Separation and Quantitation of a Mixture of Alcohols by Gas Chromatography

1. Purpose

This procedure will determine the percentage of two alcohols in an unknown aqueous mixture.

2. Background

Gas chromatography (GC) is a very common technique for both qualitative and quantitative analysis. It is used by scientists for a variety of applications, including pharmaceutical, food analysis, forensics, and environmental. For example, in food analysis GC can be used to characterize flavor components in wine. It is also commonly used in the pharmaceutical industry to determine the ethanol content of drug formulations. Environmental applications include the determination of chlorinated pesticides in water. This is but a small list of the numerous analyses that can be performed using gas chromatography.

Samples are separated in GC based on differences in vapor pressure (boiling point) and interaction with a stationary phase. Samples are injected, vaporized and separated on a column that contains the stationary phase. The samples travel through the column via an inert carrier gas. Depending upon their boiling points and relative affinities for the stationary phase, they move through the column at different rates and so, ideally, each analyte has its own retention time, the time required to move through the column. A detector at the end of the column sends a signal to the read-out device when an analyte is present. In this laboratory, the detector is a flame ionization detector (FID). The data is collected in a chromatogram, which contains peaks for the various species present in the sample. The peak area is determined by an integrator. When known reference standards are also injected, both the identity and quantity of an unknown can be determined by GC.

Because of the errors that can be introduced by sample injection volume, flow rate, and variations in column conditions, the highest precision in quantitative GC is accomplished using the internal standard method. An internal standard is a compound that is different, but chemically similar, to your analytes. Once you know how the detector responds to a known standard mixture containing your analytes and the internal standard, you can use spike your unknown with the internal standard and determine the concentration(s) of the analyte(s) in the unknown. Since the unknown in this experiment has two alcohols, a third alcohol serves as the internal standard. The ratio of analyte peak area to internal standard peak area is used as the analytical parameter.

The unknown in this laboratory will be composed of water plus two of these three alcohols: methanol, ethanol and 1-propanol. The first step is to determine the identity of the two alcohols in your unknown mixture. The third alcohol will serve as the internal standard. Using the internal standard method of quantitation, the percentage of the two unknown alcohols in the mixture will be calculated.

Materials and Equipment

| Methanol | Disposable test tube |
| Ethanol | Parafilm |
| 1-Propanol | Agilent 6850 Gas Chromatograph (GC) |
| Phenomenex ZB-WaxPlus capillary column | (30 m, 0.53 mm I.D., 1.0 µm thickness) |
| 2 Screw cap test tubes | 10 µL capillary syringe |
| 5 mL pipette | 10 mL pipette |
| Vortex mixer | Optional test tube rack |
3. **Safety / Special Handling Procedures**

Protective eyewear must be worn at all times. Many internal parts of the GC are very hot or carry dangerous voltages. Hydrogen, helium, and compressed air are run into the GC from gas cylinders. Do not go past the chain in front of the cylinders. If the gas must be turned on, notify the instructor. Notify the instructor if there is a problem with the instrument. For more general safety in the laboratory, please refer the appendix.

4. **Experimental Method**

4.1. **Preparation of the unknown for identification**

4.1.1. Ask your instructor for your unknown sample, a solution consisting of two alcohols in water. Also ask for two capped test tubes, one of which will be used for your standard mixture and the other for your spiked unknown.

4.1.1.1. Aliquot 10.00 mL of unknown into one of the test tubes. Label the test tube appropriately as it will eventually be used to make the spiked unknown. Ensure that there is at least 1-mL unknown left over to perform the next step.

4.1.1.2. Take the remainder of the unknown to the GC (see instrument operation below starting in section 4.4) and inject about 1-uL as directed in 4.4.1.1 to 4.4.1.3. You will eventually get two peaks. The chromatographic analysis (i.e., the “run”) takes 5-6 minutes and so you can begin preparing your standard mixture as discussed in 4.2 below. If you wish, return to the GC in several minutes and inject the unknown a second time just to ensure that you are getting two peaks with consistent retention times (do this while making the standard reference solution described in 4.2 below). Make a data table in your notebook of each peak number and its corresponding retention time.

4.2. **Preparation of the standard reference solution**

4.2.1. Obtain the three pure alcohols (methanol, ethanol, and 1-propanol) from the chemical cabinet.

4.2.1.1. Transfer 5.00 mL aliquots of each alcohol to the second test tube. To do so, transfer about 20-mL of the pure alcohol into a 50-mL beaker and thoroughly rinse your 5.00-mL pipette before using it to make the transfer. **Never pipette directly from the actual reagent bottles.** Doing so might possible contaminate the reagents and will result in an immediate 5-point deduction for this laboratory.

