Navigating Mass Spectrometry: A Comprehensive Guide to Basic Concepts and Techniques

Akhilesh Kumar Kuril[1]

[1]Bhagwant University, Sikar Road, Ajmer, Rajasthan, India

Abstract: Mass spectrometry (MS) is a powerful analytical technique used to determine the molecular mass and structural information of compounds by measuring the mass-to-charge ratio (m/z) of ionized molecules and their fragments. At its core, a mass spectrometer consists of three fundamental components: an ion source, a mass analyzer, and a detector. The ion source converts sample molecules into gaseous ions, typically by either removing an electron (forming positive ions) or adding an electron (forming negative ions). Common ionization techniques include electron ionization (EI), chemical ionization (CI), electrospray ionization (ESI), and matrix-assisted laser desorption/ionization (MALDI). The mass analyzer separates the ionized molecules based on their m/z ratios using electric and/or magnetic fields. Different types of mass analyzers exist, such as quadrupole, time-of-flight (TOF), ion trap, and Fourier transform ion cyclotron resonance (FT-ICR). The separated ions are then detected and their abundances recorded, generating a mass spectrum that displays the relative abundances of ions plotted against their m/z ratios. This mass spectral data provides information about the molecular weights and structural features of the sample components. Main concepts in mass spectrometry include the monoisotopic mass (calculated from the most abundant isotopes), average mass (weighted by natural isotopic abundances), and nominal mass (rounded integer mass). Tandem mass spectrometry (MS/MS) involves multiple stages of mass analysis with fragmentation steps, enabling in-depth structural characterization.

Keywords: Mass Spectrometry Basic, ESI, MALDI, TOF, Orbitrap, Monoisotopic mass, Nominal mass, Average mass

1 Introduction

Mass spectrometry (MS) is a convoluted analytical technique that separates components of a sample based on their mass and electrical charge. It is used to conduct both quantitative and qualitative chemical analyses of sample compounds. MS is used to identify unknown compounds by estimating their molecular weight, quantifying recognized compounds, and deciphering the structural and chemical features of the molecular components. Mass spectrometry yields a mass spectrum, which is a graphical representation of the mass-to-charge (m/z) ratio of the compounds in the sample, along with the relative abundance of individual ions. Ions are accelerated to high speeds and deflected at various angles in an electric or magnetic field based on their m/z ratios. The m/z ratio can be used to determine the exact molecular weight of the components in the sample.

1.1 Principles of Mass Spectrometry

The basic principle of mass spectrometry (Figure 1) involves the following steps [1,2].

Ionization: The sample is ionized in the ionization source, converting neutral molecules into charged ions.

Acceleration: The ions are accelerated by an electric field and focused into a beam.

Deflection: The ion beam passes through electric or magnetic fields, causing the ions to deflect based on their m/z ratios. Lighter ions deflect more than heavier ions.

Detection: The deflected ions reach the detector, which records their abundances and generates a mass spectrum.

Data Processing: The mass spectrum displays the relative abundances of ions plotted against their m/z ratios, providing information about the molecular weights and structures of the sample components.

2 Ionization in Mass spectrometry

Ionization is the process of producing gas-phase ions for mass analysis. The initial sample can be solid, liquid, or gaseous. Whether it is solid or liquid, it is evaporated into a gas before being ionized by the ion source. This is normally accomplished by losing one electron and forming a cation with a +1 charge. To prevent ionized molecules from interacting with air molecules, the ionization chamber is kept vacuum-tight. A positively charged metal plate propels the samples forward to the next stage of the machine. Mass spectrometers can work in either positive or negative ion mode. It is critical to understand the correct configuration in order to properly analyze the data [3]. Figure 2 depicts the basic ionization process in the sample molecule.

