Swayam Course - Analytical Techniques

Week: 9, Module 24 - Principle of Mass Spectrometry

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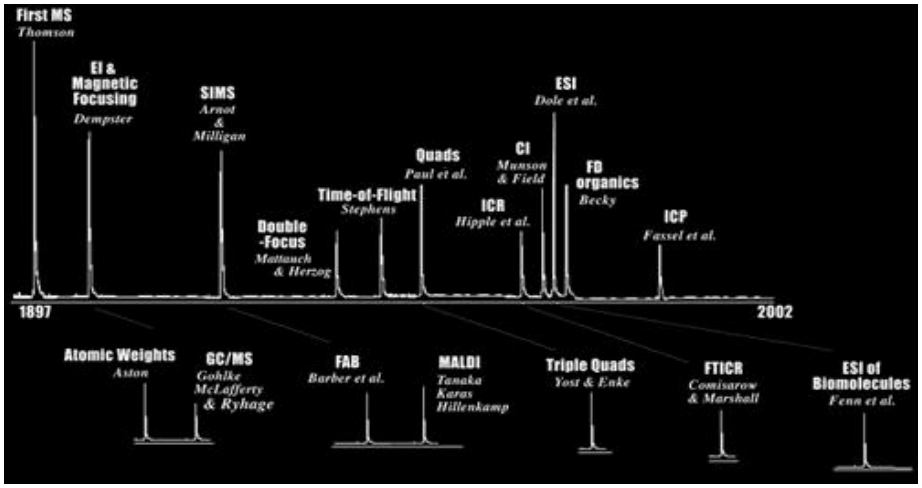
"Mass spectrometry is the art of measuring atoms and molecules to determine their molecular weight"

Introduction

It is a hyphenated instrument which utilizes the separation capability of chromatography with the mass resolving property of mass spectroscopy for the precise identification of compounds for their quantification and analysis. It is an analytical technique that can identify a substance, its chemical nature and quantity present in a sample by measuring its mass-to-charge ratio. In this technique sample is first ionized into gaseous ions by ionizing energy on molecule, further, these ions get characterized by their mass to charge ratios (m/z). For a chromatographer tandem mass spectroscopy is a detector and for the mass user HPLC is a sample feeder. Typically, mass spectrometry is employed to identify unknown compounds via molecular weight determination, to quantify known compounds, and to determine structure and chemical properties of molecules.

History of Mass spectrometers

Sir J. J. Thomson of the Cavendish Laboratory of the University of Cambridge, discovered electron based on his experiments using electrical discharges in gases in 1897. The first mass spectrometer (parabola spectrograph) was developed for the determination of mass-to-charge ratios of ions. In this spectrograph, the influence of electric and magnetic fields were utilized for the ions move through parabolic trajectories using discharge tubes. Scintillation plate or photographic plates were used to record the deflection of ions. Subsequently, Thomson received the 1906 Nobel Prize in Physics. In Thomson's group Francis **W. Aston** designed a mass spectrometer in which ions were dispersed by mass and focused by velocity. His model improved MS resolving power by an order of magnitude over the resolution better than the model developed by Thomson. Aston received the 1922 Nobel Prize in Chemistry for isotope studies carried out with this type of instrument. In 1920, Prof. A. J. Dempster, at Chicago developed a magnetic deflection instrument with direction focusing. He also developed the first electron impact source, which ionizes volatilized molecules with a beam of electrons from a hot wire filament.




JJ Thompson


Francis W Aston


Wolfgang Paul


Hans G Dehmelt


John B. Fenn


Koichi Tanaka

Noble prize winners in Mass Spectroscopy associated with the development of LC-MS/MS

Ever since, several developments took place in the development of mass spectrometers for structural determination. In tandem MS (MS-MS), a precursor ion is mass-selected and typically fragmented by "collision-induced dissociation" (collisionally activated dissociation or CAD), followed by mass analysis of the resulting product ions. The collision-induced dissociation was introduced in 1968 by Prof. Keith R. Jennings, (Univ. of Warwick, England) and Prof. McLafferty (Univ. of Purdue).

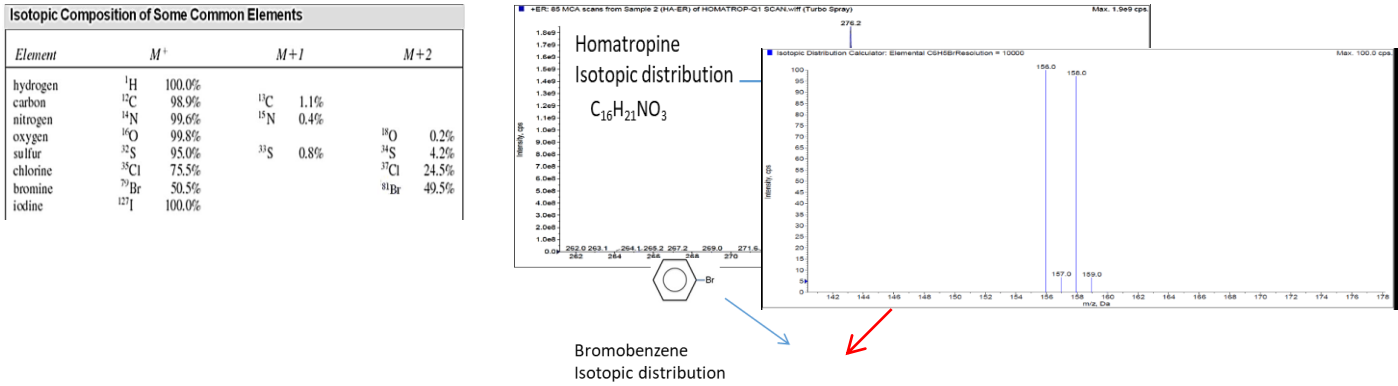
Prof. R. Graham Cooks (Univ. of Purdue) defined the combination of newer soft ionization methods with collision-induced dissociation which gives tandem MS its power in the analysis of mixtures. The tandem MS instrument (triple quadrupole MS) was invented by Prof. Richard A. Yost and Christie G. Enke (Michigan State Univ.). James D. Morrison of Latrobe University, Melbourne, Australia, helped Yost and Enke reduce the technique to practice. Tandem MS was popularized by triple-stage quadrupoles introduced first by Finnigan and Sciex (in 1980).

There are different types of mass spectrometers available for different applications. First question asked while looking for the help of mass spectrometer is what exactly the analyst wanted to know. The interest is only on molecular weight or interested in the quantity of particular molecule. Based on the requirement the choice of machine varies. Looking forward for the precise estimation and analysis of compounds for their pure structural information one would opt for MALDI (Matrix Assisted Laser Desorption Ionization) coupled Time of Flight (TOF) or Quadrupole coupled Time of Flight. Where as for pharmaceutical applications requiring quantification as well as metabolite identification one would opt for either triple quadrupole or trap type of systems. Another type of tandem mass spectrometry used for radiocarbon dating is Accelerator Mass Spectrometry (AMS), which uses very high voltages, usually in the mega-volt.

Understanding the principle of mass spectrometry

<u>Isotopic abundance of elements</u>

In the earth every element is existing along with their isotopes naturally. Isotopes have same atomic number with different molecular weights, for example hydrogen (MW=1) is having deuterium (MW=2) and tritium(MW=3),but all of them have atomic number as 1. In the standard mass spectrum, the isotopes form of compounds can be seen clearly. The mass of the most abundant peak in the mass spectrum is called mono-isotopic mass. Identifying the isotopic pattern is essential to determine the charge state of compounds in proteins which are known to have multiple charges and higher molecular weight.



Isotopic Abundance of common elements and their impact on MW of compounds

Mass Analyzer properties

Charge of a compound

When a molecule is getting protonated $(M+ H+)^+$ or deprotonated $(M-H+)^-$ it is expected to gain or lose charge +1 or -1 respectively . They follow the formula (m+z)/z (where m= mass of the molecule and z= charge).

According to this formula, singly protonated molecule with the molecular weight of 2000 would be seen in the mass spectra as (2000+1)/1= 2001/1= 2001. If the molecule is doubly protonated then, (2000+2)/2 = 1001. If the molecule protonated 4 times at 4 various positions then (2000+4)/4 = 501. In electrospray ionization technique, a peptide having the molecular weight of 2000 would be reflected as having the molecular weight as 501. Here, the resolution power of the instrument is very important to understand the charge state.

