Gas Chromatography Experiment

3/8/18

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Abstract:

This lab was looking to show how good of a method gas chromatography is for separating samples, how gas chromatographs work, and how to properly use a gas chromatograph to identify unknown samples. This lab demonstrates the importance of good injection technique through tests examining the effect of incorrect injection techniques and practices. These techniques include rinsing and wiping off the syringe before injections, injecting quickly and accurately, and not releasing the material to be injected before injecting. Additionally, this lab provides the background knowledge needed to use a gas chromatograph. This includes information about parts of the gas chromatograph, like the column and Wheatstone bridge, and the governing equations used in gas chromatography. Also, unknown samples are tested against pure samples, and the unknown samples are identified through use of these gas chromatography techniques. A consistent method for determining mass and mass percentages was found, and the mass percentages of compounds within two unknown samples were found to be 19.9% methanol, 20.5% 1-propanol, and 59.6% sec-butanol in mixture 2A, and 44.3% methanol, 25.1% 1-propanol, and 30.6% sec-butanol in mixture 2B.

Introduction:

Gas chromatography is a practice through which samples can be identified by testing known pure samples or known mixtures, and using the data recovered from these runs to find probable matches. This is done through a number of steps. First, a sample is injected into the gas chromatograph (GC). The sample is then vaporized. Then, gas used to move the sample through the GC, known as a carrier gas, picks up the sample, and carries it through the GC's column. The sample's contents then run past a thermistor, which is a resistor whose resistance varies greatly with temperature, and this allows us to collect useful data.

Two major factors used when trying to identify an unknown sample are peak area and retention time, which are two measurements taken by the GC. Area is proportional to the mass of sample inserted, and is calculated by the GC through a Wheatstone bridge. The Wheatstone bridge allows us to run our carrier gas past one thermistor, and our sample past the other. Since our sample will have a different thermal conductivity than the inert gas, this creates a voltage difference, which corresponds to the area, since the wheatstone bridge takes this voltage difference, and we integrate this over time to get an area (though the units of said area end up being millivolts times minutes, due to how this is calculated) Additionally, the greater the mass of sample flowing past the thermistor, the greater the voltage difference and corresponding area will be, because the greater the amount of sample flowing past the thermistor, the greater the change in thermal conductivity will be. Retention time is a measure of how quickly the sample goes through the column. This is dependent on a number of factors, such as temperature, boiling

point, and polarity. The reason polarity is such a big factor is because of the compound packed in the column. In our case, this material was Porapak Q, a nonpolar bead, which caused polar samples like water to go through quite quickly, while relatively large nonpolar compounds like sec-butanol took much longer.

Proper procedure is very important with GCs. Our first day of testing revealed a lot of telling data that indicated to us what the best proper procedure would be. First, the syringe used to inject the sample into the GC should be pumped a number of times (20+ ensured the best results for us). Then, after drawing the sample into the syringe, the syringe should be depressed until only the volume you wish to inject remains in the syringe. Then the syringe must be wiped, especially on the side of the needle to ensure no liquids are stuck to the side of the syringe, as this will impact the accuracy of your runs. After this, the syringe should be placed into the proper input slot in the GC, injected, and pulled out quickly.

When finding the masses or mass percentages of an unknown sample, it is important to have a well-calculated proportionality constant (k). This is typically done through testing known samples at a number of different known volumes. If the samples' densities are known, these volumes can then be converted to masses of sample injected, which can be used to calculate k. The equation used to calculate k from results is $A = k_i \, {}^{m_i}/_{Q}$ where A is the area recorded from the Wheatstone bridge, k is the proportionality constant, m is the mass injected into the GC, and Q is the volumetric flow rate of gas within the GC. When this formula is used, k values can be calculated for pure substances, and used to find masses or mass percentages of these within unknown samples.

One other concept that is key to gas chromatography is HETP, or the height equivalent of one theoretical plate. This can be calculated using the formula $HETP = {}^{L}/_{16(t/b)^2}$, where t is retention time, b is peak width, and L is the length of the column. It is also worth noting that the number of theoretical plates, N, is the denominator of the above equation. HETP is derived from a number of different factors, but it is essentially a measure of the efficiency of the column, since it measures how well materials are separated. HETP is different for each material, differs when temperature is changed, and also differs with the GC used. In short, the lower the HETP (and higher the number of plates) the better the column is at separating materials.

It is important to note how important gas chromatography is within many fields of scientific study. It is used by industries like pharmaceuticals and cosmetics to separate out mixtures, and even used in forensics to identify material from possible arsons or crime scenes.