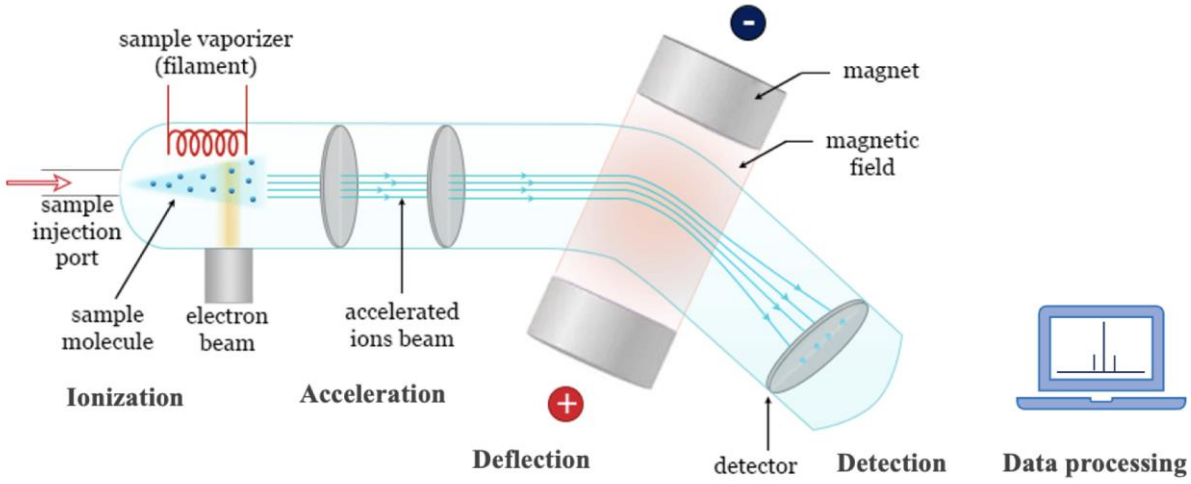

Figure 1 Schematic instrumentation of Mass spectrometer

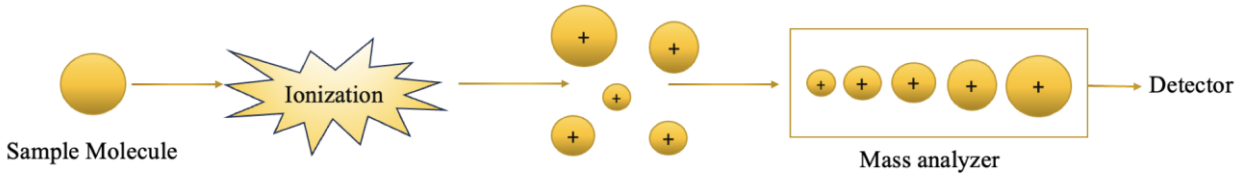

Figure 2 Schematic of ionization process

2.1 Soft ionization technique

Soft ionization techniques use less energy to ionize the sample, which results in limited fragmentation. The spectra mostly feature the most abundant molecular ion peak.

2.1.1 Atmospheric Pressure Chemical Ionization (APCI)

APCI is a soft ionization technology that utilizes gas-phase ion-molecule reactions at atmospheric pressure to generate primary ions from a solvent spray. It is often combined with high-performance liquid chromatography (HPLC). APCI yields a single-charged product, which eliminates signal overlap but provides limited structural information due to the formation of few fragment ions [3].

Analytes having molecular weight less than 1500 Da can be analyzed by this method.

Suitable for thermally stable non-polar compounds and Polar compounds.

Analysis of drugs, pesticides and non-polar lipids.

2.1.2 Atmospheric Pressure Photo Ionization (APPI)

APPI is a soft ionization technique that is commonly used in conjunction with liquid chromatography. It ionizes gaseous samples through photochemical activity. The solvent and sample from liquid chromatography combine to form a gaseous analyte, which is subsequently ionized and interacts with photons emitted by the light source at atmospheric pressure. The APPI light source may be an argon lamp or, more often, a xenon lamp. The ions are then put into the mass spectrometer and analyzed. It can ionize both polar and non-polar small molecules, allowing more compounds to be examined in a single pass [3].

APPI is used to analyze pesticides, steroids, and drug metabolites that lack polar functional groups. It is often used in security applications to detect explosives.

2.1.3 Electrospray Ionization (ESI)

ESI is a soft ionization process that uses electrospray to apply a high voltage to a liquid, resulting in an aerosol containing multiple charged ions [4,5]. ESI allows users to operate in either positive or negative ion mode. Higher molecular weight molecules in ESI tend to carry numerous charges; the distribution of charge states precisely quantifies molecular weight, resulting in precise molecular mass and structural data [4,5]. The experimental conditions and the solvent utilized must be carefully selected.

