Mass Spectrometry is a collection of techniques used information about mass. It is to first ionize the analytes, sort the ions by mass-to-charge ratio(M/Z). It requires amount of sample to obtain characteristic information the structure and molecular weight of the analyte.

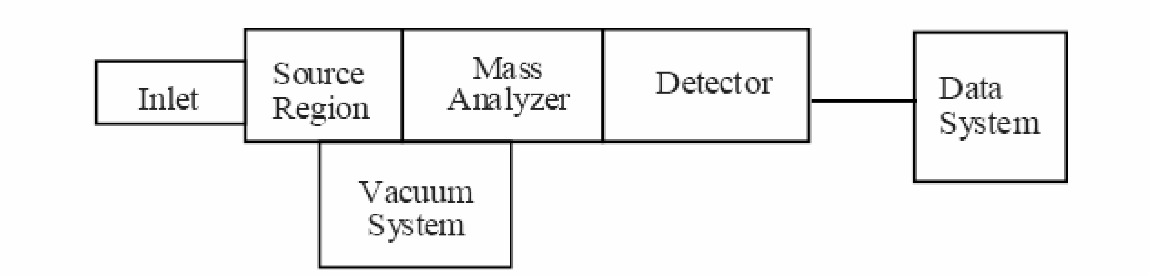
- Identification of compounds by the mass of one or more elements in a compound
- Determination of the isotopic composition of one or more elements in a compound

A **mass spectrometer** is a device used for mass spectrometry, and produces a mass spectrum of a sample to find its composition. This is normally achieved by ionizing the sample and separating ions of differing masses and recording their relative abundance by measuring intensities of ion flux. A typical mass spectrometer comprises three parts: an ion source, a mass analyzer, and a detector.

The Nature of Mass Spectra

A mass spectrum will usually be presented as a vertical bar graph, in which each bar represents an ion having a specific mass-to-charge ratio (m/z) and the length of the bar indicates the relative abundance of the ion. The most intense ion is assigned an abundance of 100, and it is referred to as the **base peak**. Most of the ions formed in a mass spectrometer have a single charge, so the m/z value is equivalent to mass itself.

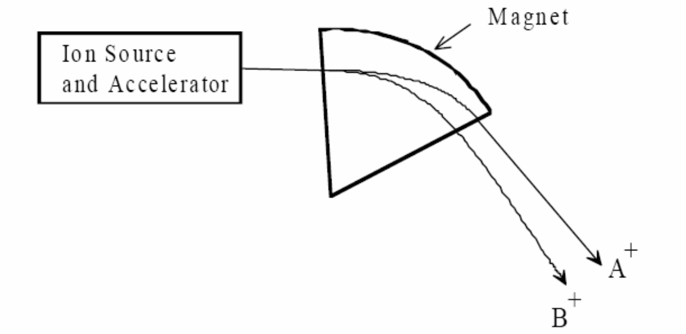
Instrumentation



Ion source

The ion source is the part of the mass spectrometer that ionizes the material under analysis (the analyte). The ions are then transported by magnetic or electrical fields to the mass analyzer.

- Techniques for ionization have been key to determining what types of samples can be analyzed by mass spectrometry. Electron ionization and chemical ionization are used for gases and vapors. Two techniques often used with liquid and solid biological samples include electrospray ionization (due to John Fenn) and Matrix-Assisted Laser Desorption/Ionization (MALDI, due to M. Karas and F. Hillenkamp). Inductively coupled plasma sources are used primarily for metal analysis on a wide array of samples types. Others include fast atom bombardment (FAB), thermospray, atmospheric pressure chemical ionization (APCI), secondary ion mass spectrometry (SIMS) and thermal ionisation.



Magnetic Sector Mass Spectrometer. The low m/z ion (B^+) is separated from the high m/z ion (A^+

Mass analyzer

The mass analyzer is the most flexible part of the mass spectrometer. It uses an electric and/or magnetic field to affect the path and/or velocity of the charged particles in some way.

