Wien crossed the mile stone in 1899 in similar experiments. He found "Kanalstrahlen" which means "canal rays" in English [6]. Wilhelm charges. He named these deflected charged anode (positive) rays perforated and deflected to cathode depending on their positive under experimental or in diseased condition conditions [5]. This particular technique has therefore, many applications starting from quantitative to qualitative analyses of metabolites under normal, experimental and diseased conditions in organisms including human being. Based on the method applied, sample preparation process, sample type, measurement process such as abundance or time of retention or flight of charged fragments etc., each MS vary with its own advantages and disadvantages. A general commentary article is written on this particular modern technique to make it understood and popular among the researchers. Present century is the era of life sciences and metabolomics is the dominating field in present day over genomics and proteomics. Powerful techniques in the later cases are useful to determine the qualitative and quantitative studies of the levels of metabolites in bio-medical samples. In past time, biochemistry had very least role because the analyses of metabolites using individual conventional biochemical techniques had been used from past centuries. Owing to this limitation, mass spectrometry (MS) was introduced in bio-medical sciences. The fundamental rule in MS is that it ionizes individual chemical species and sorts the fragmented samples at charged state (ions) on the basis of their mass to charge (m/z) ratio. Therefore, each molecule in purified form or in homogenate can be identified and quantified based on their unique m/z ratio. In other words, MS measures the masses of the fragmented samples by enabling them to be charged during measurement. This particular technique has therefore, many applications starting from quantitative to qualitative analyses of metabolites under normal, experimental and diseased conditions in organisms including human being. Based on the method applied, sample preparation process, sample type, measurement process such as abundance or time of retention or flight of charged fragments etc., each MS vary with its own advantages and disadvantages. A general commentary article is written on this particular modern technique to make it understood and popular among the researchers. Keywords: Advance technique; Modern biochemistry; Biomedical sciences; Mass spectrometry; Mass to charge ratio

Introduction

The age of metabolomics arrived in recent decades after genomics and proteomics dominated in biomedical sciences due to their applications [1]. Powerful techniques in the later cases such as transcript analysis, protein expression studies were useful to determine the molecular mechanism of biological phenomena at genomics and proteomics level [2,3]. During those past periods, biochemistry had very least role although the analyses of metabolites using individual conventional biochemical techniques had been used from past centuries. This is because; biochemist still had no powerful and reliable techniques to analyze the metabolites and proteins in biological samples. May be this is one of the major reasons why mass spectrophotometry (MS) was necessary and eventually discovered, followed and now has been established as the most advanced technique in biochemistry [4]. Due to its reliability, reproducibility, wide applicability and contribution in modern biomedical sciences, the technique is now dominating over most of the genomics and proteomics techniques. The fundamental rule in MS is that it ionizes individual chemical species and sorts the ions based on their mass to charge (m/z) ratio [5]. Therefore, having uniqueness for the above character, each molecule in purified form or in homogenate can be identified and quantified too. In simpler terms, a mass spectrum measures the masses within a sample by enabling them to be charged during measurement. Mass spectrometry is used in many applied and basic sciences and is applied to analyze isolated pure samples or complex mixtures in bio-medical sciences. This particular technique can be used to identify abnormal or disordered proteins/metabolites for quantitative and qualitative analyses of metabolites under experimental or in diseased condition conditions [5].

The history of MS goes back to 18th century when Eugen Goldstein noticed in 1886 that rays travelled under electric field are perforated and deflected to cathode depending on their positive charges. He named these deflected charged anode (positive) rays as "Kanalstrahlen" which means "canal rays" in English [6]. Wilhelm Wien crossed the mile stone in 1899 in similar experiments. He found that canal rays are deflected by strong electric or magnetic fields. Based on the results, he was able to build a machine in which positive rays were separated under parallel electric and magnetic fields on the basis of their charge-to-mass ratio (Q/m) [6]. He noticed that the type and nature of the gases used in the discharged tube influence their Q/m ratio. Then work of Wien was improved by the British scientist JJ Thomson who was able to reduce the pressure of the measuring environment to generate the mass spectrograph. The word "spectrograph" obtained by such devices was systematically introduced internationally in science and was became a part of the scientific vocabulary in 1884 [7-9]. Owing to the importance of the technique, it has evolved with new adjustments and advances with specific requirement. In this article, general information is aggregated in this article on this particular technique to make its language simpler among the researchers.