Electrospray ionization consists of three stages (Figure 3):

Droplet formation: Under the influence of the high voltage, the liquid emerging from the capillary forms a cone (Taylor cone) at the tip, from which a fine mist of charged droplets is emitted [6].
Desolvation: The charged droplets undergo solvent evaporation, leading to an increase in their surface charge density. When the Rayleigh limit is reached, the Coulombic repulsion overcomes the surface tension, causing the droplets to undergo Coulomb fission and form smaller progeny droplets [4-7].
Gas phase ion formation: As the desolvation continues, the droplets become smaller until the analyte ions are eventually released into the gas phase as multiply charged ions [8].

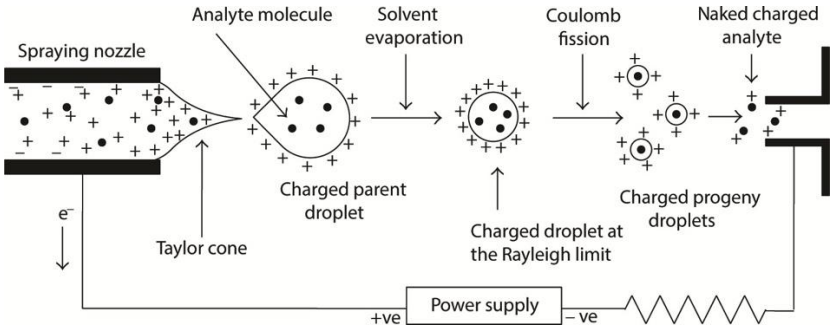

Figure 3 Schematic of the proposed ESI mechanism [3]

Advantages of ESI
Allows ionization of non-volatile and thermally labile compounds like biomolecules with minimal fragmentation [9].
Generates multiply charged ions, extending the mass range for analysis of large molecules [10].
Compatible with various separation techniques like liquid chromatography [11].
Offers positive and negative ionization modes for analysis of different analyte types [11].

Applications
ESI is widely used for the analysis of biomolecules (proteins, peptides, nucleic acids), synthetic polymers, and small organic/inorganic compounds in fields like proteomics, metabolomics, environmental analysis, and drug discovery [9-11].

2.1.4 Chemical Ionization

Chemical ionization (CI) is a soft ionization technique used in mass spectrometry that produces ions with minimal fragmentation, making it useful for determining molecular masses of analytes.
In CI, a reagent gas (e.g., methane, isobutane, ammonia) is ionized by electron bombardment to produce reagent ions. These reagent ions then undergo ion-molecule reactions with the analyte molecules in the gas phase to form analyte ions [12]. The key reactions involved are:
Ionization of reagent gas: e.g., $CH4^{+}$ e- → $CH4^{++}$ 2e-
Formation of reagent ions: e.g., $CH4^{+} + CH4 \rightarrow CH5^{+} + CH3$
Ion-molecule reaction with analyte (M): e.g., $CH5^{+} + M \rightarrow [M + H]^{+} + CH4$
The most common ions observed in positive CI are protonated molecular ions $[M + H]^{+}$ or adduct ions like $[M + CH5]^{+}$.
In negative CI, molecular anions $[M]^{-}$ or deprotonated ions $[M - H]^{-}$ are typically formed.
Advantages
Produces abundant molecular ion peaks, aiding in determining molecular weights.
Minimizes fragmentation compared to electron ionization (EI), resulting in simpler mass spectra.
Selectivity can be tuned by choosing appropriate reagent gases based on their proton affinities.
Compatible with various sample introduction techniques like GC and LC.
Disadvantages
Lower sensitivity compared to EI due to fewer ions formed.
Multiple reagent ion species can complicate spectra interpretation.
Requires additional reagent gas supply and modified ion source.
CI is widely used in organic and biochemical analysis, particularly when EI causes excessive fragmentation, obscuring the molecular ion peak. It complements EI by providing molecular weight information for structure elucidation [1,12-13].

2.1.5 Matrix-Assisted Laser Desorption/Ionization (MALDI)

Matrix-assisted laser desorption/ionization (MALDI) is an ionization technique used in mass spectrometry for analyzing large biomolecules like proteins, peptides, oligonucleotides, and carbohydrates with minimal fragmentation.

