

Title: Gas Chromatography

Matthew Hueston* and Bin Li, Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

Submitted 18 November 2011

Abstract: The method used for this laboratory experiment was gas chromatography or GC. In using this method, specifically the uses of a gas chromatograph, the retention times of alcohols were determined. From the data collected in this laboratory experiment, it was seen that an increase in retention times correlates to an increase in molecular weight (i.e. number of carbons) and boiling points for specific alcohols. Gasoline was also analyzed and was found to contain both ethanol and propanol. Retention times of different concentrations of ethanol were used to generate a calibration curve. This calibration curve was used to determine the concentration of ethanol in three unknown commercially available mouthwashes. In using the data collected from this experiment, the relationship between chemical structure, physical properties, and retention times can be seen and analyzed.

Introduction: Gas chromatography (GC) is an analytical method used for separating and analyzing compounds that can be vaporized into a gaseous state. This method is used to analyze compounds and samples that have low molecular weight and high volatility. For this method of chromatography, the mobile phase is a gas that carries the analytes through the column and is therefore referred to as the carrier gas. The stationary phase for this experiment is a silicon phase. In this experiment, the carrier gas is inert helium. Just like with a HPLC instrument, a GC is composed of several components. The components include a pressure and flow regulator, an injector, the column, and the detector.¹ The carrier gas must be regulated at a constant flow and pressure. The carrier gas system includes filters to remove water and other impurities. The samples were injected using a microsyringe described in the Experimental section of this report under Materials. The injector's purpose is not only to allow the introduction of the sample into the instrument, specifically the head of the column. The injector also vaporizes and mixes the sample with the carrier gas. The type of injector used in this instrument is a direct vaporization injector. The instrumentation contains an oven which controls the temperature of the column. The column used in this experiment is also stated in the Experimental section and described in the instrumentation. The type of column used for this experiment is a packed column in which the stationary phase is deposited or bonded by chemical reaction onto the porous support. As stated above, the stationary phase is composed of silicon, which reacts with the samples in the carrier gas, causing certain samples to be trapped in the column for longer amounts of time. The final component of this instrumentation is a thermal conductivity detector or TCD. This is a non-destructive detector. These detectors operate on the thermal conductivity of gas mixtures as a function of their composition. They have two identical thermistors, which resemble minuscule filaments. These thermistors are located within the path of the carrier gas. One is flushed by the carrier gas evolving the column, and the other is flushed by a part of the carrier gas entering the injector.² Once temperature equilibrium has been established between the thermal conductivity of the carrier gas and the electrical current through the filament. When the solute elutes, there is a change in the mobile phase composition, causing a change in the thermal conductivity. Thus, the equilibrium is disrupted and the variation of resistance of one of the filaments is proportional to the concentration of the compound in the carrier gas or the peak area. Using the above

described method of gas chromatography, different materials can be analyzed to determine the retention times. From the retention times, more specifically the retention times of methanol and ethanol in the 50:50 mixture, the capacity factor (k'_A) for methanol and ethanol, the response factor (R_f) for methanol and ethanol, the resolution (R_s) between methanol and ethanol, and the theoretical plates (N) using the ethanol peak can be determined. In order to determine the above values, the following equations are used:

$$k'_a = (t_r - t_m) / t_m \quad (1)$$

$$R_f = \text{Peak Area} / \text{Concentration of Sample (\%)} \quad (2)$$

$$R_s = 2 \times [(t_2 - t_1) / (w_1 + w_2)] \quad (3)$$

$$N = 16 \times (t_r / w) = 16 \times [(t_r(\text{min}) \times 60 \text{sec}) / w] \quad (4)$$

For these equations t_r is the retention time of the component, t_m is the dead time or retention time of the component minus the retention time of water ($t_r - t_w = t_m$), w is the width of the peak for the particular component, t_1 is the retention time of component 1 (methanol), t_2 is the retention time of component 2 (ethanol), w_1 is the peak width of component 1, and w_2 is the peak width of component 2. This method of analysis is currently being used to analyze many different samples throughout the world. Two types of research that are currently using GC to analyze samples are analyzing soil samples to test for residue of metsulfuron methyl³ and testing ways to increase n-3 fatty acid content in canines⁴. In the research being done to test metsulfuron methyl levels in soil samples, the researchers used a Shimadzu gas liquid chromatography instrument, model GC-17A. This instrument was equipped with a ⁶³Ni electron capture detector (ECD). The column used was an OV-1 megabore column (20cm×0.53mm i.d.). The instrument had a microprocessor control data system that allowed automatic calculation of detector response in terms of peak area. The mobile phase used was nitrogen gas at a flow rate of 1mL/min. The GC method as used to determine the concentration of metsulfuron methyl residues in soil. Metsulfuron methyl is an herbicide use to protect plants. From this study the structure of the derivitized product was found by GC-MS and the recovery of metsulfuron methyl from soil was above 70%. Metsulfuron methyl is a sulfonylurea herbicide with a low application rate registered for use in India. This compound cannot be determined in soil by GC because of its thermal instability and extremely low volatility. However, after derivatization to a dimethyl derivative using diazomethane it can be analyzed by GC-MS. For the research being done on how to improve a canine's dinner, scientists used GC to analyze erythrocyte lipids from blood samples of canines and tested those samples for n-3 fatty acids. N-3 fatty acids could be beneficial to certain medical conditions that occur in dogs like atopic dermatitis, cancer, and heart disease. In order to increase these fatty acids in dogs, scientists introduced them into the canine diets. Both of these studies used GC to analyze samples to determine concentration of a particular substance in the sample. This is similar to the purpose of our laboratory experiment, which is to test for the presence of ethanol in unknown samples of mouthwash.

Experimental:

Samples

The samples used for this laboratory experiment were prepared and given by the instructor. Table 1 is an inventory of the week 1 samples used in this report. Table 2 is an inventory of the week 2 samples used in this report.

Table 1: Week 1 Samples

Sample ID	MW (g/mol)	Amount Injected (μL)	Time (min)	Boiling Point ($^{\circ}\text{C}$)	# of Carbons
Ethanol	46.07	1.0	10	78	2
Methanol	32.05	1.0	10	65	1
50:50 (Ethanol:Methanol)	78.12	1.0	10	-	-
Propanol	60.10	1.0	25	98	3
Gasoline	-	1.0	25	-	-

Table 2: Week 2 Sample

Sample ID	MW (g/mol)	Amount Injected (μL)	Time (min)
5% Ethanol	64.09	1.0	10
30% Ethanol	64.09	1.0	10
50% Ethanol	64.09	1.0	10
80% Ethanol	64.09	1.0	10
Unknown 1 (mouthwash blue)	-	1.0	10
Unknown 2 (mouthwash clear)	-	1.0	10
Unknown 3 (mouthwash clear)	-	1.0	10

Materials

Ethanol (CAS #64-17-5), Methanol (CAS #67-65-1), n-propanol (CAS # 71-23-8), n-butanol (CAS# 71-36-3), n-pentanol (CAS # 71-41-0), and distilled deionized water (CAS #7789-20-0). All reagents were purchased from Sigma Aldrich in St. Louis, Missouri. The 250mL bottle was obtained from ThermoFisher Scientific in Bridgewater, New Jersey. The syringe used to inject the samples was a Unimetrics (10 μL) syringe, purchased from Unimetrics Corporation in Sherwood, Illinois. Note: n-butanol was not used in this laboratory report due to its long retention time.