Detector

The final element of the mass spectrometer is the detector. The detector records the charge induced or current produced when an ion passes by or hits a surface.

Atomic /molecular weight

- Atomic and molecular weight are generally expressed in terms if atomic mass units (amu) or dalton. The atomic mass unit is based upon a relative scale in which the reference is the carbon isotope ^{12}C, which is assigned a mass of exactly 12 amu. Thus the amu/dalton is defined as 1/12 of the mass of one neutral ^{12}C atom.

Exact mass and nominal mass

- In mass spectrometry, people are interested in the exact mass of particular isotopes of an element or the exact mass of compounds containing a particular set of isotopes. Normally, exact masses are quoted to 3 to 4 figures to the right of the decimal point, because high-resolution mass spectrometers make measurements at this level of precision. E.g., ^{12}C, 12.0000; ^{13}C, 13.0033
- The term of nominal mass implies a whole number precision in a mass measurement. The nominal mass is calculated by summing the integral atomic mass values of the lightest (most abundant) isotopes of all atoms present in a given ion.

Mass-to-Charge Ratio (M/Z)

Mass-to-charge ratio is obtained by dividing the atomic or molecular mass of an ion m by the number of charges z that the ion bears. Ions are often to be singly charged, we can use mass to represent m/z, but ions can be multiple charged, especially those ions generated by electrospray

Resolution in Mass Spectrometry

Ions with different mass-to-charge ratios are separated by mass analyzers. The capability of a MS to differentiate between masses is usually stated in terms of its resolution R

$$\mathbf{R=M/\Delta M}$$

Where $\mathbf{\Delta M}$ is the width of the peak at a height that is a specified fraction of the maximum peak. Two peaks are considered to be separated if the height of the valley between them is no more than some percentage of their height (often 10%). Resolution is mass dependent. Larger molecules require better resolution instrument to resolve.

Mass Accuracy /Exact Mass Measurement

Exact and nominal mass for methane CH_4

	Mass in amu	Abundance in %
^{12}C	12.0000	98.90
^{13}C	13.003355	1.10
^{1}H	1.007825	99.98
^{2}H	2.014102	0.015

The exact mass of CH_4 is: 12C1H4=16.031;

The nominal mass of CH_4 is 12+1x4=16

With sufficient resolution, the position of an ion signal on the mass scale can be assigned very accurately.

E.g. C_2H_5OH with nominal mass of 46
The exact mass can be measured as 46.0419,
where $^{12}C = 12.0000$
$^{1}H = 1.00782522$
$^{16}O = 15.994915$

Possible Empirical formula for m/z 46

	C	H	N	O	
CH_2O_2	1	2	-	2	46.005478
CH_4NO	1	4	1	1	46.029287
CH_6N_2	1	6	2	-	46.053096
C_2H_6O	2	6	-	1	46.041862

Molecular ion

There are molecules containing only majority isotopes and having lost or gained electrons without bond break-age and are normally designated as $M^{+\cdot}$ or $M^{-\cdot}$. Molecular ions are the intact ionized analyte molecule. Fragment ions are formed by subsequent fragmentation of molecular ions. The term also denotes a type of molecular-related ion. In order to unify the symbols with multiply charged ions, represented by M^{2+} and M^{3+}, the symbol is also expressed as $M^{\cdot +}$.

Characteristics of Molecular Ions

- The *m/z* value must correspond to a reasonable molecular formula with the proper isotope abundance.
- Most compounds have an even molecular mass. The one common exception to this is the "Nitrogen Rule".
- **The Nitrogen Rule**: Any compound with an odd number of nitrogen atoms will have an odd molecular mass. Any compound with an even number of nitrogen atoms (including zero) will have an even molecular mass. This is because nitrogen is the only common atom where the most common isotope has an odd valence and an even mass. For example: the molecular ion for CH_4 is *m/z* 16, the molecular ion for NH_3 is *m/z* 17, and the molecular ion for N_2H_4 is *m/z* 32.
- If a peak is the molecular ion, the next highest mass fragment must correspond to the loss of a possible neutral fragment. For example, a peak that corresponds to loss of 5 u from the molecular ion is highly unlikely.