MALDI is a three-step process (Figure 4)[3,13]

i. A considerable amount of acceptable matrix material is mixed into the sample and put to a metal plate. The matrix must survive with the sample while minimizing interaction between its molecules. Choosing the matrix is the most important stage in the MALDI analysis. The optimal matrix should have high electron absorption at the laser wavelength in use, lower vapor pressure, improved vacuum stability, and be miscible with the solid analyte. Matrices are typically solid and organic; however, they can generate background peaks that interfere with the characterization of sample chemicals. Compounds that have been shown to minimize background interference include inorganic compounds, porous silicon, and surfactant-blocking substrates.

ii. A pulsed laser (typically a nitrogen laser) delivers high-intensity energy to the sample/matrix material mix, allowing it to desorb off the metal plate with little fragmentation. The matrix will absorb UV light and convert it to heat energy. A small portion of the analyte/matrix will quickly heat and evaporate. The matrix absorbs UV radiation to protect the analyte from harm and then transfers that energy to the sample, vaporizing and ionizing it.

iii. The analyte molecules are ionized by being protonated or deprotonated in the hot plume of ablated gases. The matrix absorbs the laser energy, facilitating desorption and ionization of the analyte molecules into the gas phase with minimal fragmentation.

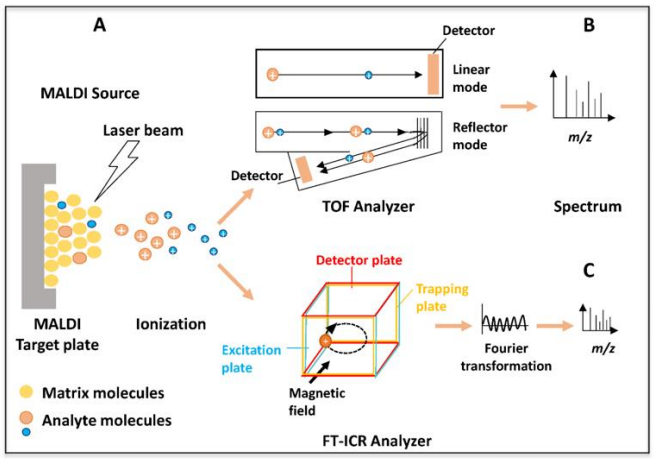

Figure 4 Schematics of the MALDI-MS preparation and the equipment in the sample preparation step, analytes are mixed with a large excess of matrix and are spotted onto a stainless-steel target plate, where they co-crystallize. In the MALDI source, (A) the co-crystals are irradiated with a laser beam, inducing the desorption and ionization of analytes. The generated ions are accelerated in an electric field, which directs them to the analyzer. In the TOF analyzer, (B) ions are separated according to their m/z in a flight tube by two different modes: linear and reflectron (C) the m/z are measured according to the cyclotron frequency of the ions, trapped in a circular orbit, and subjected to a magnetic field, subsequently generating a signal [14]

Advantages of MALDI [3]

Allows analysis of Polar, non-volatile, and thermally unstable samples, biomolecules (DNA, proteins, peptides, carbohydrates) that tend to fragment with other ionization methods.
Tolerant to contaminants like salts and buffers.
Requires only subpicomolar sample amounts.
High mass accuracy (0.1-0.01%) and precision.
Can provide structural information through post-source decay.

Applications

MALDI is widely used in proteomics for determining protein molecular weights, identifying sequences, characterizing structures, and quantification. It also finds applications in metabolomics, lipidomics, glycomics, and analysis of synthetic polymers and oligonucleotides

MALDI is commonly coupled with time-of-flight (TOF) mass analyzers, known as MALDI-TOF MS. Ultraviolet lasers like N2 and Nd:YAG are commonly used. Atmospheric pressure (AP)-MALDI allows coupling to ion traps and other mass analyzers [3].