Why Mass Spectrophotometry?

Mass spectrophotometry has numerous advanced advantages when enlisted; it seems to replace many techniques in future. Some of the newer trends in biomedical and chemical sciences those use MS as their basic tool. The vast application of this technique already removed its various limitations to be used in other areas of sciences such as geology and space science [5]. Due to the complexity of the matrix of samples for example, blood or urine, requirement of more sensitivity for low dose studies, and acquisition of data for long time point (for example for sustained release) of drugs, MS has its unique importance in pharmacokinetics. Most commonly used instrumentation in pharmacokinetics with the above needs is liquid chromatography.
MS (LC-MS) with a triple quadrupole system [5]. Tandem MS is also preferred by many workers because for ensuring added specificity in drugs. The pharmacokinetics i.e. bioavailability or level of drug samples at different time points and the clearance rate from the body can easily be monitored using blood and urine. On the other hand, the high sensitivity of analyzers present in MS clears the path of pharmacologists to study microdosing principles. Analyzing the metabolites and determining the abnormal proteins in patients extended the use of MS in medical sciences. May be these are the reasons why many world class patho-laboratories use MS as one of the major techniques for analyses of medical samples [10-12]. It is believed that respiratory gas analysis in patients using MS had been done in beginning around 1975. Some of these machines are perhaps still used in some laboratories but their manufacture is stopped [13]. Some MS instruments are intentionally modified to simultaneously report the composites of gases that are respired by a patient although the mass range for the detection is limited to ~ 120 amu [14].

Quantitative analyses, characterization and sequencing in proteomic studies are now mainly based on MS due to its high accuracy of results. The protein(s) can be fragmented and ionized with electrospray ionization and matrix-assisted laser desorption (MALDI)/ionization or protein(s) can be enzymatically digested for example with trypsin or pepsin into smaller peptides and then be analyzed for peptide mass fingerprinting. Using tandem MS de novo peptide sequencing are now phsyible to be done [4,5].

In MS, one of the major advantages is the requirement of low sample and high sensitivity. In glycobiochemistry, such application of MS than HPLC predominantly makes it preferable and reliable for characterization and elucidation of the structures and properties of glycans [15]. Several free and commercially sold softwares are available now days to interpret the MS data for structural elucidation of glycans [16,17]. Unbroken glycans are detected directly as single charged ions by using matrix-assisted laser desorption/ionization MS. Such glycans can be also better detected by permethylation/peracetylation followed by atom bombardment MS (FAB-MS) [15]. Nevertheless, electrospray ionization MS (ESI-MS) is also used to study smaller glycans and this machine gives high sensitive signals too for the smaller glycans. High voltage is applied in ESI method to produce ions from macromolecules without breaking it which then can be detected by the detectors.

Quantification and characterization of less abundant isotope with sensitivity is another uniqueness of MS. As a result, 18O/16O and 13C/12C isotope ratio can be determined in samples. Therefore, in evolutionary biology, MS is also used to analyze the age of trees or fossils by carbon dating or biogenious carbon. Labeling a particular amino acid with stable isotopes of the composite elements is also used for quantification of protein(s) under different experimental conditions. Such instruments those quantify proteins with labeled amino acids are sometimes referred as isotope ratio MS. These MS work on the principle that they use one magnet to twist the beams of ionized fragments/ particles towards a series of detectors (usually Faraday cups). The detectors then convert particle impacts as beams to electric current. Most sensitive and perfect MS for this purpose is flowing afterglow MS. These MS are capable to measure individual atoms and nuclides with a range of ~ 1018 as compared to the major stable isotope [18]. Using specific ion flow tube, reaction to transfer protons, several chemical ionization processes are used now days to analyze trace gases in air, breath or liquid headspace. Such modifications even are done by using particular reaction time and allowing the system to calculate concentrations of analyte(s) from known reaction kinetics. Such modifications will be helpful to analyze samples without using internal standard or calibration of the machine. The position of individual atoms in a compound is also mapped by combing two techniques out which one is MS. Such examples are combining atom probe based techniques such as time-of-flight (TOF) MS and field-evaporation microscopy. Mass spectrometry techniques are also widely used in space research programs to identify and measure the composition of plasmas. Exploration of the elements of other planets and moons is also being done using MS [19]. For example, in the Cassini-Huygens mission, a specialized GC-MS with Huygens probe was sent to the atmosphere of Titan, the largest moon of Saturn. The GC-MS lunched with the probe in the atmosphere of Titan and analyzed its atmospheric species and its descent trajectory. When the probe had landed on Titan, it was able to vaporize and analyze the frozen hydrocarbon samples that covered the surface. After such measurements, a comparison was made about the abundance of isotope(s) of each particle present in Titan to that natural abundance in earth [20]. Similarly, using Cassini Plasma MS in the Cassini spacecraft, the mass of ions in magnetosphere of Saturn was measured [21].