Mass resolving power

The mass resolving power is the measure of its ability to distinguish two peaks of slightly different m/z. This property is of importance while determining accurate molecular weight of compounds for their identification from libraries. Usually the highest mass number of two adjacent peaks in the mass scan is used to derive this resolving power. Triple quadrupole mass spectrometers works with unit mass resolution but with higher sensitivity in quantification. Q-TOF type of instruments is known to have low sensitivity in quantification where higher mass resolution.

Resolution (R)= $M_n/(M_n-M_m)$

Mn = lowest mass number and Mm = highest mass number of two adjacent peaks.

For example M_n=1999 and M_m=2000, then the resolution is 2000 by applying the above formula, which is seen in unit mass resolution mass spectrometers.

Other parameters used in mass spectrometry

The mass accuracy is the parameter which determine the accuracy of the instrument. It is the ratio of the m/z measurement error to the true m/z. Based on the choice of mass analysers it could be varied from ± 0.5 to ± 0.0001 m/z. Usually, ±0.5 (nominal mass measurement) is used for analytical purposes but for accurate mass ±5 ppm of the theoretical molecular weight is must.

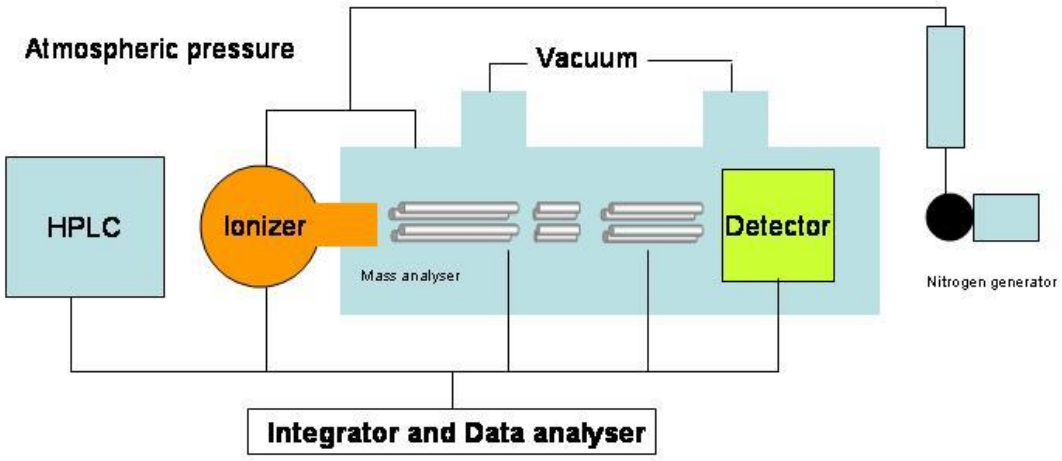
Mass range is the capability of the mass spectrometer in quantifying lower and higher values of analytes (molecular weight).

Linear dynamic range is the specification over which ion signal is linear with analyte concentration.

Speed of the mass analyser refers to the time frames of experimental data taken per unit time. Speed governs the data points covered for quantitation experiments in the continuous monitoring mode where the effluent from sample injector is fed into mass analyser.

Construction and Working of Liquid Chromatography coupled Triple Quadrupole Mass Spectroscopy or Tandem Mass Spectroscopy

This assembly consists of a HPLC machine coupled with an interface ionizer to a mass spectroscopy.



Schematic representation triple quadrupole mass spectroscopy linked with a ionizer to high performance liquid chromatography.

For mass spectrometric analysis of a compound, the sample has to be introduced into the ionization source of the instrument for the production of gas phase ions of the compound by electron ionization. The sample passes through an electron beam which knocks off some electrons from the molecules and turns them into ions. A mass spectrometer generates multiple ions from the single sample. Ionic forms of molecules of sample are advantageous as ions are easier to manipulate than neutral molecules. These molecular ions undergo further fragmentation. These ions are separated according to their mass (m)/charge (z) ratios i.e. (m/z) in the analyzer region. The resulted mass spectrum is displayed in the form of a plot of ion abundance versus mass-to-charge ratio. Thus the structure and nature of precursor molecule is elucidated.

The mass spectrometer consists of following three fundamental components.

I. *Ion Source* : An ion source is the first component of mass spectrometer that creates gaseous ions from the substance being studied. A small sample is ionized, generally to cations by loss of an electron. The two most common ion source devices are Matrix-assisted laser desorption/ionization(MALDI) and Electrospray ionization(ESI).

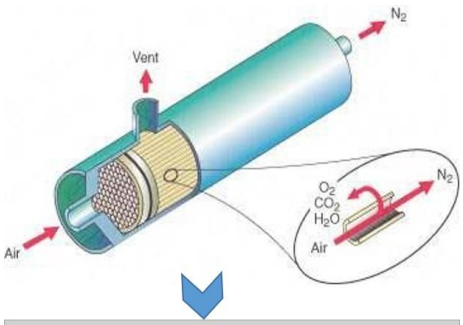
II. *Mass Analyzer*: A mass analyzer is the second component of the mass spectrometer that receives ionized masses and resolved them on the basis of charge to mass ratios and outputs them to the detector. The most common mass analyzers are Time of Flight Mass Analyzer, Quadrupole Mass Analyzer, and Quadrupole Ion Trap Mass Analyzers etc.

III. *Detector System*: It is the last component of mass spectrometer which is required for detecting the ions and recording the relative abundance of each of the resolved ionic species. The results are displayed on a chart as mass spectrum. A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio. The spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds.

Components and their functions in a mass spectrometer

Inert Gases

For the proper functioning of the mass spectrometer, a source for compressed air to produce ultrapure nitrogen and hydrocarbon free air is required. It also requires dehumidifier, sample infusion assembly or chromatography assemblies.



Follows Fick's law of diffusion and Henry's law of pressure and solubility

Vacuum System

All mass spectrometers operate at very low pressure (high vacuum). This reduces the chance of ions colliding with other molecules in the mass analyzer. Any collision can cause the ions to react, neutralize, scatter, or fragment. All these processes will interfere with the mass spectrum. To minimize collisions, experiments are conducted under high vacuum conditions, typically 10 to 10 Pa (10 to 10 torr) depending upon the geometry of the instrument. This -2 -5 -4 -7 times high vacuum requires two pumping stages. The first stage is a mechanical pump that provides rough vacuum down to 0.1 Pa (10 torr). The second stage uses diffusion pumps or -3 turbomolecular pumps to provide high vacuum.

Sample ionization and introduction

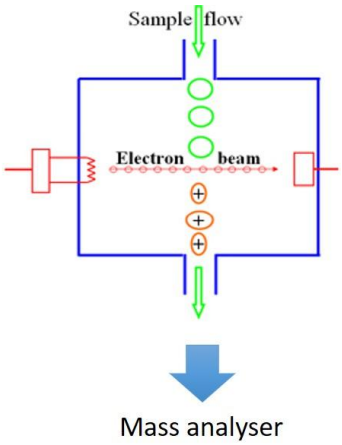
There are several methods of ionization used in mass spectrometry as given in Table 1. Among these, the electron impact (EI) and Fast Atom Bombardment (FAB) are older methods and usually required in specific needs e.g. EI for environmental work using GC-MS. The most common ionization methods employed in biological systems are Electrospray Ionization (ESI) and Matrix Assisted Laser Desorption Ionization (MALDI), and atmospheric pressure chemical

Ionization (APCI) etc. Both positively and negatively charged sample ions are generated to the proton affinity of the sample. The ionization method to be used depends on the nature of sample and the type of mass spectrometer.

Ionisation Method	Analyte Type	Mass Range	Sample Introduction
Chemical Ionisation (CI)	Small and volatile	Upto 1000Da	GC or liquid/solid probe
Electron Impact (EI)	Small and volatile	Upto 1000Da	GC or liquid/solid probe
Fast Atom Bombardment (FAB)	carbohydrates, organo-metallics peptides, non-volatile	Upto 6000Da	Sample mixed with viscous matrix
Electrospray Ionisation (ESI)	Biomolecules,proteins, peptides, non-volatlite	> 500,000Da	Liquid chromatography or capillary
Atmospheric Pressure Chemical Ionisation (APCI)	Biomolecules, proteins, peptides etc., non-volatile	>500,000Da	Liquid chromatography or capillary
Matrix Assisted Laser Desorption Ionisation (MALDI)	Biomolecules, proteins, peptides,etc.,	Upto 500,000Da	Sample mixed with solid matrix

Table 1: List and specifications of different ionization methods

Electron Impact ionization (EI)



This method is mostly used for GC-MS and considered as hard ionization technique, because it causes the fragmentation of ions. Ionization of gaseous and volatile substances can be done by energetic electrons. In this method a beam of electrons is formed by heating a filament bias at a negative voltage compared to the source(-70 volts). The electrons are further used for bombardment to the gas phase molecules. The following reaction describes the electron ionization process:

$M + e^- \longrightarrow M^{+\bullet} + 2\,e^-$, where M is the analyte molecule, e−is the electron and M+·is termed the molecular ion.

