CHEM 333L
Organic Chemistry Laboratory
Revision 1.2

Gas Chromatographic Analyses

In this laboratory exercise we will analyze, using Gas-Liquid Chromatography (GC), the Fractions collected during our Fractional Distillation of the Cyclohexane-Toluene mixture and the Essential Oil obtained as a result of our Steam Distillation. The GC analysis of the Fractional Distillation fractions will allow us to determine the relative amounts of Cyclohexane and Toluene in each fraction collected. The GC analysis of the Essential Oil will allow us to determine the number of components present in the Oil, and to identify, via comparison with appropriate standards, the major component(s) of the Oil. Chromatographic separations are ubiquitous throughout chemistry and Gas Chromatography is a particularly important type of chromatography.

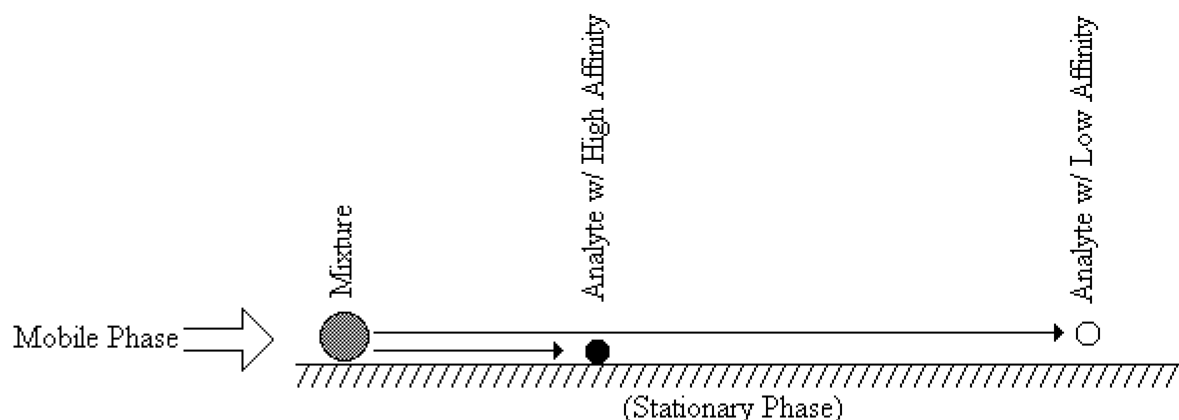
Chromatographic systems trace their origins to an experiment conducted by the Russian scientist Mikhail Semenovich Tswett circa 1906 in which he reported separating the different colored pigments of plant leaves by passing an extract of the leaves through a column of calcium carbonate (think of powdered chalk); an early form of Column Chromatography. He coined the term chromatography, from the Greek words for "color" and "to write", to describe this method of separating chemical substances.

Mikhail Tswett
(http://www.wikidoc.org/index.php/File:Tsvet_ms.jpg)

Column Being Filled With Elutent
(http://en.wikipedia.org/wiki/File:Colortest.jpg)

Presently, Chromatography is the general name applied to a series of separation methods that employ a system with two phases of matter; a mobile phase and a stationary phase.

Analytes in a mixture to be separated interact with the stationary phase with different affinities. While moving through the system, carried along by the mobile phase, those analytes with a low affinity for the stationary phase will tend to move along rapidly, while those with a high affinity will tend to lag behind. Thus, the separation of analytes in chromatographic systems is based on the differential affinity of the analyte for the stationary vs. mobile phases.



In Column Chromatography, the Stationary Phase is a solid support packed into a glass column. Liquid elutent, the Mobile Phase, is passed down the column, carrying the analytes with it. The separated analytes can be collected as they pass through the bottom of the column. If the analytes are colored, identifying when the analyte is about to pass off the column is simply a matter of watching its colored band migrate down the column. If not, then deciding when the analyte has passd off the column requires some form of detection system.