**Mass spectrometry in early times**

Early spectrometry instruments that were able to measure the mass to charge (m/z) ratio of ions and therefore they were named as mass spectrographs. These devices were capable to record the spectrum of mass values of elements onto a photographic plate [22]. A mass spectroscope is typically comparable to a mass spectrograph. However, the only exception is that beams of fragmented charged particles (ions) are directed and collected on a phosphor screen in later case [23]. The moniker “mass spectroscope” is used continuously even after the direct illumination of a phosphor screen was replaced by indirect measurements with an oscilloscope [24]. However, the term “mass spectroscopy” is now discouraged to be used to avoid confusion with light spectroscopy [25,26]. Mass spectrometry is also called as “mass-spect” or its generic name as MS [26]. Due to the accuracy in both qualitative and quantitative analyses of metabolites and proteins, MS has wide applicability in bio-medical sciences which is discussed later part of this article.

Early technique in MS was designed and modified by AJ Dempster and FW Aston in 1918 and 1919, respectively. Ernest O Lawrence then developed sector mass spectrometers that are known as “calutrons”. They were basically utilized for separating the isotopes of uranium [27]. At the Oak Ridge, Tennessee Y-12 plant, Calutron MS was used for enrichment of uranium metal during World War II. Half of the Nobel Prize in Physics was shared in 1989 by two scientists namely, H Dehmelt and W Paul for the development of ion trap technique. They developed the technique in the year 1950s and 1960s, respectively. Further, John Bennett Fenn developed electrospray ionization and Koichi Tanaka developed soft laser desorption in 2002. They demonstrated the application of their invention for ionization of bio-samples. Especially biological macromolecules mainly proteins were ionized by the machine. For their invention, they shared the Nobel prize in 2002 in Chemistry [28].

**What is Mass Spectrum?**

The mass spectrum is a plot where the ion signals are plotted against the m/z ratio of molecules [8]. To determine the signature of elements or isotopes, such spectrum is used. It is also used to determine the masses of molecules and to elucidate the chemical structures of compounds and chemical signature and sequences of peptides [29]. A typical MS procedure includes bombarding electrons to a sample that
may be in solid, liquid or gaseous form for ionization. It may result in fragmentation but charging of compounds present in samples. These fragments with charge (to form ions) are separated then as a function of their m/z ratio when they are moved under electric and/or magnetic field. The key factor is ions with same m/z ratio experience and show the same magnitude of deflection during their movements under the electric and/or magnetic field [26]. As the detector system in a simple spectrophotometer, the deflected ions are monitored and recorded by a detector capable to sense charged particles. Example of such a detector is an electron multiplier. As a result, the type and magnitude of charged compounds are shown as spectra that are directly proportional to the magnitude of the respective ions. And the relative abundance of the ions is quantified as a function of the m/z ratio. Each component in the sample after or without fragmentation will have unique spectra that will correspond to the fragmentation pattern or mass of a compound, respectively, in a purified sample or mixture. Based on such results, the purified compound or the constituents of a mixture relative abundance can be identified. It becomes possible by correlating the pattern of fragmentation or a reference compound with known characteristics and mass [5].

