Name: ___________________

Practice Lab Quiz - A very similar (perhaps shorter) quiz will be given during lab on Thursday 5/11 and Tuesday 5/16. Answers will be posted outside my office and at www.chemistry.sjsu.edu/rterrill tomorrow 5/10.

Experimental Design, HPLC Separations, Flame Atomic Absorption Spectroscopy and UV-VIS Analysis

1. Describe how to make a series of 5-calibration standards ranging from 5 to 50 ppm in caffeine concentration starting with pure caffeine and water. Use no volumetric flasks larger than 250 mL and weigh no less than 25.0 mg of caffeine. Volumetric flasks available include: 5, 10, 20, 25, 50, 100, 250 mL. Pipets available include: 1, 2, 3, 5, 10, 15, 20, 25, 50 and 100 mL.

a. Weigh about _____50.0____ mg of caffeine to the nearest 0.1 mg.

b. Dissolve in water and bring to mark in a _____250_____ mL volumetric flask. This solution (PSCF) will be __200_____ ppm caffeine.

c. Transfer by volumetric pipet __1.00____ mL of PSCF into a __50.0____ mL volumetric flask and bring to the mark with water. This will prepare a solution that is ___200x1/50 = 4.00____ ppm caffeine.

d. Similarly transfer __2.00____ mL of PSCF to a _50.0_____ mL vol flask to prepare a solution that is ___8.00____ ppm caffeine.

e. Similarly transfer __3.00____ mL of PSCF to a __50.0__ mL vol flask to prepare a solution that is ____12.0____ ppm caffeine.

f. Similarly transfer __5.00____ mL of PSCF to a __50.0_____ mL vol flask to prepare a solution that is ___20.0____ ppm caffeine.

g. Similarly transfer __10.0____ mL of PSCF to a ___50.0__ mL vol flask to prepare a solution that is ___40.0____ ppm caffeine.
2. Describe in simple terms how analytes are separated in chromatography in general and specifically reverse-phase high performance liquid chromatography (RP-HPLC). In doing so, describe the relevant chemical property of the RP-HPLC stationary and mobile phases and how the different value of this property affects retention sample molecules. Don’t forget to mention what determines which sample molecules will elute first.

In chromatography (solute vapors) solutes are carried by a (gaseous) liquid mobile phase (MP) that is flowing past a stationary phase (SP). The solutes rapidly partition to some quasi-equilibrium extent from the MP into the SP. The relative affinity of the solute for the SP determines how much time the solute will spend there relative to the MP and therefore how quickly it will travel relative to the velocity of the MP. The solutes that partition most strongly into the SP will travel the slowest.

In RP-HPLC the SP is non-polar and the MP is polar so non-polar solutes move slower, are retained longer, and elute later than polar ones.

3. Very briefly describe a typical stationary phase used for reverse-phase HPLC – material, size, shape and coating. (Answer may be very short.)

**Material:** SiO₂  
**Size:** 4 μm  
**Shape:** spherical  
**Coating:** Octadecane chemically bonded to SiO₂ surface.
4. Acetone and Benzene have the following (fictitious) molar extinction coefficients, measured in acetonitrile solvent:

a. \( \varepsilon \) values:

<table>
<thead>
<tr>
<th>( \varepsilon / M^{-1}cm^{-1} )</th>
<th>( \lambda = 300 \text{ nm} )</th>
<th>( \lambda = 261 \text{ nm} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>86</td>
<td>160</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.83</td>
<td>1300</td>
</tr>
</tbody>
</table>

b. A (fictitious) mixture of benzene and acetone in acetonitrile had the following absorbances:

1. at \( \lambda = 300 \text{ nm} \) \( A = 0.2154 \)
2. at \( \lambda = 261 \text{ nm} \) \( A = 1.0500 \)

To a good first approximation, what are the molar concentrations of acetone and benzene?

1. \( \varepsilon_{\text{benzene}} \ll \varepsilon_{\text{acetone}} \)

\[ \text{so } A_{300} \approx \varepsilon_{\text{acetone}} \cdot (M^{-1} \text{ cm}^{-1}) \cdot 1 \text{ cm} \cdot C_{\text{acetone}} \cdot (M) \]

\[ C_{\text{acetone}} = \frac{A_{300}}{\varepsilon_{\text{acetone}} \cdot (M^{-1} \text{ cm}^{-1}) \cdot 1 \text{ cm}} = \frac{0.2154}{86 M^{-1} \text{ cm}^{-1} \cdot 1 \text{ cm}} \]

\[ C_{\text{acetone}} = 0.002505 \text{ M} \]

2. \( A_{261} = \varepsilon_{\text{acetone}} \cdot 1 \text{ cm} \cdot C_{\text{acetone}} + \varepsilon_{\text{benzene}} \cdot 1 \text{ cm} \cdot C_{\text{benzene}} \)

\[ C_{\text{benzene}} = \frac{A_{261} - \varepsilon_{\text{acetone}} \cdot 1 \text{ cm} \cdot C_{\text{acetone}}}{\varepsilon_{\text{benzene}} \cdot 1 \text{ cm}} \]

\[ C_{\text{benzene}} = \frac{1.0500 - 160 M^{-1} \text{ cm}^{-1} \cdot 1 \text{ cm} \cdot 0.002505 \text{ M}}{1300 \cdot 1 \text{ cm}} \]

\[ C_{\text{benzene}} = 0.000498 = 0.00050 \text{ M} \]
5. What **spectral** quality of light **emitted** from gas phase atoms makes it possible to use this light to measure the concentrations of both Zn and Fe in the same sample without concern for interference between the Fe and Zn absorbance signals? (Very short answer.)

*Light emitted by gas phase atoms has an extremely narrow bandwidth (<0.05 nm) and thus has a very low probability of direct spectral overlap with the emission from other atoms.*

6. What is it about gas phase atoms that makes it possible to measure **low concentrations** of metals using FAAS despite the very low overall efficiency of conversion from aqueous metal ions to gas-phase metal atoms in the flame? (Very short answer.)

*The extinction coefficients for light absorption by gas phase atoms can be very large.*