Gas Chromatography - Mass Spectrometry

After this module you will be able to:

- Identify the components of a GC/MS instrument
- Describe the path molecules take from injection to detection
- Interpret GC/MS spectra

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Lesson 1: Introduction to GC/MS

GC/MS is a tandem technique meaning it is made up of two different instruments that each accomplish a different objective. The first instrument is gas chromatography (GC) which separates an analyte, the sample of interest, based on boiling point and molecular weight. The second instrument is a mass spectrometer (MS) which measures the mass-to-charge ratio (m/z) of an analyte. These two instruments are attached to form a closed system such that the sample travels from GC to MS without any interruption. A GC/MS is shown below in figure 1. The instrument on the right hand side is the gas chromatograph which will interact with the sample first. The sample will then travel directly into the MS which is on the left hand side.

Lesson 2: Gas Chromatography

Figure 1. GS/MS instrument

Principles of Chromatography

Chromatography is the process of separating a mixture into its components. There are numerous different types of chromatography with different methods proving to be more effective for different types of mixtures. The simplest type of chromatography can be seen by a marker line separating on a piece of filter paper as shown in figure 2. In this example, the ink of a brown marker is separated into its constituent colors. The ink is dissolved in a water/ethanol solution and it travels up a silica gel surface. This chromatography example showed that the brown marker is actually a mixture of at least three different colors.

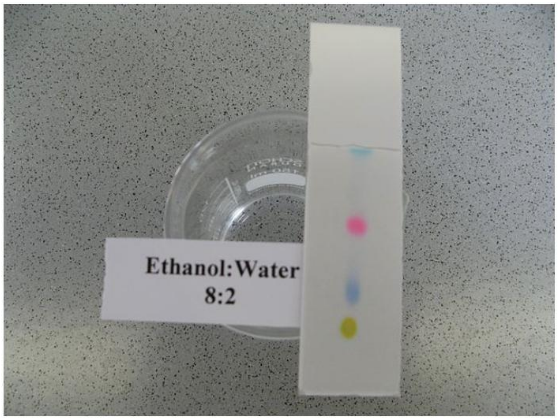

Fig. 2. Separation of ink components in a brown marker.

Chromatography is made up of two phases: the mobile phase and the stationary phase. The mobile phase contains the analyte being studied and carries it through the stationary phase which is made up of a different substance. The components travel through the stationary phase at different rates depending on specific properties. The longer it takes a substance to travel through the stationary phase, the longer its retention time.

Gas Chromatography

In gas chromatography, the mobile phase is an inert gas such as nitrogen or helium gas which ensures that the carrier gas will not react with the analyte. The stationary phase is a thin layer inside of a metal or glass column. The thin layer dictates adsorption/desorption which affects the retention time. The following steps describe how a sample travels through a GC:

1. Sample is dissolved in a solvent and injected into the GC/MS.
2. Sample is vaporized to high temperatures (up to 450ºC) and mixed with a carrier gas (N_2 or He).
3. Carrier gas moves the analyte through the stationary phase in the form of metal or glass column. The metal or glass column is coiled to increase the length of the column while keeping the instrument relatively small.
4. The analyte begins to separate into its component parts as it travels through the column.
5. The compounds leave the GC and will be detected by detectors, such as mass spectrometers (MS) or flame ionization detectors (FID).

