CHEM 5181 Laboratory #2: LC-ESI-qTOF Mass Spectrometry

Dates: October 18-19, 2011
Report Due: November , 2011

Introduction:

In this lab you will run a liquid chromatography-electrospray ionization-triple quadrupole-time-of-flight mass spectrometer (LC-ESI-qTOF-MS) from Waters Corpration, SYNAPT G2 High Definition Mass Spectrometry System (nickname: Synapt G2). In Part I of the lab you will inject solutions of pure organic compounds directly into the ESI-qTOF-MS and analyze them in both the ESI+ and ESI- modes as well as perform MS/MS (i.e., collision induced dissociation, CID). In Part II you will run an analyte mixture over UPLC (ultra performance liquid chromatography), which is coupled to detection with the ESI mass spectrometer.

You will be provided:

- Simplified instructions for using the Synapt G2
- Instructions for the lab
- A methanol solution (~10 ppm) of *trans*-1,2-Diaminocyclohexane-N,N,N',N'-tetra acetic acid (abbr. DACTAA, mw 356)
- A solution of Glu1 Fibrinopeptide (GFP, mw 1571) at 0.16 ppm in 50:50 acetonitrile (ACN)/water and 0.1 % formic acid
- Solutions of formic acid (1% in ACN) and lithium chloride (~mM in ACN)
- A mixture of five compounds for LC-MS, i.e., acetaminophen, caffeine, sulfadimethoxine, terfenadine, and reserpine, each 2 ppm weight in 10 : 90 ACN/water.

Description of Instrument Setup:

Refer to the uploaded Lecture Notes of Oct 6.

Additional information:

- ESI capillary needle: stainless steel tubing (O.D. 400 µm) with the tip approximately 10 mm apart from the counter electrode at ground potential.
- Heated nebulizer gas (nitrogen, 600 L/h, 150 – 375 °C) is flowed coaxially with the liquid spray ("pneumatically assisted ESI").
- A typical mass accuracy without calibration: +/- 0.1 in units of m/z
- UPLC column: Waters ACQUITY UPLC BEH C18 column, 2.1 mm I.D. x 50 mm in length, 1.7 µm column particles
- UPLC mobile phases: A1 = 2 : 98 ACN/water with 0.1 % formic acid and B1 = ACN with 0.1 % formic acid for reversed-phase chromatography. Column flow rate: 5 – 2000 µL/min. Maximum operation pressure: 1000 bar

Laboratory Directions:

I. ESI analysis of DACTAA and GFP

1. ESI+ MS of DACTAA

Use the Isotope Model and predict the peak position and isotope distribution for protonated DACTAA ($[M+H]^+$ and $[M+nH]^{n+}$).

Set the Capillary to 2800V, Sampling Cone voltage to 25 V, and inject the DACTAA solution at 5 µL/min. Take and print out a mass spectrum over the mass range of m/z 50 – 1200 and find the $[M+H]^+$ peak at m/z 347. What other ions do you see? Do you find multiply charged ions $[M+nH]^{n+}$?

Use the above setting for the rest of DACTAA ESI+ experiments. Prepare two samples by adding i) 2 µL of LiCl solution and ii) 20 µL of formic acid solution to each of the DACTAA solutions (ca. 200 µL) and take spectra. From the observed changes in product distributions, assign chemical formula to ions of m/z 347, 352, 353, 369, and 385.

Referring to Kebarle and Verkerk (2009) plus other papers and provided information, predict the onset voltage for methanol and compare it with the capillary voltage used.

2. ESI+ MS/MS (or CID) of DACTAA

Use the acidified DACTAA solution. Set the quadrupole resolution (LM Resolution) to 15.0 and do CID on the $[M+H]^+$ ion (Trap Collision Energy (CE) = 0, 15, and 25 V) and take spectra. Discuss the mechanism for the formation of product ions m/z = 214 and 134.

Do CID on the m/z 693 ion at Trap CE = 0, 5, and 10 V and take spectra. From the observed products and CID efficiency, what is the most likely structure for this ion?

3. ESI- MS of DACTAA

Set the Capillary to 2200V and Sampling Cone 25 V. Take a mass spectrum over the mass range of m/z 50 – 1200 and find the $[M–H]^-$ peak at m/z 345. Do you find multiply charged ions $[M–nH]^{n-}$?