Equipment:

In this lab, a Buck Scientific Model 310 Gas Chromatograph with a 3ft column, and a Wheatstone bridge as the detector was used along with the software, PeakSimple to gather data. A one-microliter syringe was used for injections, and a flow meter was used to measure flow rates within the GC. Pure samples of water, methanol, ethanol, 1-propanol, 2-propanol, and sec-butanol were used in k value calculations. Additionally, mixtures of water and ethanol were used in specific ratios (0/100, 10/90, 20/80, 30/70, 40/60, 50/50, 60/40, 70/30, 80/20, 90/10, and 99/1

ethanol to water mass percentages). Finally, two unknown mixtures (2A and 2B) containing the same three pure alcohols in different proportions were used, and the mass percentages of these were determined through use of the GC.

Experimental Procedure:

Week 1:

First, all group members made sure they knew how to properly operate the GC, and corresponding software. Then, a check was done to ensure the GC was at 150°C, and operating properly.

Next, some injection tests were done. First, all team members needed to practice injecting 0.2μL samples, until they got three consistent runs (each with similar peak areas). Then, using the 50/50 water/ethanol solution the team studied how the length of time the needle is in the injection port impacts results. This was done by injecting a zero volume by filling the syringe with solution, and then fully pressing the plunger down, and wiping the needle. Then the syringe was injected into the injection port and left it there for a measured length of time, and repeated for a few short lengths of time (1, 2, 4, 6, and 8 seconds). Finally, the results were graphed.

The next step was to observe how contamination affected runs. This was done by rinsing the syringe with water 20 or more times, then aspirate 99% ethanol into the syringe without rinsing/pumping the syringe, and then finally injecting 0.2μL into the GC. The syringe was not rinsed in between ethanol injections. Then 0.2μL of ethanol was aspirated and injected again, with the process repeated until

either there was no detectable water peak or the water peak remained constant. Percent contamination could then be graphed as a function of the number of rinses. The syringe was then rinsed with 99% ethanol at least 20 times, and this potion of the procedure was repeated with water instead of ethanol.

Another set of tests was then done to test the GC's response to linearity in sample mass. First pure water was injected in quantities from 0.00 to 0.40μL in 0.05μL intervals, including 0.00μL. This allowed us to test the GC's response to linearly increasing masses. This was then repeated for the 99% ethanol solution in place of water, to test for a linear response to ethanol. The mixture solutions, each with different water/ethanol ratios (10-90% ethanol), were then injected with constant volumes. This tested for a linear response to mass ratios of two different substances.

Three trials were then each conducted at a different temperature (120°C, 150°C, and 180°C) to assess the effect of temperature on retention time, column efficiency, and flow rate. This was done by injecting 0.2μL a 50/50 water/ethanol mixture. After these final runs, the GC was shut down for the day.

Week 2:

The two unknown solutions and all pure samples were gathered for the second week. Additionally, the same GC was used to try to make results as constant as possible, and the group member with the lowest standard deviation in peak area when injecting was chosen to take care of all injections for the week.

The GC was again set to 150°C, and ensured to be working properly before starting. First, several injections of the mixtures were done to find an optimal operating temperature to ensure that peaks were close together without touching. The temperature of 180°C was chosen, since this fulfilled these requirements.