Metastable ion (MI)

Designates ions generated in the ionization chamber that dissociate at some location in the analysis tube before reaching the collector. Normally these ions have life spans of 10-5 ~ 10-6 seconds. Dissociation of metastable ions with short life spans may be called fast metastable ion decay.

Metastable peak Peaks on the mass spectrum generated by ions created from decay of metastable ions in flight. A fragmentation reaction may be written:

$$\mathbf{M_1^+ \rightarrow M_2^+ + M_3}$$

The decomposition of a metastable ion of mass-to-charge ratio $\mathbf{m_1/z_1}$ into an ion of mass-to-charge ratio $\mathbf{m_2/z_2}$ after electric acceleration of the metastable ion and before magnetic deflection gives rise to a peak in the mass spectrum at an apparent mass, $\mathbf{m_2^2 z_1/m_1 z_2^2}$. The symbol **m*** should be used to indicate the apparent mass of the product ions giving rise to this peak.

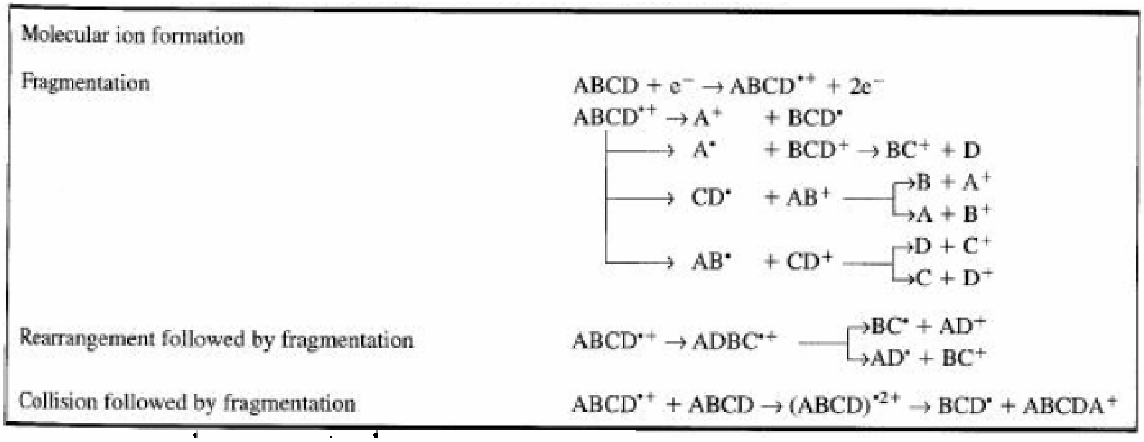
Ionization.

Various Ionization Source
- Electron Impact Ionization
- Chemical Ionization
- Field Ionization
- Field Desorption
- Fast Atom Bombardment
- Matrix Assisted Laser Desorption
- Electrospray Ionization

Electron Impact Ionization

Electrons are emitted from a heated tungsten or rhenium filament, accelerated by a potential ~70V that is impressed between the filament and the anode.

The highest peak in the mass spectrum is called BASE PEAKIt is not always possible to identify the molecular ion peak with electron impact ionization, certain molecules yield no molecular ion peak.



Nitrogen Rule

The **nitrogen rule** is not a rule, *per se*, as much as a general principle which may prove useful when attempting to solve organic mass spectrometry structures. Simply stated, the nitrogen rule for organic compounds containing exclusively hydrogen, carbon, nitrogen, oxygen, silicon, phosphorus, sulfur, and the halogens, is that an odd nominal mass indicates an odd number of nitrogen atoms are present and an even nominal mass indicates an even number of nitrogen atoms are present in the molecular ion.