Fast Atom Bombardment (FAB)

FAB is one of the first techniques used for ionization of non-volatile compounds. In FAB, the sample is mixed in a matrices like glycerol, 1-thioglycerol, a mixture of dithiothreitol and dithioerythritol, 3-nitrobenzyl alcohol, and triethanolamine to keep the sample in a liquid state as it enters the high vacuum ion source. This matrix also protects the analyte from being damaged by the high energy bombarding particle. Ionization is done by bombarding a sample with a beam of atoms, typically Ar or Xe , accelerated to 8-10 keV kinetic energy. The ions formed by FAB are adducts to the molecule , where adducts could be protons , sodium, potassium or ammonium ions etc.

Electron Spray Ionization (ESI)

Currently, ESI is one of the most popular ionization techniques and generally employed in the mass analysis of polar molecules. ESI has an advantage in its easy compatibility with LC.

In ESI probe, often mild acid conditions are used with the help of weak acids like formic, acetic acids etc. to enhance the ionization process. ESI probe ionization can be operated at positive and negative polarities to add a proton (adding a hydrogen) or removal of the proton (removal of the hydrogen) respectively. With the pressure of solvents from HPLC as well as source gas pressure the particles produced in a normal ESI probe would be having 1µm in diameter. Where as in nanospary techniques the diameter may be from 100-200nm.

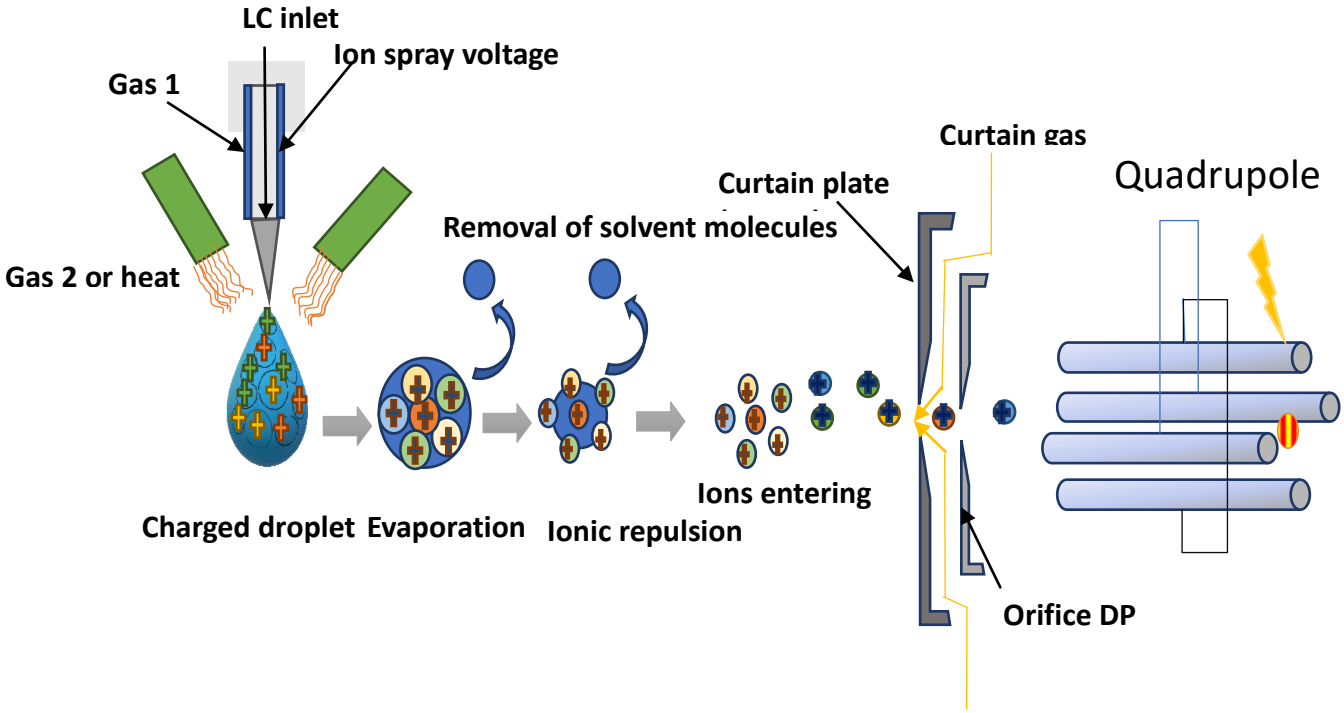


Figure showing the generation of ionized molecular species from the spray containing solvents from HPLC

Under a high electrical field, conducting fluid is getting automized each droplet leaves the needle with an electrical gradient in the shape of "Tayor Cone". The tip of the Taylor cone protrudes as a filament until the liquid reaches the Rayleigh limit where the surface tension and electrostatic repulsion are equal and the highly charged droplets are attracted to the entrance of the mass spectrometer due to the high opposite voltage at the mass analyzer's entrance. As the droplet moves towards the analyzers, the coulombic repulsion on the surface exceeds the surface tension, the droplet explodes into smaller droplets ultimately releasing ions. Typically a suitable temperature gas flow in the probe (based on the type of solvent mixture used) enhances the evaporation of solvent droplets containing the analyte of interest to increase charge overcrowding. This evaporation causes shrinkage of the droplet size leading to coulombic explosion where the molecule of interest exists in ionized form and attracted towards the mass analyzer. This method is also used for ionizing proteins, peptides and thermolabile compounds without thermal degradation as the evaporating solvent decreases the temperature reaching around the molecule. However, the process of gas as well as temperature parameters needs to be optimized for each method. This process is suitable for molecules having higher polarity. This method is also highly useful in understanding the exact molecular weight of peptides due to their nature of having multiply charged.

Atmospheric Pressure Chemical Ionization (APCI)

This method uses similar setup like ESI however, the temperature around the sample probe is heated to higher temperature where the droplets roll on the heated cylinder and causes evaporation. The heated vapor reaches down a corona discharge needle pointing towards the gas flow causes proton transfer. Since the solvent ions are present at atmospheric pressure conditions, chemical ionization of analyte molecules is very efficient; at atmospheric pressure analyte molecules collide with the reagent ions frequently. Proton transfer (for protonation MH^+ reactions) occurs in the positive mode, and either electron transfer or proton loss, ($[M-H]^-$) in the negative mode. The moderating influence of the solvent clusters on the reagent ions, and of the high gas pressure, reduces fragmentation during ionization and results in primarily intact molecular ions. This method is used for compounds which are moderately polar having ionization problem in ESI mode.