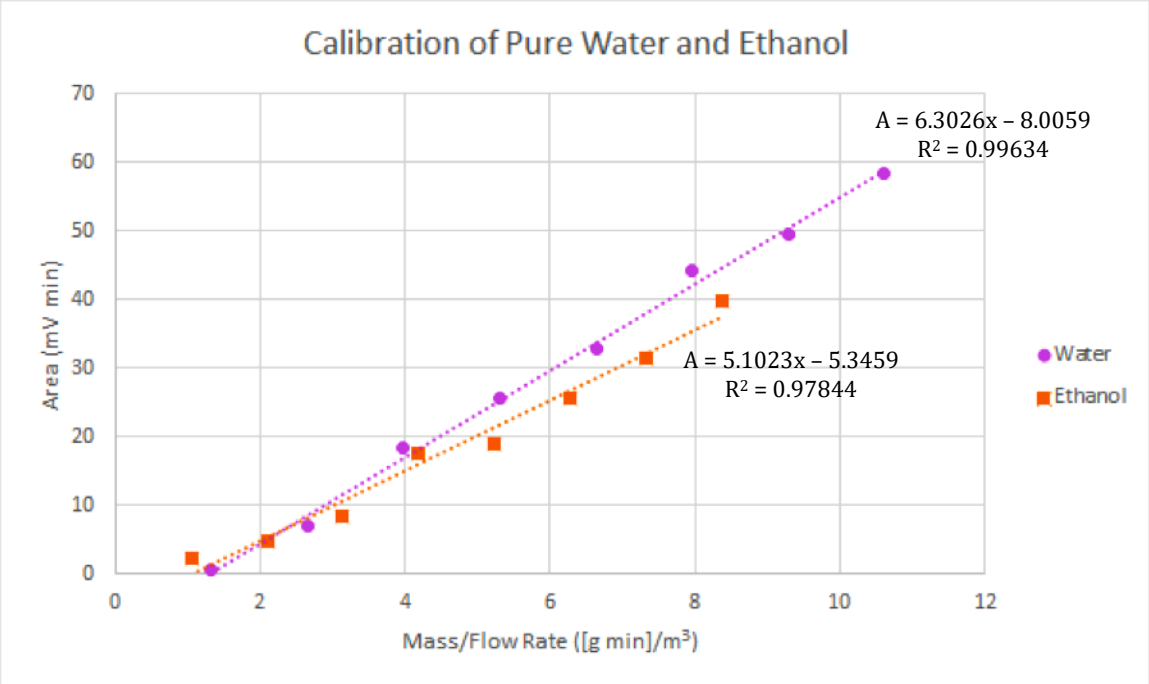
Next, injections of the pure samples were completed. For this, our chosen team member took various amounts of pure solution (0.0μL, 0.05μL, 0.1μL, 0.15μL, 0.2μL, 0.25μL, 0.3μL, 0.35μL, 0.4μL), and injected them one by one into the GC. The retention times and areas were recorded, with retention time indicating which pure sample had been injected, and area indicating how much had been injected. This was repeated for each pure sample (methanol, ethanol, 1-propanol, 2-propanol, and sec-butanol), and the syringe was pumped around 20 times between samples. These results were then graphed to find k constants for each pure sample.

Finally, peak measurements of the unknown samples were taken. The unknown samples were injected into the GC multiple times at a constant volume of 0.2μL and the retention times and areas were recorded. The GC was then shut down.

Results/Discussion:

Initial Runs (Week 1):

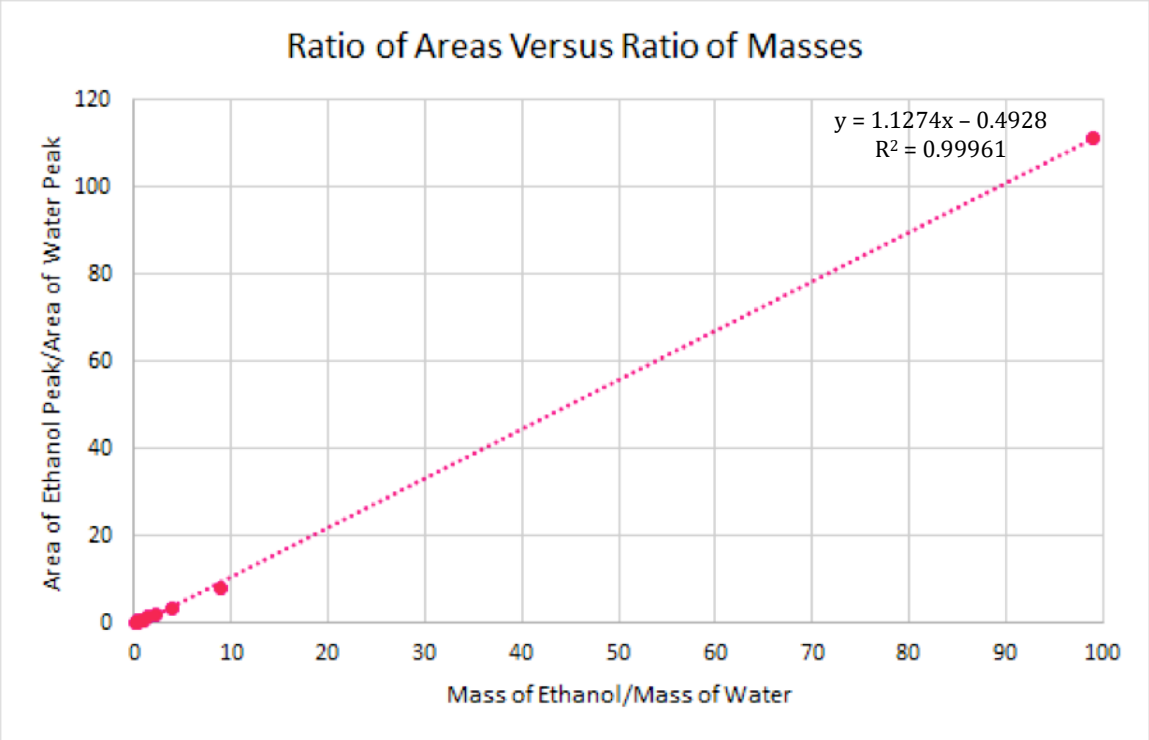
Our runs during Week 1 were mostly for the purpose of ensuring the GC was working properly, and practicing how to find values of the proportionality constant (k). We ran pure samples for water and ethanol at a number of different volumes, and the graph below demonstrates these runs. The graph is of area versus mass over flow rate, since, using the equation $A = k_i \, {}^{m_i}/_{Q}$, the slope of our graph



