# CHEM 230
# Exam 4 – Dec. 2, 2014
KEY

Short Answer/Multiple Choice Section:
Each Question worth 3 points

1. List one advantage in selecting an aerosol-based detector over a refractive index detector for universal detection in HPLC. Advantage = **1) better sensitivity, 2) gradient compatible, 3) more universal response (better for standardless analysis)**

2. List one advantage in using a fluorescence detector over a UV detector for HPLC:
Advantage = 1) better sensitivity, 2) better selectivity

3. A standard addition calibration method is desired if:
**a) the sample matrix is observed to affect response to the analyte**
b) the injection volume is imprecise
c) no standards are available for the analyte of interest
d) the detector has non-linear response

4. When using GC with manual injection for analytes with available standards, what is a typical calibration method?
a) external standard **b) internal standard**
c) standard addition d) universal calibration

5. Of the following, which type of mass spectrometer ionization results in the greatest amount of fragmentation?
a) electrospray b) atmospheric pressure chemical ionization
c) chemical ionization **d) electron impact**

6. Why is high resolution of interest in mass spectrometry?
a) to determine isotopic ratios accurately (e.g. % $^{33}S$ in specific samples)
**b) to distinguish between to compounds of the nearly the same mass (e.g. $C_6H_{12}$ vs. $C_5H_8O$)**
c) to be able to detect much lower quantities of ions
d) to be able to ionize high mass molecules

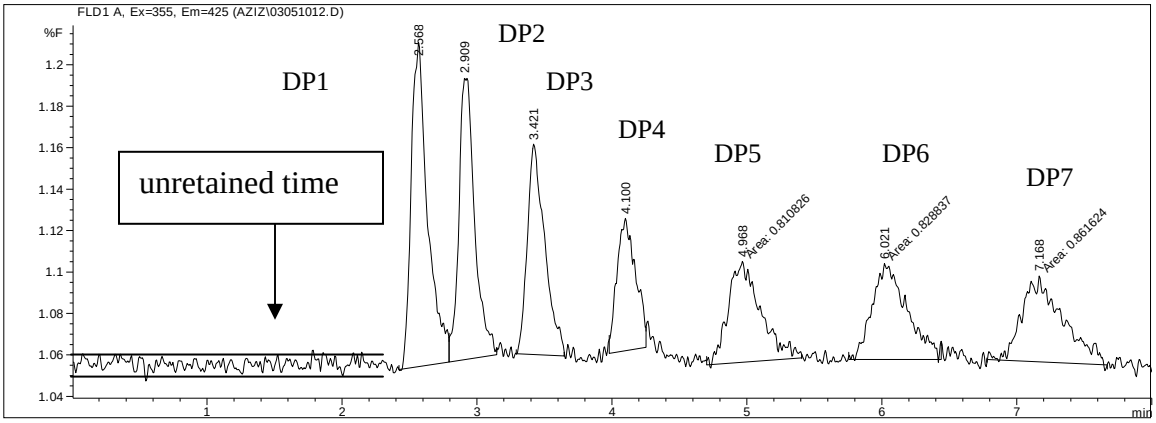
Longer Answer Questions:

1. The following mass spectrum is observed for a GC peak in GC-EI-MS. The suspected compound is believed to have carbonyl and aromatic functional groups. The 134 peak is the parent ion. Give **one useful piece of information** (e.g. identity of peak or of lost fragment, or # of C atoms in fragment) about the compound from the spectrum. Explain your work. (6 pts)

| m/z | intensity |
|---|---|
| 77 | 45.4 |
| 105 | 100 |
| 106 | 8.0 |
| 134 | 11.0 |

*1) 77 = likely (singularly substituted benzene ring (since aromatic mentioned)*
*2) 134 – 105 = 29 = -CHO or -$CH_2CH_3$ groups possible (CHO less likely because 133 peak would be observed)*
*3) 106 is likely M+1 peak 8/100 = n(1.08)/100 where n = 7.4 so likely 7 carbons*
*4) 105 – 77 = 28 = CO group (likely ketone)*

2. The following parts to this question involves the analysis of sugars derivatized with a fluorescent tag (2-amino-benzamide), followed by clean-up, and by normal phase HPLC with fluorescence detection (a concentration type detector). The chromatogram (and associated data table below) shows the separation of glucose oligomers (DP1 = glucose and DP7 = a chain of 7 glucoses) obtained on a 4.6 x 200 mm amino column with 70% acetonitrile and 30% water (water being the more polar solvent). All of the sugars were present at 10 μM before derivatization. Assume the detector gives linear response with no matrix effects.




