

4. Gas Chromatography

A. Introduction to Chromatography

1. Chromatography

Chromatography is the general name for a number of very powerful methods for separating compounds to purify and identify them. Common chromatographic methods include: thin layer chromatography, column chromatography, high-performance liquid chromatography (HPLC), and gas chromatography (GC).

All chromatography methods are based on a common principle. A **mobile phase** travels through a **stationary phase**. The components in a mixture interact differently with the moving and stationary phases and thus travel over the stationary phase at different rates. This results in the separation of the different components in the mixture.

2. Gas Chromatography

In gas chromatography, the mobile phase is a **carrier gas**, usually helium, which carries a mixture through the column. The GC column is typically packed with a fine granular solid support that is coated with a non-volatile liquid, which acts as the stationary phase.

The basis of GC is as follows: You inject your sample into a heated injection port, which volatilizes the sample. The carrier gas then moves your sample through the column (encased in an oven) where the various components are separated. These components reach the detector at different times. The detector generates an electric signal which goes out to a computer that records the signal. (Figure 1).

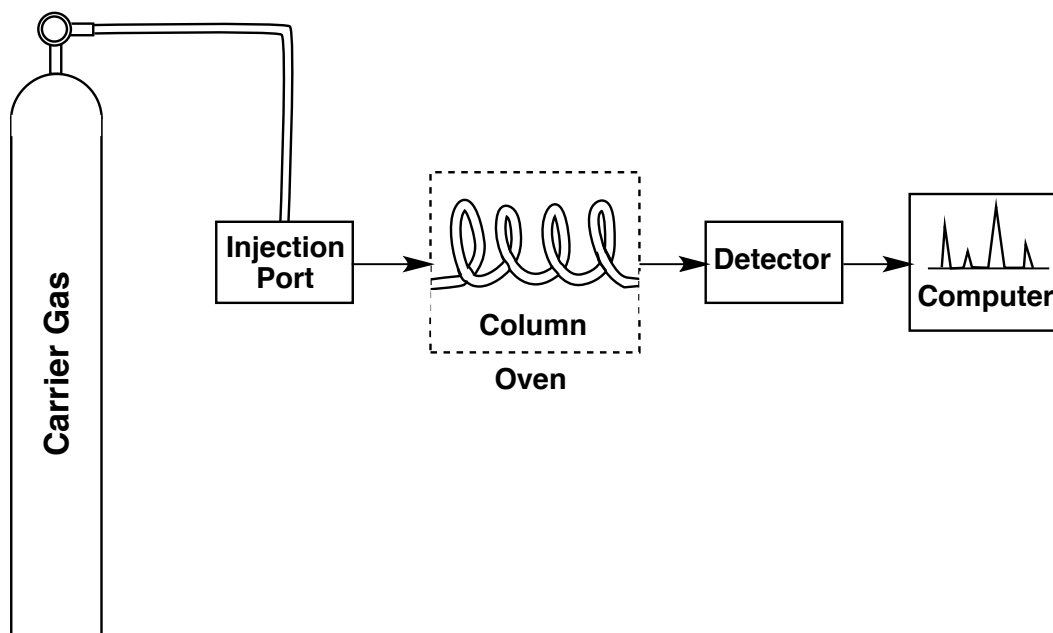


Figure 1. Gas Chromatography Block Diagram

Sample Introduction (The Injection)

When preparing to analyze a sample by CG, you must keep in mind a couple of constraints. First, the sample must be volatile enough that it will be vaporized upon injection. Second, your compound must be stable at the temperature required for vaporization.

The CG detector is very sensitive and only a small quantity of material needs to be injected for analysis. Rather than injecting your sample neat, it should be dissolved in a small volume of solvent. Typical solvents include toluene and acetone. You must insure your sample is completely soluble in the solvent, otherwise it could clog the injection port or the column.

The sample can be introduced via the injection port using a microliter syringe. The instruments in our labs are equipped with autosamplers and automated injection so you will only need to prepare your sample in a small GC vial and place it in the autosampler rack.

The injection port is a heated chamber where your sample is immediately volatilized. The injection port temperature can be adjusted on the instrument, but is typically set to between 250 and 300 °C.

The Column

Depending on the mixture to be separated, a variety of different columns are available. The column consists of a long length of steel tubing. Inside the column is a finely powdered solid support to which a non-volatile liquid stationary phase is covalently bonded. Although the solid support is coated with a liquid, it appears to be a dry powder. Some common column types include:

- Carbowax column – polyethylene glycol stationary phase (polar); best for carboxylic acids and alcohols.
- SE-30 column – polydimethyl siloxane stationary phase (non-polar); best for hydrocarbons, polycyclic aromatics, etc.
- SE-52 column – poly(phenylmethyl dimethyl) siloxane (non-polar); best for halogens and fatty acid methyl esters.