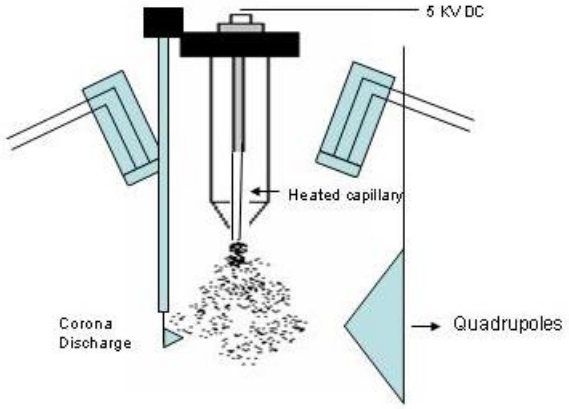


Figure showing the working principle of APCI probe

Atmospheric Pressure Photo Ionization (APPI)

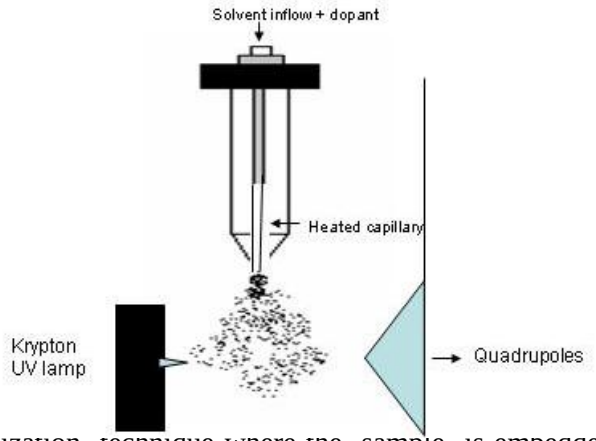
Atmospheric pressure photoionization (APPI) has recently become an important ionization source because it generates ions directly from solution with relatively low background and is capable of analyzing relatively non-polar compounds. This method uses the similar set up of APPI where the corona needle is replaced with Krypton ultraviolet (UV) lamp. The high energy UV radiation (a typical krypton light source emits at 10.0 eV and 10.6 eV) causes the Dopant to exit and transfer the charge to the adjacent analyte (Fig.4). APPI is much more sensitive than ESI or APCI and has been shown to have higher signal-to-noise ratios because of lower background ionization. Lower background signal is largely due to high ionization potential of standard solvents such as methanol and water (IP 10.85 and 12.62 eV, respectively) which are not ionized by the krypton lamp.

APPI induces ionization via two different mechanisms.

1) Direct photo-excitation - this process allows electron ejection and the generation of the positive ion radical cation (M^+). The APPI source radiation energy is higher than the ionization potentials of target molecules, where as lower than most of the ionization potentials of solvent molecules.

2) Atmospheric pressure photo-induced chemical ionization which is similar to APCI in that it involves charge transfer to produce protonation (MH^+) or proton loss ($[M-H]^-$) to generate negative ions.

In order to induce chemical ionization, a photoionizable reagent, also called a dopant, is added to the eluant. Upon photoionization of the dopant, charge transfer occurs to the analyte. Typical dopants in positive mode include acetone and toluene. Acetone also serves as a dopant in negative mode.

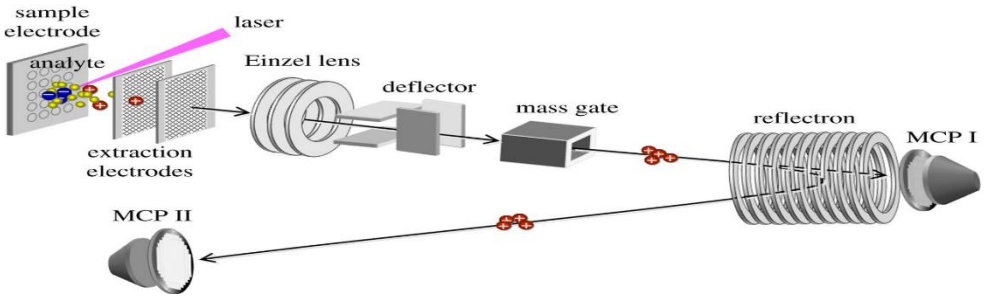


owing the working principle of APPI

Matrix Assisted

MALDI is a soft ionization technique where the sample is embedded in a solid matrix (e.g. sinapinic acid, 2,5-dihydroxybenzoic acid etc.) which absorbs energy at the wavelength of the laser (337 nm)and transfer a proton to the sample.

This technique directly ionizes and vaporizes the analyte from the condensed phase. MALDI is often used for the analysis of synthetic and natural polymers, proteins, and peptides. Analysis of compounds with molecular weights up to 200,000 dalton is possible and this high mass limit is continually increasing. In MALDI, both desorption and ionization are induced by a single laser pulse. The sample is prepared by mixing the analyte and a matrix compound chosen to absorb the laser in ultraviolet wavelength. This is placed on a probe tip and dried. A vacuum lock is used to insert the probe into the source region of the mass spectrometer. A laser beam is then focused on this dried mixture and the energy from a laser pulse is absorbed by the matrix. This energy ejects analyte ions from the surface so that a mass spectrum is acquired for each laser pulse. MALDI mostly forms single charged ions and preferentially coupled with time-of-flight (TOF)

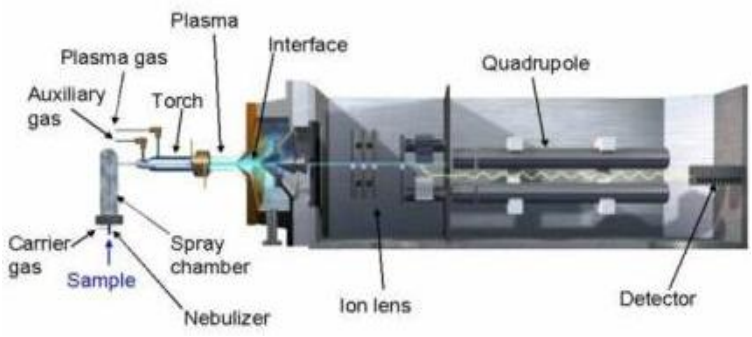


analyzers.

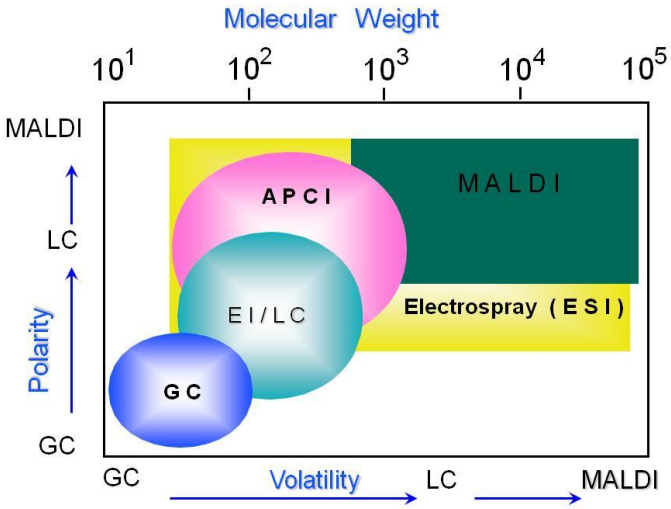
Figure showing the working principle of MALDI

Inductively coupled plasma ionisation

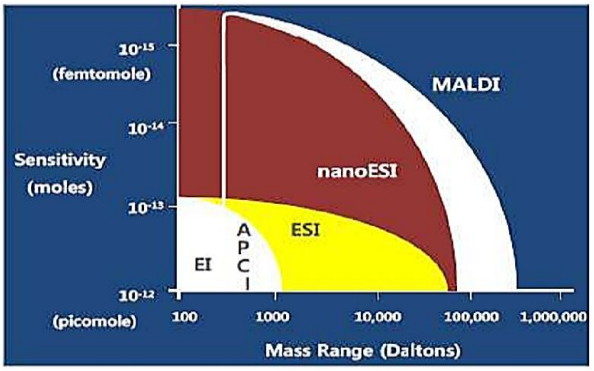
Inductively coupled plasma (ICP) is extensively used for the elemental analysis for atomic absorption and emission



spectrocopies. The ICP torch makes a plasma (high temperature flame) with the help of supporting gas carrying analyte, a radio frequency (27-41 MHz of 1-5KW) coil producesan oscillating magnetic field which in turn creates oscillating current in the ions and electrons of the support gas. This excitation create a plasma of very high temperature (7000-8000 K) in which the introduced liquid carrying elemental salts are evaporated, excited leading to the formation of elemental ions which are fed into the quadrupole mass analyzer for their accurate mass determination. This data can also be converted to quantitative measure for elemental composition analysis. Detection limits for most elements in ICP-MS are equal to or better than those obtained by using classical atomic absorption spectroscopy with graphite furnace. It is also able to handle complex matrices with low matrix interferences due to very high temperature in ICP source. It is capable of getting isotopic information, as well as, making high throughput analysis possible.



Figure



showing the choice of ionization probes according to the polarity and molecular weight of compounds & the typical sensitivity and mass ranges allowed by different ionization techniques

Curtain gas curtain plate and suction assembly

The curtain gas is a pure nitrogen which is allowed to pass through the curtain plate. As the ions enter in to the mass analyzer, the curtain gas flows against the entry of ionized species. This process reduces the inlet of uncharged solvent molecules to reduce the background noise in the mass spectrum. As in most of the applications HPLC solvents are pumped at the rate varying form 0.4-1ml/minute this process is essential to avoid solvent induced background noise. The curtain plate having a central hole is directed to the ion guide where the ions are accelerated and focused towards the Q1 mass analyzer quadrupole.