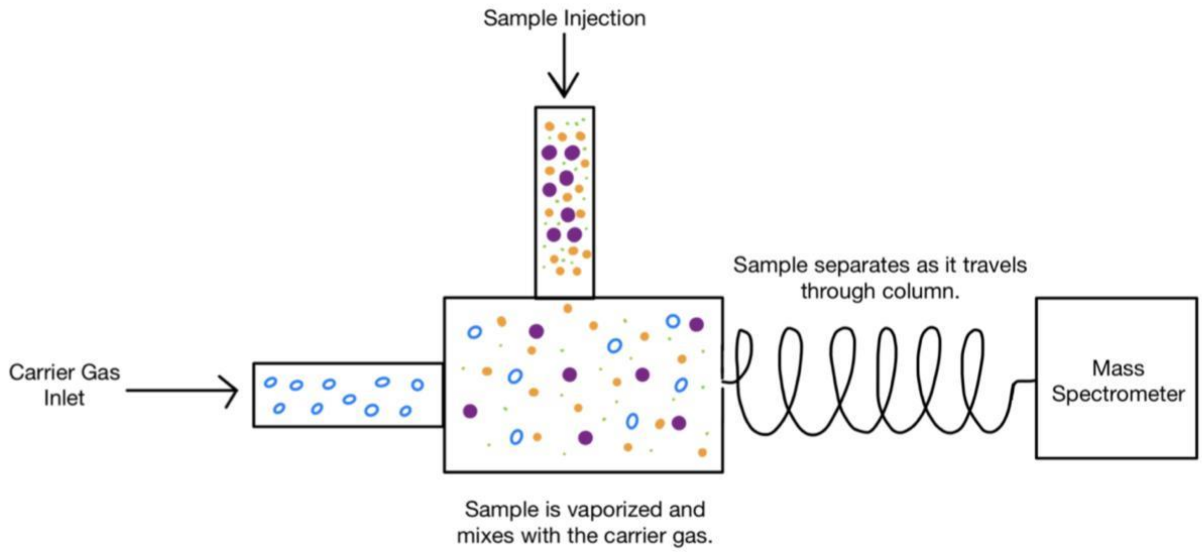

Fig. 3. Schematic of GC/MS,

Additional Resources

[Chemistry LibreTexts: Gas Chromatography]
[Video: Gas Chromatography] - (1:50 molecules are shown separating in a column)
[Khan Academy: Gas chromatography]

Lesson 3: Mass Spectrometry

Mass spectrometry is a technique that measures mass-to-charge ratios (m/z). It is made up of three parts: ion source, mass analyzer, and detector. There are numerous types of ion sources that all have their strengths and weaknesses for different experiments. The ion source that is most commonly used with gas chromatography is electron ionization (EI). In electron ionization, high energy electrons interact with the analytes in the gaseous phase under a vacuum. This interaction causes at least one electron to be expelled from the molecule. An example of this is show in the reaction below:

$$M \quad + \quad e^- \quad \rightarrow \quad M^{+\bullet} \quad + \quad 2e^-$$

Where M is the molecule in its gaseous state, e^- is a high energy electron, and $M^{+\bullet}$ is the ionized molecule after an electron is expelled.

In addition to expelling one electron from the parent molecule, Electron ionization can also cause molecules to fragment into smaller species. These pieces create a fragmentation pattern which can also be used to help identify the molecule. A visual of electron ionization is shown in figure 4. After the sample is ionized, positively charged plates repel ion towards the mass analyzer as shown in figure 5.

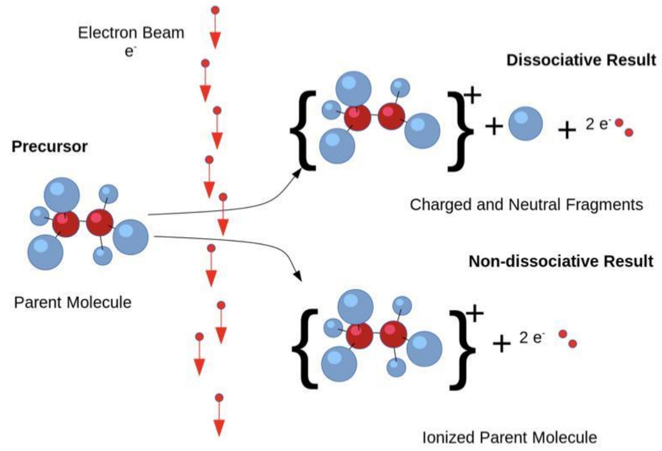

Fig. 4. Electronic ionization of a parent molecule.

