**Exercise #4b: HPLC calculations and predictions**

*Chem 6614 Chemical Instrumentation*

**4.0b TABLE OF RUN CONDITIONS:**

2 inch C-18 reverse phase guard column=`column

Flow= 1 mL/min 70:30 methanol:water mobile phase

λ(analysis)= 265 nm A(min) =0.002 fast acquire setting

Sample volume = 20.0 μL

Standard mixture is prepared by adding 2 mL volumes of non-polar compounds X, Y and Z into a 100 mL volumetric and diluting to the mark in HPLC grade methanol.

**TABLE OF COLLECTED HPLC RUN DATA :**

**Chromatogram #1: Known reference mixture data in methanol**

Ref. Component tr,obs(min) Area (pA\*s)

X 0.58 40,000

Y 1.02 67,030

Z 1.56 78,900

**Chromatogram #2: unknown mixture of above components in methanol**

Unk. Component tr, obs(min) Area (pA\*s)

X 0.59 12,450

Y 1.03 53,500

Z 1.55 87,100

**4.1b ANALYZING REFERENCE HPLC DATA TO DETERMINE REFERENCE PEAK SENSITITIES, γk**

Step 1: determine absolute volumes of reference X, Y Z (in μL) being detected in 20 μL HPLC sample

Initial dilution is 2 mL/100 mL =0.02 (2%)=> 2% of the 20 μL HPLC injection volume is composed of the components : V­o =0.02\*20=0.40 μL

\_\_\_0.40\_\_\_\_ μL = absolute sample volumes for X,Y, Z = Vo(μL)

Step 2: Determine reference peak sensitivities γk for X,Y and Z using the observed areas from the

Reference Chromatogram #1 and your computed Vo (μL) as indicated by the reciple in column

4 in the table below:

**Table 1: Computed Sensitivities γk of X,Y and Z**

Ref. Component tr,obs(min) Area (pA\*s) γk= Area/Vo (pA\*s/μL)

X 0.58 40,000 100,000

Y 1.02 67,030 167,575

Z 1.56 78,900 197,250

**4.b2. USING THE DERIVED Sk TO DETERMINE UNKNOWN MIXTURE’S ABSOLUTE Vk DETECTED AND**

**RELATIVE VOLUME %= % Vk**

Step 3: Computing the absolute Vk of the Unknown mixture

Since γk =Area/Vo reflects the relative sensitivity of the HPLC detector per μL and since we

assume that it is constant regardless of the detected volume:

**Vk (unknown) = kth unknown Area/ γk**

Compute the unknown Vk using your determined γk in Table 1 and the measured A for the unknown mix using Table 2 below:

**Table 2: Absolute Volumes, Vk, for Unknown Mixture of X,Y and Z**

Component tr, obs(min) Area (pA\*s) γk Area of kth peak/γk=Vk

X 0.59 12,450 100,000 0.125

Y 1.03 53,500 167,575 0.319

Z 1.55 87,100 197,250 0.442

**Fill in from Table 1 compute**

**Step 4: Computing the relative % volume, %V,**

Since Table 2 contains the absolute volume detected of X, Y and Z in the unknown out the total of 20 μL injected , it is a simple matter to compute the relative % volume, %V using the reciple below:

**%Vk = 100\*Vk(μL)**

**20(μL)**

**Table 3: Relative % Volumes , %Vk for an Unknown Mixture of X,Y and Z**

Component Area of kth peak/γk=Vk  100\*Vk /20=%Pk

X 0.125 0.625

Y 0.319 1.60

Z 0.442 2.21

**Fill in from Table 2 compute**