4.2: Gas Chromatography Procedure

Using a Gas Chromatograph: Identifying Unknown Compounds

Learning Objectives

- Become familiar with the concept of using chromatography to separate substances in a mixture.
- Become familiar with using chromatography to identify unknown substances.
- Learn to operate and use the Vernier Mini GC
- Measure the retention time of three different ketones and analyze each peak as they pass through a Vernier Mini GC.
- Analyze a known mixture of the ketones as they pass through a Vernier Mini GC.
- Identify the ketones in a mixture (previous knowns) based on retention times.

Safety

- Obtain and wear goggles.
- Handle the organic chemicals with care. Avoid spilling it on your skin or clothing. Do not directly smell these chemicals.
- Properly handle and store the syringe and Mini GC.
- Ensure all organic chemical containers are closed when not in use.

Materials

- Vernier Mini GC
- computer or LabQuest
- Logger *Pro or* LabQuest App
- 1 µL glass syringe
- imwipes® or paper towel
- acetone
- 2-butanone (ethyl methyl ketone)
- cyclohexanone
- ketone mixture of all 3 ketones
- unknown ketone mixture

Discussion & Review

There are many different types of chromatography: paper, thin layer, liquid, high-pressure liquid (HPLC) and gas (GC). Chromatography is applied in many fields. Biochemists use liquid chromatography to separate proteins; chemists use GC and HPLC to identify organic compounds. Technicians use GC for drug tests, toxin screens and environmental analysis. Many forensic tests involve the use of chromatography.

All chromatography approaches operate under the same basic principles. There is a stationary phase and a mobile phase. The mobile phase (a gas or a liquid) travels along the stationary phase (the column or on a plate) from a start point to an end point. Compounds can travel from the start to the end at different rates, depending on whether they tend to “stick” to the stationary phase or “float” in the mobile phase. Compounds stick to the stationary phase through intermolecular attractions such as dipole interactions, dispersion forces or ionic interactions. When a substance has properties similar to the stationary phase it will tend to stick to it along the way and move through the mobile phase more slowly.

The Vernier Mini GC uses a metal column with the inside of the column coated with the stationary phase. A sample, consisting of one or more compounds, is injected onto the column and is pushed through by air, which acts as the mobile phase. Organic compounds flowing out of the chromatography column are seen as a *peak* on a chromatograph, as seen in Figure 1. The amount of time it takes for a compound to exit the column after it is injected is called the *retention time*. With a GC, a compound can be identified from a mixture of chemicals by its retention time.

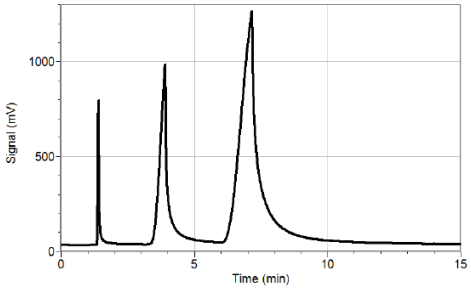

Figure 4.2.1. Sample Gas Chromatogram

Several factors can affect the interaction of a compound with the GC. More volatile compounds (i.e., compounds with a lower boiling point and weak Intermolecular forces) tend to move through the column faster because they are flowing in the mobile phase and interacting or “sticking" very little with the stationary phase. The functional groups present on the compound are also a factor. For example, alcohols may interact with a polar stationary phase more than esters because alcohols can form stronger hydrogen bonds. The molecular weight of a compound can also play a role, although it is not a simple matter of saying that the heavier the molecule, the slower it will travel through a GC column.

Part A: Procedure

> **Important**: The glass syringe is fragile and can be easily damaged. Be careful not to bend the needle or bend the plunger. If the plunger is accidentally pulled out of the glass barrel, reinserting it is extremely difficult, and sometimes impossible.

Figure 4.2.2. Syringe with liquid drawn to the 0.2 µL mark.

1. Obtain and wear goggles.
2. Obtain a glass syringe and a set of vials containing the three ketones, a mixture of all 3 ketones, and an unknown mixture of ketones to be tested. You will not only test acetone but use it to clean the syringe needle.
3. Prepare the Vernier Mini GC for data collection.
    a. Turn on the Mini GC.
    b. **Important:** Connect the USB cable of the Mini GC to the **USB port on your computer**.
    c. Start the data-collection program, and then choose New from the File menu.
    d. Click Collect in Logger *Pro*, or tap ► in LabQuest, to bring up the Temperature-Pressure profile.
    e. Set the Temperature-Pressure values to:

Start temperature	35 °C
Hold time	2 min
Ramp rate	5 °C/min
Final temperature	55 °C
Hold time	9 min
Total length	15.0 min
Pressure	5.0 kPa

    f. Select Done to initiate the Mini GC warm up. Note: A new message will appear, “Do not inject until GC is ready”, and the LED on the Mini GC is red. The Mini GC will take a few minutes to warm up and stabilize. When the Mini GC is ready for injection in Step 7, the message will read, “Inject and select Collect simultaneously”, and the LED will turn to green. Continue with Step 4 during warm up.