This rule is derived from the fact that, perhaps coincidentally, for the most common chemical elements in neutral organic compounds (hydrogen, carbon, nitrogen, oxygen, silicon, phosphorus, sulfur, and the halogens), elements with even numbered nominal masses form even numbers of covalent bonds, while elements with odd numbered nominal masses form odd numbers of covalent bonds, with the exception of nitrogen, which has a nominal (or integer) mass of 14, but has a valency of 3.

It should be noted that the nitrogen rule is only true for neutral structures in which all of the atoms in the molecule have a number of covalent bonds equal to their standard valency (counting each sigma bond and pi bond as a separate covalent bond for the purposes of the calculation). Therefore, the rule is typically only applied to the molecular ion signal in the mass spectrum.

Mass spectrometry generally operates by measuring the mass of ions. If the measured ion is generated by creating or breaking a single covalent bond (such as protonating an amine to form an ammonium center or removing a hydride from a molecule to leave a positively charged ion) then the nitrogen rule becomes reversed (odd numbered masses indicate even numbers of nitrogens and vice versa). However, for each consecutive covalent bond that is broken or formed, the nitrogen rule again reverses.

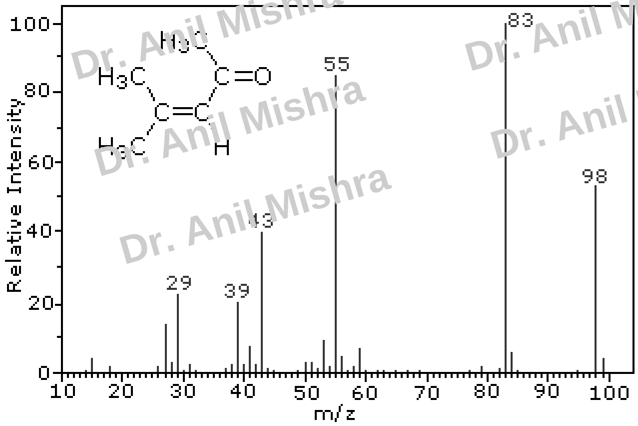
Therefore, a more rigorous definition of the nitrogen rule for organic compounds containing exclusively hydrogen, carbon, nitrogen, oxygen, silicon, phosphorus, sulfur, and the halogens would be as follows:

An even nominal mass indicates that a net even number of covalent bonds have been broken or formed and an even number of nitrogen atoms are present, or that a net odd number of covalent bonds have been broken or formed and an odd number of nitrogen atoms are present. An odd nominal mass indicates that a net even number of covalent bonds have been broken or formed and an odd number of nitrogen atoms are present, or that a net odd number of covalent bonds have been broken or formed and an even number of nitrogen atoms are present

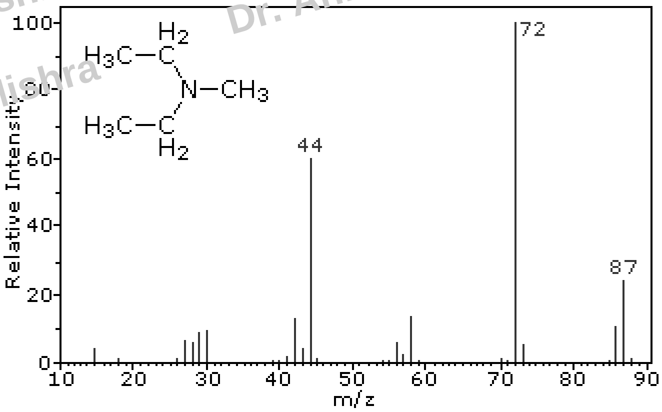
The masses of molecular and fragment ions also reflect the electron count, depending on the number of nitrogen atoms in the species.

