Chem 231 Final Exam
April 15, 2013
SOLUTIONS

Short Answer Section (answer in **bold**)

1. If a scientist is using the Agilent ChemStation software to improve integration of chromatograms that were collected previously using the 1100 series HPLC, which window should they use?
a) Method and Run Control
b) Data Analysis
c) Report Layout
d) Diagnosis

2. The Buck GCs featured which of the following columns that were used in the set 1 experiment:
a) 150 x 4.6 mm C18 columns
b) 6' x 0.25" packed columns
c) 0.10 mm x 30 m (narrow bore) DB-225 columns
d) 0.53 mm x 15 m MXT-5 (mega bore) columns

3. A chemist is using a GC to perform a separation with a temperature gradient from 150 to 225 °C. Which of the following shut down procedures is likely to lead to column degradation?
a) gases turned off with oven left on at initial ramp temperature
b) oven and FID gases (air and hydrogen) turned off but helium flow left on
c) oven cooled and both oven and gases turned off, but detector left heated
d) oven cooled and both oven and gases turned off, but injector left heated

4. List one device which can be used to trap analytes from a gas sample onto a solid substrate. These are typically used in conjunction with GC injection.
Device = **SPME or sorbant columns**

5. A solid sample is being extracted into a solvent in a round bottom flask. Following extraction, the solids are filtered, put back into a round bottom flask and extracted a second time. The concentrations of the analytes from both extractions in this case can be used to test:
a) extraction precision
b) extraction efficiency
c) extraction time needed
d) all of the above

6. Give one advantage for using thin layer chromatography over HPLC.
Advantage is **1) low cost, 2) faster to run (set up + run time), 3) no compounds "lost" due to too much or too little retention**

7. Why was an internal standard used in lab experiments using GC but not HPLC?
answer: **The GC has poor injection volume precision, the HPLC does not**

8. Which of the following procedures is the correct one for using a C18 solid phase extraction cartridge for concentrating phenols from an aqueous solution.

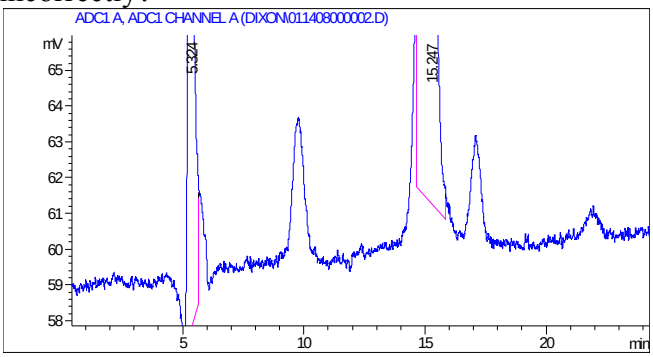
a) rinse cartridge with water, pass sample through, elute with methanol

b) rinse cartridge with methanol and then water, pass sample through, wash with water, elute with methanol

c) rinse cartridge with water and then methanol, pass sample through, wash with methanol, elute with water

d) rinse cartridge with methanol, wash with water, pass sample through, elute with methanol

9. Looking at the chromatogram below, how were the integration parameters set incorrectly?



a) too many noise peaks were picked up

b) the integration did not account for the increasing baseline

c) the integration did not pick up actual peaks

d) given the noise, the integration did as well as can be expected

10. When using an Agilent HPLC to run samples using a sequence and autosampler, what part of the software is selected to determine the sample run order:

a) Subdirectory selected in the Sequence Parameters

b) Method in the Sequence Parameters

c) Sequence Table under the Sequence drop-down menu

d) Selected as the last menu box under Edit Entire Method

Problem Section

1. A scientist is trying to optimize a new method to derivatize fatty acids to fatty acid methyl esters (FAMEs) which occurs in a methanol solvent. Since an unoptimized method will result in some fraction of the fatty acids not being derivatized, it is important to remove unreacted fatty acids from the FAMEs.

a) If using liquid – liquid extraction to remove FAMEs to a hexane solvent, describe whether you would want to add water or methanol as the polar solvent and buffered to what pH. Fatty acids have pK_a values of around 5 and FAMEs are fairly non-polar but more polar than hexane. Hexane is immiscible with both water and methanol. Explain your answer. (8 pts)

