

Analytical Chemistry**Experiment 5: Determination of Impurities in Whiskey Using Gas Chromatography**

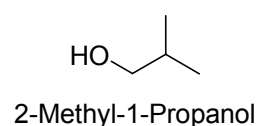
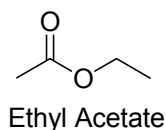
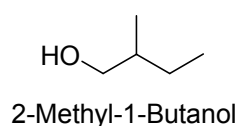
Your write-up for the lab is due by Monday, May 9 (the last day of finals).

No flowchart is required before lab. However, you are expected to provide a clear description of your experimental procedure—both volumetric and instrumental—in your notebooks.

Reference: Rice, Gary W., *J. Chem. Ed.* **1987**, *64*, 1055-1056.

Introduction

The uninitiated think that a distilled alcoholic beverage such as whiskey is simply an intense source of ethanol. However, the unique flavor of different brands of whiskey arises from a complex mixture of trace impurities. In this experiment you will determine the mixing ratios (in ppm) of three impurities found in two different whiskey samples using gas chromatography. The three analytes you will determine are shown below:



The relative amounts of these substances can serve as a fingerprint for a given brand of whiskey. You will use as your solvent a 40% solution of ethanol in water—this mimics the whiskey sample matrix. You will compensate for variations in sample injection volume by using an internal standard, 1-butanol.

Procedure**A. General Comments**

1. During the lab session on April 21, each lab team will make up solutions. You will also need to sign up for a two-hour time slot to make measurements on the Hewlett-Packard 5890A Gas Chromatograph. You should finish your measurements by May 2, the Monday of finals week. Note that you have card access to the “Infrared Laboratory” (Olin-Rice 386) where the instrument is located.
2. In the Analytical Lab, there will be a different syringe dedicated to each of the reagents. Please do not cross-contaminate the syringes.
3. There will be a stock solution of the 40% ethanol-in-water solvent.

B. Solution Preparation

You should partially fill each of the volumetric flasks with solvent before you add aliquot(s) of solute. The presence of solvent minimizes evaporative loss of solute, which would otherwise be a substantial source of systematic error.

1. *Preparation of whiskey samples.* Partially fill a 50-mL volumetric flask with one of the whiskey samples (be sure to note which one). You can fill the flask approximately halfway to the mark—the exact volume does not matter. Use a 100- μ L syringe to pipet a 25- μ L aliquot of 1-butanol into the same flask. Then dilute to the mark with the same whiskey sample. Mix thoroughly (you know the drill). Repeat for the second whiskey sample.
2. *Preparation of standard solutions.*
 - a. Put some solvent mixture (the 40% ethanol solution) into a clean 100-mL volumetric flask. Then use one 100- μ L syringe to pipet 50 μ L of 1-butanol into the same 100-mL volumetric flask. Use another 100- μ L syringe to pipet 50 μ L of 2-methyl-1-butanol into the same flask. Dilute to the mark with the solvent mixture and mix thoroughly.
 - b. Repeat step a for each of the other analytes, ethyl acetate and 2-methyl-1-propanol. Each solution should have 50 μ L of one analyte and 50 μ L of 1-butanol.
 - c. Finally, make a 100-mL solution containing a 50- μ L aliquot of each of the three analytes, plus a 50- μ L aliquot of 1-butanol. That is, this solution should contain 200 μ L total of solute.

You should have a total of four standard solutions, each in a separate 100-mL volumetric flask.
3. For your data analysis, you will need to measure the density of the stock solution of ethanol in water. Think of a simple experiment you can perform to do this.

C. Using the HP 5890A Gas Chromatograph (GC)

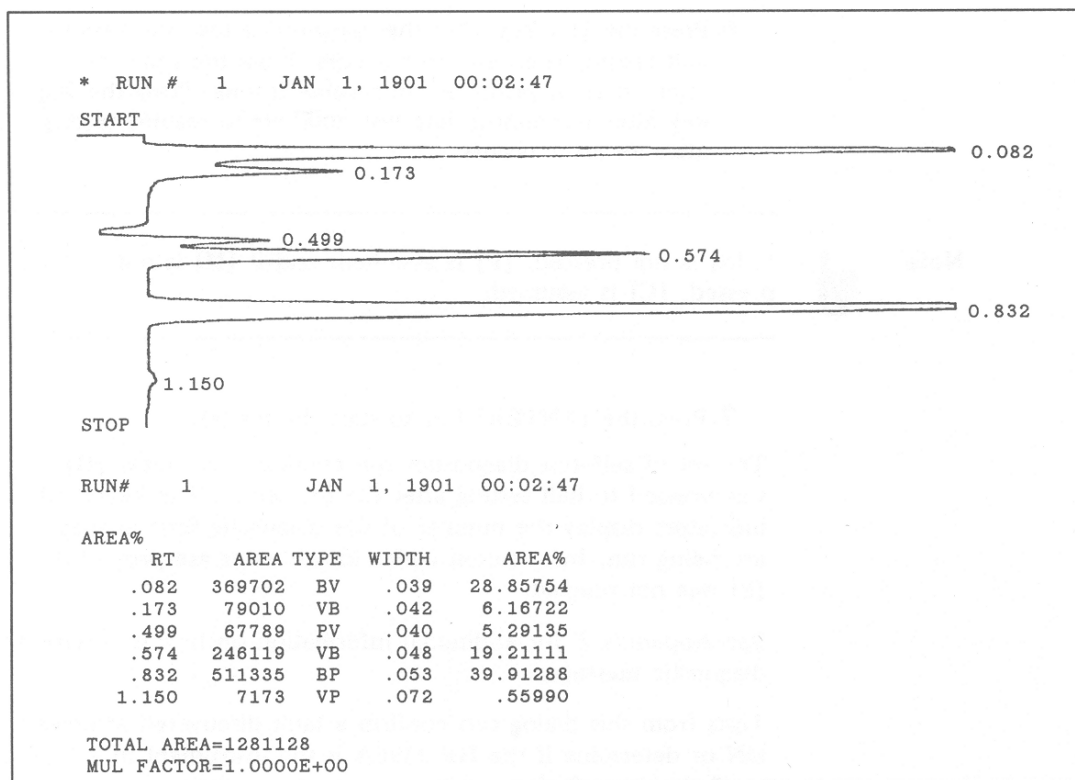
(Usually, the instructor or TA will have performed Steps 1-4.)