4. Follow the steps below to clean and flush the syringe with acetone. **Important**: The glass syringe is fragile. Be careful not to bend the needle or bend the plunger. Never pull the plunger back more than 50% of its total volume. Be careful not to bend the plunger as you press it down.
    a. Depress the plunger fully.
    b. Submerge the tip of the syringe needle into the vial of acetone.
    c. Pull back the plunger to fill the barrel about 1/3 full of acetone. Examine the barrel of the syringe and estimate the amount of acetone in the barrel.
    d. Expel the liquid onto a Kimwipe or a paper towel.
    e. Repeat Steps a–d at least two times, until you are comfortable pulling up a liquid into the syringe and measuring the volume in the syringe barrel. Use a Kimwipe or a paper towel to carefully pat around the tip of the syringe needle.
5. Collect a volume of acetone for injection.
    a. Submerge the needle into the vial of acetone one last time.
    b. Draw up approximately 0.2 µL of liquid. See Figure 4.2.2. It is not critical that the volume be exactly 0.2 µL; a tiny bit more or less volume is all right.

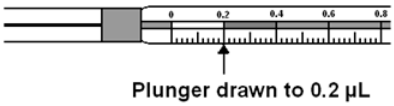

Figure 4.2.2. Syringe with liquid drawn to the 0.2 µL mark.

    c. After collecting your sample, use a Kimwipe to gently wipe the needle from barrel to tip.
6. Prepare for injection and the start of data collection. It is important for you and your lab partner to divide the tasks in this step. One person will operate the syringe and the other person will operate the computer controls.
    a. When the Mini GC has reached the correct start temperature and pressure, the message reads, “Inject and select Collect simultaneously,” and the LED on the Mini GC is green.
    b. To insert the needle of the syringe into the injection port of the Mini GC, hold the syringe with one hand and steady the needle with your other hand. Insert the needle into the injection port until the needle stop is fully seated, as shown in Figure 3. If the needle sticks, rotate it slightly while inserting. Do not move the plunger yet.

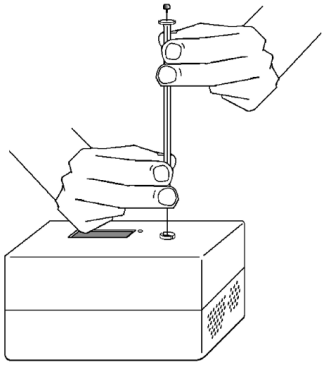

Figure 4.2.3. Injecting the liquid into the Mini GC.

    c. Simultaneously, depress the syringe plunger and select Collect to begin data collection. Pull the needle out of the injection port immediately.
7. While the data collection proceeds, repeat Step 4 with Acetone to thoroughly clean the syringe and needle. It may take more than three flushes to feel the syringe plunger move smoothly again, which is your indicator that the syringe and needle are both suitably clean.Choose Peak Integration from the Analyze menu.
8. Data collection will end after fifteen minutes.
9. Analyze your chromatogram.

a. Select and integrate the left-most peak. To do this, drag from a little before the peak to a point far enough to the right that includes all of the peak. Then choose Add.
b. Record the retention time in your data table.
c. Enter the name of the compound, if known.
d. To analyze another peak on the same graph, repeat Steps b and c.
e. When you are finished with all of the peaks, select OK.

10. (optional) You can choose to save this chromatogram and peak analysis for later use, with a unique file name, by choosing Save from the File menu.
11. Prepare another sample.
    a. Click Collect in Logger *Pro*, or tap ► in LabQuest, to bring up the Temperature-Pressure profile. This profile will be the same as for your previous run. If you are satisfied with these values, select Done to initiate the Mini GC profile.
    b. While the Mini GC adjusts to its Temperature-Pressure profile prepare the next sample.
    c. Follow the process in Step 4 using 2-butanone rather than acetone to clean and flush the syringe with 2-butanone.
    d. Repeat Steps 5–6 with a sample of 2-butanone.
    e. After the Mini GC is ready, repeat Steps 7–11 using your new sample.
12. Repeat Step 11 for the other 2 samples, cyclohexanone, mixture of the 3 ketones and the unknown ketone mixture. In total you should run 5 total samples.
13. When you have completed your final data collection run, turn off the Mini GC. Carefully put away the Mini GC and Hamilton syringe.
