Experiment 5: Determination of Impurities in Whiskey Using Gas Chromatography

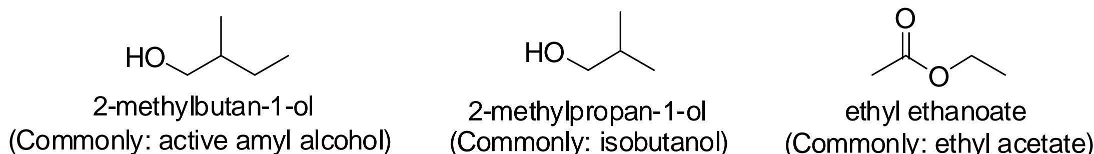
Reference: Rice, Gary W., *J. Chem. Ed.* **1987**, *64*, 1055-1056.

Your notebook for this lab is due by **Wednesday, May 6 and will not be accepted late**

Chromatograms must be collected by Wednesday, April 29

INTRODUCTION

The uninitiated generally believe 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 blend of trace impurities. In this experiment you will determine the mixing ratios 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 $40\%_{mass}$ 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.

EXPERIMENTAL PROCEDURE

General Comments

1. During the lab session on April 16, 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. (I will demonstrate its use that day in lab.) You must finish your measurements by April 29, because we will be checking out of lab the next day.
2. Each stock bottle of analyte or internal standard has a dedicated 100-µL syringe and a beaker. Pour a small amount of liquid into the beaker, and syringe out of the beaker. Please do not cross-contaminate the syringes, and please do not draw directly out of any stock bottle.
3. There will be a stock solution of the $40\%_{mass}$ ethanol-in-water solvent.

Solution Preparation

Be sure to write down both what you do and what you observe in your lab notebook.

You should partially fill each of the volumetric flasks with solvent before you add aliquot(s) of solute. (For the standards, the solvent is the $40\%_{mass}$ ethanol-in-water solution. For the samples, the solvent is the whiskey itself.) 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-µL syringe to transfer a 50-µL 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%$_{mass}$ ethanol-in-water solution) into a clean 100-mL volumetric flask. Syringe 100 µL of 1-butanol into the same flask. Dilute to the mark with the solvent mixture and mix thoroughly.

b. Repeat step (a), replacing the 1-butanol in turn with each of the three analytes: 2-methylbutan-1-ol, 2-methylpropan-1-ol, and ethyl ethanoate. Each solution should contain 100 µL of only one of the analytes.

c. Finally, make a 100-mL solution containing a 100-µL aliquot of *each* of the three analytes, plus a 100-µL aliquot of 1-butanol. That is, this solution should contain a total of 400 µL of solute.

You should now have five 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. Perform a simple, yet accurate, experiment of your own design in order to determine this.

Using the HP 5890A Gas Chromatograph (GC)

Usually, steps 1 – 5 will have been performed for you Skip these steps unless you are told otherwise.

1. Turn on the GC (the switch is on the back corner on the right hand side) and the integrator (the push-on, push-off switch is on the back left side of the integrator.)
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 2008, we use these pressures: H_2 at 30 psi, air at 20 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 closed, and never opened. Open the AIR and HYDROGEN valves completely. Press the FID IGNITOR; you should hear a pop if the FID lights. Most likely, it won't: in which case you should click the sparker on the butane lighter above the exhaust port for the FID until you no longer hear popping sounds when you do so. (No need to make the lighter provide a flame; the spark is enough.) The flame comes from the exothermic reaction of $H_2(g)$ and $O_2(g)$. To check that the FID is working, hold a watch glass over the detector vent: water vapor should condense on it. Set the oven temperature to 200°C and turn it ON.
4. See if the integrator printed anything out when you turned it on. If it didn't, the ink cartridge will need to be cleaned or replaced: see Rob. Once that's fine and it is printing, hit the buttons ATT 2^ (at the top of the keyboard), 0, and ENTER (this sets the attenuation of the peaks to $2^0 = 1$).
5. Now program the GC. The injector and detector A port temperatures should be 200˚C. The oven should start at 45˚C for 6 minutes, then rise to 160˚C at a rate of 30˚C/min, with a final time of 2 minutes. Septum purge should be off. The helium (the mobile phase in this experiment) should have a flow rate of 100 mL/min. If this has not been set for you already, here's how you set it:

   a. Make sure the COLUMN HEAD PRESSURE is set to 75 kPa (11 psi).

   b. Connect the soap film flow meter to the SPLIT VENT 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 knob above the SPLIT VENT until a bubble rises to the 100-mL mark in 60 s.

