Analysis of Blood Serum for Ethanol Using Flame Ionization Detector Gas Chromatography

September 13th, 2010

I. Introduction:

Gas chromatography requires both a stationary and mobile phase. The stationary phase consists of a packed column in which the packing support is coated with a liquid stationary phase. The mobile phase, referred to as the carrier gas, is comprised of an inert gas such as helium. When a liquid sample is injected into the injector port, it is volatilized and proceeds through the column. The components of a mixture are separated based on their interaction with the stationary phase. The stronger the interaction between the compound and the stationary phase, the more time it takes to elute from the column. As the components reach the end of the column, they pass through a detector which records the retention time and can be used to produce a graph of peak areas.

One type of detector that is commonly used in gas chromatography is the thermal conductivity detector. In this detector, the sensor consists of a filament, made of platinum or tungsten wire that is situated in the eluent gas. The filament is heated by an electric current and exists in thermal equilibrium at a given temperature when it comes in contact with the carrier gas. When a solute is eluted from the column the thermal conductivity of the gas surrounding the heated filament changes. This change results in a change in wire temperature and the system becomes unbalanced. A signal is then sent to the detector which records the change in thermal conductivity. The electron-capture detector is also commonly used in gas chromatography. In this detector, electrons are generated by plasma discharge. A constant potential is applied across the electrodes in order to collect all electrons produced. When a molecule enters the detector it can capture electrons, causing the molecule to become charged. The captured electrons do not have as much mobility as the free electrons, which causes the detector current to fall. Another commonly used detector is the flame ionization detector. Carrier gas from the column enters at the bottom of the detector. The gas is then mixed with hydrogen combustion gas in the area below the flame. This mixture is combined with air and burned just above the jet tip. A negative voltage is applied between the jet tip and a collector electrode. As electrons are formed, they move across the gap and are sent to an electrometer. The flame ionization detector is the most sensitive detector to hydrocarbons. Blood serum is comprised mostly of water however, the flame ionization detector is incapable of detecting water. In this experiment, the only other component of the blood serum will be ethanol. The flame ionization detector will be able to detect any ethanol present with great accuracy.

This experiment will employ the use of an internal standard. An internal standard is a known amount of a certain compound that is different from the analyte, which is added to the unknown. When the sample is passed through the gas chromatograph, the signal from the analyte is compared with the signal from the internal standard. The relative response of the detector to the standard and analyte is constant over a wide range of conditions. If the signal from the internal standard increases, then the signal from the analyte will increase as well. If the concentration of the internal standard is known then the concentration of the unknown can be determined using a ratio. Using an internal standard can also account for the variability in injection volume as well as sample loss.

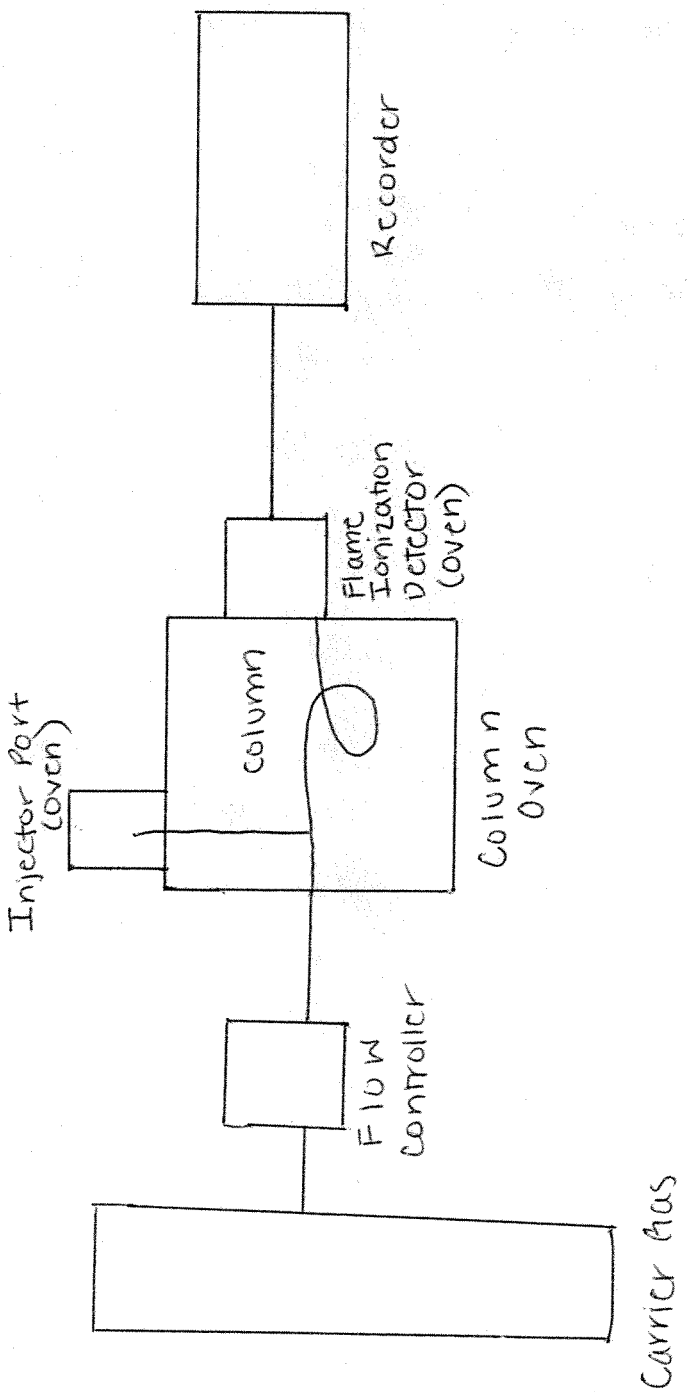
The concentrations of the samples will range from the ethanol present in a sober individual's system through that of an impaired individual. The concentration of an impaired driver is 0.08% w/v. The ethanol standards will cover concentrations both below and above the impaired limit. These concentrations will range from 0.04% to 0.20% ethanol. The same amount