The instrumentation and moniker of mass spectrometer

Like a simple spectrophotometer, a MS consists of three fundamental components: an ion source instead of light source in spectrophotometer, a mass analyzer instead of a photomultiplier tube in spectrophotometer, and a detector, which is usually a phosphor screen or an oscilloscope in MS [30,31]. The ionizer as it named, ionizes a part of the fragmented sample into ions or simple add charges to the fragmented sample. Depending on the state i.e. solid, liquid or gas of the sample, the ionization techniques may vary. It can also vary for known and unknown samples and for the latter case, it vary as per the phase of the sample. After ionization, an ion remover filters the ions and forwards them to mass analyzer followed by the detector [32]. On the basis of m/z ratio, the fragmented ions are then sorted by the mass analyzer. Based on the signals, the detector measures and provides data about the quality, characters and abundances of each fragmented ions (Figure 1). Few detectors are modified as per the need to give spatial information as well for example, multichannel plate. Therefore, ionization and detection of ionized fragments in samples seem to be the key steps in MS [32]. To clarify the instrumentation in MS, MALDI-ToF can be considered as example. Non-mass spectrometrists widely accept the MALDI-ToF moniker than MALDI or ToF individually. Occasionally, “MS” is used as the generic name that denotes a device with an extremely definite sector based analyzer and detection system, for example in Atomic Mass Spectrometry (AMS). Specific functions and applications of MS alert the monikers to refer its broad definition. Finally, instrument configurations for the particular moniker of the MS become precise. Isotope ratio mass spectrometry (IRMS) is an example of such moniker practice. Because restricted mass analyzers are used in IRMS which are sector based. Other examples of such MS are thermal ionization-mass spectrometry (TIMS), inductively coupled plasma-mass spectrometry (ICP-MS), accelerator mass spectrometry (AMS) and spark source mass spectrometry (SSMS) [5,32].

Resolving and mass determining capabilities of MS in tandem is also dependent on the separation technique that integrated with the system. Usually chromatographic techniques and exceptionally other separation techniques are used to separate the sample composite before ionization. A familiar assembling is chromatography (either gas or liquid phase) with MS, i.e., GC-MS or LC-MS. As the name indicates in GC-MS, a gas chromatographic technique is used to separate different components of a mixture before their fragmentation, ionization and detection. In smaller term, the mobile phase is a gas in GC-MS. Notably, volatile or lipoid substances can be separated by this technique but not the proteinaciuos substances. An ion source then ionizes the divided components of the sample when their flow is channelized. An ionizer is usually a metallic filament to which voltage is applied to emit electrons. Emitted electrons from the filament ionize the compounds. The ions can then further be fragmented to yield a predictable pattern(s) of the components. Finally, the unbroken ions or broken fragments are flown into the analyzer of the MS to fall on the detector [33]. Instead of gas phase in GC-MS, a liquid mobile phase is used in liquid chromatographic MS called as LC-MS [5]. As liquid phase, water and several organic solvents are usually used together as a mixture. As a source of ionizer, electrospray ionization is usually used. Atmospheric pressure chemical ionization, atmospheric pressure photoionization and laser spray are also used as other available LC/MS ion sources [5]. When a capillary electrophoresis liquid separation system in incorporated with MS it is referred as Capillary Electrophoresis-Mass Spectrometry (CE-MS) [34]. In CE-MS, electrospray ionization process is usually used as ionizing source [35]. Drift time is a measure for the radius of an ion which is influenced by its charge. In some MS, ions are first separated by their drift time under an applied electrical filed. It is done before the ions are processed into analyzer and detector units of the MS. The job is done with the help of some neutral gases. Such MS are referred as Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS or IMMS) [36]. The advantage of IMS is that it produces data in a similar way as that of a LC/MS [37]. So, it becomes easy to analyze the data by the workers who are familiar to deal with LC-MS data. Another advantage of IMS is its duty cycle of IMS is shorter in comparison to liquid or gas chromatography separation processes. So, IMS can be coupled to other MS techniques which can results in producing triple modalities such as LC/IMS/MS. However, one of the limitations in IMS is that longer time is taken by it to analyze a sample than most of the MS [38].

Biophysics of screening fragments based on m/z ratio

Basically two laws govern the dynamics of charged particles in electric and magnetic fields in vacuum. One is Lorentz force law in
which the force is measured by the equation $F=Q(E+vXB)$ and the speed is Newton's second law, i.e., $F=ma$ and this is applicable when the ionized molecules move with a velocity less than the velocity of light [5,32,39]. Here “F” indicates the force provided to ion, “m” indicates ionic mass, “a” indicates the acceleration by with which the ions move, “Q” denotes charge of the ion, “E” denotes the applied electric field, and “v x B” denotes the cross vector product between the ionic velocity and the magnetic field. When both the equations are combined it result in $(m/q)a=E+vXB$ and is widely accepted as a classic equation for charged particles to measure their movement. So, MS determines the particle’s motion in space and time based on their $m/Q$ ratio [39]. Thus MS can be otherwise referred as ‘mass-to-charge spectrometers’. The official language used for the above ratio is $m/z$, where “z” denotes the charges of the elements (e) on the ion, i.e., $z=Q/e$. Therefore, the final value although is unofficially called as $m/z$ ratio, more precisely it indicates the ratio between the mass (m) and charge number (z) [5,32,39].

