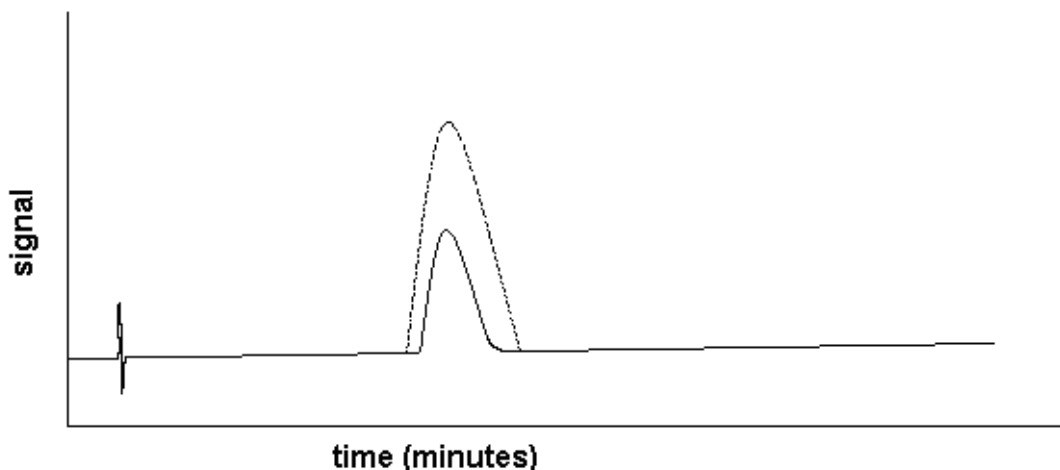<u>**Chromatography Solutions**</u>

1.   A separation on an affinity column produced the chromatogram shown below. Overtop of the chromatogram, sketch show what it would look like if a second sample with more (higher concentration) of analyte was run.

Note that the peak is in the same position, but larger.

2.   a. decrease---each substance is moving faster so can get through the system faster
        b. increase—each substance will travel a longer distance so it will take a longer time
        c. increase—each substance must travel a longer distance so more time is required
        d. no effect—the peak size will increase, but the average position should stay the same
        e. decrease—in general higher temperatures make things go faster
        f. no effect—retention time is characteristic of substance identity no size.
        g. decrease—with less difference between the polar mobile phase by using a less polar stationary phase, substances should move out of the stationary phase more readily.
        h. decrease-- with less difference between the less polar mobile phase and the nonpolar stationary phase, substances should move out of the stationary phase more readily.

3.   In reverse-phase, the mobile phase is polar, therefore polar compounds will spend more time in the mobile phase and elute first.
        a. ethylamine < butylamine < ethylene < 2-butylene
        b. trichlorophenol < chlorophenol < phenol < benzene
   If the column is polar, polar compounds will more strongly interact with the stationary phase and have longer retention times. If the column is nonpolar, nonpolar compounds will interact more strongly with the stationary phase and have longer retention times. In gas chromatography, the mobile phase does not interact to any extent with the sample and therefore does not affect retention time.
        c. butanol < methanol < water
        d. water < methanol < butanol
   In ion chromatography, ions with larger charges will interact more strongly with the resin (stationary phase) and have longer retention time. If the charges are the same, smaller ions, with higher charge densities will interact more strongly.
        e. bromide < fluoride < sulfide

4. This requires a ruler. You might want to make a relationship mm = time to convert length to time. I apologize, my scaling is not quite perfect. Do not expect to match these answers exactly. However the order and approximate values should be the same.

Dead time (t_M) will be the first signal, so in this case the BEGINNING of the first peak.

Retention time (t_R) will be measured at the highest point of each peak.

Adjusted retention time (t_R') is the retention time minus the dead time.