of internal standard will be added to each standard. The concentration of internal standard will be 0.08% w/v.

The purpose of this lab is to determine the concentration of ethanol in a synthetic blood serum using a gas chromatograph with flame ionization detection. Five aqueous standard solutions of ethanol and internal standard will be prepared. The internal standard used will be 1-propanol. The ethanol solutions will cover the expected range for both sober and impaired drivers. The internal standard concentration will be equal to the average standard ethanol concentration and will be constant in each ethanol standard. These standards will be used to determine the ethanol concentration in two 10 mL blood serum samples as well as two standard reference material solutions.

The gas chromatograph will be used in conjunction with the Flame Ionization Detector to perform this experiment.

Figure 1. Block diagram of a GC-FID



II. Procedure:

This experiment was carried out using the recorded procedure in the lab notebook. ✓

Table 1. Instrument Parameters

Instrument Parameters	
Instrument Type	Hewlett-Packard 5890 Gas Chromatograph
Detector	Flame Ionization Detector
Oven Temp	50°C
Injector Temp	120°C
Detector Temp	120°C
Flow Rate	14 mL/min
Injection Amount	1 µL

✓ +3

III. Calculations:

A stock solution of ethanol and 1-propanol were prepared using a concentration of 0.1 w/v%.

ethanol = 0.1 w/v%

$= \frac{1\,g}{100\,mL}$ → 1%

1-propanol = 0.1 w/v%

$= \frac{1\,g}{100\,mL}$

A set of six standard ethanol solutions were to be prepared using concentrations of 0.00, 0.04, 0.08, 0.12, 0.16, 0.20w/v% of ethanol and 0.08w/v% of 1-propanol and diluted to 25 mL.

0.04%

$C_1V_1 = C_2V_2$

$\frac{1000\,mg}{100\,mL}(V_1) = \frac{40\,mg}{100\,mL}(25\,mL) = 1\,mL$ of ethanol

$\frac{1000\,mg}{100\,mL}(V_1) = \frac{80\,mg}{100\,mL}(25\,mL) = 2\,mL$ of 1-propanol ✓

Standard solutions of the two unknowns and SRMs were prepared using 0.08w/v% and diluted to 10 mL.

1-propanol

$C_1V_1 = C_2V_2$

$\frac{1000\,mg}{100\,mL}(V_1) = \frac{80\,mg}{100\,mL}(10\,mL) = 0.8\,mL$ 1-propanol ✓

The ratio of response of analyte (ethanol) to internal standard (1-propanol) was calculated for the standards to be used to plot the internal standard calibration curve. The ratio of the response of unknowns and SRMs was calculated as well using the same method.

0.04 w/v% standard
Average area (Ethanol): 23546
Average area (1-Propanol): 60611

$$\frac{R_A}{R_{IS}} = \frac{23546}{60611} = 0.3885$$ ✓

The linear regression line of the internal standard calibration plot was determined using a LINEST table and found to be:

$$y = mx + b$$

$$R_A/R_{IS} = (10.28 * \text{concentration}) + -0.015$$ ✓

The concentrations of the unknowns and SRMs were calculated using the linear regression line and the response ratios.

