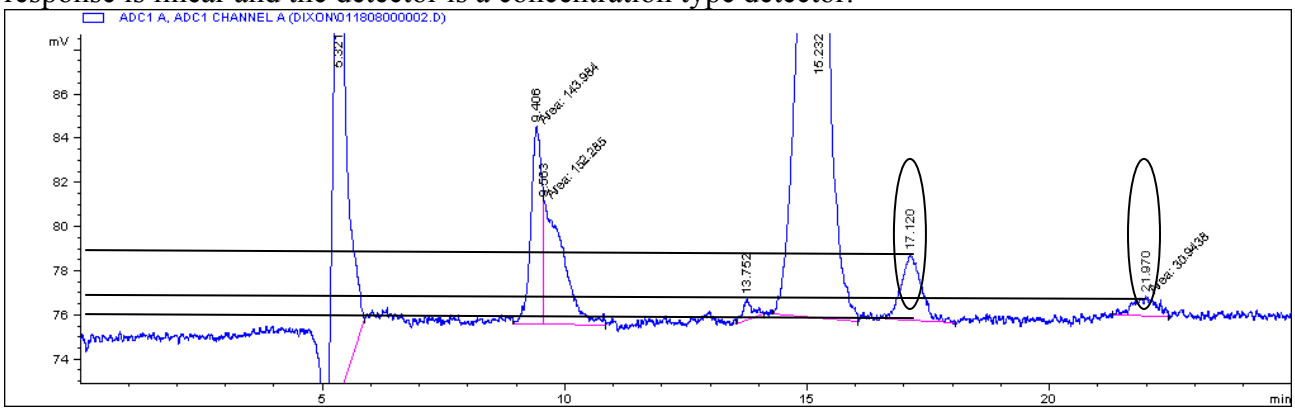
Chem 230, Fall, 2014
Homework Set # 4
LONG ANSWER SOLUTIONS

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.
minimum detectable signal = 0.28·3.3 = 0.92 mV
compound A, peak height = 79.0 – 76.0 = 3.0 mV, slope = 3.0 mV/100 = ng mL^{-1} = 0.030 mV/ng mL^{-1}
compound B, peak height = 76.9 – 76.0 = 0.9 mV, slope = 0.018 mV/ng mL^{-1}
comp. A LOD = 0.92 mV/0.030 mV/ng mL^{-1} = ***31 ng mL^{-1}***
comp. B LOD = 0.92 mV/0.018 mV/ng mL^{-1} = ***51 ng mL^{-1}***
(note: based on this definition, comp. B is at the LOD)
ADDITIONAL NOTES: we are not using 3.3N/5 because the noise give is the standard deviation – not the peak to peak noise in past examples. Also, I'm don't expect everyone to get the same baseline levels or peak levels since it requires using the plot.

b) The 3.3σ mass LOD for compound A if the injection volume was 100 µL.
mass LOD = (31 ng mL^{-1})(0.1 mL) = ***3.1 ng***

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)
If we assume LOQ = 5·LOD, LOQ min signal = 5(LOD min signal) = 5(0.92 mV) = 4.6 mV. So it is clear that a peak height of 1.5 mV is above LOD and below LOQ. It should be reported as ***below LOQ****. Others may get different answers depending on interpreation of the plot.*

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.

Assuming a linear detector, the peak area should be constant regardless of the retention time. If we further assume no extra-column broadening, the peak width should be proportional to the retention time (N = constant = $16(t_r/w)^2$ meaning a change in t_r should result in a proportional change in w). If area is the same and w is decreasing, height must be increasing (we can approximate that area = ½(h·w)). Thus height is inversely proportional to retention time. Thus $(h_2/h_1) = (t_{r1}/t_{r2}) = (22.0/12.2) = 1.80$.
The new slope would be 0.9(1.80)/50 = 0.032
and the new LOD = 0.92 mV/0.032 = ***29 ng mL^{-1}***
This also assumes the change in eluent affects neither response nor noise levels.

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.
Quantification is done by internal standard method with only one level of standards. We can calculate the concentration of C17 FAME using this method.
F = [A(C17 FAME)/A(octadecane)]/[C(C17 FAME)/C(octadecane)] – using data from standard: F = (14750/13211)/(100.0/80.0) = 0.8932
Rearranging this equation to solve for C and applying it to the spiked unknown,
[C(C17 FAME)/C(octadecane)] = [A(C17 FAME)/A(octadecane)]/F or
C(C17 FAME) = [A(C17 FAME)/A(octadecane)]C(octadecane)/F
C(C17 FAME) = (558/17,099)(80.0 µg/mL)/0.8932 = 2.923 µg/mL
Now we can calculate the mass of C17 FA:
moles (C17FAME) = (2.923 µg/mL)(5.00 mL)/(270.4 µg/µmol + 14.03 µg/µmol) = 0.0514 µmol = moles(C17 FA)*
measured mass(C17 FA) = (0.0514 µmol)(270.4 µg/µmol) = 13.9 µg
mass(C17 FA) added = (50.0 µL)(398 µg/mL)(1 mL/1000 µL) = 19.9 µg
% recovery = 13.9*100/19.9 = 69.8%