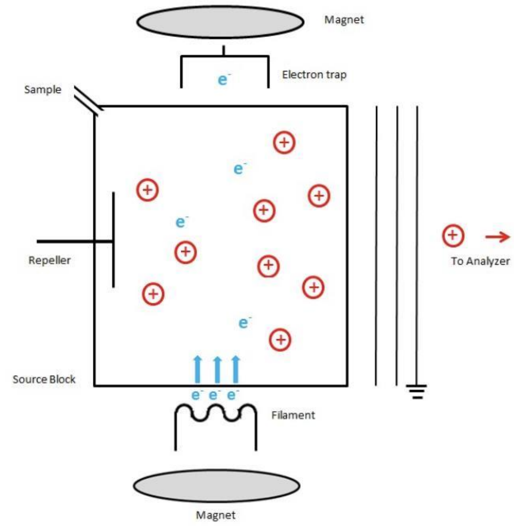

Fig. 5. Electronic ionization of molecules.

Similar to ionizers, there are a number of different mass analyzers available. The mass analyzer used in this research laboratory is a quadrupole mass analyzer. In a quadrupole mass analyzer, the ions enter an electric field created by four electromagnets. The quadrupole is directed by the computer to stabilize one m/z ratio at a time. When the electric field stabilizes an m/z ratio, molecules with that m/z ratio will continue to travel through the quadrupole and hit the detector while all other species will bounce off the rods and not be detected as shown in figure 6 below. By scanning a range of frequencies, the mass analyzer can determine the relative population of species with a specific m/z ratio in a sample. The final component, the detector, uses a computer program to convert the impulse created by molecules into two spectrums: abundance vs. retention time and abundance vs. m/z ratio which is the fragmentation pattern of each species.

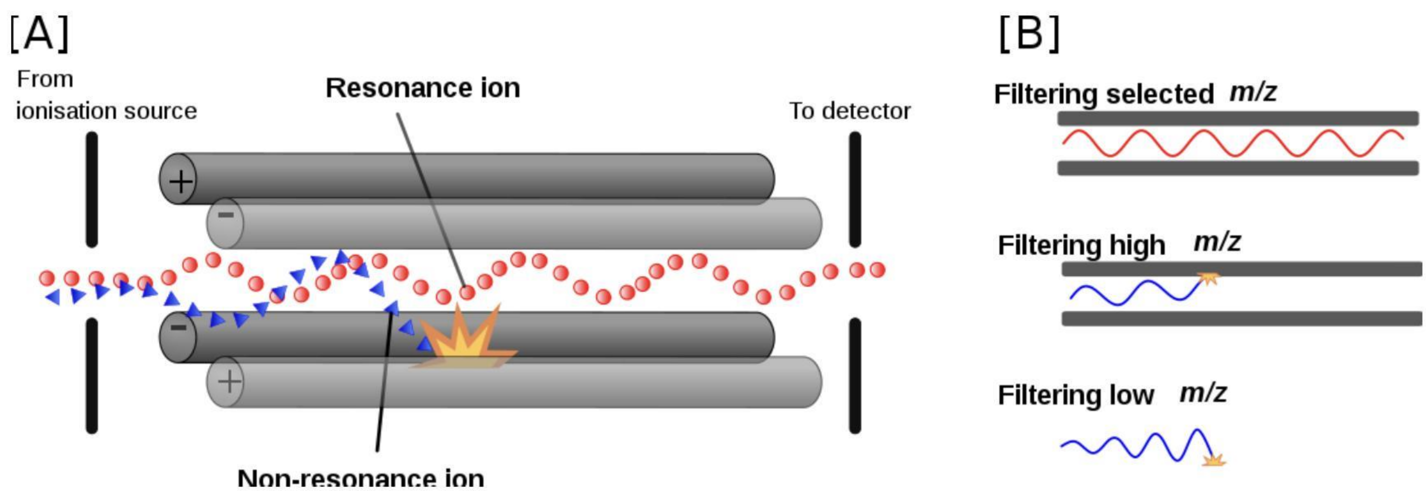

Fig. 6. [A] The four electrodes create an electric field of varying frequency. The m/z ratio of the red molecules match the frequency so they travel to the detector. [B] The effect of too high m/z and too low m/z are show. .