Water *is better than methanol because it is more polar. This will drive a greater fraction of FAMEs to hexane.* ***A pH of ~7 to ~10 would be best****. pH > pK_a is needed so that fatty acids are ionized, but high pH will result in ester bond cleavage.*

b) To test the recovery efficiency (from the reaction and extraction procedures), 499 µg of C17 fatty acid (heptadecanoic acid – MW = 270.4 g/mol) is added to 5.00 mL of a sample that contains no C17 fatty acids (most biological sources only have even numbers of carbon). The C17 fatty acid is reacted in methanol to the corresponding FAME (MW = 284.5 g/mol) and extracted to hexane resulting in 25.0 mL of hexane which also has 501 µg of internal standard butylbenzoate. A standard is also prepared that contains 19.7 µg/mL C17 FAME and 20.1 µg/mL butyl benzoate. These are run using GC with manual injection with the peak areas shown in the table below.

Sample	Peak Area – C17 FAME	Peak Area – butylbenzoate
C17 recovery test	36,208	47,921
Standard	54,121	58,359

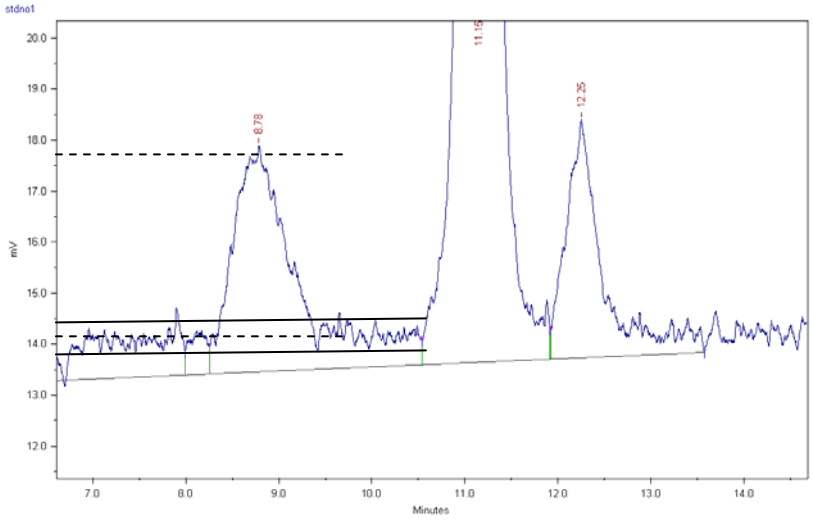
Calculate the reaction/extraction efficiency for C17 fatty acid. (12 pts)
The butylbenzoate is being used as an internal standard so we can use the internal standard calibration method: $F = \left(\frac{A_x}{A_{IS}}\right) \Big/ \left(\frac{C_x}{C_{IS}}\right) = \left(\frac{54,121}{58,359}\right) \Big/ \left(\frac{19.7}{20.1}\right) = 0.946$

Applying this F value to the C17 recovery standard where C_{IS} = 501 µg/25 mL = 20.04 µg/mL,

$$C_x = \left(\frac{A_x}{A_{IS}}\right) \Big/ \left(\frac{F}{C_{IS}}\right) = \left(\frac{36,208}{47,921}\right) \Big/ \left(\frac{0.946}{20.04}\right) = 16.00 \mu g/mL$$

mass of FAME in 25 mL = (16.00 µg/mL)(25 mL) = 400 µg
mass of FAME expected with 100% yield
= (499 µg FA)[(284.5 µg/µmol)/(270.4 µg/µmol)] = 525.0 µg
So % recovery = 400*100/525 = **76.2%**

2. (15 points) Part of a chromatogram is shown below in which the peaks at 8.78 and 12.25 min. were for compounds both present at 2 µg mL^{-1}. The peaks were integrated as shown using the programmed computer software.



a) Is the integration done correctly for the peaks at 8.78 and 12.25 min.? If not, describe what is wrong with the integrations. (6 pts)

No. There are two problems with integration of each of those peaks. The baseline is too low and the peak ends too late.