The GC column is typically 1.5-10 meters in length and is coiled in order to fit inside the instrument. The internal diameter of the column is typically 2-4 mm.

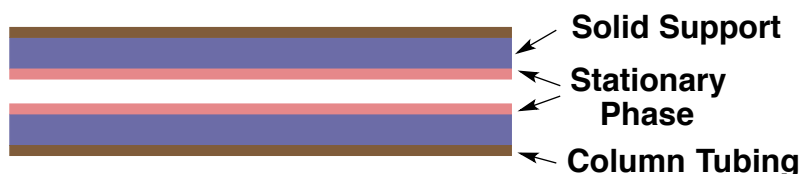


Figure 2. Cross Section of a GC Column

The column is encased in an oven where a temperature program can be set. Depending on the stability of the column packing material, columns can be heated up to 250 or 350 °C. The instrument can be set to maintain a particular column temperature or it can be programmed such that the temperature changes as desired during the course of the run. Often the temperature is set to steadily increase over the run. This process is referred to as a temperature ramp.

The Carrier Gas

While helium is the most common carrier gas, other gases or mixtures of gases can be used depending upon the instrument and the type of detector used. The flow rate of the gas can be adjusted on the instrument. Altering the flow rate will affect the separation of your components and will change the time it takes your components to exit the column. Flow rate is typically adjusted to achieve the best combination of a speedy run along with good separation.

Once your sample is injected via the injector port, it is carried onto and through the column (generally heated by an oven). The mixture separates and the various components elute from the column at different rates at which time they are detected by the detector. As each component hits the detector, a signal is generated and recorded by a computer.

Sample Detection

The various components of the mixture must be detected as they exit the column. The vaporized compounds that exit the detector have a very low concentration, thus the detector must be extremely sensitive and must have a very high temperature.

A variety of different detectors are available. Our instruments are equipped with a flame ionization detector (FID). With this type of detector, a continuously burning hydrogen flame is situated between two electrodes. When a compound exits the column, it enters the flame, resulting in immediate combustion to form CO_2 and H_2O . A few of the molecules, however, will ionize at which time the ions are detected by the electrodes and a signal is sent to the detector.

Care must be taken when working with hydrogen gas as it is highly flammable.

A common, but expensive detector that can be coupled to a GC is a mass spectrometer (MS). This instrument takes the eluted component and analyzes it for its mass. The mass data can then be used to determine the compound's molecular formula. Computers that are connected to online databases can analyze the GC-MS data and will match this data to a structure or mixture if that particular item is cataloged in the database.

Some Factors Affecting Separation of Components

- The boiling point of the compounds in the mixture. Typically, compounds with lower boiling points travel through the column faster than those with higher boiling points.
- The flow rate of the carrier gas. If the flow rate is too low, the components of interest will elute very slowly, resulting in poor separation. If the flow rate is too high, the components will not have enough time to interact with the stationary phase. In this situation, they will come off of the column too quickly and signals may overlap.
- The choice of stationary phase in the column. As discussed previously, several different types of columns are available. One column type may give excellent separation while another will not separate the components at all. Sometimes, trial and error is required to select the best column for a particular application.

3. Analysis of the Gas Chromatogram

Once all components of the mixture elute from the column and are detected, a chromatogram is produced. The chromatogram is a plot of retention time versus peak height. The retention time is a measure of how long from the time of injection it took the component to exit the column. The area under each peak can be used to determine the percentage of that component in the sample.

Retention Time (T_R)

Retention times measured from the time of injection are called “uncorrected.” In many cases, a small bubble of air is injected along with the sample. The air moves through the column at the same speed as the carrier gas. When it emerges from the column, it is detected and results in a small “bleep” at the beginning of the chromatogram. Retention times measured from the air peak are termed “corrected.” We will be using “uncorrected” retention times in this experiment, which will serve our purpose.

While retention times can be measured with a ruler, modern instruments record the retention times for each signal digitally. This data is then provided in tabular form.

Area

The area under each signal is directly proportional to the amount of component that produced it. Modern instrument, such as the ones used in our laboratories, integrate the area under each signal digitally. If, however, this data is not provided by the instrument, it can be estimated manually by the following method.

- Measure the peak width at $\frac{1}{2}$ of the peak height ($W_{1/2}$). Multiply this value by the peak height, h . **Area $\propto h \times W_{1/2}$**

Component Percentages

The area of each peak can be used to calculate the percentage of that component in the starting mixture.