Additional Resources

[Chemistry LibreTexts: Mass Spec]
[Video- Electron Ionization Animation]
[Video- Quadrupole MS]

Check Quiz: GC/MS Theory & Instrumentation

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1. Every chromatography technique is made up of two phases. Which phase contains the analyte being studied?
   a. Mobile phase
   b. Stationary phase
   c. Solid phase
   d. High-temperature phase
2. Retention time is defined as the amount of time the sample...
   a. is stored before testing
   b. spends in the reaction vessel
   c. takes to reach the GC oven temperature
   d. spends in the GC column
3. Which of the following gaseous molecules should not be used as a carrier gas?
   a. N_2
   b. O_2
   c. He
   d. Ar
4. In gas chromatography, where in the instrument does separation occur?
   a. Sample injection port
   b. Oven where sample is vaporized
   c. Column made of metal or glass
   d. Mass spectrometer
5. Mass spectrometry is a technique used to measure the _____________ of a sample.
   a. Molecular weight
   b. Molecular charge
   c. Mass to charge ratio
   d. Boiling point
6. In electron ionization, what is used to ionize a sample?
   a. Laser light
   b. Accelerated hydrogen atoms
   c. Charged water molecules
   d. High-energy electrons

Lesson 4: Interpreting GC/MS Spectra

A GC/MS will produce two different spectra. The first is a GC chromatogram which plots relative abundance vs. retention time. The GC chromatogram shows all of the components in the mixture. The second spectra is a fragmentation pattern produced by the mass spectrometer. The MS will produce a fragmentation pattern for each of the molecules in the mixture.

GC Chromatogram

The X-Axis: Retention Time

In a GC/MS spectra, the retention time is plotted along the x-axis. The retention time is the amount of time a molecule spends in the column. Each peak of a GC/MS chromatogram represents an individual compound separated from the mixture. Usually smaller molecules with lower boiling points will have shorter retention times but other molecular features may affect retention time. Additionally, there are a number of experimental factors that can impact retention time such as column type, carrier gas flow rate, type of carrier gas, injection temperature, and oven temperature. Therefore, when comparing retention times between experiments, proceed with caution as one change in experimental designs can alter the retention time.

The Y-Axis: Abundance

The y-axis represents the abundance of each molecule in a mixture. The area under the graph represents the relative amount of each component.

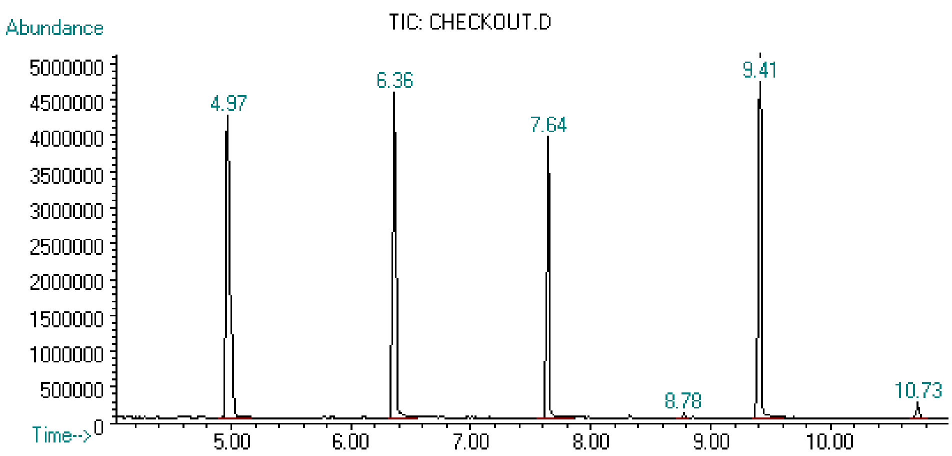


Fig 7. Chromatogram generated by MS. (source: gmu.edu)