4.2.1.2. Cap the tube tightly and invert several times before thoroughly (~6-8 seconds) mixing the tube using the vortex mixer (Figure 1). Label the tube appropriately.
4.3. Qualitative Analysis of Unknown Sample

The analyte elution order in GC is largely dependent upon the boiling points of the solutes. As such, the compounds should elute from the column in the following order: methanol, ethanol, and 1-propanol. Given this information, you must determine the identity of the two alcohols in your unknown mixture following the procedure in Section 4.4.1.

4.4. Analysis using Agilent 6850 GC system

Note: If the GC is not set up and running, ask your instructor for assistance. Each run will take 3-4 minutes. However, after each run, the GC reconfigures itself before the next run can start. If the "Not Ready" (located next to Start button – see Figure 5) LED is on, the GC is not ready to run the next sample. Do not inject the next sample until the LED is off. When the instrument is ready, it will read the "Ready for manual inj" message on the display. It takes approximately 10 minutes for the instrument to complete the run and reconfigure itself between injections. During the wait, you may want to check over your lab notebook, perform calculations, or clean up.

Prior to performing the first injection, verify the following instrument settings on the control module or control panel. Load “Method 1” if it has not already been loaded.

**Inlet**
- Temperature: 200°C
- Pressure: 9.00 psi
- Flow ratio: 200.0

**Oven**
- Temperature program: 60°C – 85°C at 10.00°C/min
- Run time: 3.50 minutes
**Column**
Pressure mode: 9.00 psi  
Carrier gas: He

**Detector**
Temperature: 200°C  
Flame gases: H₂ at 30.0 mL/min and Air at 400 mL/min

The control panel is located on the front of the instrument and the control module hangs on the side of the instrument (refer to Figure 2). Document the settings from the control panel in your lab notebook. Verify that these parameters are identical to those listed above. If they are not, notify the instructor before continuing.

**4.4.1. Sample Identification**

4.4.1.1. First, rinse the capillary syringe (see Figure 3) with the excess unknown solution several times. Then, fill the syringe with solution; expel the excess liquid to bring the volume to 1 µL.

4.4.1.2. Note: sample injection in GC can be tricky so ask your instructor for a demonstration if you have questions. Carefully insert the needle of the syringe into the center of the injector (see Figure 4), so that the hub of the needle butts up against the injector. The needle will be penetrating a rubber septum (inside the injector).

4.4.1.3. Inject the sample in one swift motion and then quickly withdraw the needle from the injection port. Press the “Start” button (Figure 5) immediately.
4.4.1.4. Repeat the procedure (steps 4.4.1.1 through 4.4.1.3) for your standard solution. Do not inject your next sample until the “Not Ready” light is off.

4.4.1.5. Once each run is complete, inspect the resulting chromatogram. Your unknown contains two alcohols and your standard contains three. (Note: the unknown also contains water, but water is not detectable). Make a data table to record the retention time and identity of each peak in your notebook. Retention time is the amount of time the substance remains in the GC column and is measured from the time the “start” button is pressed to when the top of the peak appears on the graph. The retention times should be relatively consistent, perhaps varying by only a couple seconds from run to run for a particular alcohol.

4.4.1.6. Compare the retention times of the unknown peaks with the known alcohols. From this data, determine the identity of the missing alcohol in the unknown. This alcohol will serve as your internal standard. Clearly record in your notebook the identity of your missing alcohol (internal standard).

Note: You should record the identity of the sample on the integrator print-out next to the data or chromatogram. Also, cross out any chromatograms or data that you are not using. Remember that you must document in your notebook valid reasons for rejecting data.

4.5. Quantitative Analysis of Unknown Sample

4.5.1. Inject 1 µL of the standard mixture, following the procedure in steps 4.4.1.1 through 4.4.1.4. Inspect the chromatograms and record the Area Percent (A%) for each alcohol. Inject the standard for a total of three trials (including your initial trial) and average the area percent values for each replicate. The standard deviation of the A% is the precision of the method. Your precision should be ≤ 2.5% RSD. While you are running your standard, prepare your spiked unknown as described below.

4.5.2. Spike the 10.00 mL unknown in the capped test tube (prepared in step 4.4.1.1) with a 5.00 mL aliquot of the internal standard alcohol.

4.5.3. Tightly cap the test tube and invert several times before thoroughly mixing the contents of the test tube for several seconds on the vortex mixer.

4.5.4. Run the unknown sample in triplicate. Record the Area Percent for each alcohol in each replicate in tabular format.

Note: It is important to understand that standards and unknowns MUST both be run under IDENTICAL CONDITIONS during the SAME PERIOD while the GC (or any instrument) is operating. If you run out of time between standards and unknowns, the data must be discarded and run again next period. Unknowns CANNOT be compared to standards run on different days as instruments do not always respond exactly the same way. This applies to all experiments in instrumental analysis.