represents the k value. The k values were found to be 6.30 and 5.10 $\frac{mV\ m^3}{g}$ for water and ethanol respectively. Additionally, confidence intervals were found for these k values to be 0.369 and 0.731 respectively. Also, the graph has a y-intercept, which is non-zero, indicating that the detector within the GC has trouble detecting very small volumes, leading to b values (or y-intercept values on the graph). These values are a loose measurement of the accuracy of the detector with smaller samples. If we were

to include these b values in our main equation, he equation would become $A = k_i \, {}^{m_i}/_{Q} + b$. These values are -8.01 for water and -5.35 for ethanol.

Additionally, samples were analyzed that had changing mass ratios of water/ethanol (specific ratios mentioned above on pg. 4). This lead to the graph just below which graphs area ratios against mass ratios. Utilizing our equation from

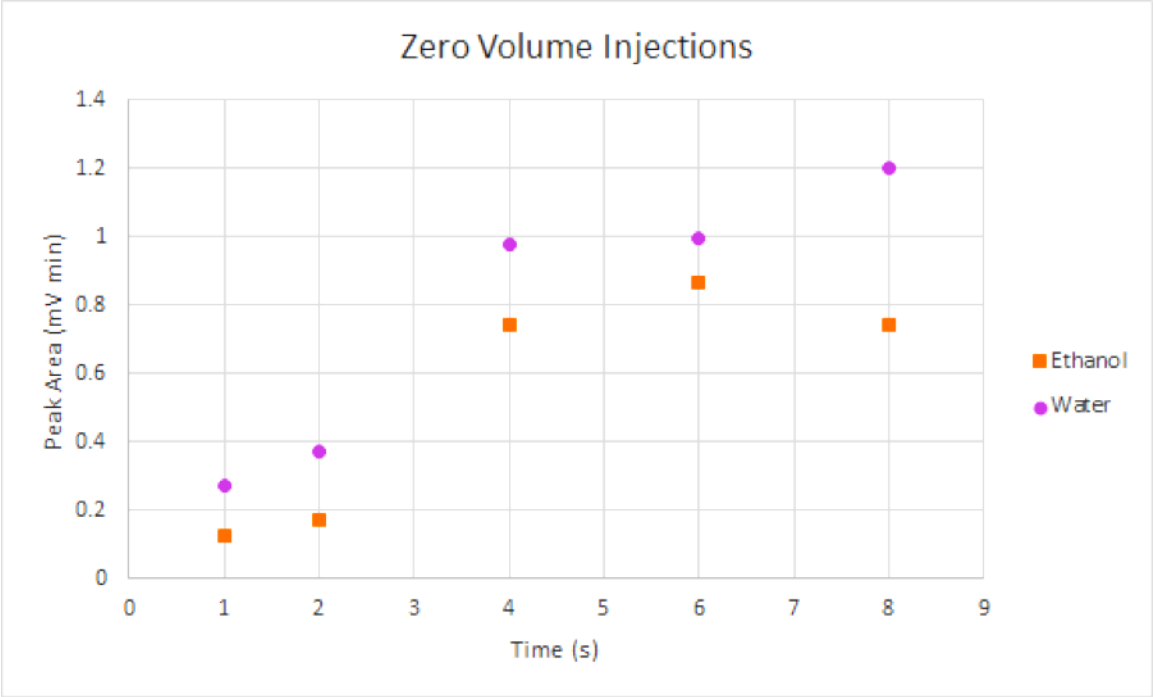


before, and combining the equations for water and ethanol, we can get $\frac{A_i}{A_j} = \frac{k_i}{k_j}\frac{\%m_i}{\%m_j}\frac{Q_j}{Q_i}$, which shows us the equation we've graphed. Within this equation, both Qs (volumetric flow rates) cancel, leaving us with the fact that area ratios plotted against mass ratios will give us a ratio of the ks as our slope. In this particular case the slope is 1.13, meaning that $\frac{k_{ethanol}}{k_{water}} = 1.13$. Of these two methods of finding k,

we decided to use the former method (varying volume of a pure substance) to determine k.

