Experiment 1

Using Gas Chromatography: Identifying an Unknown Compound

There are many different types of chromatography:

- Paper chromatography
- Thin layer chromatography (TLC)
- Liquid chromatography (LC)
- High-pressure liquid chromatography (HPLC)
- Gas chromatography (GC)

Chromatography is applied in many fields. Biochemists use liquid chromatography to separate proteins; chemists use GC, TLC, and HPLC to identify organic compounds. In forensics and other specialties, scientists use GC to perform drug tests, toxin screens, and environmental analysis.

All types of chromatography employ a stationary phase and a mobile phase. The stationary phase is immobile on the column or the plate and the mobile phase travels from a start point to an end point. Compounds travel from the start to the end at a specific rate depending on their competing affinity for the mobile gas/liquid phase versus the stationary solid phase. Compounds adhere to the stationary phase through dipole interactions, dispersion forces, or ionic interactions.

In this experiment, you will use a Go Direct Mini GC (Gas Chromatograph) to identify the specific compounds in a mixture. In the Mini GC, the stationary phase coats the interior of a metal column inside the device. When the sample, consisting of one or more compounds, is injected into the column it is carried by room air, the mobile phase, up the column. Compounds flowing out of the chromatography column are indicated as a peak on a chromatograph. The amount of time it takes for a specific compound to exit the column after it is injected is called the retention time. Each compound can be identified by its unique retention time.

Several factors can affect a compound's retention time. More volatile compounds (i.e., compounds with a lower boiling point) move through the column more quickly because they flow with the mobile phase and are not strongly bonded with the stationary phase. The surface functional groups present on the compound are also a factor. For example, alcohols may weakly bond with a polar stationary phase more than esters because alcohols are capable of forming hydrogen bonds. The molecular weight of a compound may also play a role to a slight extent, although there is not a direct relationship between molecular mass and retention time.

In this experiment, you will explore the process of identifying one or more unknown species using gas chromatography. First, you will practice using a gas chromatograph by testing several known substances. You will then use this information to identify the substances present in an unknown mixture.

OBJECTIVES

- Measure and analyze the retention time of six substances as they pass through a Go Direct Mini GC.
- Measure and analyze the retention time of an unknown mixture of the substances.
- Identify the substances present in the unknown mixture based on retention times.

MATERIALS

Chromebook, computer, or mobile device
Instrumental Analysis app
Go Direct Mini GC
1 µL Hamilton syringe
goggles and gloves
paper towel or lint-free wipe
pentane
n-hexane
ethyl acetate
butyl acetate
cyclohexane
toluene
unknown mixture

PRE-LAB ACTIVITY

A table such as Table 1 is a common starting point for understanding the behavior of a set of substances, being tested by gas chromatography, which may be found in a mixture. Fill out the table for each substance, including the bonding functional group, or organic family (e.g., alkane, alkene, alcohol, aldehyde, ester, ether, ketone).

Table 1

Compound	Boiling temperature (°C)	Molar mass (g/mol)	Bonding functional group
pentane			
n-hexane			
ethyl acetate			
butyl acetate			
cyclohexane			
toluene			

PROCEDURE

1. Put on safety goggles and gloves.

2. Set up the Go Direct Mini GC and Instrumental Analysis.
   a. Launch Instrumental Analysis.
   b. Connect the Mini GC to your Chromebook, computer, or mobile device via USB or Bluetooth wireless technology. If using Bluetooth, click or tap Connect an Instrument, connect to your Mini GC, and click or tap Done.
   c. Click or tap Gas Chromatography to begin a new experiment.
   d. Set the temperature/pressure profile to the parameters listed in Table 2. Then, click or tap Apply to initiate the temperature/pressure profile.

Table 2	
Start temperature (°C)	45
Hold time (min)	2
Ramp rate (°C/min)	5
Final temperature (°C)	95
Final hold time (min)	2
Total time (min)	14
Pressure (kPa)	11

3. While the Mini GC is warming up, follow the procedure in this step to clean and flush the syringe with n-hexane. **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.
   a. Depress the plunger fully.
   b. Submerge the tip of the syringe needle into the vial of n-hexane. **DANGER**: *n-Hexane,* C_6H_{14}: *Keep away from heat, sparks, open flames, and hot surfaces—highly flammable liquid and vapor. Do not eat or drink when using this product. Avoid breathing mist, vapors, or spray. May be fatal if swallowed and enters airways. May cause damage to organs. Causes skin and eye irritation. May cause drowsiness or dizziness. Suspected of damaging fertility or the unborn child. Do not handle until all safety precautions have been understood*.
   c. Pull back the plunger to fill the barrel with 0.4 µL of n-hexane.
   d. Expel the liquid onto a lint-free wipe or a paper towel.
   e. Repeat Steps a–d at least two times, or until you are comfortable pulling up a liquid into the syringe and measuring the volume in the syringe barrel.

4. Follow the process in Step 3 to clean and flush the syringe with pentane, the first sample to be injected into the Mini GC. **WARNING**: *Flammable liquids. Flammable liquid and vapor. Keep away from heat, sparks, open flames, and hot surfaces. No smoking. May cause drowsiness or dizziness. Avoid breathing mist, vapors or spray.*

5. Once the Mini GC has reached the correct start temperature and pressure, the software will indicate that the GC is ready for injection and the check LED will turn green. Collect 0.2 µL of pentane for injection. Insert the needle into the injection port of the Mini GC. Depress the syringe plunger; immediately following, press the button on the Mini GC to initiate data collection. Be careful not to bend the plunger as you press it down. Pull the needle out of the injection port immediately. Data collection will automatically stop after the allotted time, or you can choose to end it early if you are satisfied that all the analytes have eluted completely.

6. Name your sample appropriately. To do this, click or tap the y-axis label. Click or tap ⋯ next to the data set you wish to rename, and select Rename Data Set. Enter a name for the data set, and click or tap Rename.

7. To analyze the chromatogram, drag across the peak to select it, then click or tap Add Peak Integral, . Record the retention times in Table 3.

8. Test the remaining compounds, including the unknown mixture.
   a. Click or tap Initiate Data Collection. Then, click or tap Apply; you will use the same parameters as before.
   b. While waiting for the instrument to warm up, follow the process in Step 3 to clean and flush the syringe with the next compound you want to test.
   c. Repeat Steps 5–7. Inject 0.2 µL when testing pure compounds. Inject 0.4 µL when testing the mixture.

9. Save the file as directed by your instructor. Keep your test results open in Instrumental Analysis; you will need to refer to the various chromatograms to answer the Data Analysis questions.

10. When you are done with data collection, make sure to turn the Mini GC power switch to the off position and disconnect the Mini GC from AC power.

DATA TABLE

Table 3

Compound	Retention time (min)
pentane	
n-hexane	
ethyl acetate	
butyl acetate	
cyclohexane	
toluene	
mixture	

DATA ANALYSIS

1. Discuss the retention times of the six substances with regard to their molecular weights. Describe any pattern that emerges.
2. Discuss the retention times of the six substances with regard to their boiling points. Describe any pattern that emerges.
3. Identify the substances that are present in your unknown mixture. Support your identification.