Ions with no nitrogen or an even # N atoms	odd-electron ions even-number mass	even-electron ions odd-number mass
Ions having an odd # N atoms	odd-electron ions odd-number mass	even-electron ions even-number mass

This distinction is illustrated nicely by the following two examples. The unsaturated ketone, 4-methyl-3-pentene-2-one, on the left has no nitrogen so the mass of the molecular ion (m/z = 98) is an even number. Most of the fragment ions have odd-numbered masses, and therefore are even-electron cations. Diethylmethylamine, on the other hand, has one nitrogen and its molecular mass (m/z = 87) is an odd number. A majority of the fragment ions have even-numbered masses (ions at m/z = 30, 42, 56 & 58 are not labeled), and are even-electron nitrogen cations. The weak even -electron ions at m/z=15 and 29 are due to methyl and ethyl cations (no nitrogen atoms).



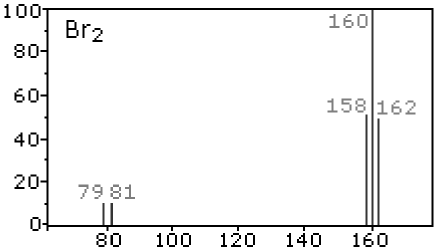
4-methyl-3-pentene-2-one



N,N-diethylmethylamine

Isotopes

Since a mass spectrometer separates and detects ions of slightly different masses, it easily distinguishes different isotopes of a given element. This is manifested most dramatically for compounds containing bromine and chlorine, as illustrated by the following examples. Since molecules of bromine have only two atoms, the spectrum on the left will come as a surprise if a single atomic mass of 80 amu is assumed for Br. The five peaks in this spectrum demonstrate clearly that natural bromine consists of a nearly 50:50 mixture of isotopes having atomic masses of 79 and 81 amu respectively. Thus, the bromine molecule may be composed of two ^{79}Br atoms (mass 158 amu), two ^{81}Br atoms (mass 162 amu) or the more probable combination of ^{79}Br-^{81}Br (mass 160 amu). Fragmentation of Br_2 to a bromine cation then gives rise to equal sized ion peaks at 79 and 81 amu.



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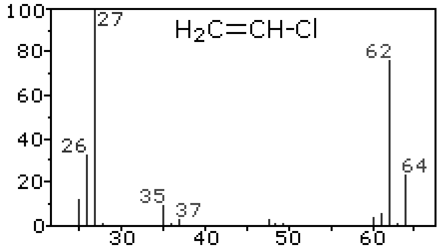
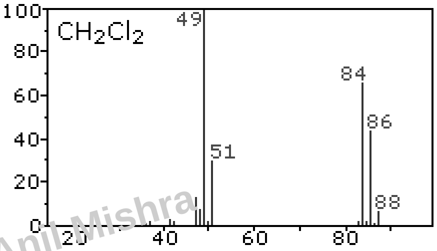
bromine



vinyl chloride



methylene chloride

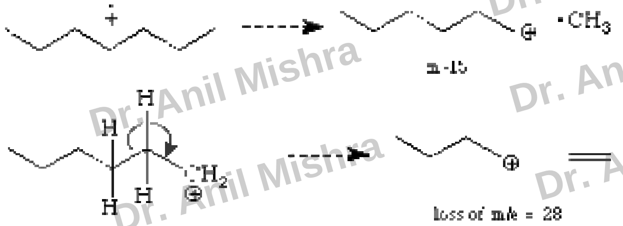
The center and right hand spectra show that chlorine is also composed of two isotopes, the more abundant having a mass of 35 amu, and the minor isotope a mass 37 amu. The precise isotopic composition of chlorine and bromine is:

Chlorine: 75.77% ^{35}Cl and 24.23% ^{37}Cl

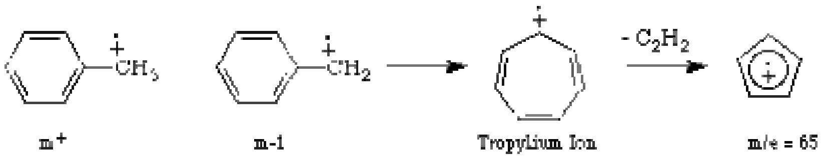
Bromine: 50.50% ^{79}Br and 49.50% ^{81}Br

The presence of chlorine or bromine in a molecule or ion is easily detected by noticing the intensity ratios of ions differing by 2 amu. In the case of methylene chloride, the molecular ion consists of three peaks at m/z=84, 86 & 88 amu, and their diminishing intensities may be calculated from the natural abundances given above. Loss of a chlorine atom gives two isotopic fragment ions at m/z=49 & 51amu, clearly incorporating a single chlorine atom. Fluorine and iodine, by contrast, are monoisotopic, having masses of 19 amu and 127 amu respectively. It should be noted that the presence of halogen atoms in a molecule or fragment ion does not change the odd-even mass rules given above.