Error Analysis/Injection Technique (Week 1):

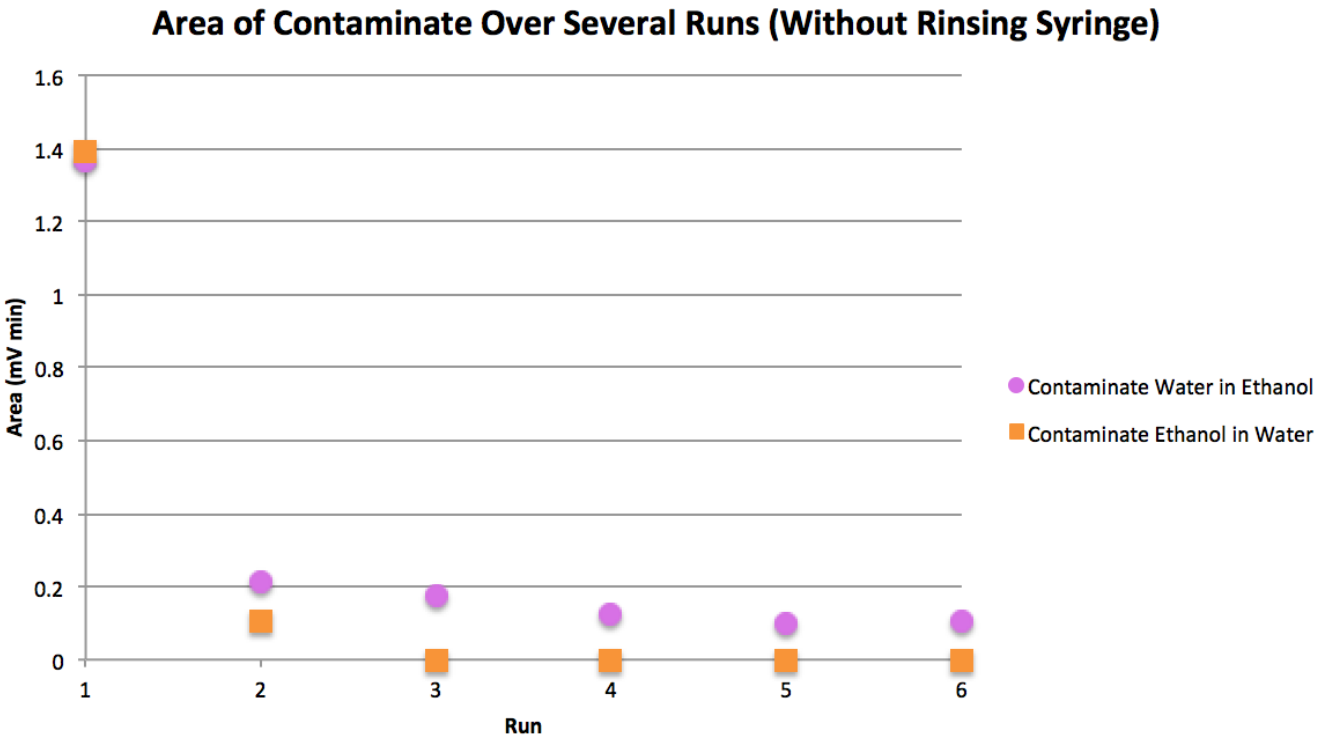
Another project that was done on the first week of experimenting was studying the effects of improper injection technique on runs, and examining runs that had bad data to understand what to improve for future runs. Overall, the intent was to minimize error in results as much as possible. The first test we did were a series of zero volume injections. In each of these, the syringe was rinsed with a 50/50 mass percent mixture of water and ethanol, and then inserted the syringe into the GC for set amounts of time. The graph just below demonstrates our runs,



showing that slight amounts of volume leech into the GC over time if the syringe is left in for more than a second or two. We took this information, and recognized that

accurate trials should involve injecting the sample quickly, and then removing the syringe just after injections.

Another type of test we did was contaminated sample tests. These were tests in which we rinsed the syringe in water, and then proceeded to inject ethanol into the GC, and vice versa. We did this over several runs to observe how many rinses it took to get rid of all of the contaminant. These runs are graphed below. We noted



that the contaminant seemed to be gone after about 5 or 6 rinses, with contaminant ethanol in water reaching 0 mV min, and contaminant water in ethanol reaching around 0.1 mV min, signaling that there were only trace amounts. These trace amounts can be explained, since the ethanol samples were only 99% pure, with the added 1% being water. From these tests, we concluded that the syringe should be rinsed at least 5 or 6 times when changing substances, though we often rinsed 20+ times to be absolutely safe. The trend within the graph is logarithmic decrease,

which makes sense, since the contaminant would be decreasing exponentially with each rinse.