1. Turn on the GC (the switch is on the back corner on the right hand side) and the integrator (the switch is on the lower right of the back panel.)
2. Open the three gas cylinders along the wall, using the main valves. The gas tank regulators are already set to the right pressures as long as you open the main valves completely. (As of Fall 2004, we use these pressures: H₂ at 20 psi, air at 40 psi, He at 40 psi.)
3. Start the flame ionization detector (FID) using the controls at the upper left of the instrument. The AUX GAS (helium) valve should be completely off. Open the AIR and HYDROGEN valves completely. Press the FID IGNITOR; you should hear a pop as the FID lights. The flame comes from the exothermic reaction of H₂(g) and O₂(g). To check that the FID is working, hold a watch glass over the detector vent; water vapor should condense on it. Then open the AUX GAS valve completely. Make sure the FID is still producing water vapor.
4. Now program the GC. The injector and detector port temperatures should be 200 °C. The oven should start at 60 °C for 5 minutes, then rise to 160° at a rate of 8

°C/min. The helium gas (the mobile phase in the experiment) should have a flow rate of 100 mL/min. If this has not been set for you already, do the following: (a) Make sure COLUMN HEAD PRESSURE is set to 100 kPa. (b) Connect the soap film flow meter to the SPLIT VALVE outlet. (c) Charge the flow meter with some fresh soap solution. (d) Squeeze the bulb at the bottom of the flow meter to get a bubble flowing. (e) Adjust the SPLIT VALVE knob until a bubble rises to the 100-mL mark in 60 s.

(Here is where your role begins.)

5. Sign the log book. Please note in the log book and in your lab notebook if you have any problems with the instrument.
6. Use a 1- μ L syringe to inject an aliquot of the solution to be analyzed. (You should never inject more than a 1- μ L aliquot—it is easy to flood the narrow column.) Insert at least 1” of the needle into the injection port at the top left of the instrument. Simultaneously depress the syringe plunger and press the START button on the right side of the GC.
7. Make sure the flame ionization detector (FID) is working by pressing the SIG1 key on the chromatograph control panel until a number is displayed on the far right. This value is proportional to the current measured by the FID. When no substance is passing through the detector, the signal should be between 5 and 10. You can watch this number jump (sometimes dramatically) as carbon-containing substances reach the detector.
8. Press the TIME key on the control panel until it displays the ELAPSED time in minutes.
9. Let each elution continue until all expected substances have been detected. Then press the STOP button, and wait until the GC is ready before you start another run.
10. So, how do you know that all expected substances have been detected? First, do runs on the three standards containing one analyte and 1-butanol. This will let you estimate the approximate retention times of all four substances. Then do runs on your standard containing all three analytes, and on your two whiskey samples.
11. Since the GC has extremely low detection limits, it is important to minimize the cross contamination between runs. Therefore, before each run, flush the injection syringe 3 times with the solution you are about to analyze before injecting it onto the column. After each injection wash the syringe three times with the 40% ethanol-in-water solvent mixture. Use a Kimwipe to clean off any droplets sticking to the tip of the needle.
12. When you have completed all your runs, press the SHIFT and ENTER keys on the integrator simultaneously to advance the paper to the next perforation.
13. Keep the instrument after you are done using it.



14. Note that on the chromatograms you will obtain (such as the one above), time is the vertical axis (increasing from top to bottom), and intensity is the horizontal axis (increasing from left to right). In the table at the bottom of the chromatogram, **RT** stands for retention time (in min), **AREA** gives the absolute peak area (which is proportional to the detector current), **WIDTH** gives the integrator's estimate of baseline peak width (in min) (so you can calculate resolution for two neighboring peaks if you wanted to), and **AREA%** gives the, well, percent area for each peak. (Ignore the **TYPE** column.)

Data Analysis

Consider the following for your write-up, which should be entirely in your notebook. Please tape in all chromatograms into one of the notebooks.

1. Determine the retention time for each component by using the chromatograms you obtained for each of the three analytes by themselves. This will allow you to assign the peaks on the standard chromatogram containing all the analytes. Anticipate that the retention time for a given substance will not be completely precise. Methanol and acetaldehyde ($t_r \approx 2$ min) are not determined in this experiment, but you may see peaks for them on your chromatograms.
2. Determine the response factor for each of the three analytes. This is easiest to do using the chromatogram for the standard solution that contained all the solutes. Note that you will need to convert your experimental volume ratios to mass ratios

- (in ppm). This will require you to look up the density of each of the pure components and to use your experimental value for the solvent density.
3. Determine the mixing ratios (in ppm) for 2-methyl-1-butanol, ethyl acetate, and 2-methyl-1-propanol in your two whiskey samples. You should assume that the mixing ratio of the 1-butanol internal standard in your two whiskey samples is equal to the 1-butanol mixing ratio in the standard solution. (This means that we are assuming that the density of all the solutions is the same.)
 4. No quantitative error analysis is required for this experiment. However, you should provide a qualitative discussion of both systematic and random sources of error.