Here is where your role begins:

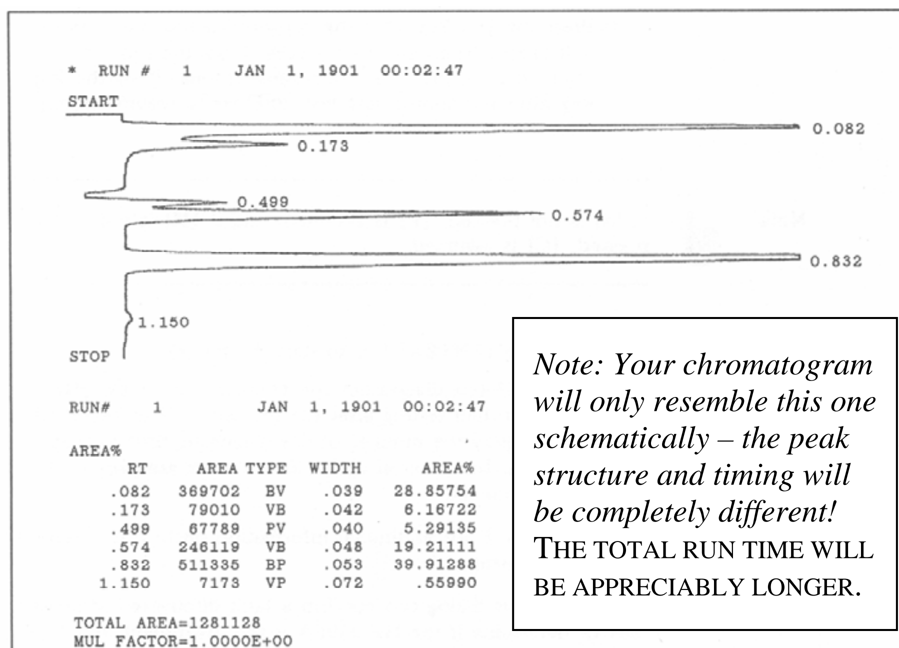
- In your lab notebook, write down the chromatograph settings noted on the white board. This is important to document, since chromatograms are very sensitive to these parameters.
- By following the specific procedures below, obtain a chromatogram of each of the four standard solutions containing one solute. The chromatogram should contain a huge peak for ethanol and a good-sized peak for the solute. (There *may* also be small peaks corresponding to trace impurities, and a big lump of unknown organic(s) eluting after ~10 min. Ignore these extra peaks in your analysis, but be sure to let them elute off the column before ending a given run.) This will let you determine the retention time of each solute.
- Then obtain a chromatogram of the standard containing all four solutes. (Based on your first three chromatograms, you will know how long you will have to wait to see all the peaks of interest, as well as how long it takes for any other components to elute off the column.) This chromatogram will let you determine the response factor for each of the three analytes, as we will discuss in class.
- Finally, obtain chromatograms of your two whiskey samples.

Here are the specific procedures you should follow (*this does not need to be written in your notebook*):

1. Sign the log book! Please note in the log book and in your lab notebook if you have any problems with the instrument.
2. Make sure the flame ionization detector (FID) is working by holding a glass watch glass over the detector vent. Water vapor should condense on it.
3. Since the GC has 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 $40\%_{mass}$ ethanol-in-water solvent mixture. Use a Kimwipe to clean off any droplets sticking to the tip of the needle.
4. Use a small (≤ 10-μL) syringe to inject a 1-μL aliquot of the solution to be analyzed. (Never inject more than ~1-μL – it is easy to overload the narrow column.) In one continuous motion, insert at least 1" of the needle into the injection port at the top left of the instrument, depress the syringe plunger, and press the START button on the right side of the GC or at the upper right corner of the integrator.
5. Press the TIME key on the control panel until it displays the ELAPSED time in minutes.
6. 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.
7. 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.
8. Please leave the instrument on when you are done using it.

WASTE DISPOSAL: All solutions can go down the drain. As you are cleaning up, take the opportunity to make sure everyone in your lab team has every item on the cabinet inventory list. Each item should be clean and undamaged. This will facilitate lab check-out!

DATA ANALYSIS



RT	AREA	TYPE	WIDTH	AREA%
.082	369702	BV	.039	28.85754
.173	79010	VB	.042	6.16722
.499	67789	PV	.040	5.29135
.574	246119	VB	.048	19.21111
.832	511335	BP	.053	39.91288
1.150	7173	VP	.072	.55990

TOTAL AREA=1281128
MUL FACTOR=1.0000E+00

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 on 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.) Use the **AREA** values as the peak intensities in your calculations of response factors and mixing ratios.

Your calculations and discussion should all be presented in your notebook. **Also, please tape in each chromatogram onto a separate page into the notebook**. Use a spreadsheet to facilitate your calculations. Include a copy in your lab notebook and e-mail a copy to me.

1. Determine the retention time for each component by using the chromatograms you obtained for each of the four solutes 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, in part due to injection/start timing, in part due to column loading.
2. Determine the response factor for each of the three analytes. 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. Be sure to write down the source of your density data.
3. Discuss the relationship (if any) between the analyte response factors you have measured and each analyte's molecular size and structure, and/or bulk physical properties.
4. Determine the mixing ratios (expressed as ppm_{mass}) for 2-methylbutan-1-ol, 2-methylpropan-1-ol, and ethyl ethanoate in your two whiskey samples. 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 approach assumes that the density of all the solutions is the same.)
5. As always, end with a brief Conclusion that addresses if the objective was achieved and mentions possible improvements. Include a qualitative discussion of both systematic and random sources of error (no quantitative discussion is required).