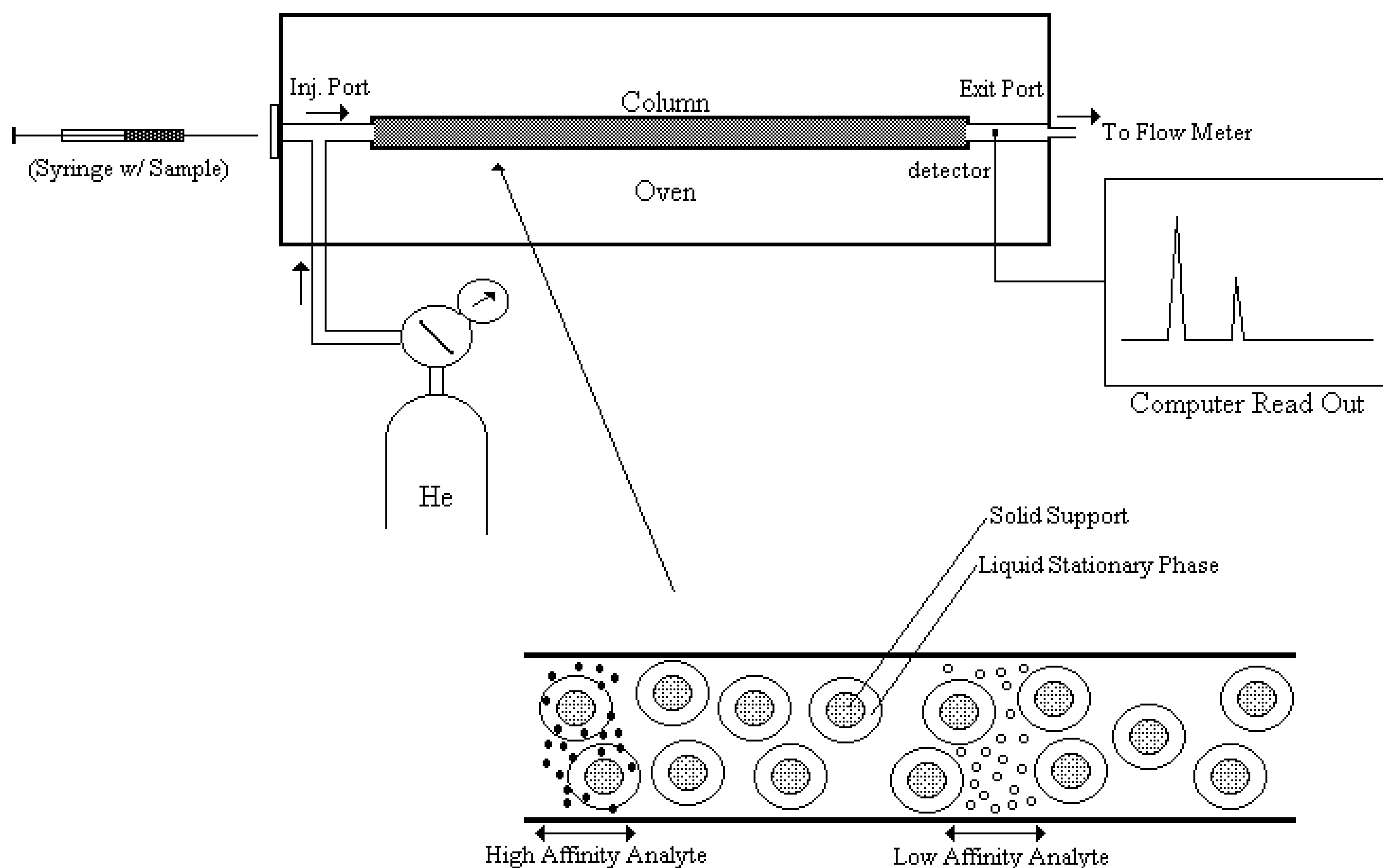
Some of the more common types of Chromatographic systems encountered in the Organic Laboratory are:

- Paper Chromatography
- Thin Layer Chromatography
- Column Chromatography
- Gas-Liquid Chromatography