In the ionizing probe bottom of the probe is usually connected with a vacuum pump to remove the vapors of the mobile phase immediately to maintain the ionization head at normal atmospheric pressure. It is usually achieved by using vacuum devices which are working with Bernoulli's Principle where a compressed air is allowed to create vacuum.

Mass Analyzers

Analyzers are typically described as either continuous or pulsed. Continuous analyzers include quadrupole filters and magnetic sectors. These analyzers are similar to a filter or monochromator used for optical spectroscopy. They transmit a single selected m/z to the

detector and the mass spectrum is obtained by scanning the analyzer so that different mass to charge ratio ions are detected. While a certain m/z is selected, any ions at other m/z ratios are lost, reducing the Signal/Noise ratio (S/N) for continuous analyzers. Single Ion Monitoring (SIM) enhances the S/N by setting the mass spectrometer at the m/z for an ion of interest. Pulsed mass analyzers are the other major class of mass analyzer.

1) Quadrupole Analyzer

It consists of 4 rods which are resistant to thermal expansion and made up of gold coated ceramic rods, or made up of molybdenum or gold coated quartz etc depending upon the place of application. These rods typically create electric fields using alternating high voltage direct current (DC) and radio-frequency(RF). Each pair of the opposing rods is connected electrically. An RF voltage is applied between other parts of the opposing rods with a DC voltage superimposed on it. This creates a quadrupole field between the rods.

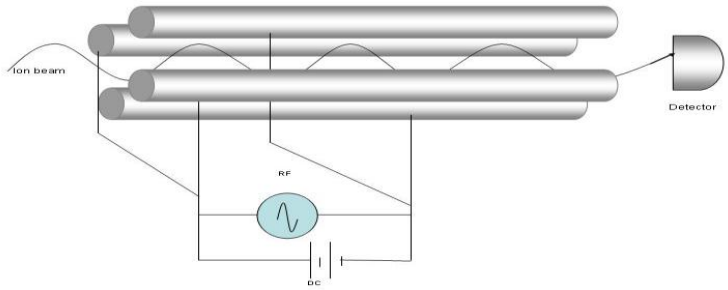
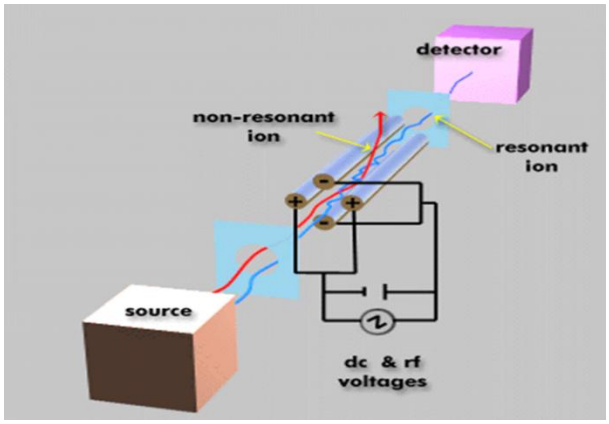


Figure showing ion-propulsion through the quadrupole



The quadrupole mass spectrometer is the most common mass analyzer. Its compact size, fast scan rate, high transmission efficiency, and modest vacuum requirements are ideal for small inexpensive instruments. Most quadrupole instruments are limited to unit m/z resolution and have a mass range of m/z 1000. Many bench-top instruments have a mass range of m/z 500 but research instruments are available with mass range up to m/z 4000. Typically, in LC-MS/MS one mass analyzer first separates a particular molecule in the vacuum using its mass to charge ratio. This is typically achieved by applying radio frequency and high power direct current alternatively between two pairs of quadrupoles. The charged molecule generated by the ionization technique is brought into the first quadrupole where the separation is typically based on the type of its resonance between radio frequency and high volt DC current, which gives momentum in spiral path and guides the charged molecule towards the second quadrupole.

Tandem Mass Spectrometry

The attachment of more than one mass analysers in series is called Tandem Mass Spectrometry. Tandem mass spectrometry enables a variety of experimental sequences. Many commercial mass spectrometers are designed to expedite the execution of such routine sequences as single reaction monitoring (SRM), multiple reaction monitoring (MRM), and precursor ion scan. In SRM, the first analyzer allows only a single mass through and the second analyzer monitors for a single user defined fragment ion. MRM allows for multiple user defined fragment ions. SRM and MRM are most often used with scanning instruments where the second mass analysis event is duty cycle limited. These experiments are used to increase specificity of detection of known

molecules, notably in pharmacokinetic studies. Precursor ion scan refers to monitoring for a specific loss from the precursor ion. The first and second mass analyzers scan across the spectrum as partitioned by a user defined m/z value. This experiment is used to detect specific motifs within unknown molecules.

Sequence of events in the typical tandem mass spectroscopy

(i) Separation of compounds (Quadrupole 1)

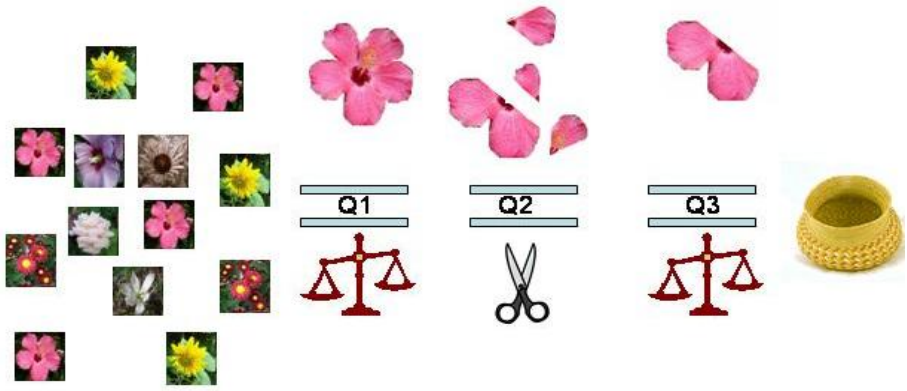
This quadrupole is set for a desired value of mass (which has been set according to the reported literature or decided as a result of mass experiments in tuning mode). Therefore, this quadrupole is set to transmit only the molecular ions having desired molecular weight towards the quadrupole-2, which is otherwise called as Collision cell.

(ii) Fragmentation using neutral gas molecules (Quadrupole-2)

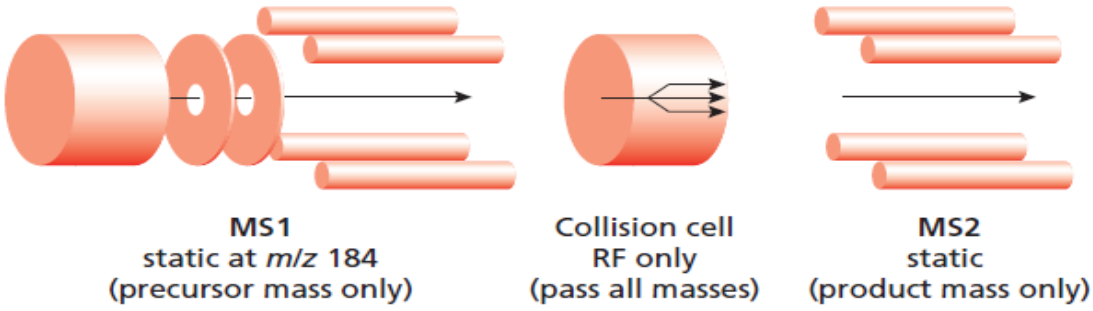
The second quadrupole collects the molecule and makes it to collide with neutral gas molecules like argon or nitrogen where the chemically weaker bonds like C→ N or C→O bonds are broken and give fragmented ions called daughter ions. This process is called CID (Collision –Induced-Dissociation). This process is enabled by using collision potentials set in the rods along with RF and DC voltages for a preset set time usually in nanoseconds. CID is popularly used in most of the modern commercial mass spectrometers. However, there are many other methods available for fragmenting the molecules, such as, electron capture dissociation (ECD), electron transfer dissociation (ETD), infrared multiphoton dissociation (IRMPD) and blackbody infrared radioactive dissociation (BIRD).