Unknown A $R_A/R_{IS} = 0.1682$

$$x = (y - b)/m$$

$$\text{concentration} = [(0.1682) - -0.015] / 10.28$$

$$\text{concentration} = 0.16 \text{ w/v\%}$$ ✓

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IV. Data and Results:

A set of six ethanol standards was prepared using varying amounts of ethanol with a constant amount of 1-propanol. These solutions were diluted to 25 mL using distilled water. The two unknown samples, A and B, and the standard reference materials were prepared by added 0.800 mL of 1-Propanol to 10 mL of solution. ✓

Table 2. Preparation of Standard Ethanol Solutions. 1% w/v ✓

Concentration (w%/v)	Total Volume (mL)	Amount of Ethanol Added (mL)	Amount of 1-Propanol Added (mL)
0.00	25	0.00	2.00
0.04	25	1.00	2.00
0.08	25	2.00	2.00
0.12	25	3.00	2.00
0.16	25	4.00	2.00
0.20	25	5.00	2.00
Unknown A	10	0.00	0.800
Unknown B	10	0.00	0.800
Standard 0.205	10	0.00	0.800
Standard 0.077	10	0.00	0.800

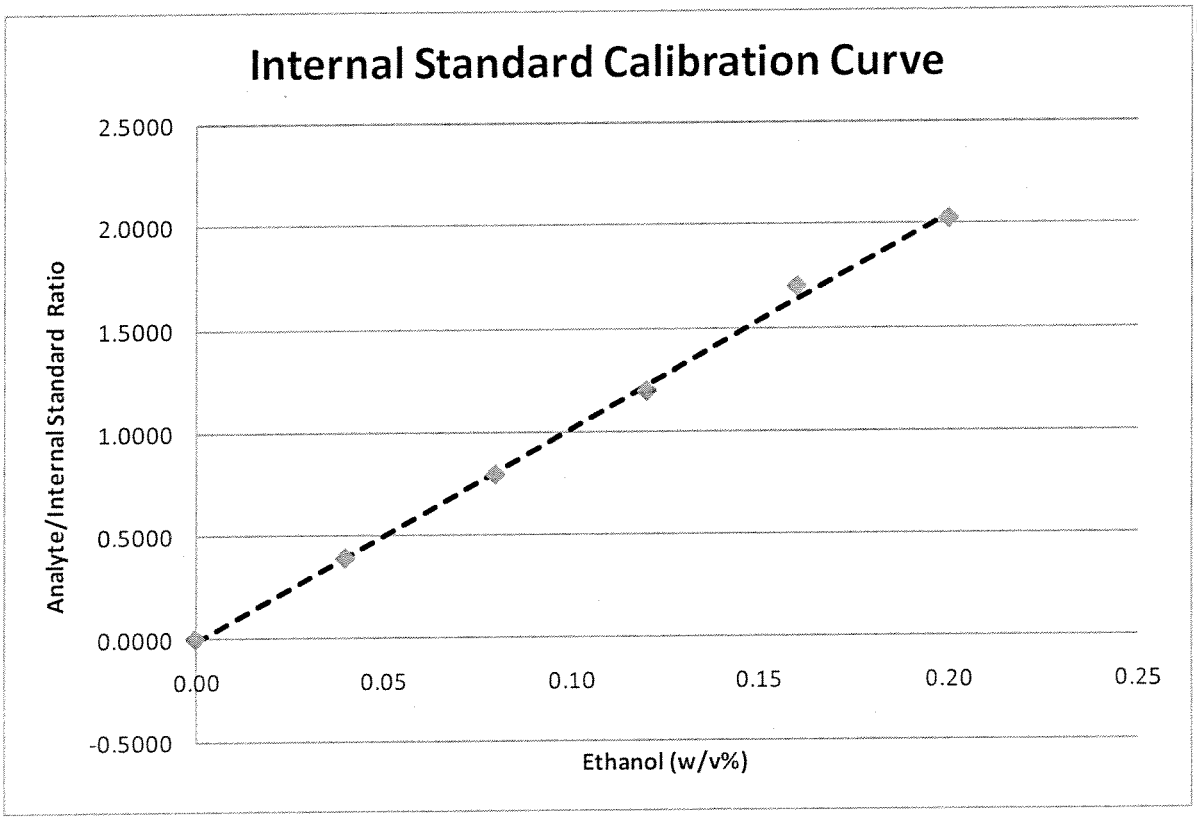
Three samples of each of the standard ethanol solutions were injected into the GC-FID. The areas for the two peaks of ethanol and 1-proponal were averaged for the three runs. The ratio of analyte response to internal standard response was calculated.