Instrumentation

The instrumentation used for this experiment is a Buck Scientific 910 Gas Chromatograph with TCD (thermal conductivity detector) detector. The TCD detector can reach a temperature of about 275 $^{\circ}\text{C}$. The instrument was purchased from Buck Scientific. Buck Scientific headquarters are located on 58 Fort Point St., East Norwalk, Connecticut, 06855. The software that controls the instrument is PeakSimple 3.88. The column for this experiment is a 6' x 1/8" HayeSep D column. The column's ID, as found in the oven of the instrument, is stainless steel, ss1583-888 C7959-1 125386 RESTEK 1/16/02. The instrument used compressed helium, which was stored in a cylinder made by and provided by Airgas, in Radnor, Pennsylvania.

Analytical Procedure⁵

For this laboratory experiment, the GC was set to 130 $^{\circ}\text{C}$ and a He gas flow at 18PSI (~30mL). This was to ensure that once the samples were injected they would vaporize into the gaseous state. The samples were all injected with the Unimetrics syringe using roughly 1 μL of sample. The syringe was rinsed with water between the injections of each sample, except the sample of gasoline, which was rinsed with acetone, due to water's polarity. A blank of water was taken first. This was to ensure that the water present in the sample could be identified. Inert helium was used as the carrier gas to carry the vaporized sample through the column and the stationary silicon phases. After each sample was analyzed using the instrumentation stated

above, print screens of each chromatogram were taken and copied into Microsoft Excel. The retention times, peak areas, and other data were copied and recorded in Microsoft Excel. n-Butanol was not analyzed in this experiment due to its lengthy retention time.

Results and Discussion: This laboratory experiment was divided into two weeks. For all chromatograms and data, an injection peak appeared. This injection peaks are void volumes or the air peak, which means that there was air in the column being pushed out. In the first week, water, methanol, ethanol, a 50:50 mixture of ethanol and methanol, propanol, and a gasoline sample were analyzed by GC. The retention time, peak area, height, full width, and half height are summarized in Table 3. All values are average taken from the two injections of each sample. The peak of water eluted first at an average retention time of roughly 1.3245min. Water being the first peak is surprising since the elution order of samples for GC is primarily based on boiling point. Therefore, based on the boiling points of water and the alcohols, water should have eluted last not first. However, once the water eluted, the other samples eluted in the right order. Methanol (B.P. 65°C) eluted second, producing an average retention time of 2.458min. Next, ethanol (B.P. 78°C) eluted and finally propanol (B.P. 98°C) eluted. The order of elution is seen in Table 3, and includes all the average data between the two injections. This order of elution is correct based on the fact that samples should elute based on boiling points. Table 4 summarizes the retention times, boiling points, and number of carbons of the three alcohol samples analyzed in this experiment. Based on these values, two plots were generated. The first plot, seen in Figure 1, is a plot of retention time versus boiling point. From this plot, it is seen that there is a linear relationship between these retention time and boiling point, which was previously stated. The second plot, seen in Figure 2, is a plot of retention time versus the number of carbons. This plot also shows a linear relationship between retention time and number of carbons. This shows that retention time is also based on molecular weight. That the higher the molecular weight of a sample, the longer it takes to elute out of the column. This is primarily due to the fact that there are more interactions generated between the sample and the stationary phase as the molecular weight increases. It is also due to that the greater the number of carbons, the longer it takes for the sample to breakdown.

Table 3: Order of Elution

Order of Elution						
Peak #	Component	Avg. r_t (min)	Avg. Peak Area	Avg. Height	Avg. Width	Avg. height _{1/2}
1	Water	1.3245	876.75775	33.023	259.5	16.5115
2	Methanol	2.458	653.0355	31.884	131	15.942
3	Ethanol	5.908	928.1465	18.9365	188	9.46825
4	Propanol	18.433	537.69575	3.973	356.5	1.9865

Table 4: Table of Alcohol Retention Times, Boiling Point, and Number of Carbons

Alcohol	Retention (min)	Boiling Point	# Carbons
Methanol	2.47875	65	1
Ethanol	6.405166667	78	2
Propanol	18.733	98	3