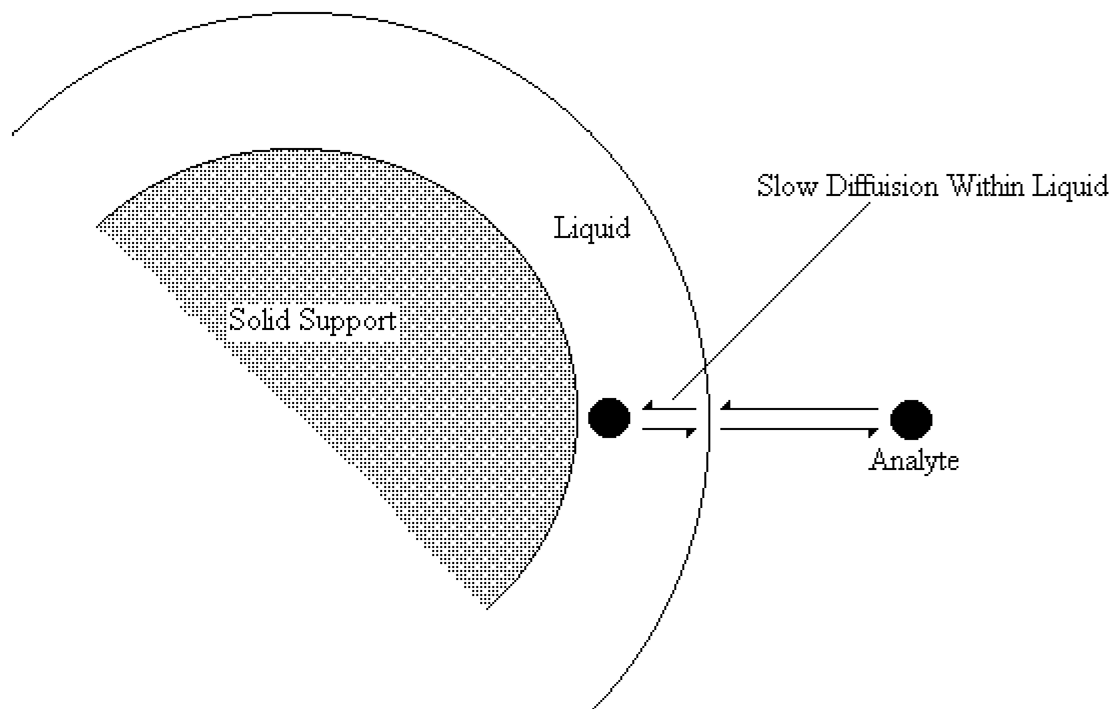
Features of a Chromatographic Column (http://en.wikipedia.org/wiki/File:P1000495.JPG)

We will employ the last three types of chromatographic separations during this laboratory course.

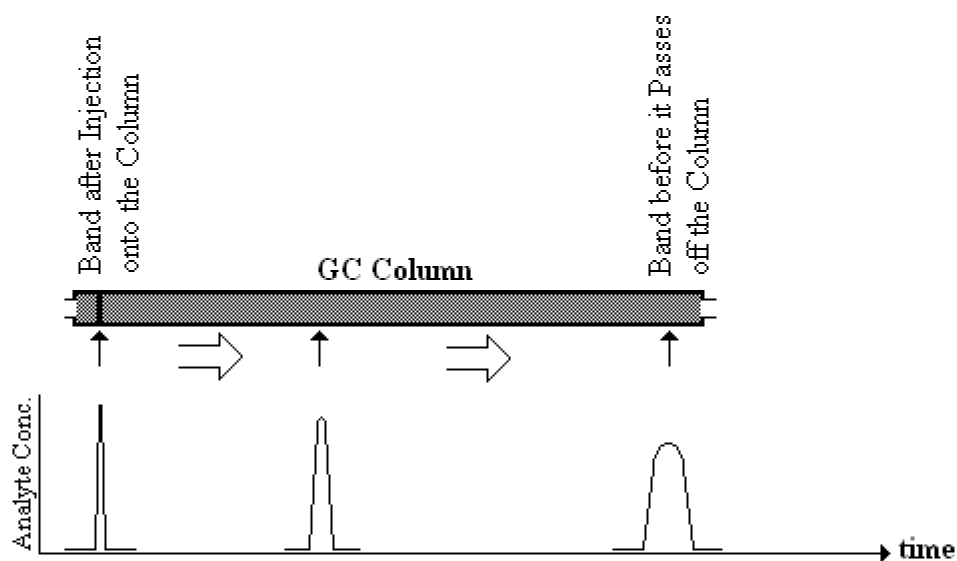
Gas-Liquid Chromatography, the most common type of Gas Chromatography, employs a Gaseous mobile phase and a Liquid stationary phase. The mixture to be analyzed is injected into a heated Injection Port where it is vaporized. The resulting vapor then mixes with an inert Carrier Gas, which is typically Helium (He). This is the mobile phase. The carrier gas then flows through a heated column filled with support beads coated in an appropriate liquid; the stationary phase. The separation occurs while the mixture moves along the column. The separated species then pass out of the column and into an Exit Port where they pass through a detector. The detector then sends a signal indicating the presence of the analyte to an appropriate Read Out device, usually a computer.