How does this process work

In this bunch of flowers you wish to select a particular flower. However, in its weight there could be many therefore fragmentation helps to double confirm the particular species only. The simple (artistic view of the process) and real working process are depicted in the figures.



An artist's view of the principle involved in Tandem mass spectroscopy



The realistic view of the working principle in Tandem mass spectroscopy

(iii) Separation of the fragmented ion (Quadrupole-3)

The third quadrupole separates a particular broken piece of fragmented ion to guide towards detector having opposite charge.

2) Ion- Trap Analyzer

These analyzers have similar working principles as that of quadrupoles, with the difference being, that the ions are "stored" in a three-dimensional electric field, by the varying RF & DC voltages within the quadrupole. Upon, stepping up the scanning mode, the ions of a specific m/z are selectively ejected. These analyzers allow MSn scans, thus, leading to enhanced sensitivity in full scan experiments.

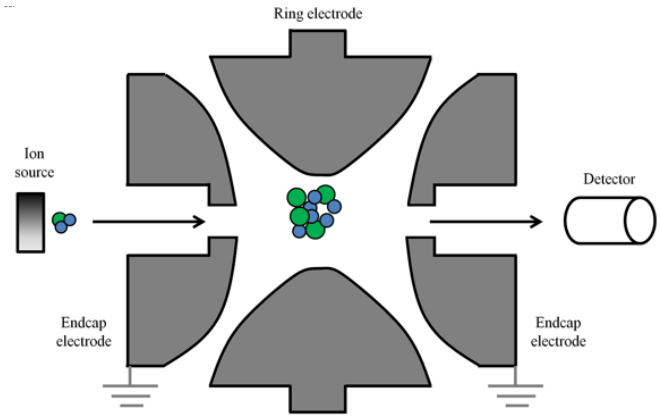


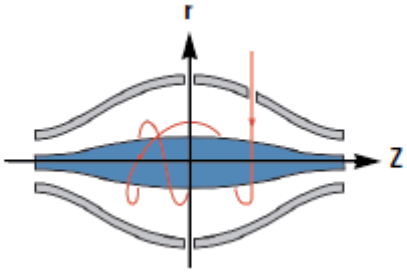
Figure showing the assembly and working of Ion-Trap analyzer

3) Time-of-Flight (TOF) Analyzer

The time-of-flight mass analyzer separates ions in time as they travel down a flight tube. This is a very simple mass spectrometer that uses fixed voltages and does not require a magnetic field. The greatest drawback is that TOF instruments have poor mass resolution, usually less than 500. These instruments have high transmission efficiency; no upper m/z limit, very low detection limits, and fast scan rates. For some applications these advantages outweigh the low resolution.

4) Orbitrap Analyzer

The Orbitrap mass analyser is an axially-symmetrical mass analyser consisting of a spindle-shaped central electrode surrounded by a pair of bell-shaped outer electrodes. It employs electrostatic fields to capture and confine ions.



Schematic of the Orbitrap cell

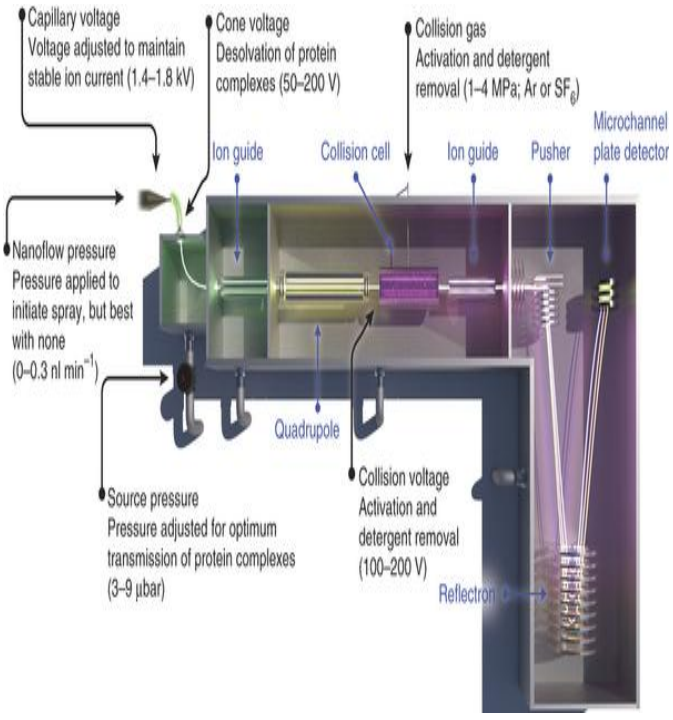
Upon entering the Orbitrap mass analyser, the ions start rotating around the axial central electrode. This rotation is combined with the harmonic oscillations of the ions that run along the axial central electrode. The frequency ω of these harmonic oscillations is a property governed solely by the m/z of the ions and the instrumental constant k.

$$\omega = \sqrt{\frac{z}{m} * k}$$

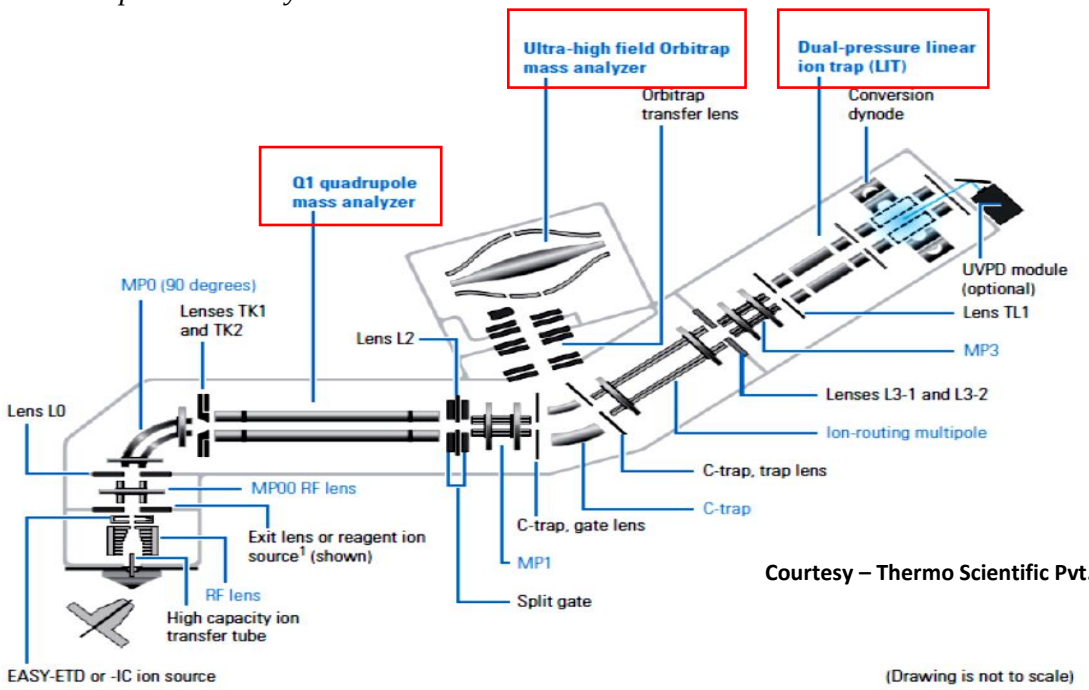
The harmonic oscillations produce an image current that is detected by the two split halves of the outer electrode. By using a fast fragment ion algorithm of the amplified image current, the instrument measures the frequencies of these axial oscillations and the m/z values of the ions.

5) Hybrid-Quadrupole-Time of Flight (Q-TOF) Analyzer

It works like triple quadrupole mass spectrometer in which third quadrupole is replaced with a long path of ion to increase the mass resolution. However, this path length is reduced by using reflectron thereby the actual length of the instrument is reduced. This instrument is the most accepted instrument commercially due to its versatility to adopt liquid samples. Electro spray ionization probe has been optimized to adopt nano flow rates and separation achieved by nano LC columns etc. It is widely used for high resolution approaches for metobolomics and enables library search option in the international database like MASCOT, GOLM METABOLOME DATABASE, NIST database etc.