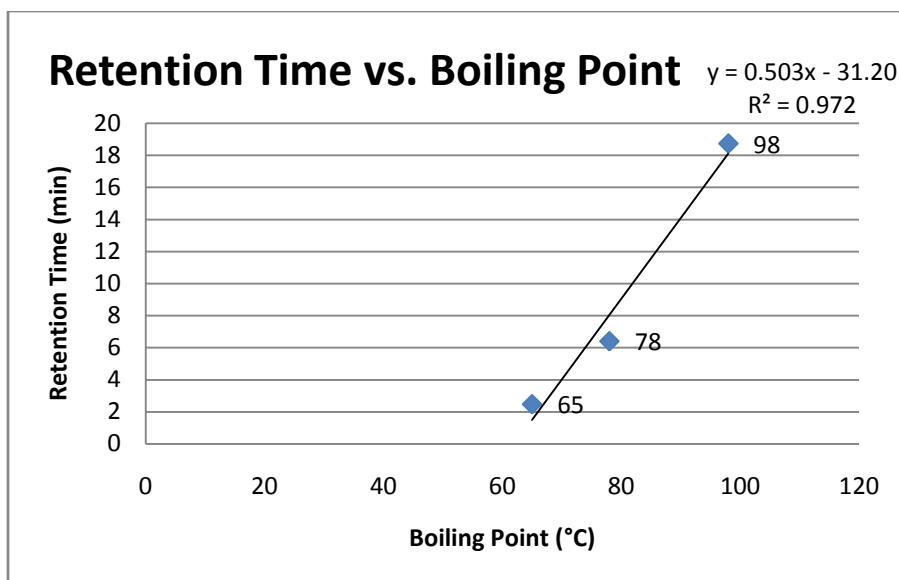


Figure 1- Retention Time vs. Boiling Point of Different Alcohols

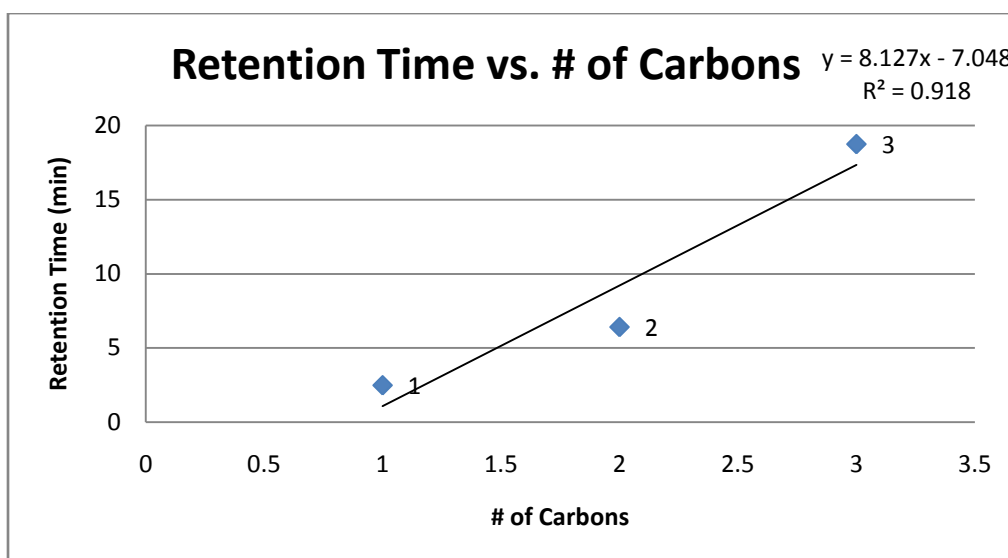


Figure 2- Retention Time vs. # of Carbons of different Alcohols

Also in week one of this experiment, a gasoline sample was analyzed. The data recorded is seen in Table 5. From this data and the chromatogram seen in Figure 3, some of the components of gasoline can be determined. From this the components of gasoline that can be definitely determined are ethanol, propanol, and a gas component that is unknown. There was no presence of methanol in the sample. However, for injection 1, there were two unknown peaks at the retention time of 3.1 and 13.466. These two peaks were either impurities caused by my previous samples or improper cleansing of the syringe or other components of gasoline that are unknown, since the only samples analyzed before the gasoline sample were water, methanol, ethanol, and propanol. However, the gasoline component of the sample is most likely butanol, due to its high retention time.

Table 5: Gasoline Sample

Gasoline				
Component	Retention	Area	Height	Width
Injection (Injection 1)	0	0	0	0
Water (Injection 1)	1.333	13.2005	1.008	83
Methanol (Injection 1)	0	0	0	0
-	3.1	0.59	0.021	56
Ethanol (Injection 1)	7.166	25.419	0.882	104
-	13.466	0.335	0.017	41
Propanol (Injection 1)	19.183	0.337	0.009	48
Gas (Injection 1)	22.733	2.817	0.057	104
Injection (Injection 2)	0	0	0	
Water (Injection 2)	1.333	12.533	0.927	76
Methanol (Injection 2)	0	0	0	0
Ethanol (Injection 2)	7.1	18.831	0.674	120
Propanol (Injection 2)	18.883	1.886	0.053	66
Gas (Injection 2)	22.166	12.88	0.146	198

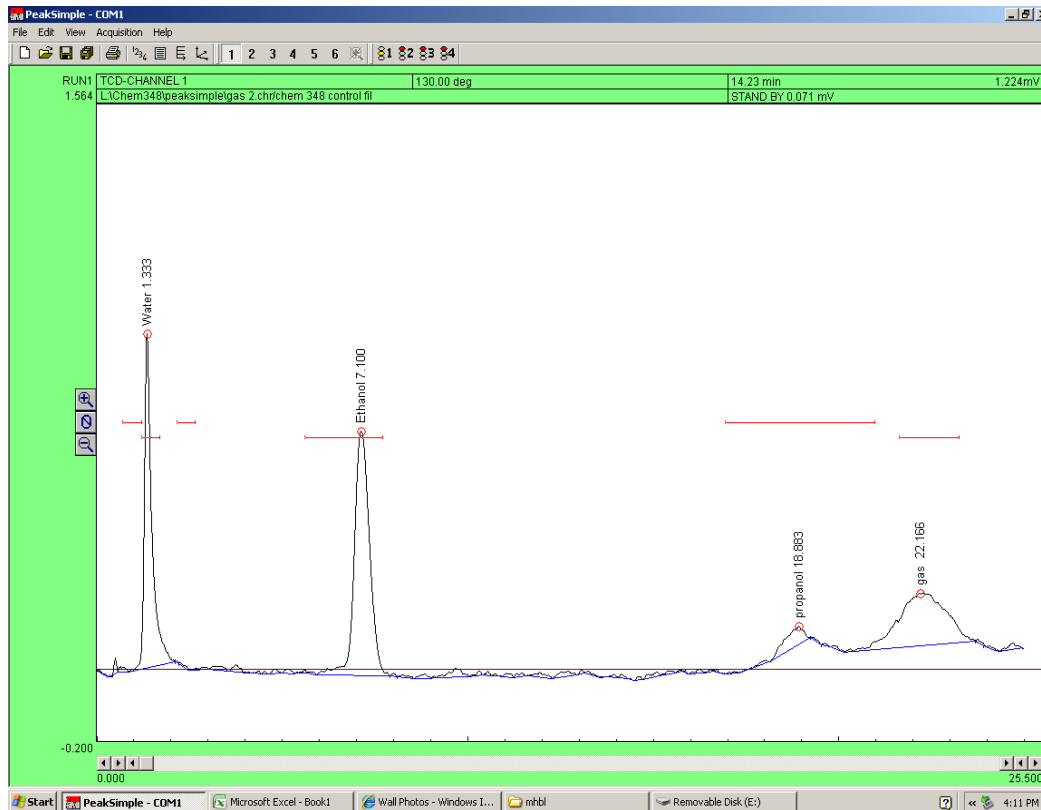


Figure 3- Gasoline (Injection 2)

As well as samples of water, methanol, ethanol, propanol, and gasoline, a sample of a 50:50 mixture of ethanol and methanol was analyzed. From this sample, the capacity factor of both methanol and ethanol, the response factor of both methanol and ethanol, and the resolution

between methanol and ethanol could be determined. The data is summarized below in Table 6. Also, in using the ethanol peak, the number of theoretical plates (N) could be determined. The number of theoretical plates was determined to be 30.169. This was determined using equation (4) seen in the introduction. The capacity factor for both methanol and ethanol was determined using equation (1) seen in the introduction, the response factor for methanol and ethanol in the 50:50 mixture was determined using equation (2). The Resolution between methanol and ethanol was determined using equation (3). The chromatograph produced from the 50:50 mixture is seen in Figure 4, and from it you can see that peaks for both methanol and ethanol are roughly the same in height and area, showing a 50:50 mixture.

