Mass Spectrometry for the Absolute Novice

WHAT IS A MASS SPECTROMETER?

- Mass spectrometers weigh atoms and molecules.[1]
- A mass spectrometer produces charged particles (ions) from the chemical substances that are to be analyzed.
- The electric charge is like a "handle" that allows the electric and magnetic fields to move the charged particles around so that we can measure the mass ("weight")[1] of the charged particles.

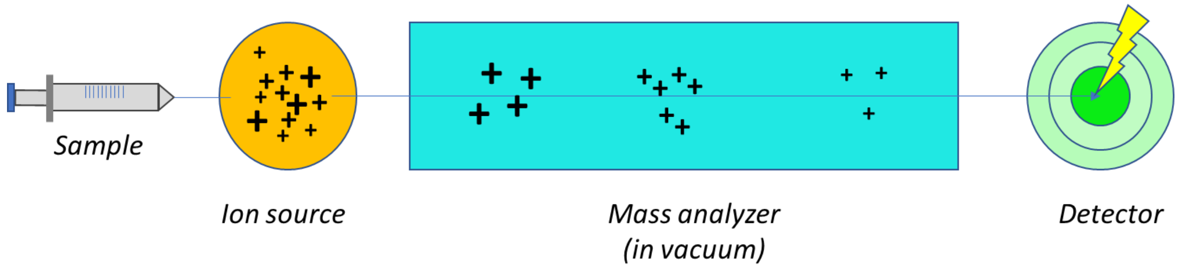
Atoms that make up chemical compounds have characteristic masses. Mass spectrometers use electric and magnetic fields to measure the masses of atoms and molecules.

WHAT ARE MASS SPECTROMETERS USED FOR?

Mass spectrometers are used to determine what chemicals are present in a sample ("qualitative analysis"), and also how much of each chemical is contained in a sample ("quantitative analysis"). Mass spectrometers have many applications in a wide range of fields including forensics, environmental analysis, biology, quality control and troubleshooting, space exploration, and art conservation.

HOW DO MASS SPECTROMETERS WORK?

All mass spectrometers have at least three components: (1) an ion source, (2) a mass analyzer, and (3) a detector. The ion source makes charged particles that are separated by the mass analyzer, and the detector records the information that is sent to the computer to be interpreted.



The three components found in all mass spectrometers

The ion source

The ion source creates electrically charged particles ("ions") from the atoms and molecules in the sample. There are many different kinds of ion sources – some operate in a high vacuum, and some create ions at atmospheric pressure. The different sources will be described in more detail in a separate article. Ions can be made from gases, liquids or solids, but the ions are always introduced into the mass analyzer in a gaseous state.

How do we make ions?

There are many ways to make ions. In a vacuum, some common ion sources are based on:

- High-energy **electron beam** (Electron ionization: **EI**)
- High-energy **electron beam in gas with chemical reactions** (Chemical ionization: **CI**)
- **Ultraviolet light** (Photoionization: **PI**)
- **Laser beam** striking the sample (Matrix-assisted laser desorption: **MALDI**)

You can also make ions at atmospheric pressure and transfer them into a vacuum to be separated in the mass analyzer. For the purposes of this basic discussion, we will only consider electron ionization (EI).