2.2 Hard ionization technique

Electron ionization (EI) is a hard ionization technique widely used in mass spectrometry. It involves bombarding gaseous analyte molecules with a beam of energetic electrons (typically 70 eV), causing the molecules to lose an electron and become positively charged radical ions [1,2,15]. The key features of electron ionization are:
High energy electrons (70 eV) are used, leading to extensive fragmentation of the analyte molecules.
Produces radical molecular ions (M+•) and many fragment ions, providing structural information.
Suitable for volatile and thermally stable small molecules (<600 Da).
Considered a "hard" ionization technique due to the high energy involved.
EI is often coupled with gas chromatography (GC-MS) since the analytes need to be volatile. The resulting mass spectra contain characteristic fragmentation patterns that can be matched against libraries for compound identification. While EI provides rich structural information through fragmentation, it can cause excessive fragmentation of labile biomolecules like proteins and oligonucleotides. For such analytes, softer ionization techniques like electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI) are preferred [15].

3 Mass Analyzers

A mass analyzer measures the mass-to-charge ratios of ions and is the central part of the mass spectrometer. Transmission efficiency, mass resolution, and mass accuracy are the key parameters for the performance of a mass analyzer. Transmission efficiency corresponds to the percentage of ions that actually reach the detector from the ion source. Transmission efficiency affects the instrument sensitivity, a parameter referring to the minimum sample amount required to produce detectable MS signals; high sensitivity is especially important for analyzing low-abundance samples. Mass resolution or mass resolving power describes the ability of a mass analyzer to distinguish ions with closely separated m/z values and can be calculated as the ratio of the m/z of a peak divided by its width, usually determined at the half maximum height. The absolute mass accuracy is the difference between the experimental and theoretical m/z values, and it is often expressed in a relative term as the ratio of the mass measurement error to the theoretical mass in parts-per-million (ppm). High mass accuracy (low mass measurement error) is crucial for confident identification.
There are several types of mass analyzers (Figure 5), each with its own operating principles, advantages, and limitations. Specifications and features of mass analyzers are presented in Table 1[16].

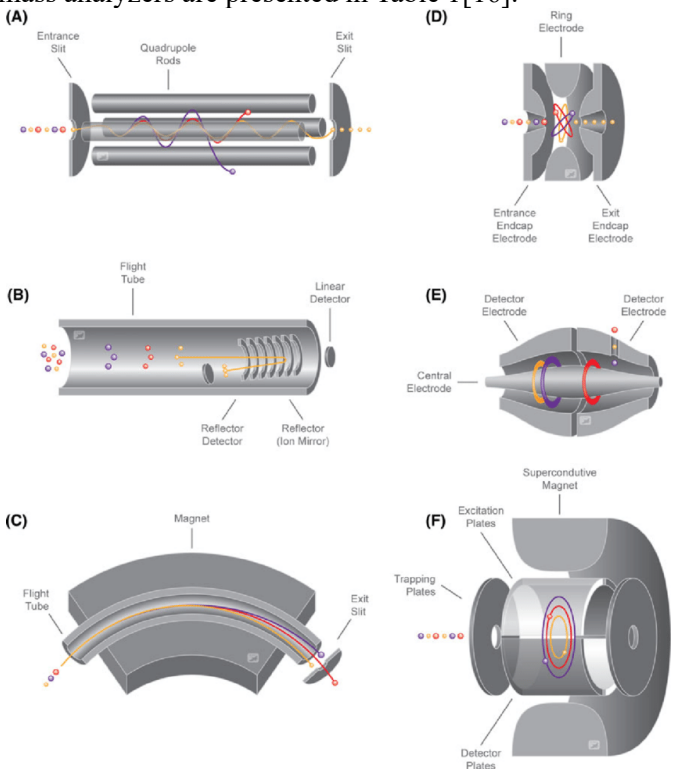

Figure 5 Schematic presentation of six mass analyzers used in mass spectrometers that are currently available on the market. a Quadrupole (Q). b Time-of-flight (TOF). c Magnetic sector (B). d Ion trap (IT). e Orbitrap (OT). f Ion cyclotron resonance (ICR) [17]

Table 1 Comparison of Specification and features of mass analyzers [16]