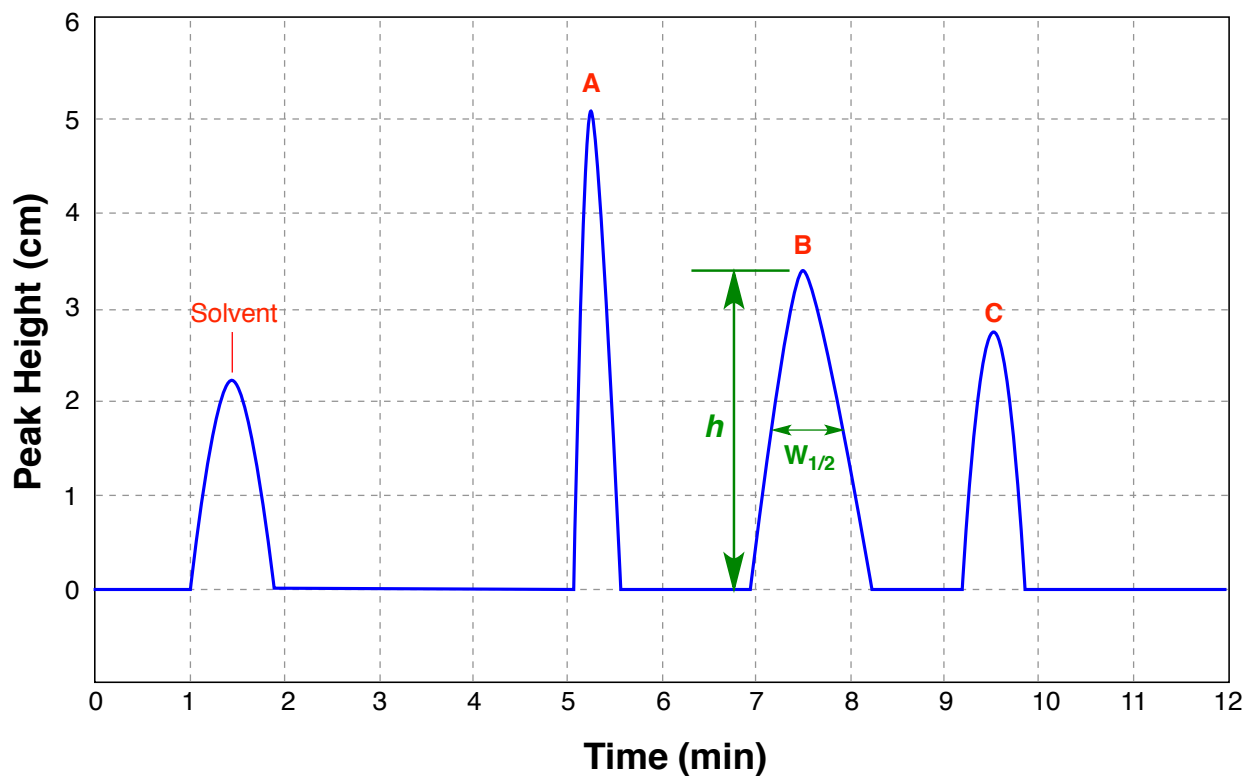
First, you will need to determine the total area contributed all of the components in the mixture. The computer software will typically provide you will the area corresponding to each peak along with the total are. You must be careful with the total area value generated by the computer however. This total area value will typically include the area of the solvent peak, which you will generally not want to include.

From the example in figure 3, there are three components (A, B, and C) in the mixture. The sum of the areas of peaks A, B, and C gives you the total area.

$$\begin{aligned} \text{Total Area} &= \text{area peak A} + \text{area peak B} + \text{area peak C} \\ \text{Total Area} &= 1.73 \text{ cm}^2 + 2.72 \text{ cm}^2 + 1.41 \text{ cm}^2 = 5.86 \text{ cm}^2 \end{aligned}$$

From here, you can determine the relative percentage for any of the components by dividing the area of that component by the total area multiplied by 100%. For example, the percentage of component B is determined as follows.

$$\begin{aligned} \% \text{ B} &= (\text{area peak B}/\text{total area}) \times 100\% \\ \% \text{ B} &= (2.72 \text{ cm}^2/5.86 \text{ cm}^2) \times 100\% = 46.4\% \end{aligned}$$



	Retention Time	Area
Solvent	1.41 min	--
A	5.27 min	1.73 cm ²
B	7.46 min	2.72 cm ²
C	9.51 min	1.41 cm ²

Figure 3. Sample Chromatogram and Computer Generated Data

Deducing the Identity of an Unknown

In most cases, GC alone cannot be used to determine the identity or structure of an unknown. However, if you have a reference sample containing a “known” you can compare the GC of the known to the GC of your unknown. If the chromatograms match, it is likely that the unknown and known are identical compounds. In order to conduct this type of analysis, the CG parameters (column, temperature, flow rate, etc.) must be identical for the “known” and the “unknown” run. If the parameters are off even slightly, the two chromatograms will not match.