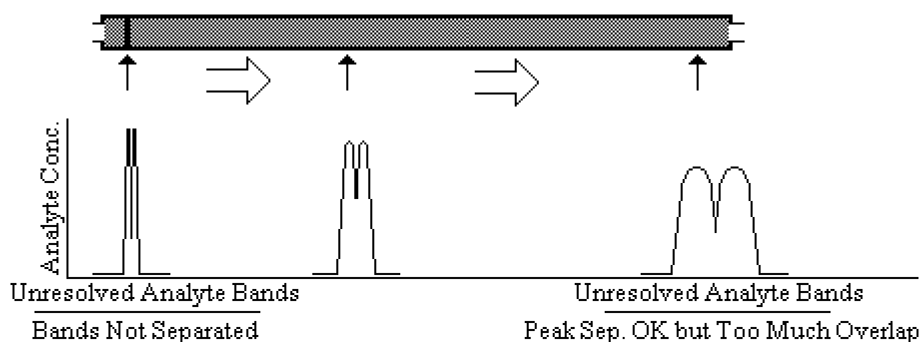
Components with a High Affinity for dissolving in the liquid stationary phase will tend to move slowly through the system; they are constantly being partitioned between the gaseous and liquid phases. Those components with a Low Affinity for the liquid support will tend not to be absorbed by the liquid and will move along fairly rapidly with the carrier gas. Thus, separation of the components in a mixture is dependent upon how well they partition and dissolve in the liquid stationary phase. This is a type of Partition Chromatography because the analyte dissolves in, and migrates into, the liquid stationary phase.



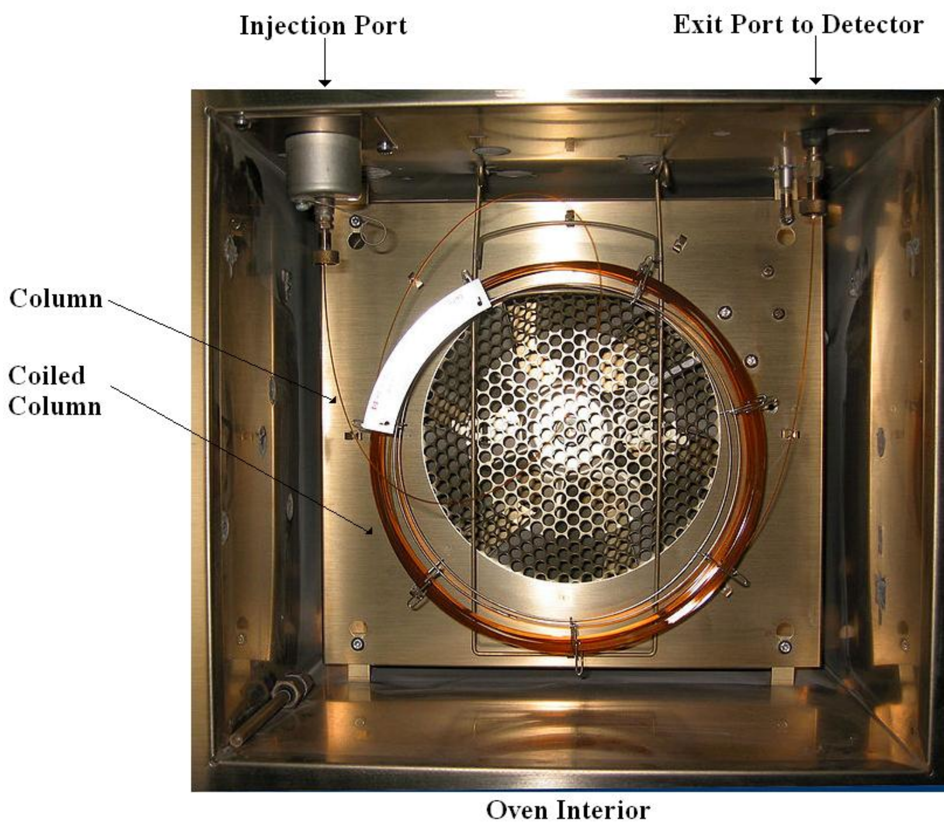
This is important to understand because the partitioning of the analyte molecules between the gas and liquid phases leads to a differential lag time for each of the molecules of a given component as they pass through the system. As the band of molecules passes through the system, each molecule will spend a different amount of time within the liquid phase. This tends to broaden the band of molecules as it moves along. Other factors that tend to increase Band Broadening are the multiple pathways molecules may take through the system, diffusion in the mobile phase, etc.