** + 14.03 g/mol is to account for addition of CH_3 and subtraction of H in going from FA to FAME)*

ADDITIONAL NOTES: 1) You cannot just assume the peak area is directly proportional to concentration in this case. If you look at the internal standard in both samples, the peak areas are not that close. This most likely means that the amount injected is not well controlled and that an internal standard calibration is needed.

2) While it is not a big change, since units given are in µg/mL , we need to account for the difference in mass between fatty acids and fatty acid methyl esters. In calculating the % recovery, we need to be sure that the top and bottom are in the same units (e.g. mass FA, mass FAME, or moles). 3) While not intended, this problem also can be solved by using a "universal" calibration method – the same type employed in problem 3.

4) Because the recovery is less than 100%, we want to account for incomplete recovery by dividing by the %recovery (once it is converted to a fraction).

b) Determine the conc. of stearic acid (C18 acid) present in the oil in mass %.

F = [A(C18 FAME)/A(octadecane)]/[C(C18 FAME)/C(octadecane)] – using data from standard: F = (14831/13211)/(100.0/80.0) = 0.8981

Rearranging this equation to solve for C and applying it to the spiked unknown,

[C(C18 FAME)/C(octadecane)] = [A(C18 FAME)/A(octadecane)]/F or

C(C18 FAME) = [A(C18 FAME)/A(octadecane)]C(octadecane)/F

C(C18 FAME) = (88,274/17,099)(80.0 µg/mL)/0.8981 = 459.9 µg/mL

mass C18FA = (459.9 µg/mL)(284.4µg C18FA/µmol C18FA/298.5 µg C18FAME/µmol C18FAME)(5.0 mL) = 2,191 µg C18FA

Accounting for the recovery, mass C18FA = (2,191 µg C18FA)(1 mg/1000 µg)/0.698 = 3.14 mg

mass percent** = 3.14*100/20.0 = **15.7%

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?

moles N in standard = (0.20 mL)(225 µmol caffeine/L)(1L/1000 mL)(4 µmol N/µmol caffeine) = 0.180 µmol N

C(caffeine in µmol N/mL)/C(compound in µmol N/mL) = A(caffeine)/A(compound)

C(compound in µmol N/mL) = A(compound)[C(caffeine in µmol N/mL)]/A(caffeine)

or (since the volume is the same) n(compound in µmol N)

= A(compound)[n(caffeine in µmol N)]/A(caffeine) = ~~1427~~793(0.180 µmol N)/~~793~~1427 = 0.1000 µmol N in compound

concentration = (0.1000 µmol N in compound)(1 µmol compound/2 µmol N)/0.00100 L

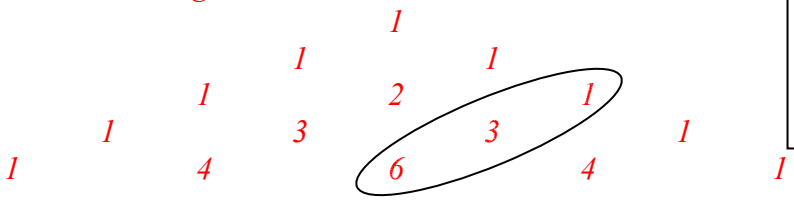
concentration = ~~162~~ 50.µM

ADDITIONAL NOTES: I got the two peak areas transposed. Also, if you calculated the concentration of compound in the 1.20 mL, you need to account for the 1:1.2 dilution.

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
main isotopes are ^{12}C, ^{1}H, ^{16}O, and ^{32}S with masses of 12.0000 amu, 1.0078 amu, 15.9949 amu, and 31.9721 amu, respectively. The mass of an electron is 0.00055
This leads to a mass of 10(12.00000) + 13(1.007825) + 2(15.9949) + 31.9721 – 0.00055 = **197.063 amu**
(masses from Harris textbook; extra H and – electron mass due to protonation)
ADDITIONAL NOTES: If you got 196.055, you forgot that you need to add $^{1}H^{+}$ to make it protonated (see the assume protonation comment above).

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.