The mass analyzer

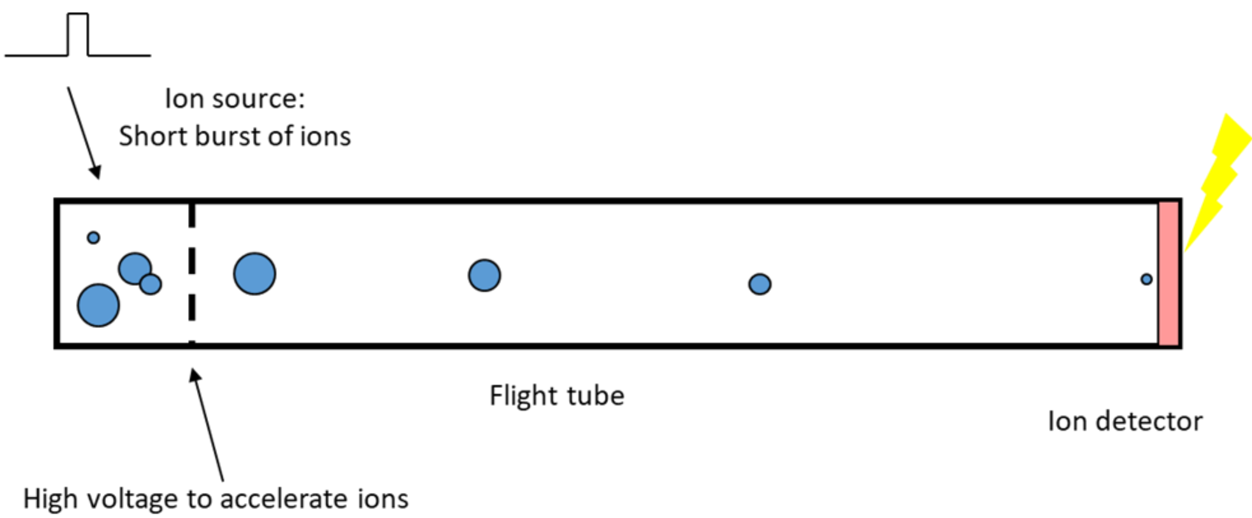
Sample ions are always introduced into the mass analyzer in a vacuum[2]. If the sample was not in a vacuum, the charged particles would bump into gas molecules, and we could not move them effectively with the electric and magnetic fields. The mass analyzer actually separates ions by their mass-to-charge ratio, symbolized as "*m/z*" (in italics), but for the purposes of this discussion, let's assume that all of the ions have a single charge. There are many different kinds of mass analyzers. The most common types are quadrupoles, magnetic sectors, time-of-flight mass spectrometers, and trapped-ion mass spectrometers.

Example: a time-of-flight (TOF) mass analyzer

The easiest mass analyzer to understand is the time-of-flight (TOF) A time-of-flight mass spectrometer uses electric fields to separate ions with different masses. You can think of it as a race track where the smaller particles move faster than the larger particles – like a sprinter racing a sumo wrestler. Both might have equal energy, but the smaller person will move faster than the larger person.

Here's another way to think about it: you can throw a baseball faster than you can throw a bowling ball.

In the TOF analyzer, a short electrical pulse is used to start the ions traveling down a tube. The smallest ions (the ones with the lowest mass) move faster than the larger ions, so they arrive at the detector first. The time it takes for an ion to travel down the "flight tube" is related to the mass of the ion.



A simplified diagram of a time-of-flight mass spectrometer.

In simplified physics terms:

If all of the ions have the same charge and are accelerated with the same electrical potential V, they will all have the same kinetic energy E_k.

$$E_k = qV$$

But kinetic energy is

$$E_k = \frac{1}{2}mv^2$$

So, if all of the ions have the same kinetic energy (qV is constant), ions with a larger mass must move slower (v is small), and ions with a smaller mass must move faster (v is large). The flight time for a given mass is inversely proportional to the square root of the mass/charge ratio.

The physics for any mass analyzer can be derived from the Lorentz force:

