Chem 230, Fall, 2014
Homework Set # 4
Do by Nov. 25

Short Answer Questions:

1. A chemist analyzing plant extracts for sugars is looking at different ways to quantify sugars using HPLC. Sugars absorb light poorly, but reagents are available to convert sugars into light absorbing or fluorescent compounds. List three possible detection methods (with or without requiring conversion of the sugars). Indicate the detector needed and one potential advantage and disadvantage to each method.

2. Besides the fact that many compounds don't fluoresce, what instrumentation features make fluorescence detection for HPLC selective?

3. Which of the following detectors (more than one) are concentration type detectors:

a) flame ionization detector b) thermal conductivity detector
c) UV absorption detector d) evaporative light scattering detector
e) electrochemical detector f) fluorescence detector

4. A GC is operated with a PID and FID in series. The PID is a concentration type detector. Describe what will happen to the peak heights and areas for each detector when the column flow rate is increased by 20%. To make the question easier, assume there is no change in H.

5. List the three most common methods of calibration. Give a specific chromatographic example where each method might be used.

6. A new HPLC-MS method is developed for determining a compound in blood plasma which uses external standard calibration. The scientist working on the method is excited about the new method since it requires fewer clean-up steps and has a shorter total analysis time (chromatogram time plus column stabilization time). Although the new method has numerous overlapping peaks, no other compounds in the cleaned-up blood has the same mass to charge ratio of the analyte. The HPLC-MS method is compared with an old method using HPLC-UV detection. The HPLC-MS is found to underpredict the analyte concentration found using the old method (which can be considered well tested). Give a possible explanation for the results and suggest a way to calibrate the HPLC-MS so that underprediction of concentration will not occur.

7. List the three main components to a mass spectrometer.

8. List a method for ionizing gases that does not cause a lot of fragmentation.

9. For an analyte whose identity is not well known, is it easier to identify it using GC-EI-MS or HPLC-ESI-MS?

10. Why is MS-MS used more often with liquid samples?

11. List two methods for ionizing liquid samples in mass spectrometry.

12. List two types of mass analyzers.

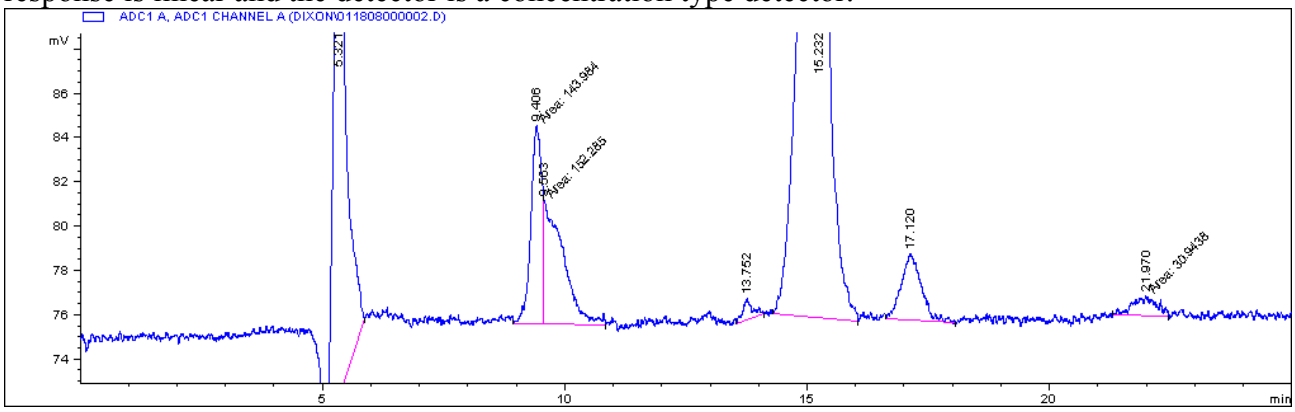
13. A modification is made in a peptide, in which one threonine residue ($NH_3CH(CHCH_3(OH))CO_2$) is replaced with cysteine ($NH_3CH(CH_2SH)CO_2$). An original peptide peak appeared at 278.25 amu, while the altered peptide peak appeared at 278.75. What was the charge on the original peptide peak?