$t_M = 0.5$ min

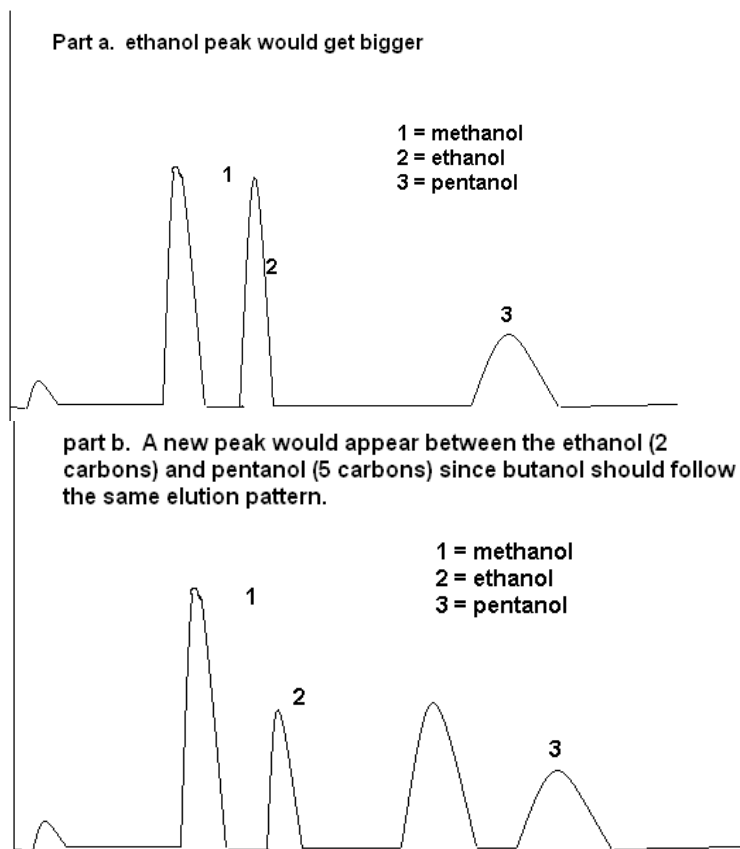peak 1: $t_R = 1.2$ min; $t_R' = 0.7$ min

peak 2: $t_R = 2.7$ min; $t_R' = 2.2$ min

peak 3: $t_R = 3.7$ min; $t_R' = 3.2$ min

peak 4: $t_R = 10.0$ min; $t_R' = 9.5$ min

peak 5: $t_R = 18.6$ min; $t_R' = 18.1$ min

5.

Part a. ethanol peak would get bigger

1 = methanol
2 = ethanol
3 = pentanol

part b. A new peak would appear between the ethanol (2 carbons) and pentanol (5 carbons) since butanol should follow the same elution pattern.

1 = methanol
2 = ethanol
3 = pentanol

Part c. There is no obvious change in the chromatogram. Water is part of the mobile phase and so will not be detected. All peaks should get smaller as you work with the same amount of a more dilute sample.

part d. An unretained species is eluted at the first signal, so
that part of the chromatogram increases in size.

1 = methanol
2 = ethanol
3 = pentanol
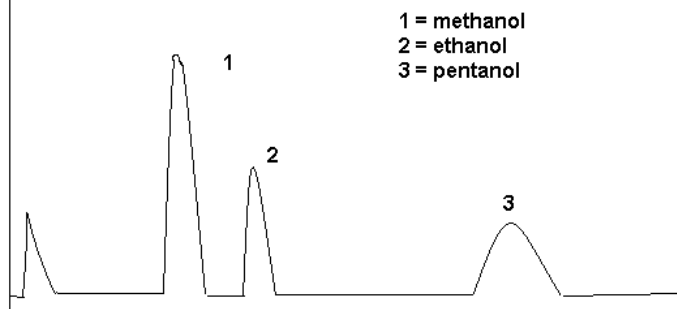
6. First calculate the concentration of each substance in each solution using the dilution equation
$$M_1V_1 = M_2V_2$$

solution	volume 100 ppm X (mL)	volume 50 ppm Y (mL)	volume 50 ppm Z (mL)	[X] (ppm)	[Y] (ppm)	[Z] (ppm)
1	5	20	10	5	10	5
2	10	15	20	10	7.5	10
3	15	10	5	15	5	2.5
4	20	5	15	20	2.5	7.5

This resulted in 4 chromatograms with the following results:

peak A		peak B		peak C	
time (min)	Area (x 10^4)	time (min)	Area (x 10^5)	time (min)	Area (x 10^4)
6.44	0.68	8.00	4.10	9.72	7.99
6.54	1.98	7.96	8.30	9.68	7.63
6.34	2.18	8.03	2.53	9.66	7.27
6.49	3.02	8.12	6.29	9.58	6.93

a. Which peak corresponds to which analyte?

