Samples: Solutions containing 100 fmol to 1 pmol of peptide were injected on column.

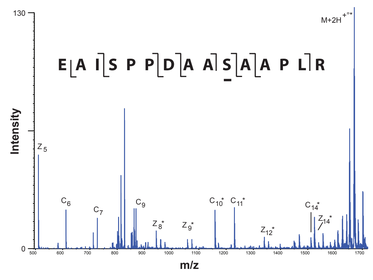
## Results - Sequencing and Localization



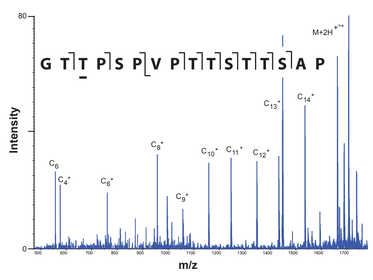

**A:** *AP-ECD spectra of sulfated hirudin*




**B:** *AP-ECD spectra of sulfated caerulein*




**C:** AP-ECD spectra of glyco EPO




**D:** *AP-ECD spectra of glyco MUC5AC*

**Figure 3 (a-d):** *AP-ECD spectra of two sulfo and two glyco peptides. The peptide sequences were determined from the extensive fragmentation. AP-ECD could also localize the modifications. Of special note was the ability to determine the location of the sulfo group on Caerulein in contrast to previously reported efforts.*

## Results - Stability

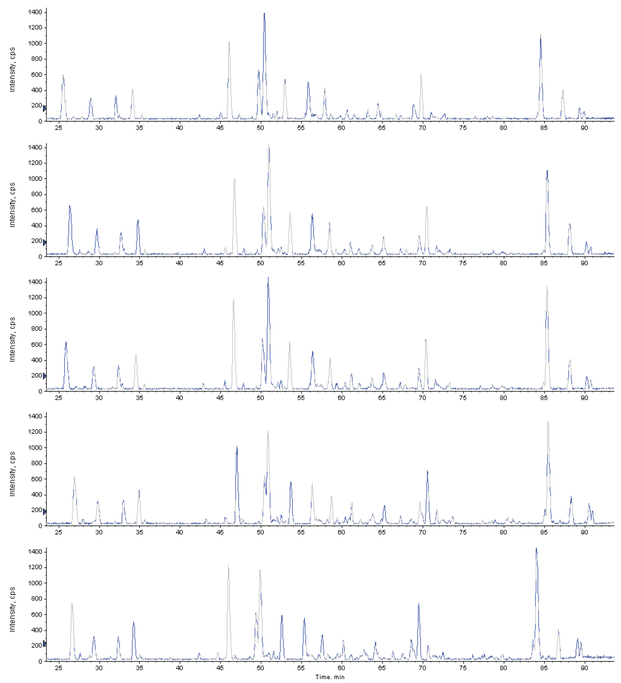



**Figure 4:** *AP-ECD base peak chromatograms from 5 consecutive 100 fmol injections of BSA tryptic digest, demonstrating overnight stability of the method*

## Conclusions

• Localization of sulfation and glycosylations and sequence determination was achieved by AP-ECD.

• The reproducibility of AP-ECD has shown with a protein digest.

• AP-ECD could be useful tool for the targeted analysis of modified peptides and proteins

## Acknowledgements

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