6) Hybrid-Orbitrap Fusion Analyzer

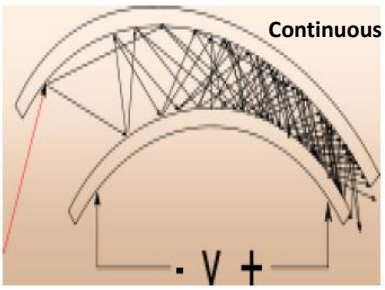


Courtesy – Thermo Scientific Pvt. Ltd.

Detectors

Various detectors are used for capturing the ionized species in the mass spectrometer, such as, phosphor coupled photomultiplier, electron multiplier, multichannel plate detectors. Solid state detectors utilize dynodes to convert into ion signal secondary to the amplification. Many detectors use an additional ion acceleration step which is called post-acceleration prior to detection. This step increases ion velocity and improves sensitivity.

Electron Multiplier



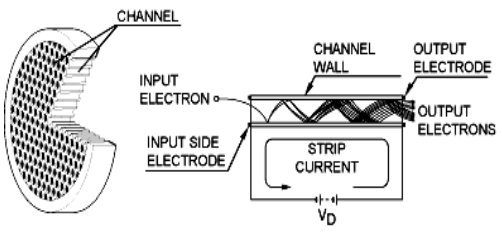
Continuous dynode

Electron multipliers provide signal detection through ion conversion and subsequent amplification. When an ion impacts on the conversion dynode, it converts the ions to secondary particles, such as electrons and these secondary particles are accelerated into an electron multiplier, on which DC voltage is applied. The conversion dynode can be configured to work in any polarity. The active lifetime of an electron multiplier is a function of surface deactivation-ion, such as caused by the absorption of water or contaminants to the multiplier surface, and can range from 1-2 years. Electron multiplier is the commonly used detectors in quadruple mass analyzers and quadruples ion traps.

Photomultipliers

The conversion dynode of a photomultiplier detector generates electrons that impinge on a phosphor, which subsequently generates photons that are detected and amplified by a photomultiplier. Photomultiplier has advantage over electron multiplier as their shelf life is around 10 years. Photomultiplier is encased in a glass and hence not susceptible from damage through water molecules or contaminants. One disadvantage of photomultiplier is, that it is very sensitive to light background and thus mass spectrometer having these detectors should be kept away from the ambient light.

Micro Channel Plate

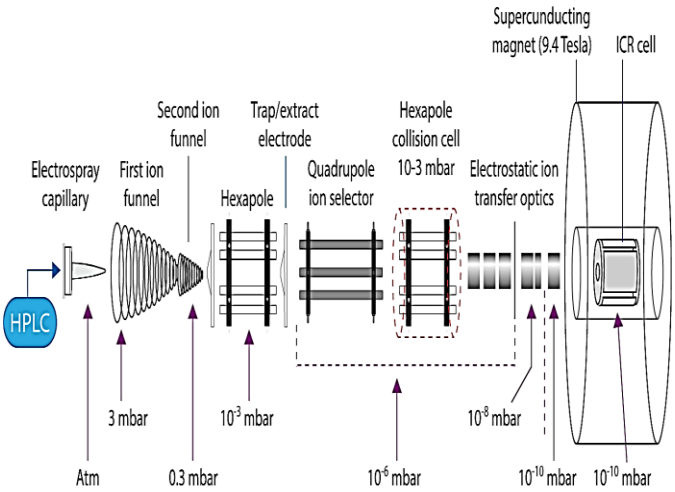


Microchannel Plate detector (MCP) is one type of the solid state array detectors commonly used in mass spectrometry. Microchannel plates are flat wafer-like detectors of leaded glass consisting of an array of 104-107 electron multipliers in parallel, each channel of which is 10-100 μm in diameter. Microchannel plate detectors are particularly useful in time-in-flight mass spectrometry, as they are flat and minimize the time spread and enhance subsequent mass resolution. In addition, they have reasonable gain and has fast response rate (100-psec time resolution). The major limitation of this detector is that it requires recovery time to recharge.

Other modified type of mass spectrometer

Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer

The Fourier-Transform Ion Cyclotron Resonance (FT-ICR) mass spectrometer uses a superconducting magnet to trap ions in a small sample cell. This type of mass analyzer has extremely high mass resolution and is also useful for tandem mass spectrometry experiments. These instruments are very expensive and are typically used for specialized research applications. The ICR traps ions in a magnetic field that causes ions travel in a circular path. This is similar to the path of an ion in a magnetic sector, but the ions are not traveling as fast and the magnetic field is stronger. As a result the ions are contained in the small volume of the trap.



The relationship between ion's m/z and its rotational frequency is,

$$m/z = B/2f_e$$

B = Strength of magnetic field

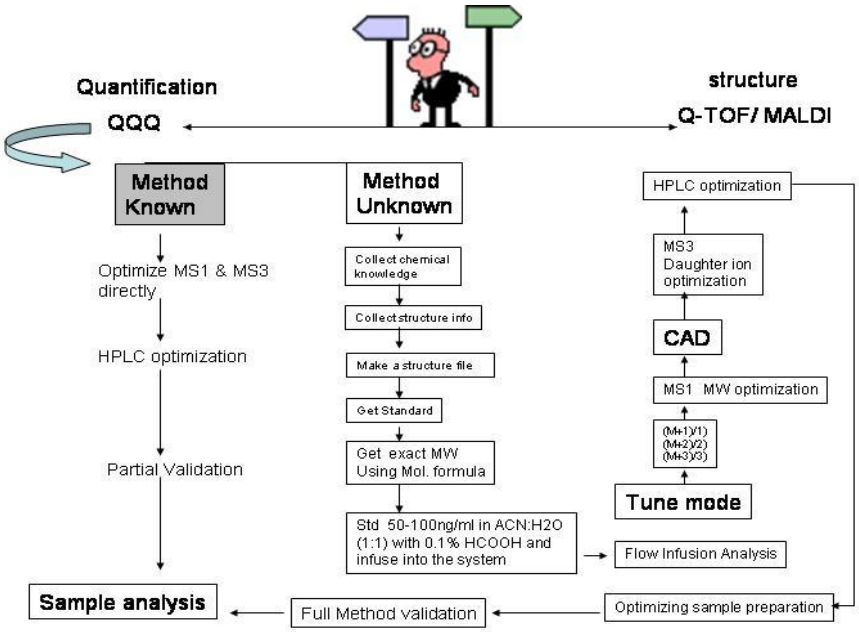
f_e = rotational frequency

By applying a variable frequency to the ion storage compartment that is resonant with fe, the radius of a given window of ions can be made to increase as the ions absorb the resonant energy.

	Quadrupole	Ion Trap	Time-of-Flight	Time-of-Flight Reflectron	Magnetic Sector	FTMS	Quadrupole-TOF
Accuracy	0.01% (100 ppm)	0.01% (100 ppm)	0.02 to 0.2% (200 ppm)	0.001% (10 ppm)	<0.0005% (<5 ppm)	<0.0005% (<5 ppm)	0.001% (10 ppm)
Resolution	4,000	4,000	8,000	15,000	30,000	100,000	10,000
m/z Range	4,000	4,000	>300,000	10,000	10,000	10,000	10,000
Scan Speed	~a second	~a second	milliseconds	milliseconds	~a second	~a second	~a second
Tandem MS	MS^2 (triple quad)	MS^n	MS	MS^2	MS^2	MS^n	MS^2
Tandem MS Comments	Good accuracy Good resolution Low-energy collisions	Good accuracy Good resolution Low-energy collisions	Not generally applicable	Precursor ion selection is limited to a wide mass range; growing number of applications	Limited resolution High-energy collisions	Excellent accuracy and resolution of product ions	Excellent accuracy Good resolution Low-energy collisions High sensitivity
General Comments	Low cost Ease of switching pos/neg ions	Low cost Ease of switching pos/neg ions Well-suited MS^n	Low cost	Good accuracy Good resolution	Instrument is massive Capable of high resolution	High resolution, MS^n high vacuum, super conducting magnet, expense	Known for high sensitivity and accuracy when used for MS^2

Comparative analysis of mass spectrometers

Developing method for a particular compound in triple quadrupole tandem mass spectrometer



Let us consider that we don't know any conditions regarding the ms/ms profile of a particular compound of our interest. We want to estimate its concentration in the biological samples like tissue, urine, blood, plasma, microdialysis fluid, tear fluid, aqueous humor, plant matrix or any other matrix. A classical algorithm to deal with the situation goes with the schematic diagram shown above.