Sample Preparation for MS Studies

As different samples such as proteins, lipids, carbohydrates or purely chemical samples needs different ionization techniques, analyzer and detector systems, it is noteworthy to mention that preparation of samples will differ based on the above mentioned parameters. For example, solid samples need to be ionized by filed disposition/plasma disposition/fast atom bombardment/MALDI methods. Whereas solution are ionized by electrospray, secondary ions, atmospheric pressure chemical ionization methods. Similarly, gas samples are ionized by electron ionization, photoionization and chemical ionization methods [39]. Based on the ionization sources, methods of preparation of samples will vary. For example for protein samples, lystate preparation (by lysis using buffered detergents, fractionation, depletion, enrichment and dialysis), in solution digestion (by reduction, alkylation, enzymatic digestion using trypsin and other enzymes), peptide enrichment and cleanup followed by MS is done [32]. The lysates can also be separated in 1 D or 2 D gels before digestion and enrichment steps. The object here is to clean the unwanted fractions from the sample to increase the robustness of the data. Chemical samples can directly fed into GC if is in one solvent but with proper dilution [5,32]. For metabolomics studies, volatilization, solid phase extraction, liquid-liquid extraction and direct injection are few methods of sample preparation [40].

Ionization and detection of samples using various MS methods

The process of ionization will be better understood in MS systems with an example. NaCl if needs to be analysed, it is vaporized first, and ionized as Na⁺ and Cl⁻ ions. Sodium atoms have ionic mass of 23 amu and its ions are mono-isotopic, i.e., it has no isotopic form in NaCl. However, Cl atoms are di-isotopic having atomic masses of ~ 35 amu and ~ 37 amu. Under both electric and magnetic fields of the analyzer of MS, the ions travel. The speed of a charged particle is controlled when it moves through an electric field, and the magnetic field possibly governs its direction of movement. The degree of movement and deflection from its path of the moving ion therefore are altered based on its $m/z$ ratio. Magnetic force deflects the lighter ions more than heavier ions (governed by Newton’s second law of motion, $F=ma$) [39]. As a result, moving ions from a mixture can be sorted due to their different speed and degree of deflection which are dependent on their $m/z$ ratio. The flows of the ions that are sorted as described above are then reached on the detector from analyzer. Since, each ions for example ions of Na and Cl along with its isotopic form will have different pattern of speed and deflection on the basis of their different $m/z$ ratio, different respective mass spectrograph are recorded which again correlate with their relative abundances. Such fundamental or raw data are analyzed to establish element composition and the chemical nature of samples, for example Na and Cl in the present case. That means the amount of both Na and Cl along with the isotopic forms of Cl, i.e., $^{35}$Cl to $^{37}$Cl ratio in a sample can be quantified. Gases and vapors are ionized using electron ionization (EI) and chemical ionization (CI) methods. In the later case, the analyte is ionized by chemical ion-molecule reactions during its collisions with the ion source. Similarly, for ionization of liquid and solid biological samples, two techniques such as electrospray ionization and MALDI are used [41,42].

Advantages and disadvantages of ionization processes

Each ionization process has its own advantages and limitations. For example, EI process fragments the sample with higher rate degree as a result it yields highly detailed mass spectra (Figure 2). These spectra can be skillfully analysed to obtain important information for structural elucidation/characterization. Finally, from the data, detection of unidentified compounds can be done by comparing to libraries such as Mascot in matrixscience.com that store mass spectral data of various compounds and elements. On the other hand, electron ionization is used primarily in GC-MS, where the whole system is run under high vacuum. However, this method of ionization is incompatible to combine with instruments such as HPLC, i.e., LC-MS, because under normal atmospheric pressure, the filaments used to produce electrons burn out quickly. To avoid such limitations, hard ionization techniques are employed. In hard ionization techniques, high quantities of residual energy are imparted that results in fragmenting the sample at higher rate. The reason attributed for the above is, excess energy ruptures chemical bonds orderly so that it restores the constancy of the produced ion(s). The produced ions tend to have lower m/z ratio in comparison to their respective molecular masses at original condition. In contrast, soft ionization process imparts very less residual energy on the sample and it therefore result in less fragmentation rate of the compounds present in sample. Fast atom bombardment, chemical ionization, atmospheric-pressure chemical ionization, electrospray ionization and matrix-assisted laser desorption/ionization is few examples of soft ionization techniques [39]. Among the other ionization techniques, field desorption (FD), fast atom bombardment (FAB), photoionization, inductively coupled plasma, glow discharge, thermospray, desorption/ionization on silicon (DIONS), direct analysis in real time (DART), atmospheric pressure chemical ionization (APCI), secondary ion mass spectrometry (SIMS), spark ionization and thermal ionization (TIMS) are also being used in various MS as per the requirement(s) [43]. Nevertheless, for proteinomics MS