$$F = \mathrm{m}a = \mathrm{q}(E + v \times B)$$

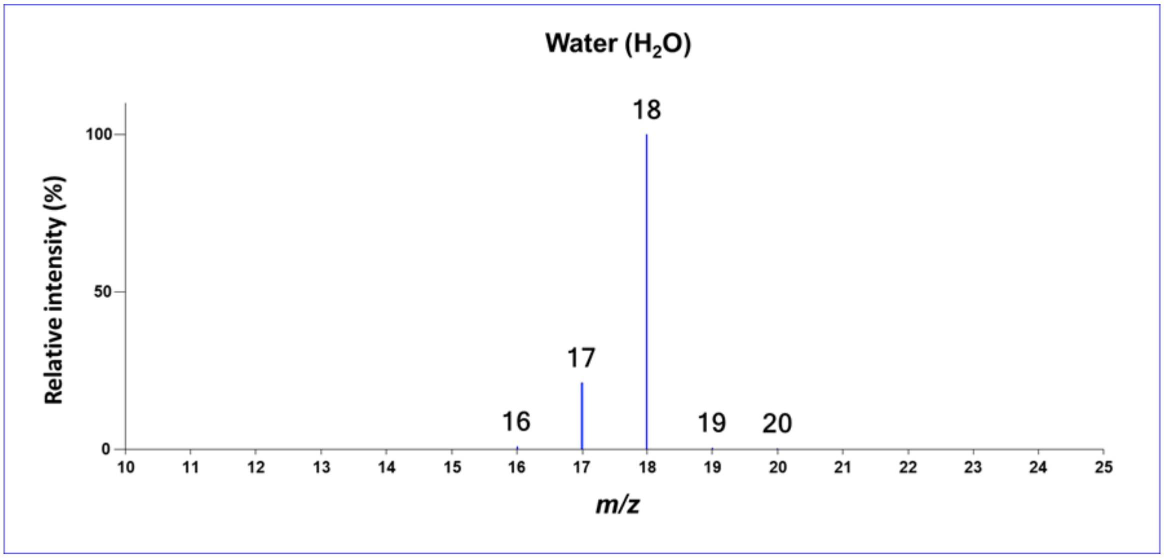
Where *F* is force, m is mass, *a* is acceleration, q is the electric charge, *E* is the electric field, and *B* is the magnetic field. Mass analyzers may use an electric field only, a magnetic field only, or both.

The detector

The detector records the signals from the ions and sends that information to the computer. In a time-of-flight mass spectrometer, the detector creates an electrical signal every time an ion strikes the detector. The information about the time the ion strikes the detector and the number of ions that strike the detector at each time interval is used by the computer to create the mass spectrum.

WHAT DOES A MASS SPECTRUM LOOK LIKE?

The mass spectrum is a graph that displays the mass-to-charge ratio (m/z) on the x-axis and the relative intensity (number of ions detected) on the y-axis. An electron ionization (EI) mass spectrum of water is shown below. We'll explain what this tells us in a minute.



An electron ionization mass spectrum of water.

WHAT DOES THE MASS SPECTRUM TELL US?

Mass

Atoms have characteristic masses that represent the amount of matter in an atom of each element. For example, the most common forms of carbon, hydrogen, and oxygen on earth have integer masses of 12, 1, and 16, respectively, but the masses aren't exactly integers[3].

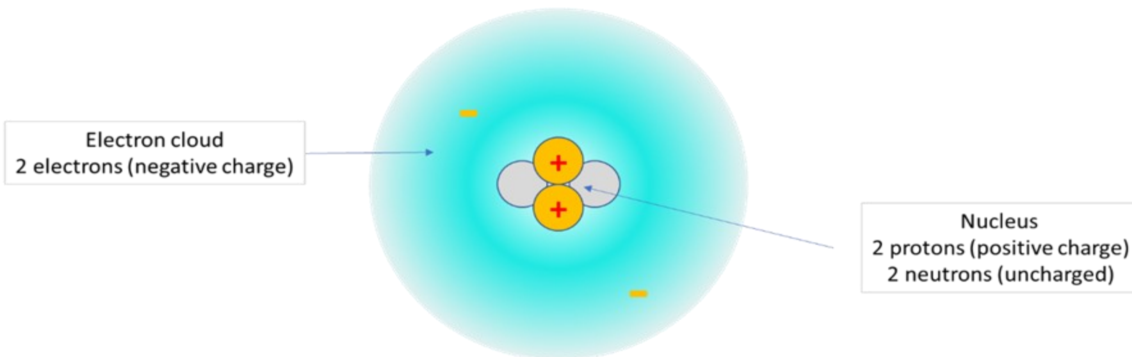
Carbon (^{12}C) has a mass of 12 (12.0000)
Hydrogen (^{1}H) has a mass of 1 (1.0078)
Oxygen (^{16}O) has a mass of 16 (15.9949)
Nitrogen (^{14}N) has a mass of 14 (14.0031)

