

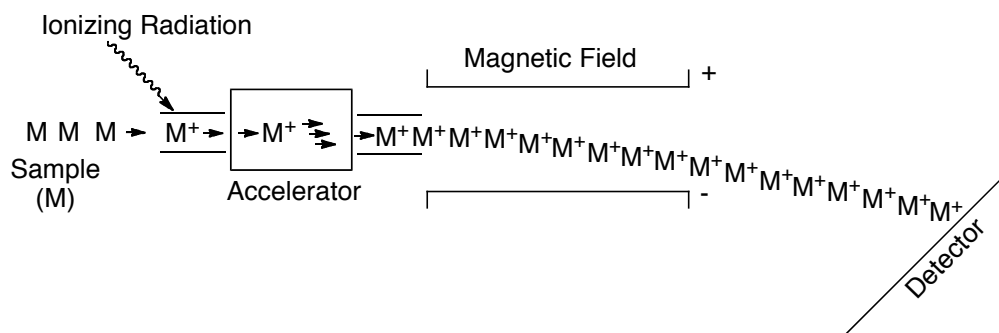
# Introduction to Spectroscopy V: Mass Spectrometry

## Basic Theory:

Unlike other forms of spectroscopy used in structure elucidation of organic molecules mass spectrometry does not involve absorption/emission of electromagnetic radiation. Hence it is spectrometry (spectrum measurement) not spectrophotometry (spectrum light measurement). Also unlike IR, NMR and UV/Vis, mass spectrometry destroys the sample, however only a very small amount of material is needed (only a few  $\mu\text{L}$  of a .01 M solution) so that is not typically a problem.

Mass spectrometry starts with a sample that is volatilized in some way, usually by heating a sample or drying an injected solution. The sample is then ionized. There are many ways of forming ions once the sample is in the vapor phase. In electron ionization (EI) a sample will be bombarded with X-rays or electricity to excite the molecule, which will then eject an electron to form a charged radical to give the molecular ion (parent ion)  $[M^{*+}]$  a.k.a.  $[M^+]$ . This often results in a high energy radical that can fragment. Alternatively a sample may be bombarded with ionized methane (Chemical Ionization or CI) to give the pseudo-molecular ion  $[M+H]^+$ , which is low energy and will not tend to fragment. If the sample was a solution that was evaporated in the spectrometer (such as with ESI-MS) ions such as  $H^+$ ,  $Na^+$ ,  $K^+$ , or  $NH_4^+$  can be added to the initial sample to give the pseudo-molecular ions  $[M+H]^+$ ,  $[M+Na]^+$ ,  $[M+K]^+$ , or  $[M+NH_4]^+$ .

The ions are then accelerated and sent through a magnetic field and since they are charged particles they will bend in that field. The amount of bend is dependent on the strength of the magnetic field and the mass of the ion. Since the field is constant, heavier ions bend less and lighter ions bend more ( $f = ma$ , therefore more mass gives less acceleration at a constant force). The amount of bend can be detected by where the ion strikes a detector plate and thus the  $m/z$  (mass/charge) ratio is measurable. The charge is a known amount as the ions formed will either be loss of an electron or gain of a single positive charge ( $H^+$ ,  $Na^+$ ,  $K^+$  etc), thus the mass of the ion can be calculated. From there the mass of the original sample can be calculated.



Early mass spectrometers ionized samples by excitation and removal of an electron resulting in a cation, so to this day most mass spectroscopy is done with cations. However, the principle works the same (with the opposite bend) for anions and anion spectroscopy is particularly useful for species that produce easily lose  $H^+$  (i.e. carboxylic acids form carboxylate anions).

Mass spectra show the relative abundance of ions at particular  $m/z$ . The most abundant ion is labeled as having an abundance of 100 and all lesser peaks are measured relative to that peak. Carbon has 98.93%  $^{12}C$  and 1.07%  $^{13}C$ , which will show up in a mass spectrum as a 100:1.11 relative abundance. Often the molecular ion has a low relative abundance, due to fragmentation and may even not be seen at all.

### Exact Masses and Abundances of Common Isotopes

Element (atomic mass amu)	Isotope	Exact Mass (amu)	Relative Abundance
Hydrogen (1.00794)	$^1H$	1.00783	100
	$^2H$ (deuterium)	2.01410	0.016
Carbon (12.01115)	$^{12}C$	12 (std.)	100
	$^{13}C$	13.00336	1.11
Nitrogen (14.0067)	$^{14}N$	14.0031	100
	$^{15}N$	15.0001	0.38
Oxygen (15.9994)	$^{16}O$	15.9949	100
	$^{17}O$	16.9991	0.04
	$^{18}O$	17.9992	0.20
Fluorine (18.9984)	$^{19}F$	18.9984	100
Chlorine (35.4517)	$^{35}Cl$	34.9689	100
	$^{37}Cl$	36.9659	32.5
Bromine (79.9094)	$^{79}Br$	78.9183	100
	$^{81}Br$	80.9163	98.0
Iodine (126.9045)	$^{127}I$	126.9045	100

NOTE: The relative abundances and masses of common isotopes do not need to be remembered, but it is helpful to remember three approximate ratios:  $^{12}C:^{13}C = 100:1$ ,  $^{35}Cl:^{37}Cl = 3:1$ , and  $^{79}Br:^{81}Br = 1:1$ .