Additionally, we did a bit of extra analysis to ensure our results for week two would be as accurate as possible. One of these was testing each group member's injection technique by injecting 0.2μL several times, and calculating the standard deviation in each group member's area, and letting the member with the lowest standard deviation conduct all injections during the second week. Additionally, we looked at a few odd results we got, and figured out what we did wrong in each run, to make sure we weren't making the same mistakes in week two. Two such results are shown below. The first (Figure 1) was from not thoroughly cleaning off the

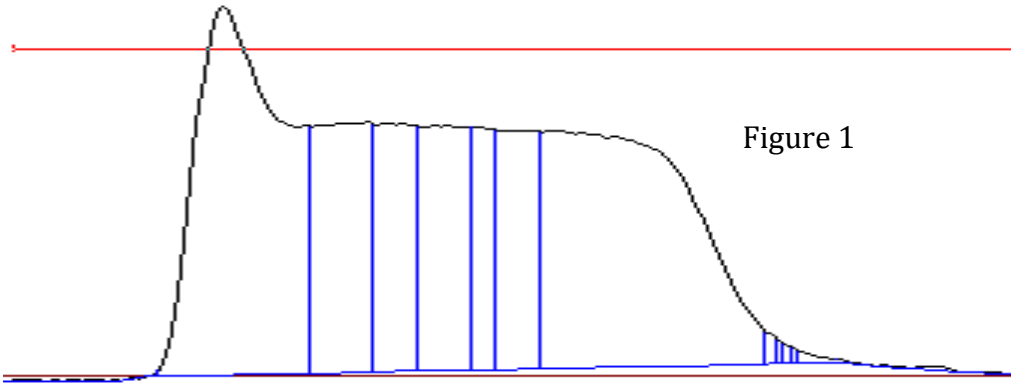


Figure 1

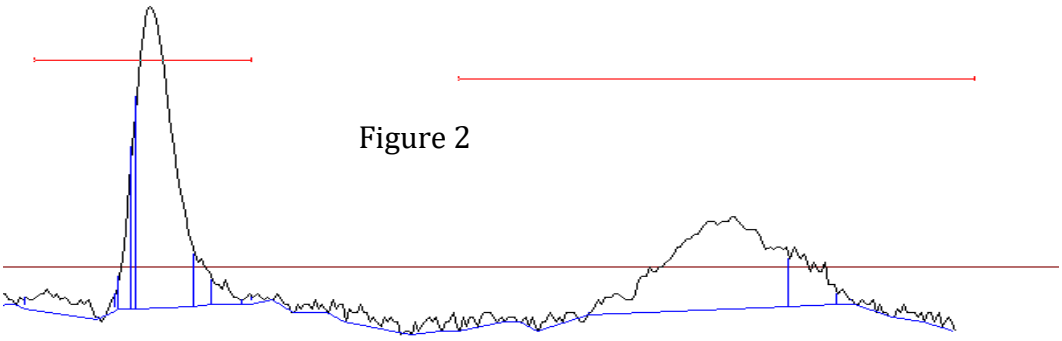


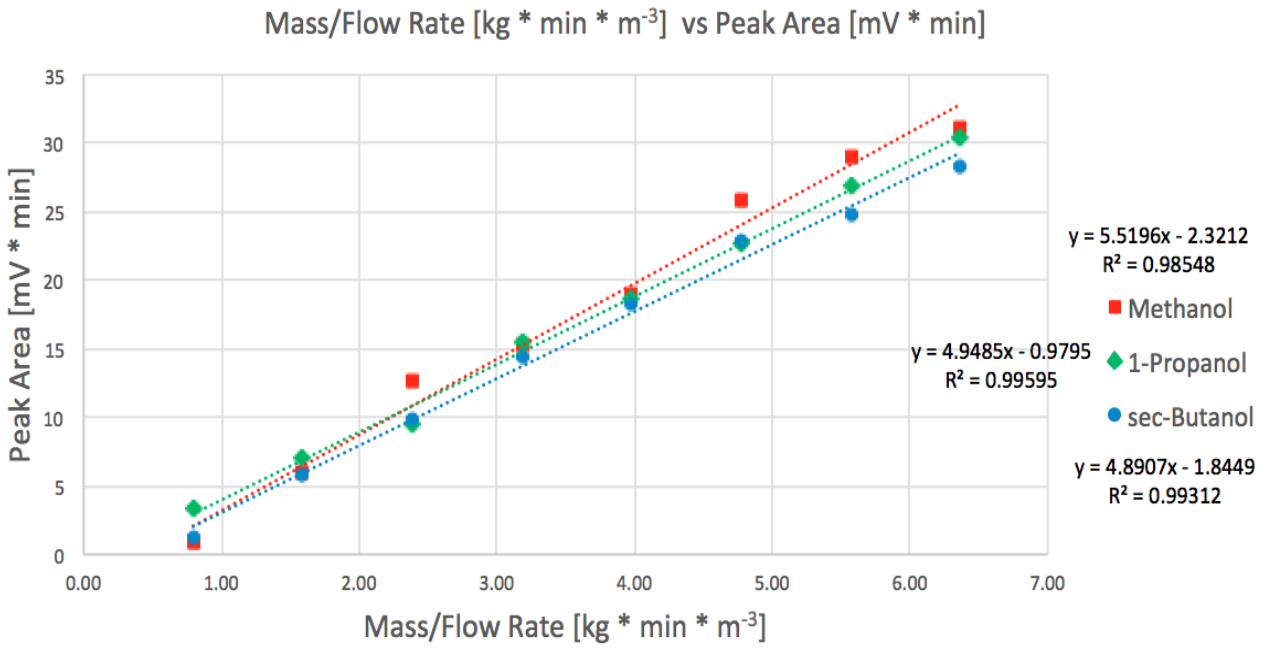
Figure 2

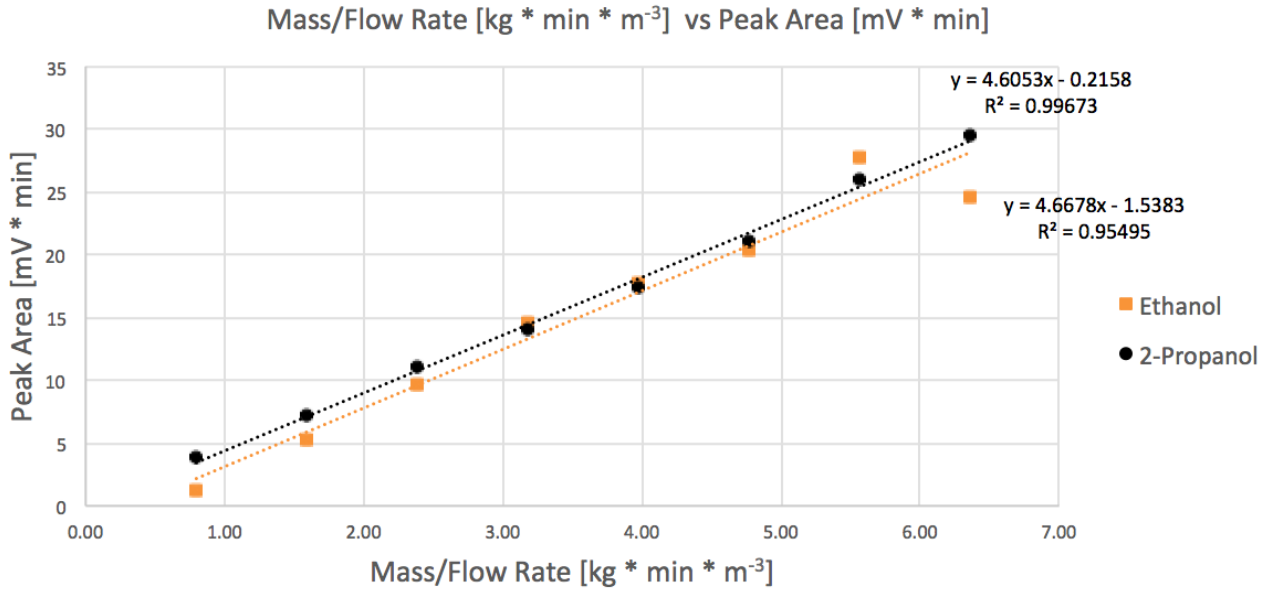
outside of the syringe before injecting, leading to a long peak for water, since water on the outside of the syringe was also injected into the GC. The second (Figure 2) was due to releasing the syringe's contents before injecting, leading to a close to zero volume injection with low, imprecise areas.

Unknown Mixture Analysis (Week 2):