Water (H_2O) has two hydrogen atoms and one oxygen atom, so the integer mass of water is

$$\text{Mass of water} = (2 \times 1) + (16 \times 1) = 18$$

We can see that the largest peak in the water mass spectrum is at *m*/*z* 18. What are the other peaks in the mass spectrum?

Isotopes



Helium Atom Diagram

2 protons (+): mass = 1.00783

2 electrons (-): mass = 0.00055

2 neutrons (0): mass = 1.00866

Nominal (integer) mass = 4

Exact mass = 4.002600

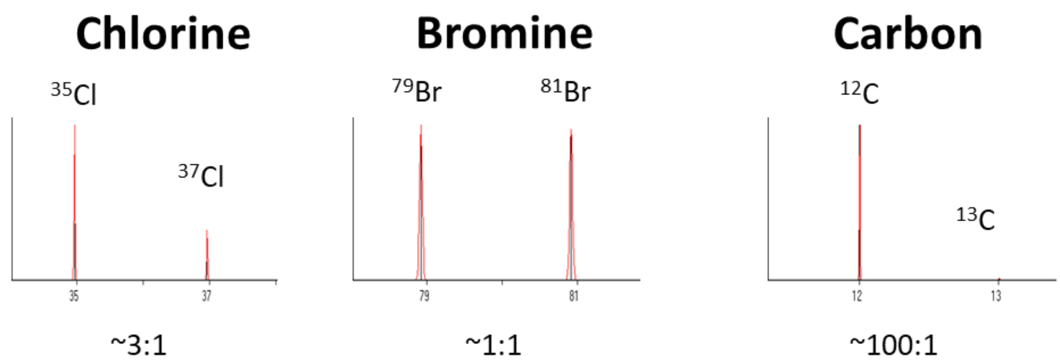
Charge: 2 protons (+) + 2 electrons (-) = 0 charge

The mass of the helium atom is approximately 4, but it isn't exactly an integer. The *exact mass* of the helium atom is 4.002600. The exact mass of the atom is smaller than the sum of the masses of the protons, neutrons and electrons because of the nuclear binding energy.

Not all atoms of a given element have the same number of neutrons, so some atoms of a particular element can have different masses. Atoms of an element that have different numbers of neutrons are called *isotopes*. Helium has another stable isotope, helium-3 (^{3}He), that has one proton and 2 neutrons and an exact mass of 3.016029. Helium-3 is very rare on earth, with a relative abundance of only about 0.000137%.

Most carbon on earth has 6 protons and 6 neutrons, so it has a mass of 12 (exactly). About 1.1% of the carbon on earth has 7 neutrons, and an integer mass of 6+7 = 13. Carbon-13 (^{13}C) atoms have an exact mass of 13.003355. The relative amounts of the different isotopes are additional information we can get from a mass spectrum.