Table 6: 50:50 Mixture of Ethanol and Methanol including k'_A , R_f , and R_s

Component	r_{t1} (min)	r_{t2} (min)	Avg. r_t	Avg. Peak Area	Avg. Width	Capacity Factor	Theoretical Plates	R_f	R_s
Methanol	2.483	2.516	2.500	488.505	123.500	0.887	-	9.770	0.023
Ethanol	6.116	6.233	6.175	602.884	199.000	3.647	30.169	12.058	0.023

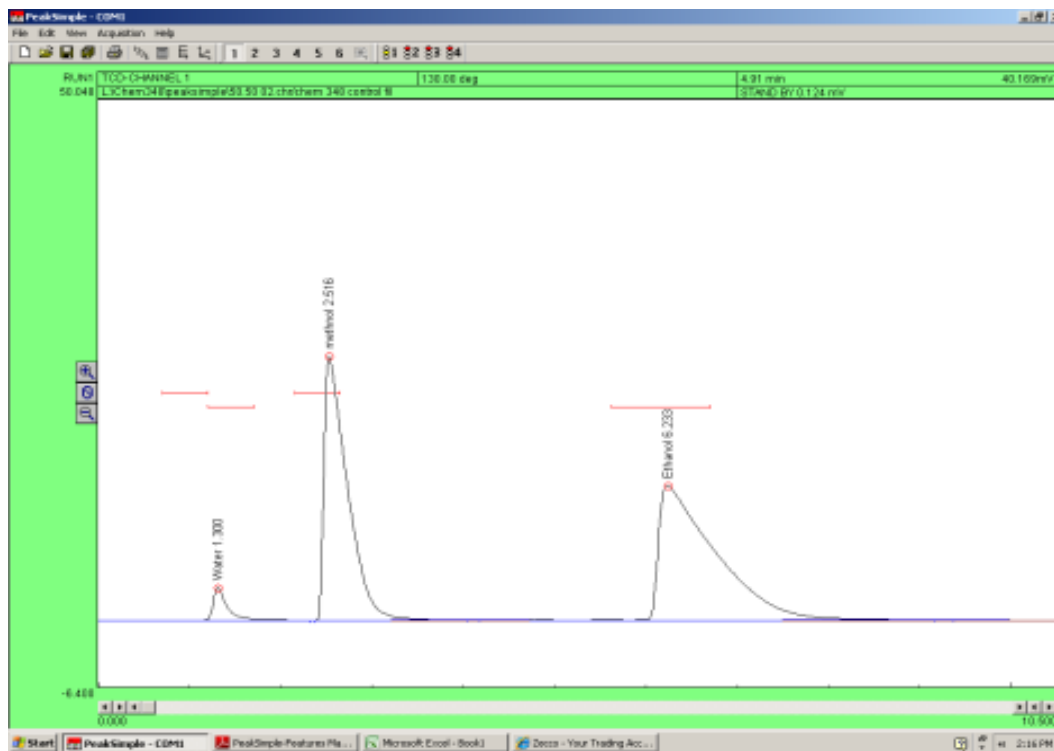


Figure 4- 50:50 ethanol/methanol mixture (injection 2)

For week 2, different concentrations of ethanol were analyzed. In Figures 5, 6, 7, 8, and 9 show the chromatograms for the ethanol concentrations of 5%, 30%, 50%, 80%, and 100% (week 1 ethanol sample), respectively.

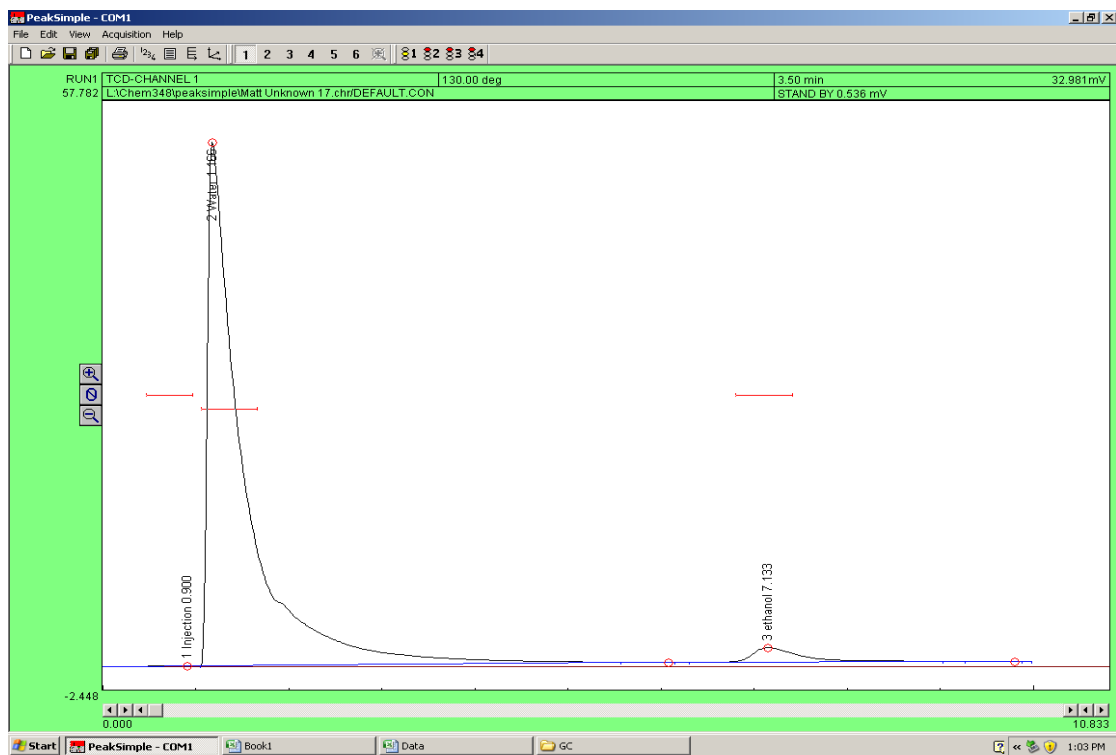


Figure 5- 5% Ethanol (Injection 1)