14. Compounds A and B are determined by GC analysis. Because the amount injected is variable, an internal standard, IS, is added to samples. 5.00 mL of an unknown solution is diluted to 10.0 mL with the addition of 1.00 mL of 60.0 μg mL^{-1} B. Given the table below, determine the concentration of A and B in the unknown:

Compound	Conc. (μg mL^{-1})	Peak Area
A (Standard 1)	2.00	9,130
B	2.00	8,760
IS	6.00	32,070
A (Standard 2)	8.00	30,010
B	8.00	28,640
IS	6.00	26,200
A (Diluted Unknown)		21,080
B		15,310
IS	6.00	29,900

Longer Problems – to be turned in

1. Given the HPLC chromatogram below and the fact that compounds A and B, eluting at 17.1 and 22.0 min, respectively, were present at concentrations of 100 and 50 ng mL^{-1}, respectively, calculate the following the requested quantities below. The σ noise was determined from chromatography software to be 0.28 mV. Assume that the detector response is linear and the detector is a concentration type detector.



a) The 3.3σ concentration limit of detection (LOD) for each compound.

b) The 3.3σ mass LOD for compound A if the injection volume was 100 μL.

c) A sample contains a peak at 17.1 min. with a peak height of 1.5 mV. How should the concentration be reported? (below limit of detection, below limit of quantification, or with the concentration given)

d) If the mobile phase is changed so that compound B now comes out at 12.2 min, estimate compound B's new detection limit. Assume H is constant.

2. Fatty acids in sesame oil were being analyzed as fatty acid methyl esters (FAMEs). To test for recovery, 50.0 μL of a 398 μg/mL $CH_3(CH_2)_{15}CO_2H$ (C17) standard (molecular weight = 270.4 g/mol) was added to a 20.0 mg. sample of hydrolyzed sesame oil. No C17 fatty acids are present in sesame oil (only fatty acids with even numbers of carbon are present). The sample was then reacted to produce methyl esters and analyzed by GC with quantification done with an octadecane ($CH_3(CH_2)_{16}CH_3$) internal standard (molecular weight = 254.5 g/mol). A standard was made from octadecane and commercial FAME standards. Following the reaction to methyl esters and extraction, the processed 20.0 mg. sample was present in 5.00 mL of solvent.

The following peak areas were measured in chromatograms made by injecting ~1 μL:

Sample:	Compound	Concentration (μg/mL)	Peak Area
Standard	octadecane	80.0	13,211
	C17 FAME	100.0	14,750
	C18 FAME	100.0	14,831
Spiked Unknown	octadecane	80.0	17,099
	C17 FAME		558
	C18 FAME		88,274

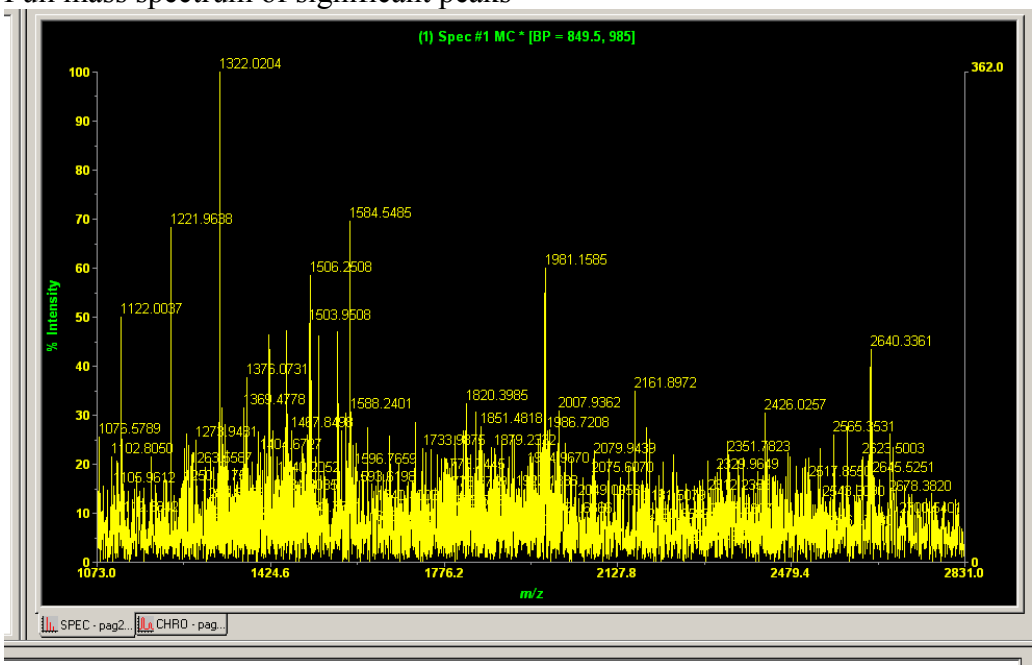
a) Calculate the percent of the C17 fatty acid which was recovered by this method.
b) Determine the conc. of stearic acid (C18 acid) present in the oil in mass %.