![Figure 2: Mass spectrograph of a fragmented protein.](image-url)
Types of MS Based on Mass Analyzers and their Limitations

Based on requirements, several types of mass analyzers are used in MS. Although, different MS that uses static or dynamic fields magnetic or electric fields has different mode of actions, all follow the typical equation \( m/q = E + v 	imes B \) \[5,32,39\]. Many MS use two or multiple analyzers for tandem measurements (MS/MS). Practically “mass resolving power” is the criterion to assess the ability to discriminate multiple or at least two peaks with a little dissimilar m/z ratio values. The mass accuracy is calculated by dividing the m/z value error to the correct m/z ratio. The value obtained in such calculations is generally calculated in milli mass units or parts per million units. The range of mass to calculate m/z should be within the limit of the analyzer. The “linear dynamic range” is measured over the range of the linear signal given by the ions and quantity of analyte. Speed of measurement is determined by the time taken by a MS to produce the number(s) of spectra per s/min/h \[5,32,39\].

Sector field mass analyzer is another one in which the pathway and/or speed, more accurately the velocity of the charged particles or fragmented ions are measured under an electric, magnetic or both under electric and magnetic field(s). Such analyzer is used to analyze a limited range of m/z ratio or to examine within a range of m/z ratio that can enlist the ions present in the sample \[44\]. Different ionized particles have different charges and hence they bear different but fixed kinetic energies because velocities of each ion will be as per their masses. Therefore, the detector will obtain the lighter ions first as they will reach faster than the heavier one. The time taken by the particles to hit the detector often is called as “the time-of-flight” and such analyzers are named accordingly as time-of-flight (TOF) analyzers. The principle is that under the same electric potential, different ions are accelerated to hit the detector at different time based on their m/z value. And hence the time to hit the detector is different for different ions which become the unit of measurement \[45\].

Another MS analyzer called as quadrupole mass analyzers that use oscillating electrical fields to categorically influence the path of ions. The applied electric field either stabilizes or destabilizes the path of ions travelling under a radio frequency quadrupole field. The radio frequency quadrupole field is usually created among 4 rods arranged parallel to each other. Such analyzers allow only the ions within limited range of m/z ratio via it at any particular time point \[46\]. Subsequently, when the potentials in the quadrupole rods changes, it allows fragmented ions within suitable range of m/z ratio values to be moved or cleared rapidly each time. The movements can be either of continuous or in a sequence of separate trips \[47,48\]. Following the identical techniques and rules as the quadrupole mass analyzer, another quadrupole ion trap is developed in which ions are trapped and directed to eject in sequences. Ions are trapped generally under quadrupole field i.e. within a space confined by the ring electrode that present between two endcap electrodes. The later electrodes are usually connected to direct current or auxiliary alternative current potentials. The fragmented samples are ionized inside by a beam of electron or laser, or are ionized on the outside. Then the ions are directed via a slit present in the endcap electrode. Resonance excitation technique is also used to direct to eject ions in which the endcap electrodes are supplied with an additional oscillatory excitation voltage. The varied magnitude of the entrapping voltage along with or alone the frequency of the excitation voltage brings ions into a resonance condition for separation based on their m/z ratio \[49,50\]. Quadrupole ion trap is further modified at the electrode level. The electrodes are designed in the form of flat rings instead of hyperbolic shaped electrodes. Such MS are called as cylindrical ion trap MS. As a result, the dimension of a trapping area is reduced and the shape of the electric field is occupied close to the core of the trapping area \[51\]. Similarly to a quadrupole ion trap, a linear quadrupole ion trap is also made. Such device traps ions in second dimension also inside the quadrupole field rather in third dimension quadrupole field \[52\].