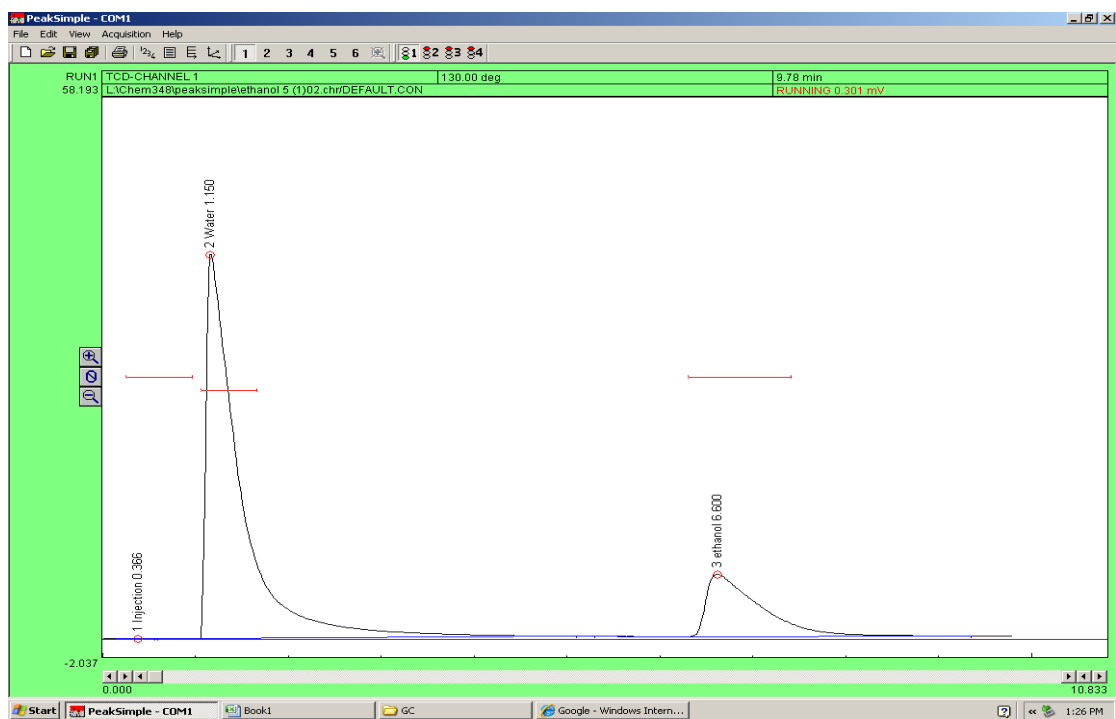


Figure 6- 30% Ethanol (Injection 1)

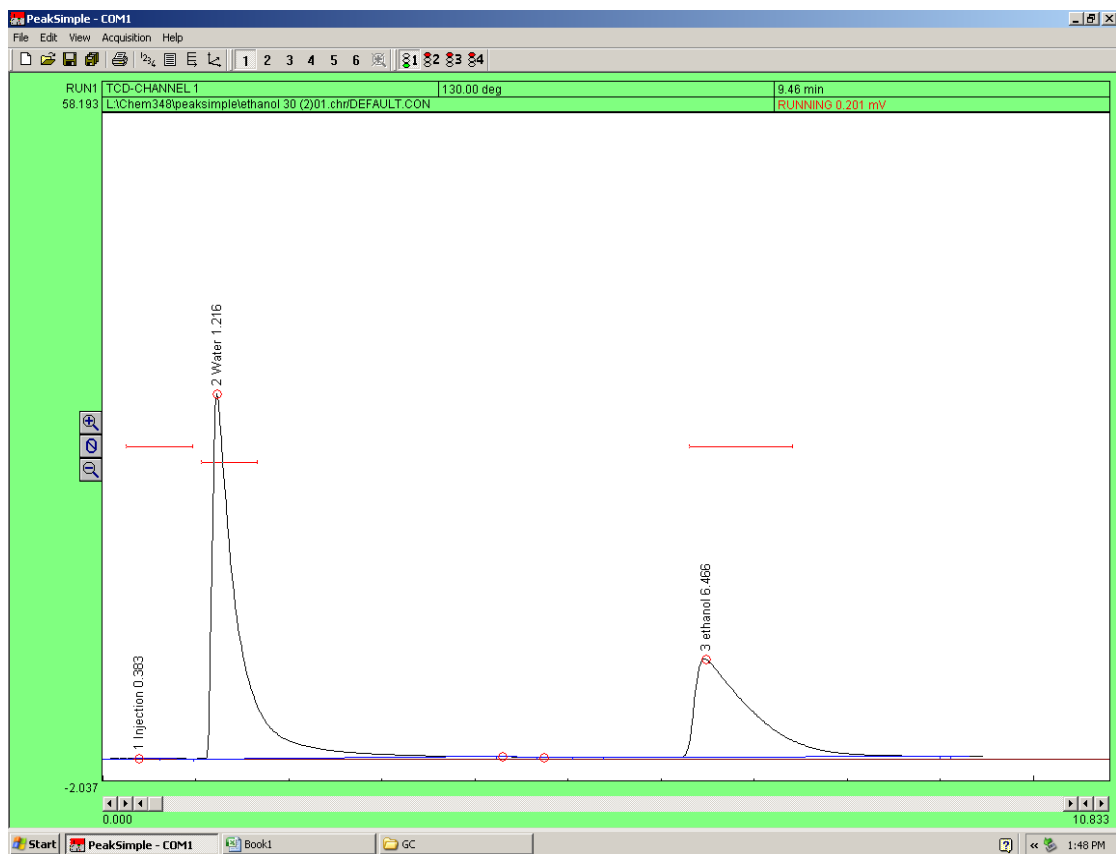


Figure 7- 50% Ethanol (Injection 1)