Mass Analyzer	Mass resolution[1]	Mass accuracy (ppm)	m/z range	MS/MS capability	Ion Source	Sensitivity
IT	4000	100-1000	50–2000; 200–4000	MS^n	ESI	Picomole
LTQ (LIT)	2000	100-500	50–2000; 200–4000	MS^n	ESI	Femtomole
Q-q-Q	1000	100-1500	50-4000	MS/MS	ESI	Attomole
TOF	10000-20000	5-50	No upper limit	n/a	MALDI	Femtomole
TOF-TOF	10000-40000	5-50	No upper limit	MS/MS	MALDI	Femtomole
Q-q-TOF	10000-40000	5-50	No upper limit	MS/MS	ESI;MALDI	Attomole
LTQ-FTICR	50000-800000	1-2	50–2000; 200–4000	MS^n	ESI;MALDI	Femtomole
LTQ-Orbitrap	50000-800000	<5	50–2000; 200–4000	MS^n	ESI;MALDI	Femtomole

[1]Mass resolution at full width half maximum

3.1 Quadrupole Mass Analyzer

Quadrupole mass analyzers consist of four parallel metal rods with opposing pairs connected electrically. Ions travel down the quadrupole axis, and only ions of a certain m/z ratio will reach the detector for a given DC and RF voltage applied to the rods [1,2]. They offer good scan speed, sensitivity, and allow high-speed polarity switching but have relatively low mass resolution when used alone [18,19].

3.1.1 Principle

A combination of radio frequency (RF) and direct current (DC) voltages is applied between the rod pairs, creating oscillating electric fields. Ions travel down the quadrupole axis, undergoing a complex spiral motion. Only ions of a certain m/z ratio will have a stable trajectory and reach the detector for a given RF/DC voltage ratio. Other ions with unstable trajectories will collide with the rods and be filtered out [18,19].

3.1.2 Key Features

Compact size, fast scan rates, and high transmission efficiency. Unit mass resolution, typically limited to m/z range of 1000 or lower. Acts as a mass filter, allowing one m/z value at a time to reach the detector as voltages are scanned. Widely used due to simplicity, robustness, and low cost [1-4].

3.1.3 Hybrid Models

Triple quadrupole (QQQ) instruments use two quadrupoles as mass filters with a collision cell in between for MS/MS analysis [19-21]. Quadrupole time-of-flight (Q-TOF) hybrids combine a quadrupole with a TOF analyzer for higher resolution. Linear ion traps can be coupled to quadrupoles for improved capabilities.

3.1.4 Application

Quadrupole mass analyzers are versatile and commonly used in various applications like environmental analysis, food safety, clinical studies, and targeted analysis of compounds due to their ability to selectively filter and monitor specific m/z values.

3.2 Magnetic Sector Mass Analyzer

Magnetic sector analyzers use a magnetic field to deflect ion trajectories based on their m/z ratios [17,20]. Only ions of a specific m/z will follow the curved path to the detector for a given magnetic field strength. They provide high resolution and mass accuracy but have a limited mass range.

3.3 Linear Quadrupole Ion Trap (LIT/LTQ)

A linear ion trap (LIT) is a mass analyzer built with three sets of RF-onlyquadrupoles arranged in a linear configuration (Figure). A differential RF potential is applied to all three sets of quadrupoles to trap the ions along the x and y directions

(or radially), whereas a DC potential is superimposed on the two end quadrupoles to confine ion motion along the z direction (or axially). During an MS scan cycle, a packet of ions isfirst injected into the LIT and trapped there. A gas such as helium is commonly used to cool down the ions without introducing fragmentation, and focus them to the center of theLIT. The amplitude of the main RF potential, V, is then ramped up, which moves the ionsout of their stability regions, and ejects the ions radially, from low m/z ions to high m/z ions. An auxiliary alternate current (AC) voltage at a fixed frequency is often applied to the x-rods during the main RF ramp to facilitate rapid resonant ejection of the ions, whichnot only improves the scanning speed and mass resolution, but also increases the upper mass detection limit. Besides functioning as a mass analyzer, an LIT can also be used to perform CID experiments. During an MS/MS event, ions with a specific m/z value are isolated by applying resonance ejection voltage to x-rods at the secular frequencies of all ions except for the ion of interest, and this leads to the ejection of all unwanted ions from the trap. The ions selected as precursors are then excited by collision with the neutral gas molecules inside the trap to produce fragment ions. Note that there is a trade-off between the extent of fragmentation and the observation of low mass product ions, as a higher energy deposition in the precursor ions requires the main RF to operate at a higher amplitude, and this unfortunately leads to an increase in the low-mass cutoff of the LIT and the loss of low mass fragment ions from the trap [21].

