CHEM 5181 Laboratory #2 – ESI-quadrupole-TOF Mass Spectrometry

CHEM 5181, Laboratory #2
Dates: September 28 – 30, 2009
Reports Due: October XX, 2009

Introduction:

In this lab you will run an electrospray (ESI)-triple quadrupole-time-of-flight (TOF) mass spectrometer from Applied Biosystems, PE SCIEX/ABI API QSTAR Pulsar i Hybrid LC/MS/MS (nickname: Pulsar). In Part I of the experiment, you will analyze an organic compound in both the ESI+ and ESI- modes as well as perform MS/MS (i.e., collision induced dissociation). You also investigate effects some instrument parameters (ESI voltage and nebulizer gas flow rate) have on the signal intensities. In Part II you will analyze a polymer sample and detect and identify multiply charged ions.

You will be provided:

- Simplified instructions for using the Pulsar
- Instructions for the lab
- A methanol solution (~100 ppm) of *trans*-1,2-Diaminocyclohexane-N,N',N'-tetra acetic acid monohydrate (Aldrich, abbr. DACTAA)
- A methanol solution (~100 ppm) of polypropylene glycol (PPG), doped with an ammonium salt
- Solutions of formic acid (1% in ACN) and lithium chloride (~mM in ACN)

Description of Instrument Setup:

Refer to the uploaded Lecture Notes of Sept 22 and the course website/"MDS SCIEX/Applied Biosystems API Q-Star Pulsar"/"Instrument Description".

Additional information relevant to the lab:

- ESI capillary: Polymicro Technologies, fused silica capillary tubing TSP075150 (O.D. 154 um, I.D. 74 um), contained in a stainless steel needle, the tip being 9 mm apart from the orifice plane.
- A coaxial flow of nebulizer gas (N_2) is used for the spray needle ("pneumatically assisted ESI").
- A typical mass accuracy for the detection: +/- 0.1 in units of m/z

****** NOTE – The only ion source parameters you can change are Ion Source Gas 1 (GS1), Ion Spray Voltage, Collision Gas (CAD), and Collision Energy (CE). The Ion Spray Voltage can go up to +5,200 V (ESI+) and down to -4,500 V (ESI-).**
Do not accidentally save changes to the instrument methods.

Laboratory Directions:

I. ESI-MS analysis of DACTAA:

1. Effects of Spray Voltage and Nebulizer Gas flow rate on the ESI+ signal

Using the instrument method for ESI+ with GS1 = 8 and Spray Voltage = +4500 V, take a mass spectrum for the range of m/z 50 – 800 and find the MH^+ peak at m/z 347. Do you find multiply charged ions?

With GS1 = 8, change the spray voltage by 500 V and record the TIC (total ion current). If you suddenly lost the signal, increase the voltage to get it back and lower the voltage more slowly. Determine the onset of electrospray for this sample in methanol.

Referring to Kebarle and Verkerk (2009) and the information above, compute the onset voltage for methanol and compare it with the data. Note that the formula in Kebarle/Verkerk is an approximation with respect to the original in Smith (1986), which gives a more general formula with the variable A_1 parameter.

With Spray Voltage = +4500 V, change GS1 around 8 (from near zero to 25) and record the TIC. Discuss how the TIC changes.

2. Effects of Additives on the ESI+ product distribution

Set GS1 to 8 and Spray Voltage to +4500 V (use this setting for the rest of DACTAA ESI+ experiments.) Prepare two solutions by adding i) 1 uL of LiCl solution and ii) 20 uL of formic acid solution to DACTAA (200 uL each) and take spectra. From the observed changes in product distributions, assign chemical formula to ions of m/z 347, 352, 353, 369, and 385.

3. CID of positive ions

Use the acidified DACTAA solution. Do CID on the MH^+ ion (Collision Energy, CE = 10, 20, and 30 V with CAD = 10) and take spectra. Discuss mechanisms for the formation of product ions m/z = 214 and 134.

Do CID on the m/z 693 ion at CE = 5, 10, and 15 V and take spectra. From the observed products and CID efficiency, what is the most likely structure for this ion?

4. ESI- of DACTAA

Using the instrument method for ESI- with GS1 = 8 and Spray Voltage = -4000 V, take a mass spectrum for the range of m/z 50 – 800 and find the $[M – H]^-$ peak at m/z 345. Do you find multiply charged ions? Do CID on the $[M – H]^-$ ion (CE = 10, 20, and 30 V with CAD = 10) and take spectra. Discuss mechanisms for the formation of product ions m/z = 327 and 301.

II. ESI-MS analysis of PPG:

Go back to the ESI+ mode and set GS1 to 8 and Spray Voltage to +4800 V. Take a spectrum of PPG in the range of m/z 300 – 2000 and observe product distributions consisting of several bands. What charge state characterizes each band?

Use the Isotope Distribution calculator and compute and print out theoretical spectra for $C_3H_8O_2(C_3H_6O)_{31}(NH_4^+)_{m=1,2,3,4,5}$. Look for these species in the spectrum. If there is a limit to the observed number of m, what could be a reason for that? (Take into account the DACTAA results as well.)

Note: Because PPG produces a number of identified mass peaks over a wide range of m/z, it is conveniently used for calibrating the instrument and performing Accurate Mass analysis (typically to within 5 ppm of mass accuracy).

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)

Clean up your laboratory space, and turn off equipment, as necessary.

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