Some elements, such as chlorine and bromine have very distinctive isotope patterns that are easy to recognize in a mass spectrum:



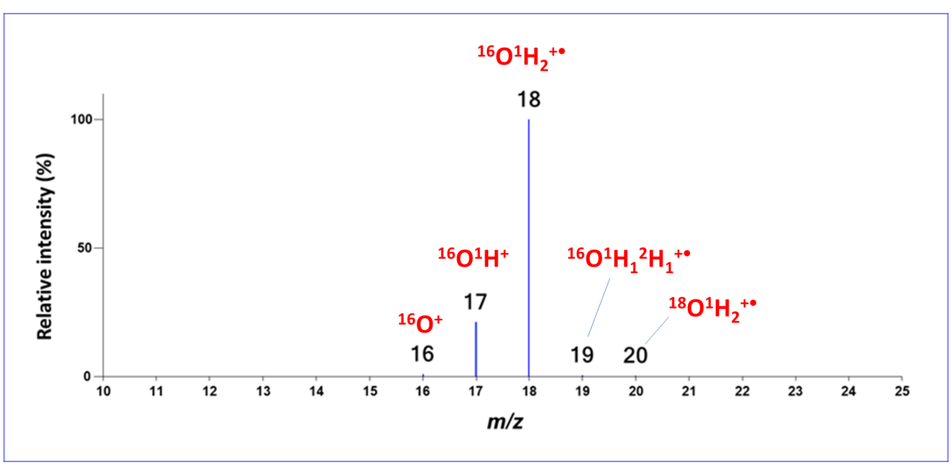
Isotope peaks for chlorine, bromine, and carbon

A tiny fraction of the hydrogen on earth has an extra neutron, giving it an integer mass of 2 (let's ignore the exact mass for now), and a fraction of the oxygen on earth has two extra neutrons, giving it a mass of 18. That's why we see small peaks at *m*/*z* 19 and 20 in the mass spectrum of water.

What are the other peaks at *m*/*z* 16 and 17?