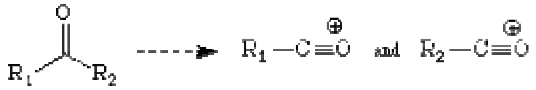
Alkanes: Simple alkanes tend to undergo fragmentation by the initial loss of a methyl group to form a (m-15) species. This carbocation can then undergo stepwise cleavage down the alkyl chain, expelling neutral two-carbon units (ethene). Branched hydrocarbons form more stable secondary and tertiary carbocations, and these peaks will tend to dominate the mass spectrum.



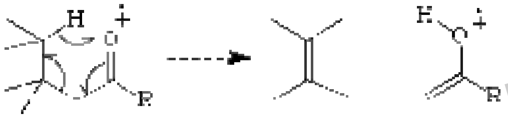
Aromatic Hydrocarbons: The fragmentation of the aromatic nucleus is somewhat complex, generating a series of peaks having m/e = 77, 65, 63, etc. While these peaks are difficult to describe in simple terms, they do form a pattern (the "aromatic cluster") that becomes recognizable with experience. If the molecule contains a benzyl unit, the major cleavage will be to generate the benzyl carbocation, which rearranges to form the tropylium ion. Expulsion of acetylene (ethyne) from this generates a characteristic m/e = 65 peak.



Aldehydes and Ketones: The predominate cleavage in aldehydes and ketones is loss of one of the side-chains to generate the substituted oxonium ion. This is an extremely favorable cleavage and this ion often represents the base peak in the spectrum. The methyl derivative ($CH_3C{\equiv}O^+$) is commonly referred to as the "acylium ion".



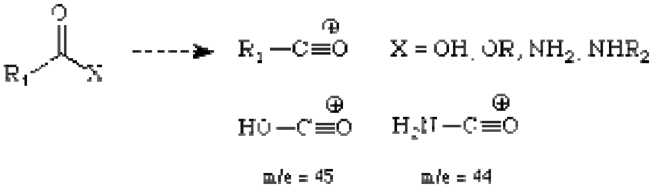
Another common fragmentation observed in carbonyl compounds (and in nitriles, etc.) involves the expulsion of neutral ethene *via* a process known as the *McLafferty rearrangement*, following the general mechanism shown below.



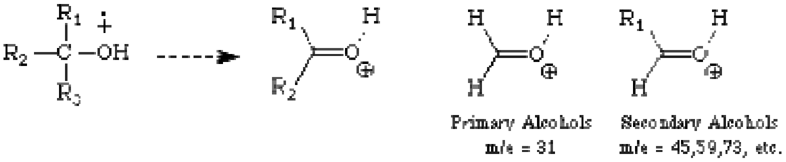
McLafferty rearrangement

This is the β-Cleavage with concomitant specific transfer of a γ-hydrogen atom in a six-membered transition state in mono-unsaturated systems, irrespective of whether the rearrangement is formulated by a radical or an ionic mechanism, and irrespective of the position of the charge.

Esters, Acids and Amides: As with aldehydes and ketones, the major cleavage observed for these compounds involves expulsion of the "X" group, as shown below, to form the substituted oxonium ion. For carboxylic acids and unsubstituted amides, characteristic peaks at m/e = 45 and 44 are also often observed.