Consider the example in Figure 4. You have a reference sample containing four saturated hydrocarbons (C8-C11). The chromatogram of this reference mixture is shown in figure 4a. You also have an unknown compound that you know to be a saturated hydrocarbon. GC analysis of this unknown produces the chromatogram shown in figure 4b. Assuming the instrument parameters and column were identical for both runs, you can conclude that the identity of your unknown is nonane because it has the same retention time (4.3 min) as that of nonane in the reference chromatogram.

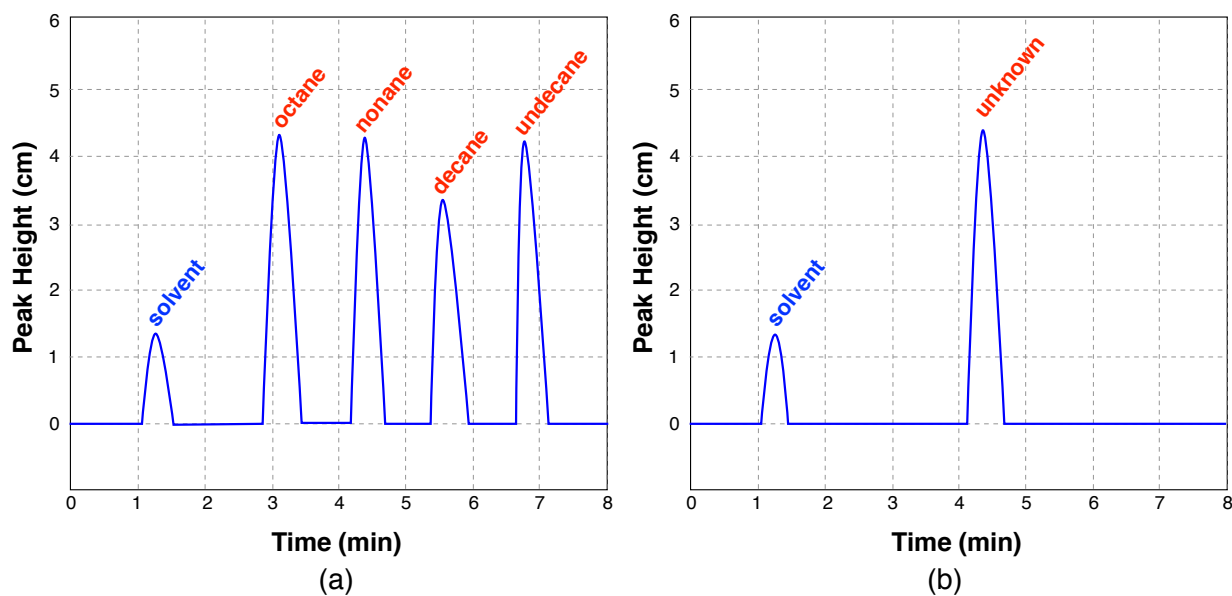


Figure 4. Reference and Unknown Chromatograms

B. Experimental Procedure

The GC experiment will use the same unknowns that you used in last week's distillation experiment.

Analysis of Distillation Products

The gas chromatograph should be up and running prior to your arriving to lab. The GC is equipped with an auto-sampler and is already programmed to give the best separation for the mixture of esters. The most difficult part of this experiment is preparing the sample in a GC vial and ensuring you place the vial in the correct place in the auto-sampler.

Obtain a small GC vial with a screw cap septum. Fill this vial 1/3 to 1/2 full with your unknown sample. *Be sure to use the same unknown that you used last week!* Cap the vial and place it in the auto-sampler rack when instructed by your TA. Once your sample has passed through the GC, a printout of the results can be obtained. Your TA will also provide you a copy of a standard chromatogram that contains a mixture of all three esters.

Alternatively, your TA may provide you with a pre-ran gas chromatogram that corresponds to your unknown sample. If this is the case, it will not be necessary to prepare the unknown sample for GC analysis.

Use the report sheet to guide you in analyzing the chromatogram.