Peak A is the peak for X, since its area is consistently increasing.
Peak C is the peak for Y, since its area is consistently decreasing.
Peak B is the peak for Z, since its area increases in the first two trials then decreases and increases again, as the pattern for the concentrations.

b. **Make a calibration curve for each analyte**
See link to spreadsheet. There will be three curves. The resulting equations are

$A_a = (1444 \pm 286)[X] + (1600 \pm 3914)$

$A_c = (1416 \pm 14)[Y] + (65700 \pm 95)$

$A_b = (43000 \pm 24556)[Z] + (39000 \pm 1793)$

c. A unknown solution was analyzed with the same method. Based on the results below, what is the composition of the sample?

retention time (min)	peak area
6.59	33419
8.05	554193
9.68	76416

$33419 = 1444[X] + 1600$
$31819 = 1444[X]$
$22.0 = [X]$

$$\frac{e_x}{22.0} = \sqrt{\left(\frac{286}{1444}\right)^2 + \left(\frac{3914}{31819}\right)^2} = 0.233$$

$e_x = (22.0)(0.233) = 5.1$

[X] = 22.0±5.1 ppm

$554193 = 43000[Z] + 39000$
$515193 = 43000[Z]$
$12.0 = [Z]$

$$\frac{e_z}{12.0} = \sqrt{\left(\frac{1793}{39000}\right)^2 + \left(\frac{24556}{515193}\right)^2} = 0.0662$$

$e_z = (12.0)(0.0662) = 0.8$

[Z] = 12.0±0.8 ppm

$76416 = 1416[Y] + 65700$
$10716 = 1416[Y]$
$7.57 = [Y]$

$$\frac{e_y}{7.57} = \sqrt{\left(\frac{14}{1416}\right)^2 + \left(\frac{95}{10716}\right)^2} = 0.0133$$

$e_y = (7.57)(0.0133) = 0.10$

[Y] = 7.57±0.10 ppm

7. Part a.

568336 = 139997[analyte] + 913
4.05311 ppm = [analyte]

$e_x/(4.05311) = [(5949/139997)^2 + (554/567423)^2]^{1/2}$
$e_x/(4.05311) = [(0.0424938)^2 + (0.0009763)^2]^{1/2} = [0.00180572 + 9.5 \times 10^{-7}]^{1/2}$
$e_x/(4.05311) = 0.0425$
$e_x = 0.1722$

The best answer uses the error to determine significant figures as the number of significant figures in the peak area is really unknown. The instrument tends to report whatever it measures without regard to precision. The units on concentration are given in the slope of the line.

Answer = 4.0±0.1 ppm

Part b.

2765422 = 139997[analyte] + 913
19.747 ppm = [analyte] in the <u>measured</u> solution

Use the dilution factor to figure out the concentration of the original solution
(19.747 ppm)(1000 μL) = [original](10.00 μL)
1974.7 ppm = [original solution]

Assume the dilution is not a significant source of error and use the equation of the line to determine the overall area. As before, report significant figures based on the error.

$e_x/(1974.7) = [(5949/139997)^2 + (554/2764509)^2]^{1/2}$
$e_x/(1974.7) = 0.0424$
$e_x = 83.9$

answer = (1.97±0.08) x 10³ ppm

part c.

6729661 = 139997[analyte] + 913
48.0635 = [analyte] in the <u>measured</u> solution

This solution was created by concentrating the sample using solid phase extraction. The solution that was measured has a volume of 5.00 mL. This 5.00 mL came from 1.00 L (1000 mL) of sample. It is still solved with the dilution equation.

[analyte](5.00 mL) = [original](1000 mL)
(48.0635 ppm)(5.00 mL) = [original](1000 mL)
0.240 ppm = [original]

Using the error from the equation of the line
$e_x/(0.240) = [(5949/139997)^2 + (554/)^2]^{1/2}$
$e_x/(0.240) = 0.425$
$e_x = 0.010$

answer = 0.24±0.01 ppm

note: The relative error was essentially the same each time because the y-intercept error was SO much smaller that it was nearly negligible. It would be more important as you have smaller peak areas/concentrations.

8. Standard addition assumes that signal (S) is proportional to concentration (c) or that you are in the linear portion of the calibration curve. It also assumes either that the blank is zero or the signal is adjusted for the blank. Therefore,

$$S = kc$$

If c_A is the concentration of sample A, the signal associated with that concentration is the peak area (S_A) = 24237396.