5. Data Analysis / Calculations

5.1. Historically, alcohol concentrations are often measured by volume percent (e.g., 80 proof vodka is 40% ethanol by volume; beer is typically 3-7% ethanol by volume). Your goal is to find the volume percent (V%, percentage by volume) of each alcohol in the original unknown prior to dilution with the internal standard.
5.2. The greater the concentration of an alcohol, the larger its peak area and thus the greater the area percent (A%) of the corresponding peak. As such, A% can be used to find the V% of the two alcohols in the unknown. However, one critical issue with an internal standard (IS) is that the detector will almost always respond differently to the IS than the unknown alcohols. The flame ionization detector used in this experiment is “mass sensitive” and generates a signal based upon the number of oxidizable carbons. It will produce proportionally more signal for propanol than it will for ethanol, and more signal for ethanol than methanol. This introduces the concept of a response factor, RF, that compares the response for an unknown to that of the internal standard.

5.3. To determine the RF for an unknown analyte, begin with the usual basic assumption that signal (A%) is linearly proportional to concentration (V%). Therefore:

\[ A%_u = k_u \times V%_u \] where \( k_u \) is a constant and “u” stands for “unknown”

\[ A%_{is} = k_{is} \times V%_{is} \] where \( k_{is} \) is a constant and “is” stands for “internal standard”

Note that the larger the value of \( k \), the more signal (A%) is obtained for a given amount (V%) of some compound (e.g., propanol’s \( k \) is larger than ethanol’s \( k \)). The response factor, RF, is a measure of how the detector responds to an unknown analyte versus the internal standard and thus is the ratio of the constants, \( k_u/k_{is} \). The above equations can be rearranged to solve for RF:

\[ RF = \frac{k_u}{k_{is}} = \frac{A%_u}{V%_u} \div \frac{A%_{is}}{V%_{is}} \]

\[ RF = \frac{A%_u \times V%_{is}}{V%_u \times A%_{is}} \]

5.3.1. To find the response factor for each analyte, first calculate and tabulate the average A% for each alcohol in the standard mixture (at this time, you can also average the A% for your spiked unknown and use that data in 5.3.3).

5.3.2. Determine the V% of each alcohol in the standard mixture made by mixing 5.00-mL of each alcohol. Use the standard mixture data and the RF equation to calculate response factors for both of the unknown alcohols. Clearly box and label these RF’s.

5.3.3. Once you know the RF’s based upon the standard mixture, it is possible to find the V% of each alcohol in the spiked unknown. Rearrange the RF equation to solve for the volume percent of each alcohol in your unknown (V%u). Since you know the RF and the V%is of your internal standard, it is possible to solve for the V%u for the spiked unknown using averaged A% values.

5.3.4. After you have calculated the V%u for each alcohol in the spiked unknown, you must then find the original concentration of each alcohol prior to being spiked with the internal standard (i.e., account for dilution factors).

5.4. Hand in the integrator print-outs (with your name written on them) with your lab notebook pages. Tear off the perforated edges of the printouts before attaching them to the report.

6. Reporting Requirements

Report the volume percent of each alcohol in the original sample to one decimal place.
7. Waste Disposal

Discard all solutions down the drain. Discard all test tubes to the glass waste container.

8. References

**Instrumental Analysis Laboratory Safety Rules**

A. **Instructions**: Carry out all manipulations in accordance with instructions and the safety rules and procedures given herein.

B. **Eye Protection**: All students and staff working in the laboratory must wear safety glasses at all times.
   If a student needs to be reminded more than three times to wear goggles, she/he will be dismissed from lab for the remainder of the day, and will not be given an opportunity to make up the work.

C. **Apparel**: The clothes you wear in lab are an important part of your “safety equipment,” and should offer protection from splashes/spills. Closed toed shoes (sneakers are fine), Full-length pants or a full-length skirt, and A shirt that completely covers your torso (i.e. at minimum, a t-shirt).
   In other words, you must NOT wear shorts to lab. You must NOT wear flip-flops, sandals, or crocs. You must NOT wear tank tops, halter tops, spaghetti-strap tops, or low cut jeans to lab. Exposed abdomens, hips, and backs are not safe in the lab.

D. **Gloves**: Gloves are an important part of personal protection. Gloves will be available at all times in the laboratory. Your instructor will require their use when appropriate.

E. **Food**: Food, drinks, and gum are not allowed in lab. None at all, not even water bottles.

F. **Sanitation Issues**: Be sure to wash your hands before leaving lab, before you eat anything outside of lab, and before you answer your cell phone.

G. **Music**: Individual headphones are not allowed. Your may choose to play music for the entire class.

H. **Cell Phones and Other Electronic Devices**: Cellular phones and other electronic devices that you do not need to perform your laboratory work should be put away.

I. **Other**: All students are explicitly prohibited from:
   1. conducting any unauthorized experiments.
   2. removing chemicals or apparatus from the laboratory for any reason.
   3. working in the lab alone, or at other than regularly scheduled lab periods.
   4. smoking in the laboratory or within 20 feet of any doorway.
   5. impeding movement in aisles or through doorways with bags, skateboards, etc.