Band Broadening directly affects the Resolution of analyte bands as they exit the system. Two closely spaced bands will begin to separate and resolve themselves as they pass along the column. However, while passing through the system the bands begin to broaden, and this may cause a significant overlap of closely spaced bands, thereby reducing the Resolution of these analytes.



The efficiency of Packed Columns increases considerably as the size of the solid particular support decreases. As an extreme, Open Tubular Columns, an open tube coated on the inside with the liquid phase, provide a high resolution alternative to packed columns. These columns are more expensive and more fragile to use than packed columns. However, because of much improved construction techniques, open tubular columns are now the most popular type of GC column in use. They can be constructed as capillaries as thin as 0.32 – 0.25 mm with lengths as long as a 100 meters. They are typically tightly coiled to fit within the confines of the GC oven.



Oven Interior

The trick in any chromatographic separation is to select a liquid stationary phase, an oven temperature, and a carrier gas flow rate so that all the analyte bands are fully resolved in a minimum amount of time. These selections are frequently empirically based, as much art as science, and are sometimes contradictory.

In general, the polarity of the liquid used for the stationary phase should be consistent with that of the analyte. Separating polar compounds will require a polar column liquid and separating non-polar compounds requires a non-polar liquid. Common stationary phases for Gas Chromatography are listed below:

Phase	Applications
Polydimethyl Siloxane	Non-polar phase; hydrocarbons, drugs, steroids, PCBs.
5% Phenyl-polydimethyl Siloxane	Fatty acid methyl esters, alkaloids, drugs, halogentated compounds.
50% Phenyl-polydimethyl Siloxane	Drugs, steroids, pesticides, glycols.
Polethylene glycol	Free acids, alcohols, ethers, essential oils, glycols.
50% Cyanopropyl-polydimethyl Siloxane	Polyunsaturated fatty acids, free acids, alcohols.

If the column and analyte polarities are reasonably consistent, then the order in which the analytes come off the column will be according to their boiling points.

Control of the GC oven's temperature is also critical for good resolution. As a rule of thumb, lower temperatures are better, however, this usually increases the time required for elution of the analytes. At a minimum, the temperature should be set just slightly higher than the boiling point of the analyte. A programmed temperature sequence may be required to separate many analyts. This starts with a fairly low temperature and then ramps up to elute the higher boiling components of the mixture.

Although GC chromatography is most commonly used to provide a qualitative analysis of a mixture in question, quantitative information about the relative composition of the mixture can be obtained from the chromatogram. In generic GC systems, as the analyte passes into the exit port, it then passes through a detector that responds to the presence of the analyte. Most detectors in these systems are non-specific; they respond to the presence of the analyte but do not identify the analyte itself. The detector response is then sent to a readout device which produces a peak representative of the analyte concentration. We expect:

$$\text{Peak Area} \sim \text{Concentration of Analyte} \qquad \text{(Eq. 1)}$$

The relative concentrations of the different analytes in the mixture can be determined by comparing their different peak areas. And, most computerized readout systems are capable of integrating the analyte peaks to provide the needed peak areas. But, these non-specific detectors

do not respond to all analytes equally; a given concentration of one analyte may produce a larger detector response than an equivalent concentration of another analyte. Therefore, we must determine each analyte's detector Response Factor, f_i:

$$A_i \sim f_i C_i \qquad \text{(Eq. 2)}$$

where A_i is the peak area and C_i is the concentration. Typically, this is done by performing an analysis on a Standard Solution where the concentrations of the analytes are known. The Relative Response Factor of component *i* to *j* (f_i/f_j) is then determined:

$$(C_i / C_j) = (A_i / f_i) / (A_j / f_j) \qquad \text{(Eq. 3)}$$

Once the Relative Response Factors are known, the relative concentrations of the analytes in the unknown mixture can be determined from their relative peak areas.