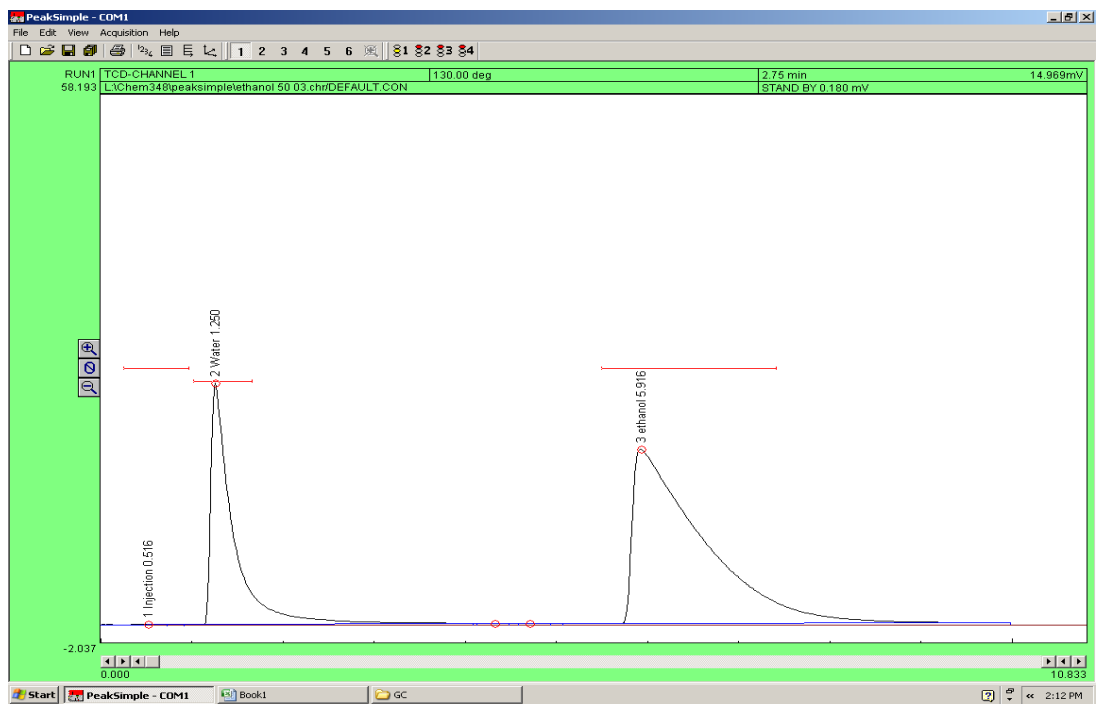


Figure 8- 80% Ethanol (Injection 1)

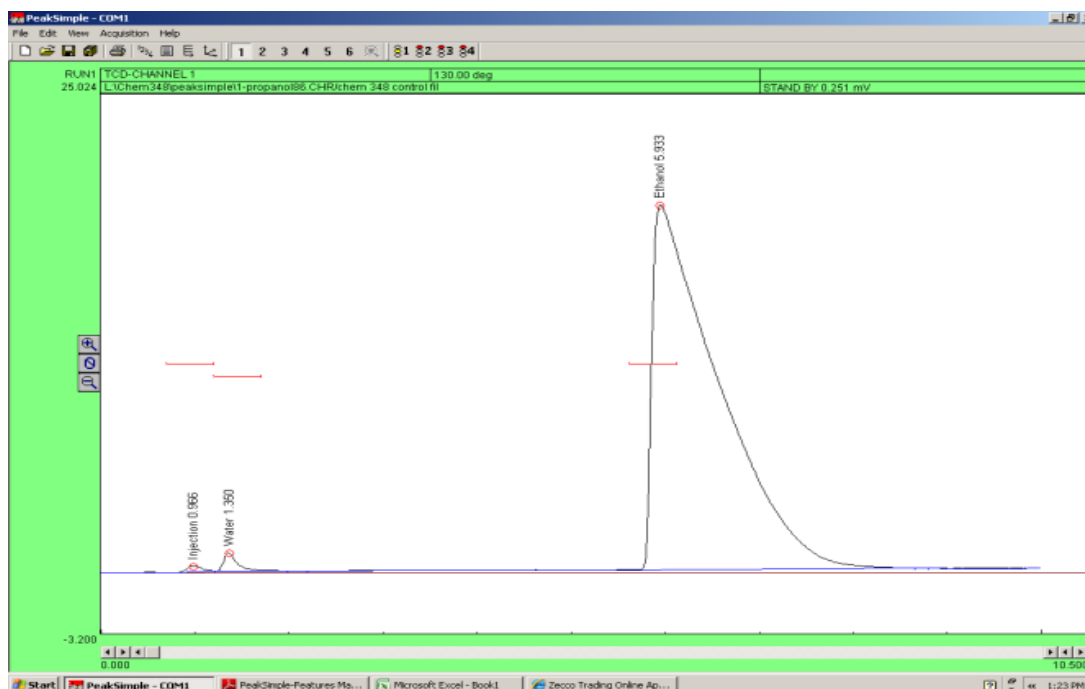


Figure 9- 100% Ethanol (Injection 1) Ethanol Sample from Week 1

As can be seen from the chromatograms above, as the concentration increases, the height, width, and peak area increase. This occurs because as the concentration increases, there is a greater amount of ethanol relative to water. Therefore as the concentration increases, the peaks of ethanol increases while the peak of water linearly decreases. In week 2, different concentrations of ethanol were measured and the retention times and peak areas were determined. This was done in order to create a calibration curve of ethanol by plotting peak areas (y-axis) versus concentrations (x-axis). Table 7 summarizes the data used to generate the calibration curves. The average peak areas of injection one and two were used, as well as the peak areas of undiluted ethanol from week one. The calibration curve of ethanol can be seen in Figure 10.