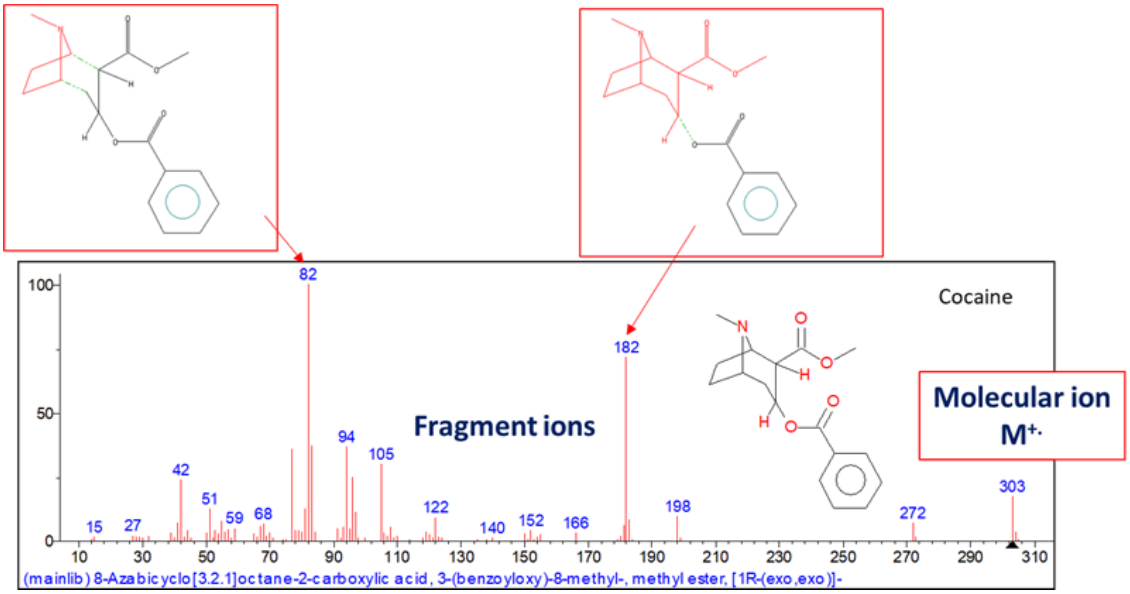
Fragment ions

As we explained above, an electron ionization source shoots a high-energy beam of electrons into a cloud of gas in a vacuum chamber. This knocks an electron off of the atom, creating an ion. If we knock an electron off of the helium atom in the diagram above, we have removed a negative charge. That means that we have two protons (+) and one electron (-), so there is a net positive charge. We show this with a plus sign, for example He^+. The electron beam doesn't have enough energy to break apart the nucleus of the atom, but it does have enough energy to break apart atoms in a molecule. Some of the water molecules were shattered when the electron beam hits them, so some of the ions are H_2O^+ (*m*/*z* 18), but some are OH^+ (*m*/*z* 17) and some are O^+ (*m*/*z* 16). Some are also H^+ (*m*/*z* 1), but they aren't shown in the water mass spectrum in the figure below.



Electron ionization mass spectrum of water with isotopes and fragments labeled.

Here's an EI mass spectrum for a more complicated molecule: a drug of abuse.



An electron ionization mass spectrum of a drug of abuse.

The unfragmented molecule ($C_{17}H_{21}NO_4$) is at m/z 303. This is called the **molecular ion**. The fragment ions represent different parts of the molecule. You can try to piece these "puzzle pieces" together to figure out the chemical structure. The most common way to identify molecules from EI mass spectra is to search databases of mass spectra to look for compounds with matching fragment patterns[3]. Databases are available with mass spectra for hundreds of thousands of chemical compounds.

High resolution and exact masses

The most common mass spectrometers can only separate and detect ions with integer masses, as shown in the mass spectra above. These are called "low-resolution" mass spectrometers. "High-resolution" mass spectrometers such as the JEOL time-of-flight mass spectrometers can separate ions that have the same integer mass, but different exact masses. Having information about the exact mass can be very useful. Here's an example:

Nitrogen (N_2^+) has an exact mass of 28.0061.

N: 14.003074

+ N: 14.003074

= N_2: 28.006148

The exact mass of carbon monoxide (CO^+) is 27.9949.

C: 12.0000000
\+ O: 15.99491464

= CO: 27.99491464

If you were given a gas with a mass of 28 and asked if you would be willing to inhale it, you might find yourself in the hospital if you made the wrong choice! However, if you were told that the gas has a mass of 28.0061, you'd be fine *as long as you only inhaled it for a very short time*.

Software programs can use the exact mass and isotope data from a mass spectrum to calculate the elemental composition of an unknown molecule. This can be very useful, especially if the compound you are trying to analyze does not have a mass spectrum in the databases.

WHAT ABOUT MIXTURES?

Mass spectrometers don't separate mixtures by themselves[6]. Mass spectrometers are often combined with equipment that separates compounds in mixtures before the compounds are introduced into the mass spectrometer. Gas chromatography (GC) and liquid chromatography (LC) are two of the most common separation methods that are combined with mass spectrometry in GC-MS and LC-MS systems. Compounds that are separated by a GC or LC are introduced into the mass spectrometer at different times. The mass spectrometer data system records a series of mass spectra as the different compounds are eluted from the chromatograph.

NOTES

[1] We really aren't measuring weight of course, because gravity isn't playing a role in the measurement. We're measuring mass (actually, the mass-to-charge ratio). Mass is independent of gravity.

[2] There is another kind of analyzer called an ion mobility spectrometer that uses electric fields to measure ions at atmospheric pressure, but that's a different topic.

[3] The exception is carbon. The exact mass of carbon is defined to be exactly 12 unified atomic mass units (u), and all other atomic masses are defined relative to carbon.

[4] Energy and mass are related -- think of the famous Einstein equation $E = mc^2$.

[5] Database searching isn't always enough, because not every molecule has a mass spectrum in a database, and some molecules have similar fragmentation patterns. The best approach is to combine all of the information you can get about the molecule, including database searching, to identify an unknown.

[6] There's a method called tandem mass spectrometry (MS/MS) that can separate mixtures if the compounds have different masses, but that's beyond the scope of this introduction.