4. ESI- MS/MS of DACTAA

Perform CID on the $[M–H]^-$ ion (LM Resolution = 15.0; CE = 0, 15, and 25 V) and take spectra. Discuss the mechanism for the formation of product ions m/z = 327 and 301.

5. ESI+/– MS and MS/MS of Glu1 Fibrinopeptide (GFP)

In the ESI- mode set the Capillary to 2200V, Sampling Cone to 40 V, and inject the GFP solution at 10 µL/min. Take a spectrum of GFP over m/z 50 – 2000. What $[M–nH]^{n-}$ ions do you observe? Set LM Resolution to 15.0, Trap CE to 32 V, and take a CID spectrum of the m/z 783.8 ion over m/z 50 – 2000.

Similarly, in the ESI+ mode with the Capillary at 2800V, what $[M+nH]^{n+}$ ions do you detect? Take a CID spectrum of the m/z 785.8 ion over m/z 50 – 2000.

In the ESI+/– MS experiments, how do you rationalize the observed charge state distributions? Compare the results with the charge states for DACTAA and discuss.

In the GFP MS/MS experiments, what are the charge states of the product ions? Are they different between ESI+ and ESI–? If so, why? How do some of the product ions have larger m/z values than the precursor ions?

II. LC-ESI-MS analysis of a small molecule mixture

Use the Isotope Model and calculate theoretical peak positions and isotope distributions for the $[M+H]^+$ ions of the five compounds.

In this experiment the LC gradient is programmed so that it runs from hydrophilic (mainly A1) to hydrophobic (mainly B1) over the 4-minute period. A blank solvent (10 : 90 ACN/water, 5 µL) is first run as reference and a sample mixture next (dissolved into 5 µL of the blank solvent). The ESI+ data acquisition occurs in the MS and MS/MS modes, which alternate rapidly during the LC run. Real-time mass calibration is performed during the run by intermittently spraying a "LockMass" calibrant solution of Leucine Enkephalin through the second ESI nozzle.

On the Sample List window, you notice three instrument setup files, i.e., MS Tune File, Inlet File, and MS File. Inspect the content of each file and describe how they work jointly to facilitate the LC-ESI-MS measurement. **(*Do not save a change to these files!)*** Specifically, you would collect these parameters:

MS Tune File: Capillary voltage, Sampling Cone voltage, and Desolvation gas temperature/flow rate (under ES+). LockSpray infusion flow rate (under Fluidics).

Inlet File: Run Time and Gradient profile (under Inlet). Run Time, Column temperature, and Sample temperature (under Autosampler).

MS File: Acquisition times, Source, Polarity, Mass ranges for Low Energy (MS) and High Energy (MS/MS) scans, Scan Time, Collision energies for Low Energy (Function 1) and High Energy (Function 2), Cone Voltage setup, and Scan Time/Interval for the LockSpray.

Run the blank and sample solutions and acquire data. Print out the chromatograms for MS and MS/MS scans for the sample run (on the Chromatogram window, Display → Tic… → Function 1 for MS and Function 2 for MS/MS). Print out the MS and MS/MS spectra for each of the five compounds and compare the MS spectra with the predictions. Are the retention times consistent with what you expected? Do you see any (apparent) outliers? What is the controlling factor for the retention time in the reversed-phase chromatography?

Note: You may find the LC peak intensities to be vastly different. Chemical properties of analyte, ES ionization efficiency, and mass-dependent ion transmission/detector efficiency all contribute to the signal intensity. In addition, ionization is occurring in the continuously changing environment (i.e., mobile phase) during the LC gradient. Running standard solutions is essential for quantitative studies.

References

P. Kebarle and U.H. Verkerk, *Mass Spectrom. Rev.*, DOI 10.1002/mas.20247 (2009) (course website)
N.B. Cech and C.G. Enke, *ibid*, **20**, 362 (2001) (course website)
D.P.H. Smith, *IEEE Trans. Ind. Appl.*, **IA-22**, 527 (1986)

For your lab report, please turn in answers to each of the lab questions, including all relevant graphs, calculations and discussions.