3. An unknown N-containing natural product is quantified using GC with a NPD. This detector gives equal response per mole of N injected. A 1.00 mL sample is spiked with 0.20 mL of 225 μM caffeine ($C_8H_{10}O_2N_4$). Assume the volumes are additive (e.g. total volume = 1.20 mL). If the natural product has a molecular formula of $C_{11}H_{14}O_5N_2$ (obtained by high resolution MS) and gives a peak area of 793 area units and the caffeine gives a peak area of 1427 area units, what was the concentration of the natural product in μM in the 1.00 mL sample?

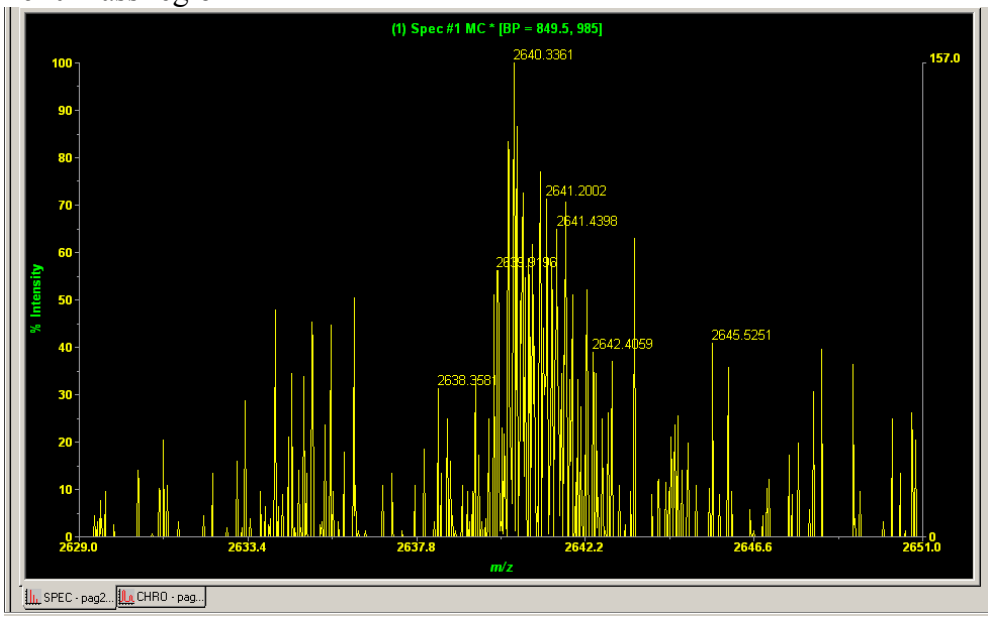
4. A compound with suspected molecular formula of: $C_{10}H_{12}O_2S$ is analyzed using positive ion mode electrospray (assume protonation of the compound occurs).
a) calculate the expected mass of the main ion expected (e.g. from most common isotopes) to 0.001 amu using weights
b) calculate expected ratios of ion intensities for peaks due to 1 mass unit heavier isotopes to most common isotope (M+1/M) and due to 2 mass unit heavier isotopes (M+2/M). For the M+1/M calculation, consider contributions from ^{13}C, ^{2}H, and ^{33}S (you can ignore contributions from ^{17}O) and for M+2/M the calculations, consider contributions from ^{13}C, ^{18}O, ^{33}S, and ^{34}S only.

5. The following positive ion ESI Mass Spectrum is observed when a large molecule is analyzed that contains multiple alkyl amines. The peaks XX22 (or near those numbers) are calibration standards. Peak "clusters" observed at around 1585, 1980, and 2640 (see zoomed in regions below).