Fragmentation Patterns

In addition to the chromatogram produced by the GC, , mass spectrometers are capable of producing fragmentation patterns. When the parent molecule comes into contact with the high-energy electrons, it will fragment into a number of smaller charged molecules. Each of these smaller molecules is detected and projected on a graph of abundance vs. m/z ratio. The fragmentation pattern of a molecule remains fairly constant and so it essentially creates a fingerprint of that molecule that can be used to help identify it. Figure 8 shows the fragmentation pattern of acetone. As you can see in the image, there are three major fragments. It is also important to note the M+1 peak which represents the mass of ^{13}C, an isotope of carbon with an abundance of 1.1% of naturally occurring carbon.

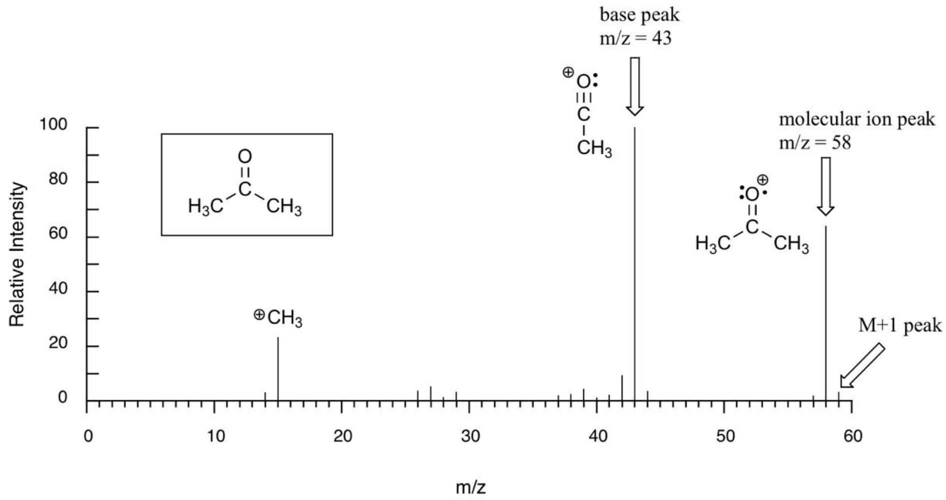


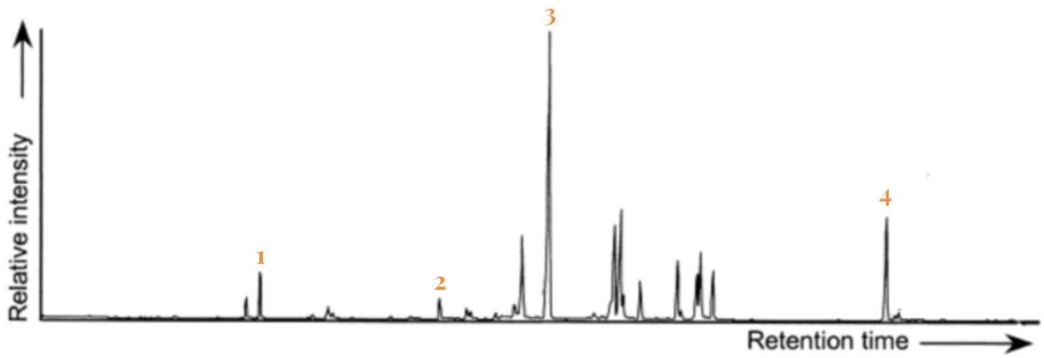
Figure 7. Spectra produced by the mass spectrometer.

