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

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
- A mixture of five compounds, i.e., acetaminophen, caffeine, sulfadimethoxine, terfenadine, and reserpine, each 2 ppm in 10:90 ACN/water.
- Solutions of formic acid (1% in ACN) and lithium chloride (~mM in ACN)

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 = 1:49 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

Summary of Experiment:

I. ESI-MS analysis of DACTAA and GFP

You will examine both ESI+ and ESI- to observe molecular ion signals of DACTAA and study the effect of additives (formic acid and LiCl) on the peak distributions. CID experiments will be performed to obtain information about the structures of the molecular ions.

Similarly, you will run the GFP sample on ESI+ and ESI- as well as perform CID and discuss the charge state distributions for GFP and DACTAA.

II. LC-ESI-MS analysis of small molecules

Using the UPLC auto-sampler and provided instrument methods, you will inject the five-compound mixture into the UPLC and analyze them on ESI+ detection. You will learn how the instrument operates and how these compounds behave on the reversed-phase LC-MS.

Prelaboratory Questions:

1. Show the structures and molecular formula of DACTAA and GFP (give the complete molecular structure for GFP, not just the amino acid sequence). Which of the ESI modes would work, ESI+ or ESI-, or both, and why? What ions do you expect from these samples?

2. On what parameters does the electrospray onset voltage depend?

3. How does the nebulizer gas work to help improve the ESI signal?

4. The UPLC is a fairly new technology that was developed some years ago. Briefly describe the characteristics and advantages of UPLC over the conventional HPLC (high performance liquid chromatography).

References

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