For our analysis we will be using a basic Shimadzu GC-17A system:

This includes ovens for the injection port, detector and column. The exit port is coupled to a Flame Ionization Detector (FID). The FID works by burning the eluted gas from the column in an H_2 and Air mixture. This produces CH species in proportion to the number of Carbon atoms in the flame. These CH fragments then react with Oxygen atoms in the flame and produce electrons:

$$CH + O \longrightarrow CHO^{+} + e^{-} \qquad \text{(Eq. 4)}$$

These electrons then alter the current within the detector system. This current is then amplified and converted to a voltage for readout. The carrier gas for our system is Helium, the most common inert carrier gas used in these systems. Software controlling the Schimadzu GC is capable of programming the oven temperature for maximum resolution of the analytes in a mixture.

We will first analyze the fractions from our Fractional Distillation of Cyclohexane and Toluene. This involves initially running samples of the individual components, Toluene and Cyclohexane, through the chromatographic system. This allows us to determine Adjusted Retention Time (t_r') for each component; the time it takes the component to migrate through the system, or Retention Time (t_r), versus the time it takes the mobile phase to do the same (t_m):

$$t_r' = t_r - t_m \qquad \text{(Eq. 5)}$$

Once the retention times are known, we will be able to identify the appropriate peaks in a chromatogram of a mixture. Then, we will run a 50:50 mixture of the hydrocarbons through the system. This will allow us to determine the Relative Response Factor of the detector for our components. Finally, we will run each fraction from the Fractional Distillation through the system, measure the appropriate peak areas and quantify how much of each component is present in the mixture. This will allow us to determine the efficiency of our Fractional Distillation for separating the Cyclohexane and Toluene.

We will then turn to the analysis of the Essential Oil produced during our steam distillation procedure. We will be somewhat more qualitative in our approach here. Our desire is to simply identify how many components comprise the Oil and their likely identity by comparison with appropriately selected standards.

Pre-Lab Questions

1. When 1.06 mmole of 1-Pentanol and 1.53 mmole of 1-Hexanol were separated by Gas Chromatography, they gave relative peak areas of 922 and 1570 units, respectively. When an unknown mixture of these two alcohols was likewise separated, the relative peak areas were 843:816 (Pentanol:Hexanol). What was the relative concentration of these species in the mixture?

2. Most GC columns are rated with an upper temperature. Why do you think this is?

Procedure

Your always helpful laboratory instructor will demonstrate the operation of the GC and how to inject a sample using the autosampler. Typical instrument parameters you should note are:

Column Type
Injection Port Temperature
Column Temperature
Detector Temperature
Detector Type
Carrier Gas and Flow Rate

Collectively the class should run samples of:

1. Cyclohexane
2. Toluene
3. 50:50 mixture of Cyclohexane-Toluene
4. Essential Oil standards. (These will be selected by your instructor)

Analyze these results for the retention times for each pure compound. Determine the relative detector response for Cyclohexane *vs*. Toluene; f_{tol}/f_{cyclo}.

Individual samples should be run of:

1. Each fraction from the Fractional Distillation.
2. Essential Oil

Determine C_{tol}/C_{cyclo} for each Fractional Distillation fraction. Determine the number of, and probable identity of, the components in the Essential Oil.

Post Lab Questions

The following questions will require a short search of sources such as Wikipedia or a good Instrumental Analysis book.

1. What is a Theoretical Plate, in the context of Gas Chromatography? How many theoretical plates is possible for a typical open tubular column?

2. What is Gas-Solid Chromatography and why is it less used than Gas-Liquid Chromatography?

3. What is a Thermal Conductivity Detector and how does it work?

4. If you injected a wet sample of an otherwise pure compound onto a gc column connected to a flame ionization detector, only a single peak would be recorded. Why?

5. Extremely narrow bore open tubular columns require a "splitter" at the injection port. What does the splitter do and why is it used?