As discussed above, LIT is an ion trapping device. Precursor ion isolation, fragmentation, and analysis of product ions can be conducted sequentially in one place in the LIT, whereas precursor and product ion analyses are performed in two physically separated mass analyzers in other tandem instruments, e.g., triple quadrupole instruments.Consequently, it is possible to perform multi-stage tandem MS analysis (known as an MS^n experiment) in an LIT. An LIT also has a large ion trapping capacity and space charge effect that is reduced compared to a 3D ion trap [22]. An LIT can either serve asa stand-alone mass spectrometer, or be combined with another mass analyzer to form a hybrid MS instrument such as the LTQ-Orbitrap.

Ion traps are particularly appropriate for the identification and targeted investigation of molecules and chemical reactions in domains such as metabolomics, lipidomics, and post-translational modification studies [23].

3.4 High Resolution Mass Spectrometry

High-resolution mass spectrometers (HRMS) are analytical instruments capable of measuring the mass-to-charge ratio (m/z) of ions with extremely high precision and accuracy, typically to 4-6 decimal places [24].

Orbitrap mass analyzers provide exceptional resolution (>100,000) and mass accuracy (<5 ppm).

Time-of-flight (TOF) analyzers offer virtually unlimited mass range and fast acquisition rates.

Fourier transform ion cyclotron resonance (FT-ICR) MS provides ultra-high resolution (>1,000,000)

3.4.1 Time-Of-Flight (TOF)

As its name indicates, a time-of-flight mass analyzer determines the m/z value of an ion by measuring its transit time from the ion source to the detector. A TOF analyzer requires a pulsed ion beam, and thus it is usually coupled with a MALDI ion source, but it can also be coupled with an ESI source via orthogonal ion injection, as implemented in Q-o-TOF and FT MS instruments. The MALDI-TOF MS is one of the most commonly used MS instruments, due to its low cost, ease of sample preparation, user friendly operation, and relatively high sensitivity and mass resolution. In a MALDI-TOF MS measurement, a burst of ions extracted from the MALDI plate are accelerated by a static electric field and all ions carrying the same charge (z) will gain the same kinetic energy (E_k), which is expressed as:

$$E_k = \frac{1}{2}mv^2 = zeV$$

where m and z are the mass and charge of the ion, respectively, v is the velocity of the ion as it exits the ion source, e is the elemental charge, and V is the accelerating potential. After acceleration, all ions traverse a field-free flight tube in vacuum to reach the detector. Assuming the length of the tube is L, the flight time of an ion can be calculated as:

$$t = \frac{L}{v}$$

$$t^2 = \frac{L^2}{2eV} \times \frac{m}{z}$$

The equations above outline the principle of operation for a TOF mass analyzer: ions with a higher m/z travel with a lower velocity and thus more slowly than ions with a lower m/z, so they take a longer time to reach the detector.

For a given accelerating potential (V) and distance of flight (L), the m/z value of an ion scales quadratically with its time of flight [25].

The linear MALDI-TOF MS design, as described in Figure 6, suffers from its poor mass resolution because ions produced from a MALDI source have different initial velocities as well as a certain degree of temporal and spatial distributions, and therefore ions with same m/z do not all arrive at the detector simultaneously; this leads to peak broadening. Two techniques have been widely implemented in modern MALDI-TOF instruments to improve mass resolution: pulsed-delayed extraction and the reflection's geometry. Figure illustrates the schematic of a MALDI-TOF/TOF instrument with pulsed-delayed extraction and reflection. The pulsed-delayed extraction is accomplished by two-stage ion acceleration. The extraction voltage (U_e) is applied to the sample plate following a short time delay (usually several hundred nanoseconds) after ions are produced by the pulsed laser desorption/ionization. The underlying principle is that slower ions will not travel as far from the sample plate during the delay, and, when the extraction voltage is applied, they will stay in the extraction electric field longer and obtain more kinetic energy. When the delay time and accelerating voltage are properly chosen, ions with a lower initial velocity will emerge from the extraction field with a slightly higher final velocity, allowing them to catch up, at the detector, with ions with a higher initial velocity. The optimal extraction voltage is mass-dependent and linear to the m/z of ions being focused the user has to adjust the voltage and delay time in order to optimize the results [25]. Use of a reflectron, rather than a simple linear flight tube, is another strategy to compensate for the initial kinetic energy spread of ions. A reflectron is an electrostatic device that sits between two stages of the flight tube that can be physically the same but traversed in opposite directions. It acts as an ion mirror that creates a retarding electric field where ions can be deflected and sent into the second stage of the flight tube. Ions that enter the reflectron are subjected to deceleration by the electric field. Their velocities will eventually reach zero, at which point ions begin to move in the opposite direction and regain the lost kinetic energy before they are expelled from the reflectron. Ions with higher kinetic energies penetrate more deeply into the retarding field and spend more time in the reflectron than ions with lower kinetic energy, thus compensating for a shorter flight time outside of the reflectron and leading to improved mass resolution.

