Mass Spectrometry: Isotope Effects

This module focuses on the effects of isotopes on mass spectra. It also addresses practical applications of isotope measurement using a mass spectrometer.

Introduction

The ability of a mass spectrometer to distinguish different isotopes is one of the reasons why mass spectrometry is such a powerful technique.The presence of isotopes – a presence that is ubiquitous in nature – gives each fragment a characteristic series of peaks with different intensities.These intensities can be predicted based on the abundance of each isotope in nature, and the relative peak heights can also be used to assist in the deduction of the empirical formula of the molecule being analyzed.

Isotopes

An 'isotope' of any given element is an atom with the same number of protons but a different number of neutrons, resulting in a different overall mass. Almost all elements have a variety of naturally occurring isotopes – some notable exceptions are fluorine, phosphorous, sodium, and iodine. For example, Carbon has 6 protons. In its most common isotope, it also has 6 neutrons, with a total number of protons +neutrons of 12 (called the 'Mass Number'). This carbon atom, with a mass number of 12, is written: ^{12}C. Carbon has two other naturally occurring isotopes, ^{13}C and ^{14}C, which have 7 and 8 neutrons, respectively. Table 1 below gives the most abundant naturally occurring isotopes for a variety of important elements. The abundance for secondary isotopes is reported in number of atoms of secondary isotope for every 100 atoms of the most abundant isotope[1].

Element	Most Abundant Isotope	Secondary Isotope	Abundance/100 atoms of Primary Isotope
Hydrogen	^{1}H	^{2}H	0.015
Carbon	$^{1}2C$	^{13}C	1.080
Nitrogen	^{14}N	^{15}N	0.370
Oxygen	^{16}O	^{17}O	0.040
		^{18}O	0.200
Sulfur	^{32}S	^{33}S	0.800
		^{34}S	4.400
Chlorine	^{35}Cl	^{37}Cl	32.50
Bromine	^{79}Br	^{81}Br	98.00
Silicon	^{28}Si	^{29}Si	5.100
		^{30}Si	3.400

Table 1. Abundance of several naturally occurring biologically important isotopes[1].

Qualitative Peak Analysis

Mass spectrometry distinguishes elements based on a mass to charge ratio, m/z. Because of this, isotopes play an important role in mass spectra. Each isotope will show up as a separate line in any mass spectrum with good enough resolution. The Y-axis on a mass spectrum is relative intensity. Therefore, the height of each of the peaks will correspond to the relative abundance of each isotope in the sample. As an example, examine Figure 1. This is a mass spectrum of a natural sample of atomic carbon. ^{12}C, with a natural abundance of 98.89%, naturally has a very high peak. ^{13}C, with a natural abundance of only 1.11%, has a very low peak. The radioactive ^{14}C has such a low natural abundance that it is not even seen in relation to the other two carbon isotopes[2]. The ^{12}C peak in this spectrum would be called the Base Peak, and would be labeled M^+. The base peak is the largest peak in a spectrum, and the intensity of every other peak is reported in comparison to the base peak. The ^{13}C peak, with an atomic mass of 1 greater than ^{12}C, would be labeled $(M+1)^+$. ^{14}C, if it were present, would be $(M+2)^+$.

Quantitative Peak Analysis

We can see from our qualitative example and Figure 1 that the intensity of each isotope peak is proportional to the abundance of the isotope in the sample. Because of this, it is relatively easy to predict the relative heights of each isotope peak for any given molecule. We can use the natural abundance of each isotope to predict how large each isotope peak will be in comparison to the base peak. Looking at the Carbon atom example in Figure 1 and the isotope abundance data from Table 1, we can see that there are 1.08 ^{13}C atoms for every 100 ^{12}C atoms, and the ^{13}C peak will be 1.08% as large as the ^{12}C peak.

The example above is simple, but the same methods can be applied to determine isotope peaks in more complicated molecules as well. The molecule $C_4Br_1O_2H_5$ has several isotope effects: ^{13}C, ^2H, ^{81}Br, ^{17}O, and ^{18}O all must be taken into account. First we will look at the $(M+1)^+$ peak in comparison with the M^+ peak. Only isotopes that will increase the value of M by 1 must be taken into consideration here – since ^{81}Br and ^{18}O would both increase M by 2, they can be ignored (the most abundant isotopes for Br and O are ^{79}Br and ^{16}O). Like the previous example, there are 1.08 ^{13}C atoms for every 100 ^{12}C atoms. However, there are 4 carbon atoms in our molecule, and any one of them being a ^{13}C atom would result in a molecule with mass $(M+1)$. So it is necessary to multiply the probability of an atom being a ^{13}C atom by the number of C atoms in the molecule. Therefore, we have:

$$4C * 1.08 = 4.32 = \text{molecules with a } ^{13}C \text{ atom per 100 molecules}$$

We can repeat this analysis for ^2H and ^{17}O:

$$5H * 0.015 = 0.075 = \text{molecules with a } ^2H \text{ atom per 100 molecules}$$

$$2O * 0.04 = 0.08 = \text{molecules with a } ^{17}O \text{ atom per 100 molecules}$$

Any of the three isotopes, ^{13}C, ^2H, or ^{17}O occurring in our molecule would result in an $(M+1)^+$ peak. To get the ratio of $(M+1)^+/M^+$, we need to add all three probabilities:

$$4.32 + 0.075 + 0.08 = 4.475 = (M+1)^+ \text{ molecules per 100 } M^+ \text{ molecules}$$

We can say then that the $(M+1)^+$ peak is 4.475% as high as the M^+ peak.

A similar analysis can be easily repeated for $(M+2)^+$:

$$1Br * 98 = 98 = \text{molecules with an } ^{81}Br \text{ molecule per 100 molecules}$$

$2O * 0.2 = 0.4 =$ molecules with an ^{18}O molecule per 100 molecules

$98 + 0.4 = 98.4 = (M+2)^+$ molecules per 100 M^+ molecules

The $(M + 2)^+$ peak is therefore 98.4% as tall as the M^+ peak.

This method is useful because using isotopic differences, it is possible to differentiate two molecules of identical mass numbers.