| | # | Time | Area | Height | W |
|---|---|---|---|---|---|
| **DP1** | 1 | 2.568 | 1.300 | 0.160 | 0.212 |
| **DP2** | 2 | 2.909 | 1.100 | 0.140 | 0.183 |
| **DP3** | 3 | 3.421 | 0.910 | 0.100 | 0.209 |
| **DP4** | 4 | 4.1 | 0.610 | 0.064 | 0.205 |
| **DP5** | 5 | 4.968 | 0.810 | 0.049 | 0.471 |
| **DP6** | 6 | 6.021 | 0.830 | 0.047 | 0.506 |
| **DP7** | 7 | 7.168 | 0.860 | 0.042 | 0.586 |

Time is the peak retention time and W is the baseline width and both have units of minutes. Height is calculated in % of maximum fluorescence. An unretained peak (only observed using UV detection) appeared at 1.570 min.

a) Which of the peaks appears to be integrated less well? Indicate how you know. (5 pts)

*DP1 and DP2 are about as good as normally expected but will have errors due to split peak and tailing (full credit)*

*DP4 stops early and has a high baseline (improper integrations) (best answer)*

*DP5 to DP7 have worse signal to noise ratios meaning the minor integration errors will have a larger percent effect (not full credit)*

b) Calculate the detection limit ($2N_{peak\text{-}to\text{-}peak}$) for the compound DP3. The bars covering the noise in front of the first peak can be used to estimate the peak to peak noise. (10 pts)

*$N_{peak\text{-}to\text{-}peak}$ = 1.06 – 1.05 = 0.01%F. Slope = m = peak height/conc = 0.100/10 μM = 0.01%F/μM*

*LOD = 2N/m = 2(0.01%F)/0.01%F/μM =* ***2 μM***

c) A commercially derivatized DP5 standard (comes as derivative) is purchased and used to create a 10 μM standard, which is injected and found to give a peak area of 1.33 (under identical conditions). Using that information and the data above, estimate the % recovery (indicative of derivatization/clean up losses) for the DP5 standard (which starts as a sugar). (5 pts)
*There are two standards: a DP5 sugar standard that was then derivatized and a commercially derivatized (and isolated) DP5 sugar. We are calculating the percent yield of the derivatization (or the % recovery). We can calculate this by using the derivatized DP5 sugar as the "true" standard. The concentration of the self derivatized standard can be calculated as:*
$C_{SD} = (A_{SD}/A_{CD})C_{CD}$ *where CD is for commercially derivatized and SD is for self derivatized*
$C_{SD} = (0.810/1.33)(10\ \mu M) = 6.09\ \mu M$. *The percent recovery (or yield) = (6.09 μM/10 μM)*100*
*% recovery (or yield) =* ***61%***

d – bonus) indicate if you think the % recovery is constant, or increases or decreases with DP number. (2 pts)
*We would expect that the derivatized sugars would give nearly equal response because they all have the same molar concentration and each has one fluorescent tag. The area, however, is seen to decrease from about 1.3 (nearly equal to the derivatized DP5 standard) for the DP 1 to around 0.8 for DP5 to DP 7. This implies* ***a decreasing % recovery for increasing sugar DP number****.*

e) A sample is known to only have even number sugar chains (DP2, DP4, DP6) present. How could you change the eluent to improve the method sensitivity? Explain your answer. (5 pts)
*With only even DP numbers, there are larger gaps between peaks, and it would be possible to decrease retention, sacrificing resolution for increased sensitivity by earlier eluting and narrower peaks.* ***This would be done by increasing the % strong solvent which is water.***

3. A peptide in a liquid sample is analyzed using electrospray ionization mass spectrometry in the positive mode. Assume all observed peaks are due to $H^+$ addition and that no fragmentation occurs. The largest peaks are observed at 595.5, 446.9, and 357.7 Daltons. (11 pts)
a) What charge is associated with each of the above peaks?
b) Determine the molecular mass of the peptide?
*a) and b) can be determined using the following approach:*
*Let 595.5, 446.9, and 357.7 correspond to peaks for an n, n+1, and n+2 charge.*
*We can use the approximate relationship: (M+n/n)/(M+n+1)/(n+1) = n+1/n = 595.5/446.9*
*or n+1/n = 1.33 or n = 3. Then we know for 446.9, n = 4 and for 357.7, n = 5.*
*We also can use a more complete way in which Δ(m/z)/(m/z) = 148.6/595.5 = 1/(n + 1) or 0.250 = 1/(n+1) or n+1 = 4 or n = 3.*
*Once we know the charges for each peak, then we can calculate M in the following equations: 595.5 = (M + 3)/3 or M = 1783.5 and M = 1783.6 (for n = 4 peak) and M = 1783.5 for n = 5 peak. So average* ***M = 1783.5***

c) Where will an M+1 isotopic peak come out relative to the 595.5 amu peak (e.g. for one $^{13}C$ in place of $^{12}C$?
*595.5 is a +3 charge. Switching one to one results in Δm = +1 and Δm/z = +1/3 or + 0.33*