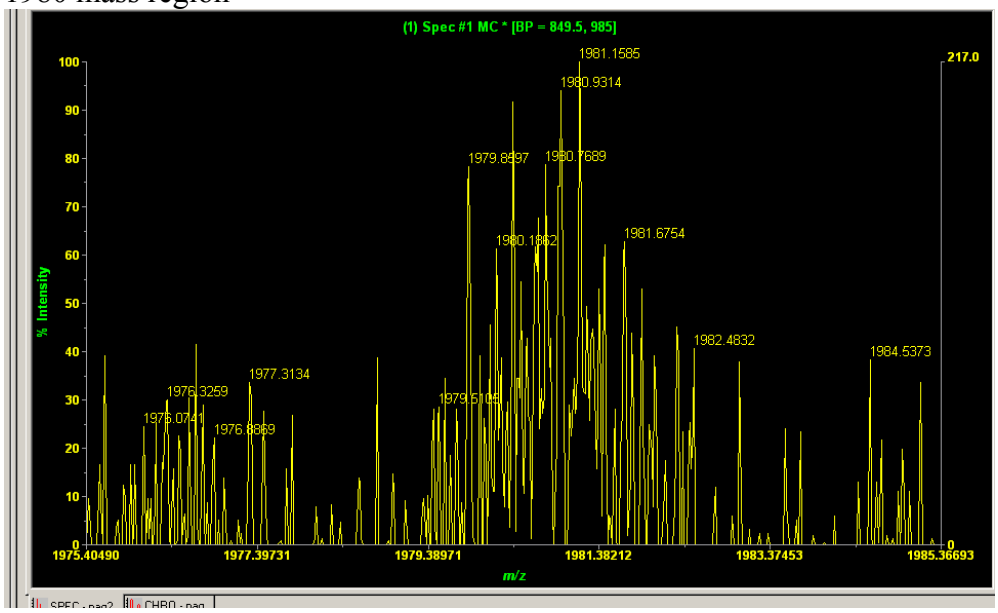
Full mass spectrum of significant peaks



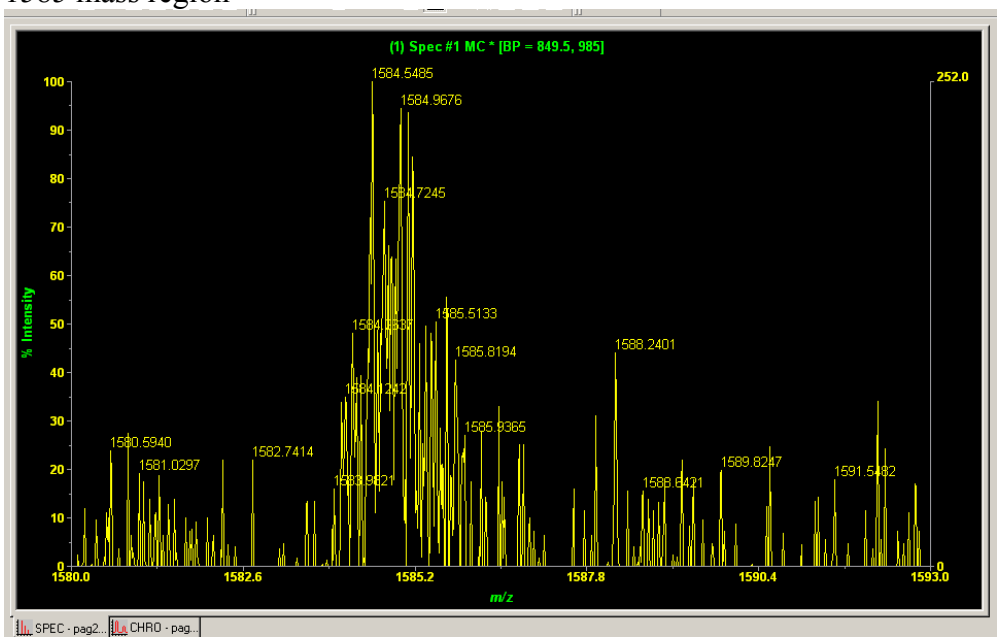
2640 mass region



1980 mass region



1585 mass region



a) Determine if you can identify the mass of the large molecule responsible for the zoomed in mass regions (hint: they are all the same) and also the charge associated with each peak cluster.

b) Separate peaks in the peak cluster are observed (best seen for 1980 mass region), but they are not baseline separated. What do these individual peaks correspond to and why are they not baseline resolved?

c) Predict another region where a mass cluster peak would be expected. Is it observed?