Table 3. Average Peak Areas and Response Ratio of Standards.

Concentration (w%/v)	Average Area for Ethanol	Average Area for 1-Propanol	R_A/R_S
0.00	N/A	70942	0.0000
0.04	23546	60611	0.3885
0.08	50944	64329	0.7919
0.12	75160	63071	1.1917
0.16	104235	61621	1.6915
0.20	141353	70073	2.0172

The average recorded peak areas of the standard ethanol solutions were used along with the response ratio of analyte to internal standard to construct an internal standard calibration curve. The linear regression line was found to be $R_A/R_{IS} = (10.28) * \text{Concentration} + -0.015$.

Figure 2. Plot of Internal Standard Calibration Curve Using Standard Ethanol Solutions.



Three samples of the Unknowns A and B and the two SRMs were injected into the GC-FID and the average peaks for ethanol and 1-propanol were recorded. The ratios of analyte

response and internal standard response were calculated. The internal standard calibration curve was used to calculate the concentrations of the two unknowns and the SRMs. The standard deviation values were calculated and recorded as well.

Table 4. Average Peak Areas, Response Ratios of Unknown Samples and SRMs, and Calculated Concentrations.

Concentration (w%/v)	Average Area for Ethanol	Average Area for 1-Propanol	R_A/R_{IS}	Calculated Concentration (w/v%)	Standard Deviation
Unknown A	116364	71495	1.6276	0.160	0.0080
Unknown B	38509	73550	0.5236	0.052	0.1126
0.205	147520	70659	2.0878	0.204	0.0051
0.077	48764	69667	0.7000	0.070	0.0010

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V. Conclusions:

The Hewlett-Packard 5890 GC-FID was used in this experiment to determine the amount of ethanol present in each solution. The flow rate was shown to be 14 mL/min and the sample injections were each 1μL. The oven temperature was set at 50°C while the injector port and the detector were set at 120°C. Three samples of each solution were injected and the peak areas averaged.

Using the internal standard calibration curve and the linear regression line, the calculations of Unknown A, Unknown B, and the two SRMs were determined. The concentration of Unknown A was found to be 0.16 w/v% ± 0.0080 with three measurements. This was expected because the R_A/R_{IS} was 1.623 which was comparable to the R_A/R_{IS} value of the 0.16w/v%, which was 1.692. The concentration of Unknown B was found to be 0.05 w/v% ± 0.1126 with three measurements. This was expected because the R_A/R_{IS} was 0.524 which was in between the R_A/R_{IS} value of the 0.04w/v% and the 0.08w/v%, which was 0.389 and 0.792 respectively. The concentrations of the two SRMs were calculated using the linear regression line as well. The first SRM stated that the concentration was 0.205w/v% which was very close to the calculated concentration of 0.204w/v% ± 0.0051 with three measurements. The second SRM stated that the concentration was 0.077w/v% which was also close to the calculated concentration of 0.070w/v% ± 0.0010 with three measurements.

While most of the calculated concentrations were comparable to what was expected, there was some deviation noticed. This could be accounted for through several sources of error. If the samples were not properly mixed before the injections, there could be some error in the response areas. Also, the samples took several days to run which could have allowed for some evaporation of ethanol to occur. This could have skewed the data somewhat, causing the standard deviation values to be what they were.

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VI. Discussion:

When alcohol is consumed, it passes from the stomach to the intestines before entering the blood. Enzymes are the main contributor to eliminating alcohol from the body. When the alcohol enters the liver, an enzyme called alcohol dehydrogenase converts the alcohol into acetaldehyde. The acetaldehyde is then converted to acetate by other enzymes in the liver, and is eventually metabolized into carbon dioxide and water.

One common field alcohol measurement technique is the breathalyzer. This technique works on the principle that while the liver metabolizes most of the alcohol consumed, some remains unmetabolized and can be measured in the breath and urine. When a breathalyzer is used, a person exhales into it and any ethanol present in the breath is oxidized into acetic acid. Any atmospheric oxygen present is reduced. This leads to the presence of acetic acid and water. An electrical current is produced by this reaction and is then measured, processed, and displayed as an approximation of the blood alcohol content.

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VII. Literature Cited:

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