b) Estimate the detection limit concentration (defined using twice the peak to peak noise) for the peak at 8.78 min. (8 pts)

peak to peak noise ~ 0.6 mV (see plot). m = peak height/conc. and peak height ~ 17.6 – 14.1 = 3.5 mV or m = 3.5 mV/2 μg mL^{-1} = 1.75 mV mL μg^{-1}

LOD = 2N/m = 1.2 mV/(1.75 mV mL μg^{-1}) = ***0.7 μg mL*** *(0.5 to 1.0 is acceptable based on estimating values)*

3. A scientist is using reversed phase HPLC to separate phenols that are dissolved in methanol. She is using a 150 x 4.6 mm C18 column with an eluent of 55% acetonitrile and 45% water, a flow rate of 1.0 mL/min and a UV detector. Initially, she was using 10 μL injections, but to try to increase the sensitivity of the method by a factor of 10, she switched to 100 μL injections. The table below shows the results (peak width is baseline width):

10 μL injection results

Compound	Retention Time (min)	Peak Width (min)	Peak Height (mAU)	Peak Area (mAU·min)
unretained	1.35	---	---	---
4-hydroxybenzaldehyde	1.98	0.118	27.1	2.00
phenol	2.74	0.160	19.7	1.97
2-methoxyphenol	3.01	0.176	22.1	2.43
4-methylphenol	5.03	0.285	14.7	2.61

100 μL injection results:

Compound	Retention Time (min)	Peak Width (min)	Peak Height (mAU)	Peak Area (mAU·min)
unretained	1.35	---	---	---
4-hydroxybenzaldehyde	1.98	0.164	192	19.7
phenol	2.74	0.191	163	19.5
2-methoxyphenol	3.01	0.198	197	24.4
4-methylphenol	5.03	0.290	143	25.9

a) Did the signal to noise (assume the baseline noise is the same in each chromatogram) increase by a factor of 10? Why or why not? (5 pts)

No. Although the peak area is ~10X larger, the peak height (used to measure signal to noise) is not – due to peak broadening.

b) What was the most undesirable result (of those shown in the table) from the increase in injection size in terms of the chromatogram? (5 pts)
The resolution of phenol and 2-methoxyphenol dropped from baseline resolution with 10 μL to worse than baseline resolution with 100 μL.
10 μL resolution = 0.27 min/0.168 min = 1.61, while for 100 μL resultion = 1.39

c) Why do you think that the greatest broadening of peaks occurs from the first eluted? What might this say about whether the injection volume size or solute overloading is contributing more to peak broadening? (5 pts)
Extra column broadening tends to affect the earliest eluting peaks the most, because otherwise, they would be expected to have narrow peaks. The extra column broadening looks to be caused by the increase in injection volume, particularly because the solvent, methanol, is a stronger solvent than the eluent. Also, the alternative, solute overloading, would be expected to lead to earlier eluting peaks, which was not observed.

bonus) What solvent for the phenols would have resulted in less peak broadening when injecting a 100 μL volume? (3 pts)
100% water solvent would have been better.

4. For both the SPME and SPE labs, use of MS detection (in the SPME – GC analysis) or photodiode array detection (in the SPE – HPLC analysis) allows qualitative information to be provided on the analytes originating from unknown samples. Answer the following questions for either GC-MS or HPLC-DAD (but not both):
a) What information is given from the detector and how is it useful in determining the identity of compound. (5 pts)
1) For GC-MS, the MS gives the molecular weight of (usually) the parent ion and major fragment ions. This will indicate the molecular weight and possible parts of the molecule.

2) For HPLC-DAD, the DAD allows one to get UV spectra for unknown compounds. The UV spectra gives information about the presence of additional double bonds beyond the benzene ring in phenols. Additional double bonds, especially those conjugated to the benzene ring or in aldehydes will shift UV maxima to longer wavelengths.

b) If a peak is observed in a "real" sample analyzed (of the types you examined) that does not match any standard, what are the limitations in using the detector to determine its identity? (5 pts)
1) GC-MS: Just the MS alone does not always make it possible to identify the compound, especially in the case of flower volatiles where many of the compounds are monoterpenes of the same molecular weight and similar common MS fragment ions. Identification usually also requires the use of standards or MS libraries.

2) HPLC-DAD: Many compounds will have similar UV spectra, so that the UV spectrum is more useful in identifying the type of compound rather than the exact compound.