Table 7: Data Used to Generate Calibration Curves

Peak Area	Conc. %
45.0175	5
262.78125	30
392.47575	50
901.794	80
928.1465	100

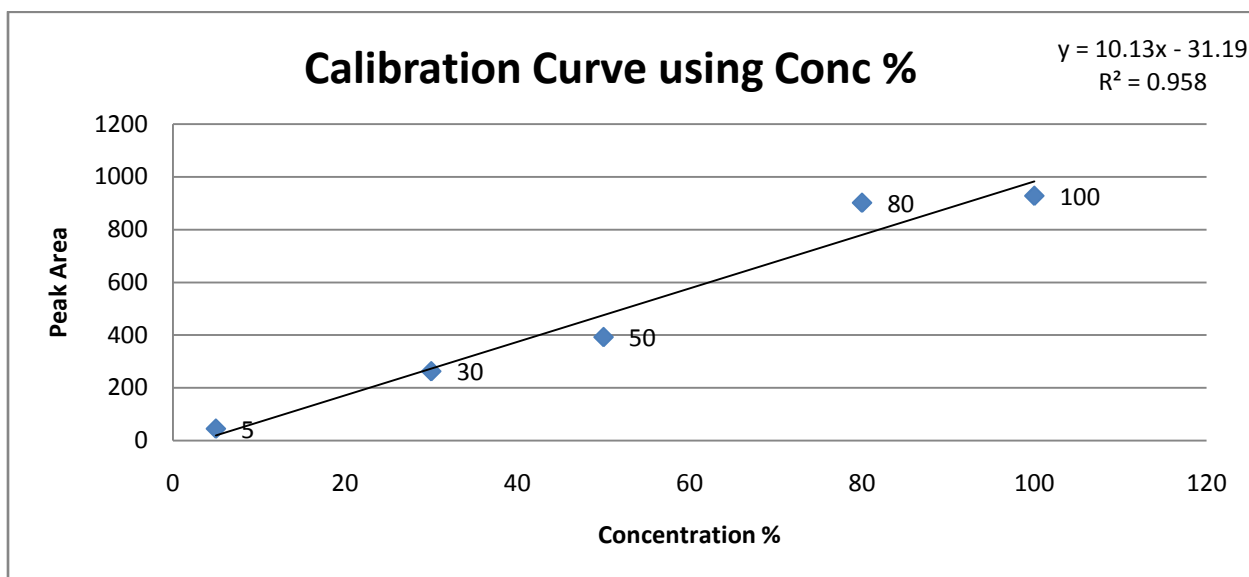


Figure 10- Calibration Curve of Ethanol

From the calibration curve above in Figure 10, the equation of the trendline generated is $y=10.13x-31.19$, with a R^2 value of 0.958. y represents the peak area and x represents the concentration. From the calibration curve generated above, the % volume of ethanol in the unknown mouthwash samples could be determined and compared to the values in commercially available mouthwashes. This was done by using the equation of the trendline rearranged ($(y+31.19)/10.13=x$), this was done because the value we are looking for is the % volume or concentration which is represented along the x axis. So to find the % volume we need to solve for x not y . The linearity of the detector from the ethanol calibration curve is the concentration range over the detector output. The linearity is represented by the R^2 value, which is 0.958, which shows that the results are almost perfectly linear. The rearranged equations generated from the calibration curve are seen below in equations (5), (6), and (7).

$$\text{Unknown 1 Ethanol Conc.} = (0.470+31.19)/10.13 = 3.125\% \quad (5)$$

$$\text{Unknown 2 Ethanol Conc.} = (54.130+31.19)/10.13 = 8.423\% \quad (6)$$

$$\text{Unknown 3 Ethanol Conc.} = (548.467+31.19)/10.13 = 57.222\% \quad (7)$$

A table summarizing the above data for Volume % as well as the peak areas and average peak areas of unknown ethanol peak are seen below in Table 8. The chromatograms for Unknowns 1, 2, and 3 can be seen in Figures 11, 12, and 13, respectively.

Table 8: Peak Area, Avg. Peak Area, and Volume % of Unknown Samples

Sample ID	Peak Area 1	Peak Area 2	Avg. Peak Area	Volume %
Unknown 1	n/a	0.470	0.470	3.125
Unknown 2	59.863	48.398	54.130	8.423
Unknown 3	513.415	583.519	548.467	57.222

From the Volume % values, the unknowns can be compared to known values of ethanol in commercially available mouthwashes to determine the unknowns. Unknown 1 is mostly likely Listermint, which has an alcohol content of 6.6%, compared to the volume % determined of 3.125%. Unknown 2 is most likely Act, which has an alcohol content of 10%, compared to the

volume % determined of 8.423%. Unknown 3 is most likely Listerine, which has an alcohol content of 26.9%, compared to the volume % determined of 57.222%. This value is very high, but the probability that is Listerine is high do to the fact that Listerine has the highest alcohol content available. There were most likely some impurities or other factors that caused the high % volume value. However, since most consumer mouthwashes have various active ingredients besides water and ethanol, the concentrations could not be as accurate as stated.

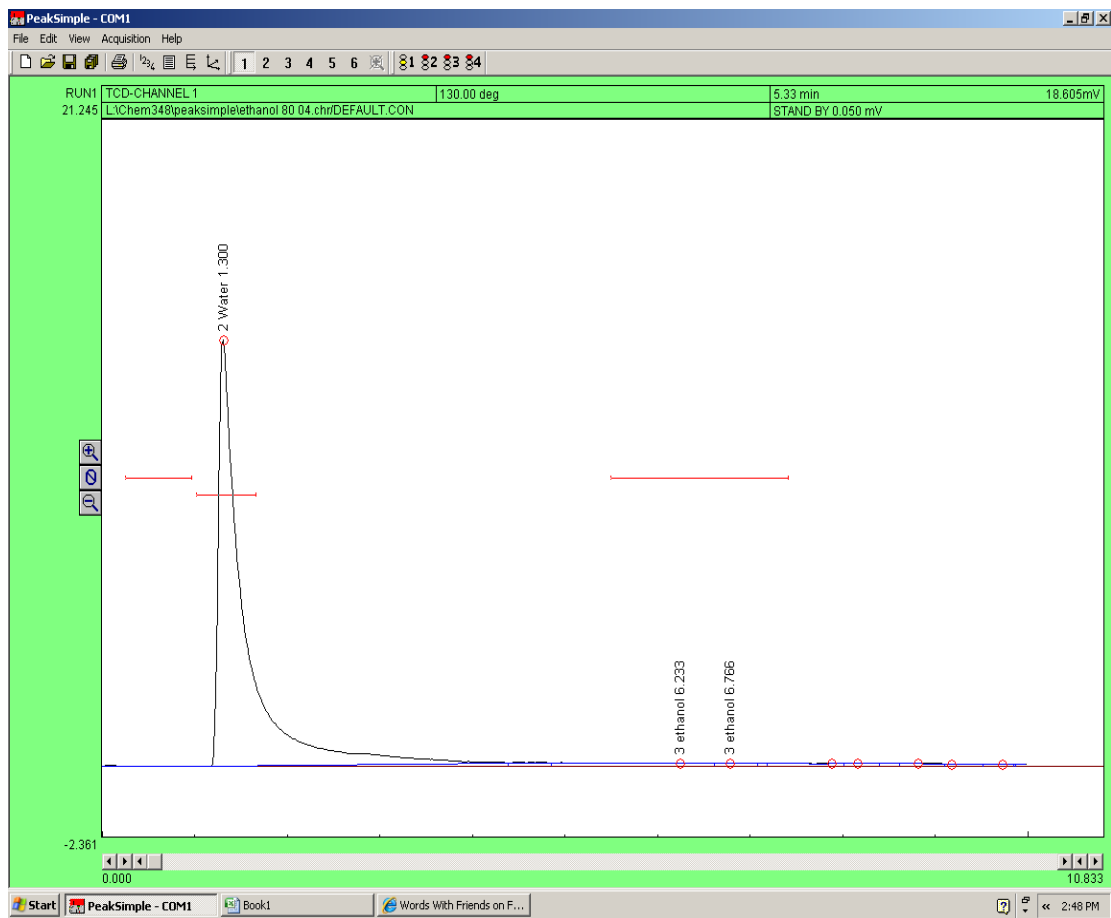


Figure 11- Unknown 1 (Injection 2)