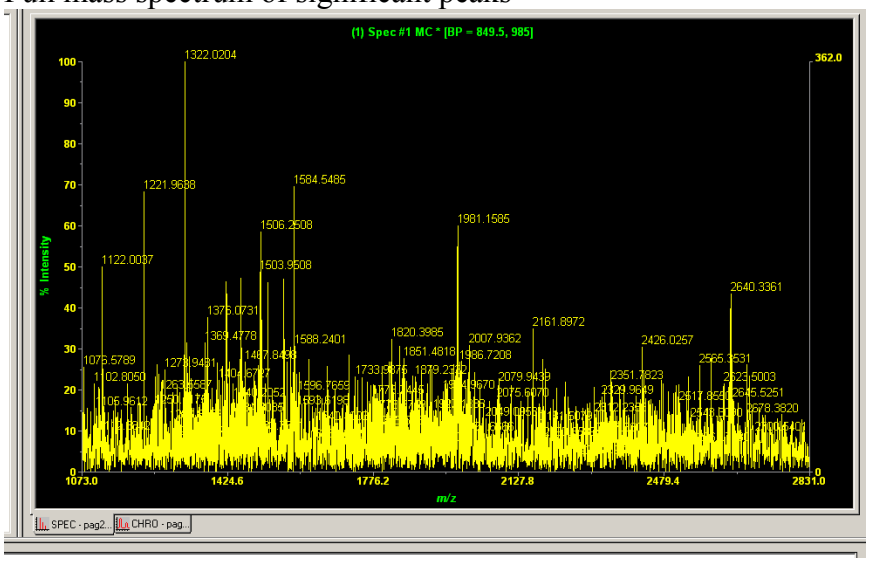
M+1/M = 10(1.08) + 13(0.012) + .801(1) = ~~**11.0**~~**11.8/100**
M+2/M sources: 2 ^{13}Cs, 1 ^{18}O, 1 ^{13}C + 1 ^{33}S, or 1 ^{34}S
2 ^{13}Cs: [(10)(9)/2](1.08)^2/100 = 0.52
1 ^{18}O: 2(0.205) = 0.41
1 ^{13}C + 1 ^{33}S: (10)(1)(1.08)(.801)/100 = 0.09
1 ^{34}S: 4.52
sum = **5.54/100**
ADDITIONAL NOTES: In the M+1/M, I made a math error. I also didn't cover all of the numbers calculated in front of the isotopic ratios (see below).
In the M+1 calculation, the 10 in front of the 1.08 comes from 10 Cs.
In the M+2 calculations, we also use n for ^{18}O and ^{34}S because only one of those isotopes increases the weight by 2 mass units. Another option is to have 2 ^{13}Cs – first line above. The (10)(9)/2 comes from (n)(n-1)/2. This will match the "second from the left" numbers in a Pascal's triangle for probability calculations (see below). The last option is to have 1 ^{33}S and 1 ^{13}C (we are ignoring ^{2}H and only have 1 S). In this case the 10 and 1 come from nm where n = # of Cs and m = # of Ss (these are independent).
*Also, the number 0.801 comes from the ratio of percent ^{33}S to percent ^{32}S where ^{32}S is set to 100 (e.g. 0.76*100/94.93 = 0.801) It is also possible to do the calculations as fractions (in place of ratios), but that method is harder so not explained.*
Pascal's Triangle



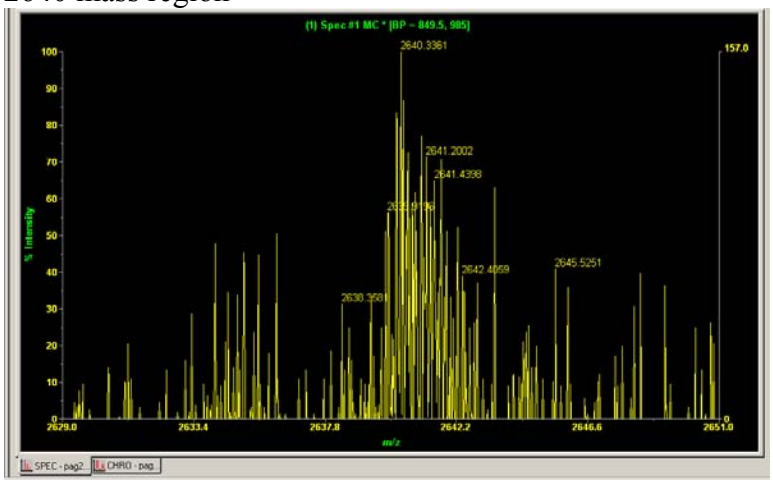
These #s used when calculating # with two non-main isotopes. For example, if 4 Cls, there are n(n-1)/2 or 6 (5th line) more combos giving 2 ^{37}Cls than all ^{35}Cls.

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).