Additional Resources

[Chemistry LibreTexts: Fragmentation Patterns in Mass Spectrometry]
[AIST: Spectral Database]

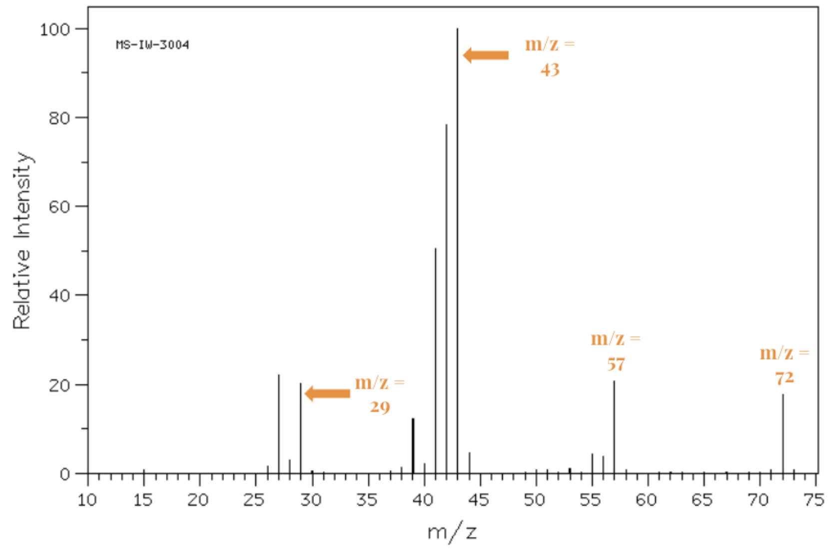
Check Quiz: Interpreting GC/MS Spectra

Using the chromatogram shown, identify which of the numbered peaks that most closely matches the description.



1. The compound is the greatest abundance. __________
2. The compound with the longest retention time. __________
3. The second compound to elute from the column. __________
4. A minor product of the reaction. __________

Use the mass spectrum for pentane shown below, to answer the following questions.



1. If the m/z = 72 peak represents the ionized pentane molecule $[CH_3CH_2CH_2CH_2CH_3]^+$, what ion is most likely represented by the highlighted m/z peaks?
    a. m/z = 57 __________________
    b. m/z = 43 _________________
    c. m/z = 29 _________________

Answer Key

Check Quiz: GC/MS Theory & Instrumentation

1. **Answer: A;** Chromatography techniques have a mobile phase and a stationary phase. The analyte is contained in the mobile phase. It is separated into its components as it travels along the stationary phase.
2. **Answer: D;** Retention time is the time the analyte spent separating into its components. The sample is separated as it moves through the GC column.
3. **Answer B;** The carrier gas should be an inert gas that will not react with the analyte being studied.
4. **Answer C;** The column contains the stationary phase.
5. **Answer C;** The mass-to-charge ratio is measured using an MS. If the charge of the ion is +1 then this value is equal to the mass.
6. **Answer D;** Electron ionization uses high energy electrons to dislodge electron(s) from the analyte.

Check Quiz: Interpreting GC/MS Spectra

1. **Compound 3;** The area under peak is the greatest. The y-axis measures relative intensity. Typically the higher the intensity the greater the concentration of that compound.
2. **Compound 4**; Retention time refers to the amount of time the compound spent in the column. The x-axis measures retention time.
3. **Compound 1**; This is asking you to identify the compound with the second smallest retention time out of all the compounds present. Compound 1 is the second peak along the x-axis.

4. **Compound 2**: The area under the peak is the smallest when compared to the numbered peaks. The y-axis measures relative intensity. Typically the lower the intensity the smaller the concentration of that compound.

1. This question is asking you to determine the fragmentation patterns created by the mass spectrometer.
    a. $[CH_3CH_2CH_2CH_2]^+$; A methyl group fragmented from the parent ion.
    b. $[CH_3CH_2CH_2]^+$; An ethyl group fragmented from the parent ion.
    c. $[CH_3CH_2]^+$; A propyl group fragmented from the parent ion.