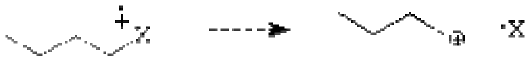


Alcohols: In addition to losing a proton and hydroxy radical, alcohols tend to lose one of the α-alkyl groups (or hydrogens) to form the oxonium ions shown below. For primary alcohols, this generates a peak at m/e = 31; secondary alcohols generate peaks with m/e = 45, 59, 73, etc., according to substitution.



Ethers: Following the trend of alcohols, ethers will fragment, often by loss of an alkyl radical, to form a substituted oxonium ion, as shown below for diethyl ether.

Halides: Organic halides fragment with simple expulsion of the halogen, as shown below. The molecular ions of chlorine and bromine-containing compounds will show multiple peaks due to the fact that each of these exists as two isotopes in relatively high abundance. Thus for chlorine, the $^{35}Cl/^{37}Cl$ ratio is roughly 3.08:1 and for bromine, the $^{79}Br/^{81}Br$ ratio is 1.02:1. The molecular ion of a chlorine-containing compound will have two peaks, separated by two mass units, in the ratio ≈3:1, and a bromine-containing compound will have two peaks, again separated by two mass units, having approximately equal intensities.



Gas chromatography-MS

A common form of mass spectrometry is gas chromatography-mass spectroscopy (GC-MS). In this technique a gas chromatograph is used to separate compounds. This stream is fed into the ion source, a metallic filament to which voltage is applied. This filament emits electrons which ionize the compounds. The ions can then further fragment, yielding predictable patterns. The stream then passes into the detector.

Liquid chromatography-MS

Similar to gas chromatography-mass spectroscopy (GC-MS) liquid chromatography-mass spectroscopy (LC-MS) separates compounds chromatographically before introduction to the ion source and mass spectrometer. The difference being that the mobile phase is liquid, usually a combination of water and organic solvents, instead of gas in liquid chromatography. Most commonly an electrospray ionization source is used in LC-MS.

MS for large molecules

For large molecules typical of biological applications, special techniques are used. The ion source subjects a sample of material to an Electric charge that causes the material to be ionized. Types of ion sources include electrospray ionization (ESI), chemical ionization (CI), fast atom bombardment (FAB), matrix-assisted laser desorption ionization (MALDI), Thermal ionisation (TI), Secondary ionisation (SI), and inductively coupled plasma ICP-MS.

Chemical ionization MS

The term chemical ionization comes from the fact that the analyte is ionized by chemical ion-molecule reactions during collisions in the source. One form of chemical ionization is atmospheric pressure chemical Ionization (APCI) which allows for the high flow rates typical of HPLC to be used directly, often without diverting the larger fraction of volume to waste. Typically the mobile phase containing eluting analyte is heated above 400 degrees Celsius, sprayed with high flow rates of nitrogen and the entire aerosol cloud is subjected to a corona discharge that creates ions. Often APCI can be performed in a modified ESI source.

Several techniques use ions created in a dedicated ion source injected into a flow tube or a drift tube: Selected Ion Flow Tube (SIFT-MS), and Proton Transfer Reaction (PTR-MS), are variants of CI dedicated for trace gas analysis of air, breath or liquid headspace using well defined reaction time allowing calculations of analyte concentrations from the known reaction kinetics without the need for internal standard or calibration.

Tandem MS

Tandem mass spectrometry involves at least two steps of mass selection or analysis, usually with some form of fragmentation in between. A tandem mass spectrometer is one capable of multiple rounds of mass spectrometry. For example, one mass analyzer can isolate one peptide from many entering a mass spectrometer. A second mass analyzer then stabilizes the peptide ions while they collide with a gas, causing them to fragment by collision-induced dissociation (CID). A third mass analyzer then catalogs the fragments produced from the peptides. Tandem mass spectrometry can also be carried out in a single mass analyzer over time as in a quadrupole ion trap. Several methods of fragmenting molecules for tandem mass spectrometry exist including collision-induced dissociation (CID), electron capture dissociation (ECD), infrared multiphoton dissociation (IRMPD) and blackbody infrared radiative dissociation (BIRD).