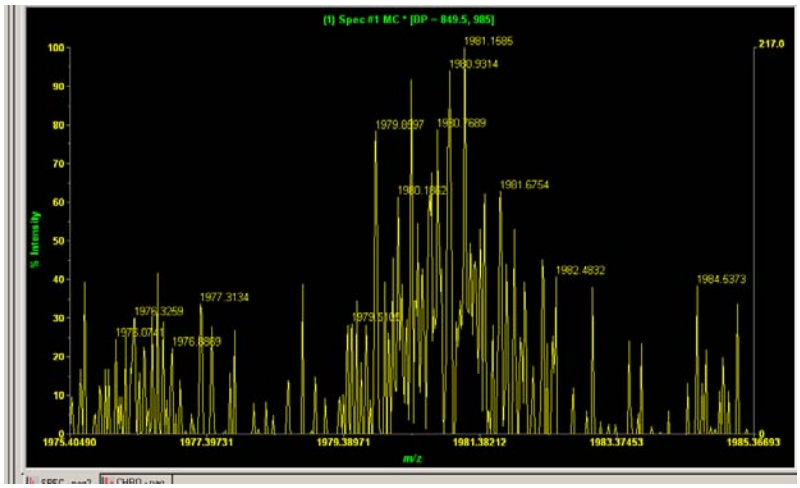
Full mass spectrum of significant peaks



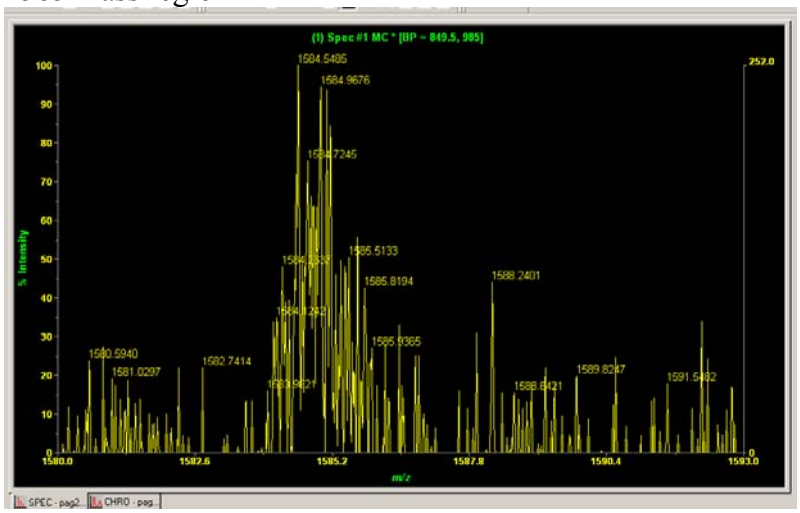
2640 mass region



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.

Looking at the mass cluster peaks, we can construct a table: 1) estimate a mass cluster peak average mass (using this instead of the highest peak), 2) calculate ratios of m/z to next lowest m/z, solve the rough calculation (m/z)/(m/z)' = n+1/n (where the prime is for the neighboring mass cluster with smaller mass, 3) solve for n in the above equation, 4) find closest whole number n, and 5) calculate M (where M+n/n+ = m/z)

meas. m/z	*(m/z)/(m/z)'*	*calc n*	*whole n*	*calc. M*
2641.1	*1.3334*	*2.9994*	***3***	*7920.3*
1980.8	*1.2498*	*4.003*	***4***	*7919.2*
1584.9	*NA*		***5***	*7919.5*
average M				***7919.7***

calc. n: (m/z)/(m/z)' = R (ratio) = n+1/n or nR = n + 1 or n(R-1) = 1 or n = 1/(R – 1)
calc. M: M + n/n = m/z or M = n(m/z) – n

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? [BONUS Points only]
These appear to be due to different isotopes. They should be separated by ~1 mass unit. In the 1980 peak cluster, we can see neighboring peaks at: 1981.158 and 1980.931 as well as 1980.186 and 1979.860. These are separated by 1981.158 – 1980.931 = 0.227 and 1980.186 – 1979.860 = 0.326. Some of the gaps are roughly consistent with a charge of 4 (0.25 would be expected). They are not fully resolved because: a) there are different possible ways to add whole mass units (e.g. 2 ^{13}C or 1 ^{18}O) which will have slightly different masses, and b) the instrument resolution may not be high enough. A resolution of over 7920 is needed to resolve the peaks. Typically, time of flight resolution is similar.

c) Predict another region where a mass cluster peak would be expected. Is it observed?
We could go to either a greater or lesser charge. Going to +2 would give a peak at (7919.7 + 2)/2 = 3961 (offscale so not in observed spectrum). Going to +6 would give a peak at (7919.7 + 6)/6 = 1321. A peak is observed at 1322, but this is also an added internal standard. So we would need to zoom in to see if there is a real peak cluster there also.