A special MS that detects the mass of the fragmented ions by detecting the current image generated by ions cyclotron movement under a magnetic field is called as fourier transform mass spectrometry or fourier transform ion cyclotron resonance MS. Instead of detecting the deflection of fragmented ions with a detector such as an electron multiplier, the ions are ejected into a stationary electric/magnetic ion trap. Finally, they effectively become the part of a complete circuit. Detectors positioned in space detect the electrical signal of such ions which pass close to them over time. Finally, it generates a periodic signal. The frequency of cycling of an ion is measured by its m/z value. The periodic obtained signal thus can be deconvoluted by doing a fourier transform. In this system, each ion is taken into account for counting multiple times. Therefore, high sensitivity with greatly superior resolution is obtained in data. As a result, high precision is another plus point in such machines \[53,54\]. Ion cyclotron resonance is another older mass analysis method which is similar to the above MS technique. In such machines, ions are measured with a conventional detector system. Ions are trapped under a static electric/magnetic ion trapping system and then are excited until they collide on the wall of the detector. Based on the impact time of ions of different mass, the samples are resolved and detected \[55\].

Similar to Fourier transform ion cyclotron resonance mass spectrometers, Orbitrap MS are developed. In such MS, ions are trapped under a static electric field. Hey are trapped electrostatically in an orbit around a central, spindle shaped electrode. The ions confined by the electrodes so that they can orbit around the central electrode and oscillate back and forth along the long axis of the central electrodes. This frequent oscillation is m/z ratio dependent of ions and produces an image current on the detector which is finally recorded. The advantages of such MS are providing high mass accuracy of ions, high sensitivity and a good dynamic range to trap ions \[56\].

Finally, in all above types of instruments, the detector winds up the work by capturing the properties (as discussed in above techniques) of ions those are basically dependent on their unique m/z ratio. Normally, various types of electron multiplier are utilized nevertheless other detectors including Faraday cups and ion-to-photon detectors are also chosen in several MS devises. Many times, the number of ions those leave the mass analyzer to detector at a particular time point is rather normally less. Therefore, substantial magnification of the signal imparted by the ions is regularly essential to obtain recordable signal. Microchannel plate detectors are generally used in contemporary marketable MS devices to avoid such issues \[57\].

Tandem MS

One limitation in the MS that are discussed above is that all the machines are capable to read the entire sample once per time. Means all the machines have only one mass analyzer. Owing to remove this limitation tandem MS was developed. A tandem MS is capable of reading samples multiple rounds that are generally separated after fragmentation \[29\]. For example, he first mass analyzer can separate one peptide out of many injected into the MS machine and combined with electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) and for metabolomics.
a second mass analyzer stabilizes the separated peptide ions next to the first when they hit a gas make them to parts by collision-induced dissociation processes. The third mass analyzer fitted with the MS then sorts the fragments produced from the peptide. Tandem MS is also done in a single mass analyzer a different time points as seen in a quadrupole ion trap [51]. The central application of tandem MS is for protein identification and quantification under different experimental conditions. One more kind of tandem MS utilized for radiocarbon dating is accelerator MS that uses very high voltages. The range of he voltage used by such MS devices are usually in the mega-volt range to accelerate negative ions for the tandem MS. Various methods are used to fragment molecules for tandem MS. For example collision-induced dissociation, electron capture dissociation, electron transfer dissociation, infrared multiphoton dissociation, blackbody infrared radiative dissociation, electron-detachment dissociation and surface-induced dissociation [58].

