Interpretation

Mass spectral interpretation is not a trivial process. Presented below are some basic terms and examples designed to provide a background on which to build further knowledge through additional research and study.

**A molecular mass spectrum**

A molecular mass spectrum is a plot of relative abundance of an ion vs. the m/z ratio of the ion (Figure 1). The most intense peak in the spectrum is often given a value of 100 and the intensity of smaller peaks are reported as relative percentages. Often times the heaviest significant m/z peak represents the molecular ion (m/z = 120 in Figure 1) and will provide the molecular weight of the analyte molecule. However, the molecular ion peak may not be the most abundant and at times may not even appear in the spectrum. The base peak is the ion with the highest abundance (m/z = 105 in Figure 1), suggesting this is the most stable ion or ion fragment produced during ionization of the analyte. Analysis of the mass spectral pattern can provide structural information about the molecule and can be compared to national databases to confirm the identity of the analyte.

![Acetophenone](image)

**Figure 1.** Mass spectrum of acetophenone with a molecular weight of 120. Fragmentation of a CH₃ results in the transition from the molecular ion (m/z=120) to the base peak (m/z=105). Further fragmentation of a C=O leads to a shift from 105→77. Using this fragmentation pattern caused by the excess energy in the EI source can help lead to identification of an analyte.
The mass spectrum of acetophenone in Figure 1 is characterized by a molecular ion peak of m/z=120 corresponding to the C<sub>8</sub>H<sub>8</sub>O<sup>+</sup> ion. The base peak (the largest relative abundance) is found at m/z=105 and is likely due to the loss of a methyl group during ionization resulting in the ion C<sub>7</sub>H<sub>5</sub>O<sup>+</sup>. Other peaks provide structural information as well.

Further examination of the mass spectrum of acetophenone shows a small peak at 121, which is higher than its molecular weight of 120. The 121 peak occurs because carbon found in nature has two primary isotopes; carbon-12 (about 99% abundant) and carbon-13 (about 1% abundant). In some acetophenone molecules, one of the eight carbon atoms will be a carbon-13 isotope, and this molecule will weigh 121 instead of 120. It will even be possible to have an acetophenone with two carbon atoms that are C-13 instead of C-12, which would have a weight of 122, but the probability of this species is quite low.

Another example of isotopic abundance can be seen using the mass spectral analysis of bromobenzene (Figure 2). Bromine is comprised of two different isotopes: 79<sup>Br</sup> and 81<sup>Br</sup>. The abundance of each of these isotopes is roughly 50%, though a more precise abundance can be found from several online databases. Because of this nearly equal isotope ratio, the average atomic weight for bromine is listed at 79.9amu. However, in a mass spectrum with decent mass resolution (at least 1 amu resolution) both isotopes are detected and displayed on a mass spectrum. Assuming that the isotopes have similar ionizability, comparing the relative abundance of the mass peaks will provide an isotopic ratio for the bromine.

The spectrum for bromobenzene in Figure 2 shows two significant peaks at m/z=156 and m/z=158 with a Δmass of 2 amu. The slightly more abundant peak at m/z=156 represents the ion that is comprised of the 79<sup>Br</sup> isotope while the other peak is due to the 81<sup>Br</sup> isotope.

![Figure 2. Mass spectrum of bromobenzene with a molecular weight of 157. The molecular ions at 156 and 158 represent species that contain either the lighter or heavier isotope of bromine, respectively, demonstrating that the natural abundance of each isotope is roughly 50%. The peak at 77 represents the loss of bromine during fragmentation to leave behind the C<sub>6</sub>H<sub>5</sub> ion.](image-url)
In addition to bromine, carbon isotopic analyses are often performed to determine the number of carbon atoms present in a molecule, and deuterium is often used in cases where reactions are monitored using isotopic labeling. Furthermore, an isotopically labeled molecule can serve as an excellent internal standard when performing quantitation using mass spectrometry. In a chromatographic separation, a non-labeled and isotopically-labeled compound will likely co-elute, but the mass spectrum will allow the quantitation of each because of the different masses of the non- versus isotopically-labeled compound. For example, GC-MS is commonly used for the analysis of polycyclic aromatic hydrocarbons (PAHs). One PAH often found in samples is chrysene (MW = 216). Chrysene-$d_{12}$ (MW = 228) is commonly used as an internal standard when analyzing PAHs by GC-MS.

Other examples where isotope analysis by mass spectrometry is employed include geological studies, forensic analyses, and food analysis. In one such study, Osorio and co-workers\textsuperscript{1} employed isotopic ratio analysis to determine whether beef cattle were fed pasture grasses, barley concentrate, or silage prior to processing. Their examination concluded that the ratio of $^{13}$C/$^{12}$C and $^{34}$S/$^{32}$S were the best predictor of pre-slaughter diet.

Fragmentation and its importance in determining structural information
Fragmentation occurs within certain ion sources provided the ionization process imparts significant energy to the analyte molecules. Due to differences in the electronic structure and stability of molecules, different fragmentation routes occur, which give rise to differing mass spectra. For example, $p$-nitrotoluene and 3-ethoxyaniline both have a molecular weight of 137. Both have some similar structural elements as well including a methyl group and aromatic ring. An examination of the two mass spectra in Figures 3 and 4 show that the molecular ion ($m/z = 137$) does not allow one to distinguish $p$-nitrotoluene from 3-ethoxyaniline. However, the fragment ions for the two compounds are quite different. Note that 3-ethoxyaniline has significant fragment ions at $m/z$ 80 and 109 whereas $p$-nitrotoluene has significant fragment ions at $m/z$ 91 and 107.
Figure 3. Mass spectrum of 3-ethoxyaniline with a molecular weight of 137.

Figure 4. Mass spectrum of p-nitrotoluene with a molecular weight of 137. The loss of the NO$_2$ is depicted in the 137→91 transition.

By using the fragmentation patterns one can elucidate the structure of each molecule, and determine which compound belongs to which spectrum. A soft ionization technique which only produces the molecular ion cannot accomplish this task. In an exact mass spectrum, the molecular ion would be able to distinguish between these two compounds, but exact mass measurements require expensive equipment.
**Parent and Daughter Ions**

It is also possible to attach two mass spectrometers in tandem as shown in Figure 5. A parent ion is the same as a precursor ion when discussing tandem mass spectrometry (MS-MS). After creation of ions in the ion source, they are separated by m/z in the first mass analyzer. Setting specific conditions in the first mass analyzer allows selection of ions with a single m/z. These ions can then undergo additional fragmentation before analysis in the second mass analyzer. The ion selected in the first mass analyzer is known as the parent or precursor ion.

![Figure 5. Schematic of a tandem MS-MS instrument.](image)

Further fragmentation of the parent ion takes place in a collision or reaction chamber. The ions extracted from the collision cell are called product ions or daughter ions.

**Citations used in this section include**