Getting the molecular weight

A compound has a distribution of individual isotopic molecular weights based on the relative abundance of its constituent ions, this is important when expressing the molecular weight of a compound. Mostly, the molecular weight of a compound is expressed as a weighted average of its constituent isotopes and is called as the average molecular weight. As a mass spectrometer is typically capable of resolving the constituent isotopes of a compound, so it is convenient to define molecular weight not by average but by exact mass. It is done by expressing the molecular weight based on a single isotope, most commonly the lowest molecular weight isotope and is called as monoisotopic molecular weight. The exact molecular weight of a compound is calculated from its molecular formula.

Preparation of stock solution for MS tuning

100 ng/ml equivalent of pure compound in 50% acetonitrile with either 0.1% formic acid or 5mM Ammonium acetate is prepared. 1ml of the solution is loaded in the Hamilton syringe and kept in position on the Harvard infusion pump. The solution in syringe is connected to the mass spectrometer by peek tubing. The flow of the solution is maintained at 5µl/min.

Setting the quad to get the compound of interest

Quadrupole is set in the tune mode. When operating mass analyzer in full scan mode (start and stop scan mode), the RF/DC voltage scans between two extremes, thereby generating window of m/z stability that ultimately generates a mass spectrum.

(i) Mass Scanning Mode

All MS techniques rely on the fragmentation of a parent ion in the mass spectrometer. Assuming ion to be singly charged, upon fragmentation in the collision cell both a neutral fragment and ion of lower m/z is generated.

(ii) Precursor Ion Scanning

It provides information about the parent ion i.e the ion to be fragmented. This is done by configuring Q3 of the triple quadrupole MS system as a static mass filter and Q1 as a scanning mass analyzer, thereby detecting only the precursor ions. The precursor ion scan provides the qualitative information on the structure of an ion, with specific applicability determined by the characteristic of ion fragmentation or more specifically, depending on what part of the ion retains the charge after fragmentation.

(iii) Product Ion Scanning

It gives information about the daughter ion i.e the fragmented ions. For this, Q1 of the quadrupole is configured as a static mass filter and Q3 as scanning mass analyzer, therefore detecting only the fragmented ion.

(iv) Neutral Loss/Gain Scanning

It provides information about the neutral or uncharged fragment. If upon fragmentation, the functional group of interest is lost as a neutral molecule, then another approach called as neutral loss scan is required. During neutral loss scan, both Q1 and Q3 of the mass spectrometer act as scanning mass analyzers.

Applications of mass spectrometry techniques

Analysis of bioactive compounds from natural products

Along with the advancement in separation technologies like HPLC, HPTLC, UPLC and flash chromatography, isolation and identification of natural products increased tremendously. Identification, isolation, quantification and structural identification of natural products are achieved by ion trap, triple quadrupole, time-of-flight, Orbitrap, ion mobility and other hybrid instruments. Extensive literature regarding their application can be seen in Pubmed and other data bases.

Omics studies using tandem mass spectroscopy

Normally, quantitative and high accuracy mass studies in Proteomics, lipidomics and metabolomic studies are accomplished by using LC coupled Q-TOF instruments and triple quadrupole instruments. Post-translational modification of proteins is another area in which use of mass spectrometers are extensively utilized. Vast amount of literature is available in this area in the common databases like pubmed and embase. High resolution obtained by quadrupole is capable of calculating the charge state of multiple protonation in the proteins which helps in determining the molecular weight accurately.

New born metabolism errors

A drop of blood is used for the identification of developmental, genetic and metabolic disorder in the newborn baby within 48 hrs of birth. This allows steps to be taken before symptoms develop in the later. A panel of metabolites are quantified with the help of triple

quadrupole mass spectroscopy to check for40 disorders. However, because phenylketonuria (PKU) was the first disorder for which a screening test developed, some people still call the newborn screen "the PKU test".

Drug metabolism and pharmacokinetic studies: In new drug discovery and drug interaction

Mass spectrometers are extensively used in new drug discovery preclinical and clinical studies to evaluate the fate of drug in the animal and human body. Metabolites of the lead compounds are identified and analysed for their fate in the body. Route of excretion, effect of drug interaction, effect of formulational parameters, impact of the presence of food and route of elimination are identified using liquid chromatography coupled mass spectrometers.

Identification of organism using mass spectroscopy finger print

Continuous monitoring of pathogenic microorganisms in food, water and infections is essential for taking appropriate measure to safe guard people. Moreover, it is also very important in the era of bioterrorism where the rapid, high throughput and accurate prediction of microorganism is essential. MALDI-TOF MS developed as a standalone technique for the identification based on microbial finger printing for intact cells. Library of fingerprints have already been developed for the identification of bacteria, fungi and viruses. Many quantitative approaches are in use for analyzing finger prints of microorganisms. However, the reproducibility of fingerprints (due to phenotypic variation in gene expression) of intact cells is a matter of concern and limitation associated with this approach for normal diagnostic applications.

Environmental pollution monitoring

Environmental contamination of high volume chemicals such as pharmaceuticals, phytoestrogens, endocrine disrupting chemicals and all of their degradation products are of increased concern in the phase of modernization. Presence, antimicrobial agents, pesticide residues, herbicides, organometalic compounds, toxins from algal and cyanobacterial orgin, are also carry similar burden for the environment which in turn affect living organisms in various levels of life in all the forms. Electrospray and atmospheric pressure chemical ionization methods are often employed with triple quadrupole tandem mass spectrometers give adequate sensitivity for the analysisof the trace amount of these agents from environmental samples such soil, water, live stocks and processed foods. Analysis of newer information regarding the structural variety of humic substances are made possible by using FT-ICR-MS due to its ultrahigh mass resolution. Moreover, the presence of micro-organisms in processed food are rapidly detected by the aforesaid MALDI and LC-MS methods.

Physiological parameters

Biological research involves, analysis of various metabolites involved in biochemical pathways of physiological importance. At times, the amount of sample like tears, humors of the eye, synovial fluid, cerebrospinal fluid and tissues samples taken by endoscopy procedures. Presence of metabolites and their contribution to pathological conditions are analysed by mass spectrometers. Quantification of neurotransmitters in brain tissues by using microdialysis techniques are largely supported by the use of LC-MS.

Forensic analysis

Presence and concentration of toxic chemical substances and therapeutic drugs at toxic doses in bodily fluids and tissues are very important while deciding the reason for the death. This information is vital at times whether the death happened by suicidal or homicidal and intentional or unintentional . With the modern liquid chromatography coupled tandem mass

spectroscopy is capable of identifying and quantifying diverse toxic compounds in shortest possible time.

NASA's space explorations

The inclusion of the SAM (sample analysis at Mars) instrument suit for Mars mission. NASA constructed the Mars Science Laboratory for Curiosity Rover with unique features for sample analysis in Martian ecosphere. This probe includes three instruments including a mass spectrometer, gas chromatograph and tunable laser spectrometer. This instrument would be collecting samples on Martian surface with its robotic arms and will subject it for evaporation and analysis by mass spectroscopy. This will measure the abundances of light elements such as hydrogen, oxygen and nitrogen which are associate with life.

Summary

Over the time of last two decades the tremendous development in the field of mass spectroscopy made the possibility of quantifying the compounds of interest using molecular weight. A mass spectrometer is typically capable of resolving the constituent isotopes of a compound, thus, making it possible to identify every single isotope of a compound, based on its mass-to-charge ratio. For mass spectrometric analysis of a compound, the sample has to be introduced into the ionization source of the instrument for the production of gas phase ions of the compound. The choice of ionization method depends upon the polarity and molecular weight of the compound. Different types of mass analyzers are available, for transmitting a single m/z to the detector. They range from quadrupole, ion trap for small molecules to time-of-fight, orbitrap for macromolecules. Various detectors are used for capturing the ionized species in the mass spectrometer, such as, phosphor coupled photomultiplier, electron multiplier, multichannel plate detectors. Different mass scanning modes such as Precursor Ion Scanning, Product Ion Scanning, Neutral Loss/Gain Scanning enable accurate detection of the compound of interest, while it is being tuned in the mass spectrometer. The robustness and sensitivity of mass spectrometry has resulted in diverse applications such as, analysis of bioactive compounds from natural products, omics studies using tandem mass spectroscopy, drug metabolism and pharmacokinetic studies, environmental pollution monitoring, forensic analysis and even in NASA's space explorations.