**Data analysis of MS spectra**

The most common type of data researchers look for is mass spectrum with peak values (Figure 2). Particular kinds of MS data are best presented as a mass chromatogram. For example, in selected ion monitoring, total ion current and selected reaction monitoring [59]. Three-dimensional contour map is another form of MS data in which m/z ratio are presented on the x-axis, intensity on the y-axis, and any other experimental parameters, such as time, is represented on the z-axis. However, general categorizations of data are primarily done in order to understand every MS data [59]. The key factor is to determine whether the charge of the obtained ions, i.e., either positively or negatively charged. This is because any MS can work in either negative ion mode or in positive ion mode. It is therefore more vital in determining the neutral mass of the fragments because it implies somewhat regarding the nature of the elements. Several kinds of ion sources result in diverged arrays of ionized fragments. An electron ionization source generates a lot of fragments and typically single-charged (+1) radicals (with odd charge numbers), whereas an electrospray source generally generates non-radical quasimolecular ions that are often with multiply charged condition [60]. Purposefully, parts of fragmented ions post-source is produced by tandem MS and therefore, it can considerably modulate the type of data obtained in any research. Prior to data analysis, information on the source of a sample can offer insight into its constituent molecules and their possible fragmentation products. Simply, plant/animal/synthetic/industrial sample will have their unique and expected molecular fragmentation pattern. Some variation may also be obtained based on sample preparation and running processes and how the sample is introduced. For example, data can vary depending on which matrix is used for MALDI spotting, since a lot of the energetics of desorption/ionization incidents are controlled by the matrix rather than the laser power [61]. Occasionally, samples are spiked with sodium or any other ion-carrying element to create adducts instead of a protonated kind of ions. Further different experimental procedures are also required if the objective(s) is to measure molar mass, molecular structure, sample purity and successful interpretation [62]. Primary approach to distinguish and identify an unfamiliar or unknown complex from its raw data pattern is to compare it against a known library. The same procedure is followed for analysis of MS spectra. Without similar matches from the search results, manual and/or software assisted interpretation method is followed for interpretation of MS spectra [63]. In silico simulation is done as the key tool for assigning chemical makeup or sequence of peptides. Such simulation is commonly supported by a fragmentation data bank that has known patterns of decomposition reactions [64]. Mass-to-charge ratio values can symbolize huge amount of supposedly ion structures. However, more accurate mass figures considerably decrease the number of actual molecular formulas. An in silico algorithm called “formula generator” calculates all molecular formulae that theoretically fit a given mass with particular tolerance [65]. Precursor ion fingerprinting is one of the most recent techniques for structure elucidation in MS. In this method, identification of individual pieces of structural information is possible. It can be done by investigating the tandem MS spectra of the molecule under examination with a library of the product-ion spectra of structurally known precursor ions [66].

**Softwares Used for MS Data Analyses**

For data acquisition, analyses, representation, different web based online servers or offline softwares are used in mass spectrometry. For example, for protein metrics Byonic, mascot, massMatrix, ProteinPilot Software, Protein Prospector, RAId, SEQUEST, SIMS (for post translational modification studies), SimTandem, SQID, XTandem, for genomics and MassWiz, for MS/MS peptide quantification MarkerView Software, MassChroQ, MaxQuant, MultiQuan Software, OpenMS, Spectronaut and SWATH Software 2.0, for computational analysis Grelag, InsPec T, MyriMatch, pFind, for biotechnological information OMSSA, for de novo sequencing Phenyx, CycloBranch, DeNovoX, DeNoS, Lutefisk, Novor and PEAKS and for Homology searching MS-Homology and SPIDER are frequently used. Similarly few other softwares such as Advanced Chemistry Development, Analyst, AnalyzerPro, Chromeleon, LabSolutions LCMS, Mass++, MassBank.jp, MassBank.eu, MassBank, Mass Frontier, MassLynx and massXpert are also used in MS studies.

**Conclusion**

As stated in the beginning of this article, increased sensitivity in comparison to available analytical techniques with efficient analyzer, m/z filtering, reduced background interference, measuring retention time and ToF properties of elements, excellent specificity from characteristic fragmentation patterns to discover unknowns or verify the existence of assumed complexes, information about molecular weight and isotopic abundance of elements and temporally resolved chemical data made this technique worldwide adorable [5,32,39]. MS data can be used for both qualitative and quantitative analyses of physical, chemical, and biological properties of samples. Objectives such as identifying unknown compounds, determining the isotopic composition of elements in a molecule or mixture, determining the structure of a compound, quantification of different known/unknown components of sample under different experimental conditions and ascertaining the sequences of proteins can be fulfilled by observing MS spectrum of fragmented samples. Although this technique has its own advantages in bio-medical sciences, the technique has also some drawbacks especially in chemical analyses. Its scope is limited in identifying hydrocarbons that produce similar fragmented ions. It also fails to distinguish between optical and geometrical isomers and the positions of functional groups in ortho, meta and para positions in an aromatic ring. However, it has still lists of its own advantages in biomedical, chemical and geological sciences.

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