### Utility:

There are three major pieces of information that can be found from a mass spectrum:

- Fragmentation Patterns
- Isotope Ratios
- Molecular Formula (HRMS)

### Fragmentation:

Ions are high-energy species that will tend to break apart in an attempt to form more stable structures. Especially in older systems that use high-energy radiation to ionize samples, fragmentation of the radical-ions is common. The exact fragmentation pattern allows for identification of functional groups. For example: loss of  $\text{CO}_2$  (-44  $m/z$ ) is common for carboxylic acids, loss of  $\text{CH}_2\text{CH}_2$  (-28  $m/z$ ) is common for long chain alkanes, and loss of  $\text{NO}$  (-30  $m/z$ ) and  $\text{NO}_2$  (-46  $m/z$ ) is common for compounds with nitro groups. For large molecules the parent ion  $[\text{M}^+]$  may not be seen as nearly all of it decomposes into fragments.

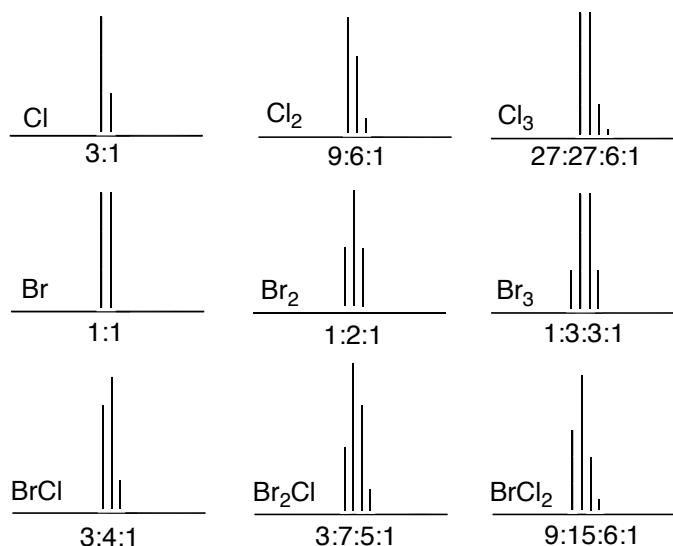
The fragmentation pattern will also allow for matching of a compound to a known sample. The fragmentation pattern of a particular compound is like a fingerprint. An unknown structure may be identifiable by matching it to the fragmentation pattern of a known sample, but this only works if there is a known sample to match too.

Modern mass spectrometers often use gentler ionization techniques that result in less fragmentation. This allows for a larger fraction of the sample to stay as the parent ion  $[\text{M}^+]$ . If fragmentation is desired inert gas such as helium may be added to collide with the ions and help them fragment.

### Isotope Ratios:

Many elements consist of multiple different isotopes, which have varying mass numbers. Mass spectrometry can differentiate between molecules that contain each different isotope and the ion peaks show up accordingly. In organic molecules isotope effects are commonly used to determine the presence or absence of halogens (Cl and Br) and occasionally to get an estimate of number of carbons.

Chlorine and bromine both have two isotopes with high abundances.  $^{35}\text{Cl}$  and  $^{37}\text{Cl}$  exist in an approximately 3:1 ratio and  $^{79}\text{Br}$  and  $^{81}\text{Br}$  exist in an approximately 1:1 ratio.



Compounds with either a chlorine or a bromine will show a significant  $[M+2]^+$  peak for any ion (molecular or fragment) that contains the halogen and these are the only common elements with significant  $[M+2]^+$  peaks. Thus the presence of an  $[M+2]^+$  ion indicates the presence of either chlorine or bromine. Also since the ratios vary for chlorine and bromine it is possible to tell how many of each atom is/are present by looking at the ratio of peak heights of the molecular ion.

The number of carbons in a molecule can be estimated by the size of the  $[M+1]^+$  peak. Since  $^{13}\text{C}$  is approximately 1% of the abundance of  $^{12}\text{C}$ , the chance of having one  $^{13}\text{C}$  increases as the number of carbons in the molecule increases. The  $[M+1]^+$  ion only gives an approximation of number of carbons as other elements that have a +1 isotope also affect the abundance of  $[M+1]^+$  and differences in  $^{12}\text{C}/^{13}\text{C}$  ratio due to carbon source and isotope effects in the ion fragmentation process. The approximate abundance of the  $[M+1]^+$  peak compared to the molecular ion can be calculated for molecules containing only C, H, N, O, F, Cl, Br, I, P, and S as follows (only C, N, and S have appreciable amounts of +1 isotopes).

$$\%[M+1] \approx (1.1 \times \#C) + (0.36 \times \#N) + (0.78 \times \#S)$$

*Formula:*

Different atoms have different masses and with sufficient precision mass spectrometry the exact mass can be used to find the molecular formula of an ion. For example at two significant figures of precision a  $\text{CH}_2$  group and a N atom are both 14 amu, but at 6 significant figures of precision the  $\text{CH}_2$  group is 14.0157 amu and the N atom is 14.0031 amu. The .0126 amu difference is significant enough to distinguish between a formula with a  $\text{CH}_2$  group and one with a N atom.

In order to be sufficiently precise to determine molecular formula a mass spectrum must have four decimals of precision (XX.XXXX amu). Spectrometers with this level of precision are called high-resolution mass spectrometers (HRMS) and the measured mass can be compared to a table of known formula masses. Alternatively the formula can be calculated using a computer program once the mass is known.

Even without HRMS some information about the formula can be found from the mass spectrum. The presence or absence of halogens can be found using isotope ratios. And the presence of an odd number of nitrogen atoms will give an odd formula mass due to the addition of an extra hydrogen to give nitrogen three bonds.

**Key Points:**

- Mass spectrometry measures the mass to charge ratio of an ion ( $m/z$ ).
- Fragmentation patterns for mass spectra act like fingerprints and can be used to identify certain functional groups.
- Isotope ratios show the number of Cl and Br atoms present.
- Odd numbers of nitrogen atoms give odd formula masses
- Exact molecular formula can be calculated from HRMS molecular ions.