Chem 230, Fall, 2014
Homework Set # 4
Short Answer Solutions

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.
1) Refractive Index Detection (no derivatization needed).
advantages: a)will detect all sugars, b) no derivatization needed, c) relatively inexpensive detector
disadvantages: a) can't use gradients, b) poor sensitivity
2) Aerosol-Based Detectors (any of three + no derivatization needed)
advantages: a) will detect all sugars (none are volatile), b) no derivatization needed, c) moderately sensitive, c) universal response
disadvantages: a) not the cheapest (CAD and CNLSD), b) not the most sensitive (ELSD), c) universal detectors are not very selective if other compounds are present in plant extracts
3) Electrochemical Detection (no derivatization needed)
advantages: a) very sensitive, b) no derivatization needed, c) somewhat selective
disadvantages: a) requires solvent with higher ionic strength, b) less commonly used detector (may take longer to learn to use properly)
4) UV Detector (requires derivatization)
advantages: a) moderately sensitive, b) universal response (provided derivatization is uniform)
disadvantages: a) requires derivatization, b) moderately sensitive
5) Fluorescence Detector (requires derivatization)
advantages: a) very sensitive, b) universal response (provided derivatization is uniform), c) very selective
disadvantages: a) requires derivatization, b) moderately expensive detector

2. Besides the fact that many compounds don't fluoresce, what instrumentation features make fluorescence detection for HPLC selective?
One can discriminate against interfering molecules by selecting both excitation and emission wavelength.

3. Which of the following detectors (more than one) are concentration type detectors:
a) flame ionization detector
b) thermal conductivity detector (but signal also depends on flow rate)
c) UV absorption detector
d) evaporative light scattering detector
e) electrochemical detector (most often when not run in depletion mode)
f) fluorescence detector
conc. type in bold

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.
When the flow rate increases by 20% (and assuming no change in H), for concentration type detectors (PID), the peak height won't change, but since the peak elutes faster, the width and area will decrease. The FID, which is a mass flow detector, will see an increase in peak height and no change in area with an increase in flow. There is no change in area because the same mass of analyte is reaching the detector, just over a shorter time period.

5. List the three most common methods of calibration. Give a specific chromatographic example where each method might be used.
There are actually 4 listed in the textbook.
a) External Standard Calibration – used commonly with HPLC-UV detection
b) Internal Standard Calibration – used commonly with GC with manual injection
c) Standard Addition Calibration – used commonly with complicated samples analyzed by LC-MS.
d) Area Normalization Calibration – used with universal detecors (GC-FID and HPLC with aerosol-based detectors) when standards of analytes are not available.

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.
A matrix effect is occurring where non-observed compounds that co-elute with the analyte affect the ionization efficiency. Use of standard addition should improve the analysis.

7. List ~~the~~ three main components to a mass spectrometer.
1) Ionization source, 2) analyzer, and 3) ion detector, 4) data processor

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

9. For an analyte whose identity is not well known, is it easier to identify it using GC-EI-MS or HPLC-ESI-MS?
Generally, GC-EI-MS is much better because it gives ion fragments allowing better library searches or determination based on adding together components.

10. Why is MS-MS used more often with liquid samples?
With most liquid ionization methods little or very little fragmentation occurs. Also, HPLC generally doesn't have as much peak capacity as GC. Thus, there is a need for the fragmentation between the MSs and more need for discerning overlapping peaks of the same molecular weight.

11. List two methods for ionizing liquid samples in mass spectrometry.
Electrospray ionization, atmospheric pressure chemical ionization, and atmospheric pressure photoionization are the most common currently.

12. List two types of mass analyzers.
ion trap, quadrupole, time of flight, Fourier transform ion cyclotron, orbitrap

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?
Δm = mass cysteine – mass threonine = mass(CH_2SH) – mass($CHCH_3(OH)$)) =
Using most common isotopes
Δm = [12.000 + 3(1.0078) + 31.972] – [2(12.000) + 5(1.0078) + 15.994] =
Δm = 46.995 amu – 45.033 amu = 1.962 amu
*$\Delta(m/z)$ = 278.75 – 278.25 = 0.50 amu = Δ m/Δz or Δz = 1.962/0.5 = 3.92 ~ **4***

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

The standards are normally plotted as an area ratio to concentration as follows:

Compound	*Area ratio (X/IS)*
A (Standard 1)	*0.285*
IS	*NA*
A (Standard 2)	*1.145*
B	*1.093*
A (Diluted Unknown)	*0.705*
B	*0.512*

Compound A line equation: Aratio = 0.1433C_A – 0.00167
Compound B line equation: Aratio = 0.1367C_B – 0.00033
Now, we plug in the unknown area ratio and solve for C_X, we get:
C_A = (0.705 + 0.00167)/0.1433 = ***4.93 µg mL^{-1}***
C_B = (0.512 + 0.00033)/0.1367 = ***3.75 µg mL^{-1}***