Assuming that volumes are additive, the concentration of the second solution can be expressed as

$$c_B = (10*c_A + 5*20)/6$$

its signal (S_B) = 121220801.

$$S_A = 24237396 = kc_A$$
$$\text{-----------------------------}$$
$$S_B = 121220801 = k(10c_A+100)/6$$

The proportionality constants (k) cancel. So

$$0.19994 = 6c_A/(10c_A + 100)$$
$$1.994c_A + 19.994 = 10c_A$$
$$19.994 = 8.006c_A$$
$$2.4987 = c_A$$

Since the concentrations and volumes all have three significant figures and concentration was given in units of ppm…. **C_A = 2.50 ppm.**

You cannot get error from this type of experiment.

9.   Part a. Since the peaks are different widths, the heights will not be representative of the entire analyte.

        Part b. Since the peak at 10.8 minutes increases in area (and height), it must represent the analyte peak. Since the addition of the spike dilutes the other components of the sample, it is natural that their areas (heights) will decrease.

        Using gram-fraction (g analyte/g sample) as the concentration unit, the concentration of the spiked solution can be expressed as

$$C_S = (c_B*4.34 + 3.98)/8.32$$

$$934148 = k*c_B$$
$$\text{-------------------}$$
$$1099110 = k*(c_B*4.34 + 3.98)/8.32$$

$$0.8499 = 8.32c_B/(c_B*4.34+3.98)$$

$$3.688c_B + 3.383 = 8.32c_B$$

$$3.383 = 4.632c_B$$
$$0.730 = c_B \text{ in gram-fraction or 73.0\%}$$

10. In a standard addition graph, the concentration of the diluted standard (x-axis) is graphed against signal (y-axis). To calculate the concentration of diluted standard

$$(35.21 \text{ ppm})(\text{vol std}) = (\text{diluted std})(10.0 \text{ mL})$$

Volume std (mL)	Diluted std (ppm)	Peak area
0.00	0.00	135235
0.50	1.8	396313
1.00	3.52	764095
1.50	5.28	1050673
2.00	7.04	1523699

See Excel spreadsheet for graph and regression analysis.

The equation of the line from regression analysis is

Peak area = (195352±11658)[fluoride] = (84802±50295)

In standard addition, you see the signal to zero and solve for analyte concentration. You will get a negative number. The positive value is the concentration of analyte in the diluted samples

0 = 195352c + 84802
0.434 = c

To figure out the amount in the original sample, recall that 5 mL of sample was added to each solution, with a total volume of 10 mL, so
(5.00mL)[original] = (10.00 mL)(0.434)
[original] = 0.868 ppm

Use the line error equation to get the error for this answer

$e_x /0.868 = [(11658/195352)^2 + (50295/848020)^2]^{1/2}$
$e_x /0.868 = [0.00356133 + 0.003517]^{1/2} = 0.0841$

$e_x = 0.7303$

Since the concentration values have fewer significant figures than peak area, significant figures are based on concentration. Although one value has two sig figs, the rest have three. Since the answer is an average, the most common number of sig figs, 3, can be used.

Answer = 0.868 ±0.730 ppm.

11. Since the area of peak one is mostly constant and the area of peak 2 increases as sulfate concentration increases, the peak one must be due to fluoride and peak 2 to sulfate.

With an internal standard, the concentration of analytical standard (sulfate) is graphed on the x-axis against the ratio of signals (signal analyte/signal internal standard = peak area sulfate/peak area fluoride) on the y-axis. Thus the information needed for the graph is

Vol SO_4^{2-} (mL)	$[SO_4^{2-}]$ (ppm)	Area peak 1	Area peak 2	Ratio of peak area
1.00	6.44	62817	96234	1.531974
2.00	12.9	68075	204377	3.002233
3.00	19.3	61262	274731	4.484525
4.00	25.8	67054	402935	6.009112

Using the data above produces a linear graph with a line equation of

Peak ratio = 0.2316±0.0014[sulfate] + 0.0285±0.0242

For the sample, the peak ratio is 278748/63281 = 4.405.
Using this value in the equation

4.405 = 0.2316[sulfate] + 0.0285
4.376 = 0.2316[sulfate]
18.896 = [sulfate]

Since the sample was diluted to add the internal standard, this must also be taken into account

(5.00 mL sample)[original] = (10.00 mL)(18.896 ppm)
[original] = 37.8 ppm

Three significant figures, since that is what the volumes and concentrations had.

Error from the error of the line:

$e_x/37.8 = [(0.0014/0.2316)^2 + (0.0242/4.376)^2]^{1/2} = [0.0060449^2 + 0.00553^2]^{1/2} = 0.0819$
$e_x = 0.309$

answer = 37.8±0.3 ppm sulfate