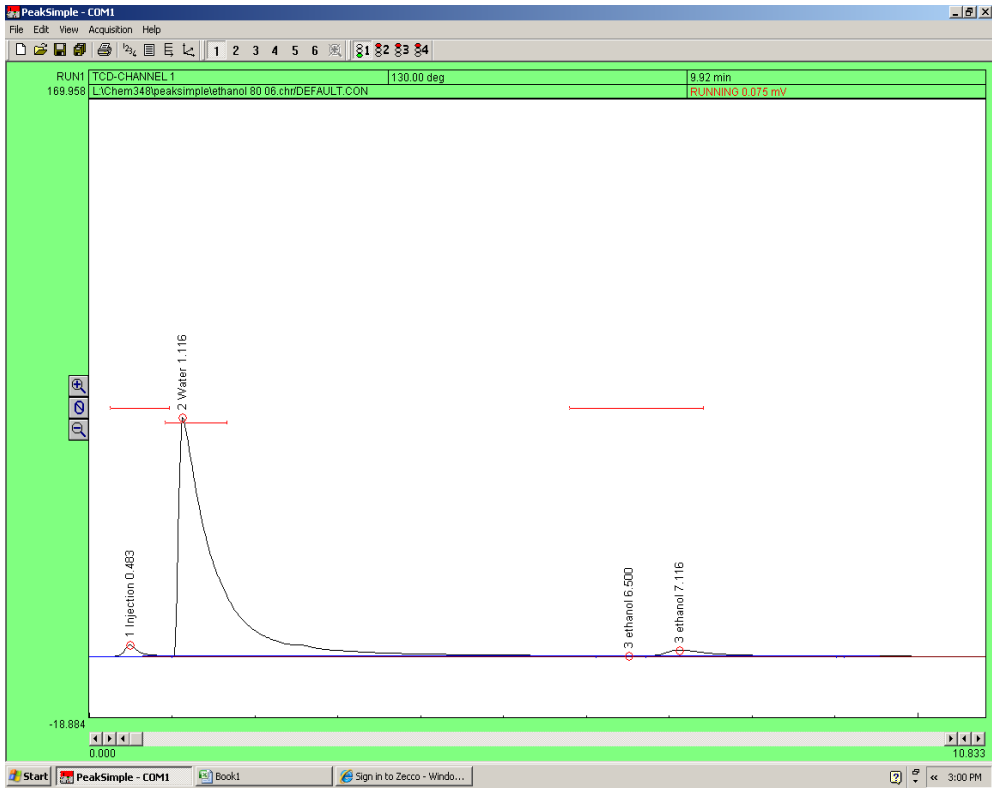


Figure 12- Unknown 2 (Injection 1)

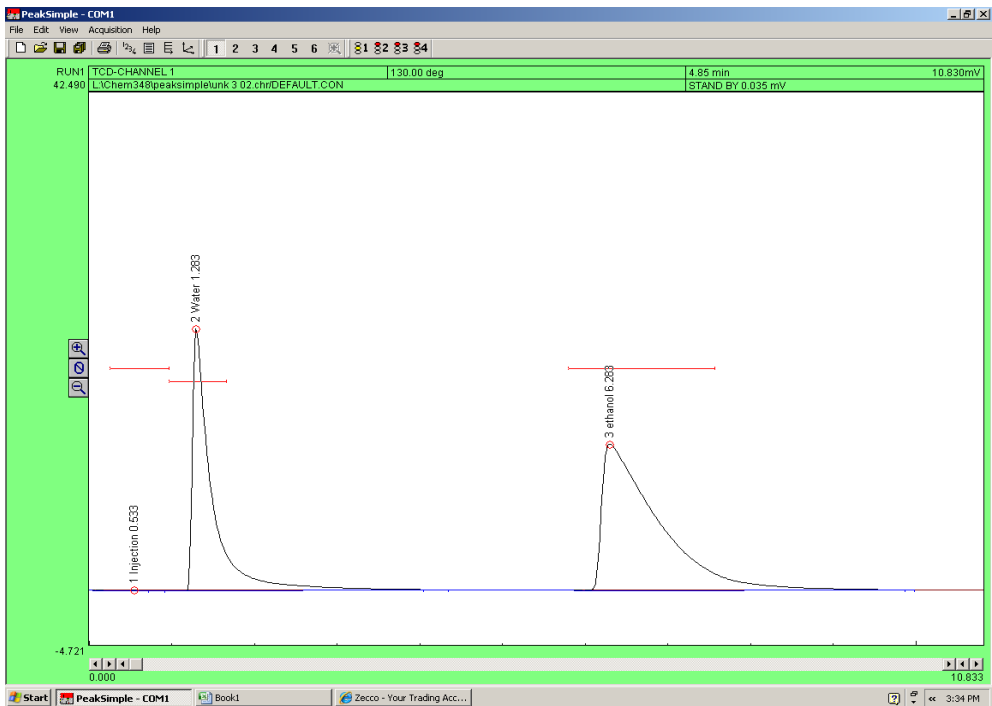


Figure 13- Unknown 3 (Injection 2)

Conclusion: For this laboratory experiment, the average retention times, the capacity factor, the resolution between methanol and ethanol, the number of theoretical plates, and the response factors for methanol and ethanol were determined. The Unknowns % Volume of ethanol was also determined. The average retention times for water, methanol, ethanol, and propanol were found to be 1.3245min, 2.458min, 5.908min, and 18.433min respectively. The gasoline sample was found to have components of ethanol, propanol, and a few unknown components not analyzed by them to determine the correct retention time. In using the 50:50 mixture of ethanol and methanol, the capacity factor of methanol and ethanol was found to be 3.647 and 0.887, respectively. The resolution between methanol and ethanol was found to be 0.023. The number of theoretical plates using the ethanol peak was found to be 30.169. The response factor for methanol and ethanol was found to be 9.770 and 12.058, respectively. The % Volumes of Unknowns 1, 2, and 3 were found to be 3.125%, 8.423%, and 57.222%, respectively using the calibration curve generated from the different dilutions of ethanol. Overall, this laboratory experiment produced relatively well results and all values were able to be determined.

References:

¹ Rouessac, Francis; Rouessac, Annick. (2007). *Chemical Analysis: Modern Instrumentation Methods and Techniques* (2nd Edition). West Sussex, England: John Wiley and Sons, Ltd. pg 31.

² Rouessac, Francis; Rouessac, Annick. (2007). *Chemical Analysis: Modern Instrumentation Methods and Techniques* (2nd Edition). West Sussex, England: John Wiley and Sons, Ltd. pg 47.

³ Singh, S. (n.d.). Gas Chromatographic Method for Residue Analysis of Metsulfuron Methyl from Soil. *Bulletin of Environmental Contamination and Toxicology*, 86(2), 149-51.

⁴ Chromatography Today. *GC Used to Improve Dogs' Dinner*. 28 October 2011. Web.

⁵ Course document for Chemistry 348, Instrumental Analysis, "Gas Chromatography", Professor Gene Hall, Fall 2011 semester, available on Sakai → Resources → Laboratory Experiments → Chem348 GC Experiment LAP.pdf, pgs 1-7.