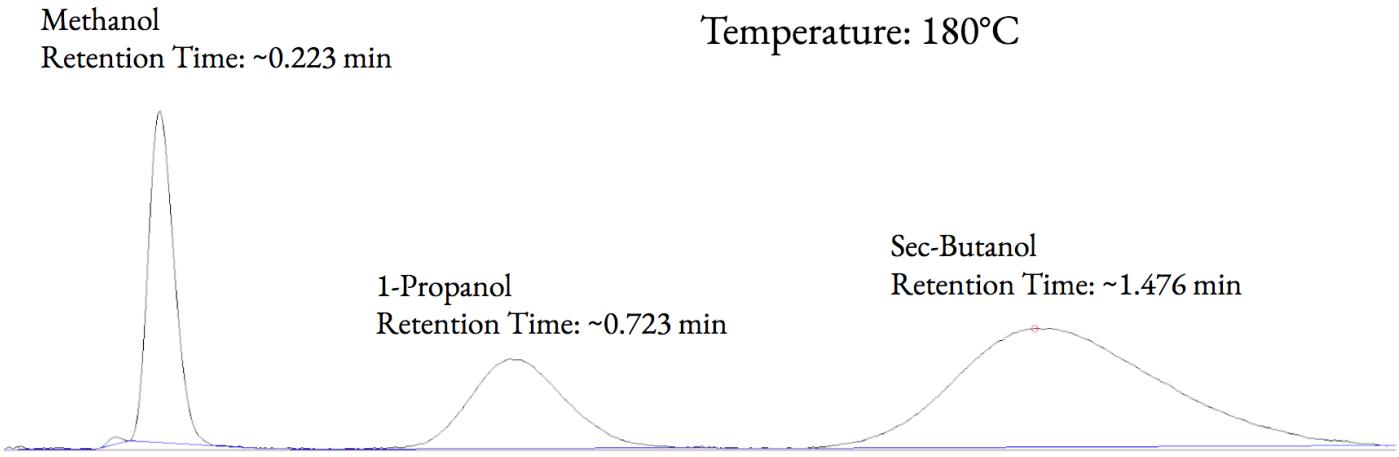
During week two, we tested five pure samples, and two mixtures composed of three of these pures at 180°C. The first tests we did were on the pures to get k values for all five of them. This was done the same way it was in week one, with area, mass injected, and flow rate measured. Then area was plotted against mass over flow rate to obtain the k values. The k values and graphs can be found below, along with the b (y intercept) values, and confidence intervals for these k and b values.

Substance	**k ($mV\ m^3/g$)**	**k Confidence Interval**	**b (mV min)**	**b Confidence Interval**
Methanol	5.52	0.65	-2.32	2.60
Ethanol	4.67	0.98	-1.54	3.93
1-Propanol	4.95	0.30	-0.98	1.22
2-Propanol	4.61	0.25	-0.22	1.02
Sec-Butanol	4.89	0.39	-1.84	1.58


Mass/Flow Rate [kg * min * m-3] vs Peak Area [mV * min]
Peak Area [mV * min]
Mass/Flow Rate [kg * min * m-3]
y = 5.5196x - 2.3212
R² = 0.98548
Methanol
y = 4.9485x - 0.9795
R² = 0.99595
1-Propanol
sec-Butanol
y = 4.8907x - 1.8449
R² = 0.99312


Mass/Flow Rate [kg * min * m-3] vs Peak Area [mV * min]
Peak Area [mV * min]
Mass/Flow Rate [kg * min * m-3]
y = 4.6053x - 0.2158
R² = 0.99673
y = 4.6678x - 1.5383
R² = 0.95495
Ethanol
2-Propanol

The pure samples then allowed us to identify the alcohols in our unknown mixtures. The main method we used to determine which alcohols were in our mixtures was to look at retention time, since the retention time of each alcohol was different, and allowed us to tell them apart. It was then determined, using the GC reading below for sample 2A, that our sample contained methanol, 1-propanol, and



sec-butanol.

Once we had identified that the alcohols making up our peaks, we were then able to use the formula we used to obtain k to find k ($A = k_i \, {}^{m_i}/_{Q}$), and solved for mass and then divided these by the total mass of each sample injected to get mass percentages. These can be seen in the table below, along with average areas and mass/flow rate for each.

2A	Average Area (mV min)	Mass/Flow Rate ($g\ min/m^3$)	Mass Percent
Methanol	6.5232	1.18	19.9
1-Propanol	6.0492	1.22	20.5
Sec-Butanol	17.3360	3.54	59.6
2B	**Average Area (mV min)**	**Mass/Flow Rate ($g\ min/m^3$)**	**Mass Percent**
Methanol	14.1477	2.56	44.3
1-Propanol	7.18137	1.45	25.1
Sec-Butanol	8.6393	1.77	30.6

Conclusion:

Our findings conclude that unknown samples can be relatively easily be analyzed and categorized through gas chromatography. This lab highlights this importance of gas chromatography as a separation technique, as well as an analysis technique, since it can both separate and analyze samples to a fairly high degree of accuracy. It is important to utilize proper procedure when utilizing GCs and to always remember good injection technique to ensure good results, and understand how gas chromatographs work, in case something goes wrong, or you need to troubleshoot. Finally, we found the masses percentages within our two unknown samples. These were 19.9% methanol, 20.5% 1-propanol, and 59.6% sec-butanol in mixture 2A, and 44.3% methanol, 25.1% 1-propanol, and 30.6% sec-butanol in mixture 2B.