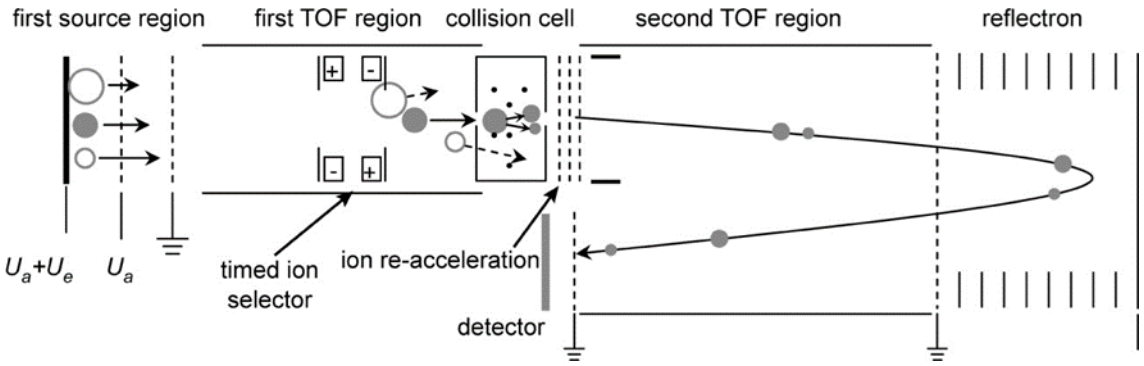

Figure 6 Schematic of a MALDI-TOF/TOF instrument [8]

3.4.2 Orbitrap

An Orbitrap is an electrostatic ion trap with a spindle-like inner electrode and a barrel- like outer electrode which is split in the middle (Figure 5E). Ion trajectories inside an Orbitrap consist of three periodic motions: rotation around the z axis, radial oscillation, and axial oscillation along the z axis. Whereas the rotational frequency (ω_φ) and the radial oscillation frequency (ω_r) are affected by the initial ion velocity and position, the axial oscillation frequency (ω_z) only depends on the m/z values of the ions and the instrument parameters as shown in the equation below:

$$\omega_z = \sqrt{\frac{k}{m/z}}$$

where k is the field curvature. Thus, ions with the same m/z form a packet and oscillate harmonically along the z direction in the shape of a thin ring around the inner electrode, and this motion produces a small alternating image current between the two halves of the outer electrode, the frequency of which can be obtained by performing FFT on the recorded transient, and thereafter used to generate the mass spectrum [26,27].

The Orbitrap has become the mass analyzer most frequently employed in proteomic studies, owing to its high sensitivity, very high mass resolving power and accuracy, and wide mass range. Figure 7 illustrates the schematic of a commercially available Orbitrap instrument, known as the LTQ-Orbitrap Velos™ hybrid mass spectrometer [28]. It is made up of an ESI source, a series of ion transfer optics, a dual pressure LTQ/LIT, a C- trap, an Orbitrap, and a higher-energy collisional dissociation (HCD) collision cell. Ions pass through a series of ion lenses and multipoles, that are operated

with stepwisedecreasing pressures, to the LTQ where they can be stored, isolated, and fragmented. The resultant ions are either expelled radially for LTQ detection or injected axially into the C trap. The C trap is a curved linear quadrupole ion trap which cools the ions and focusesthem within a small volume, then injects them tangentially into the Orbitrap, as a tight packet. The inner electrode potential is then increased, and the ion packets are squeezed to the center of the electrode and begin coherent axial oscillations at various frequencies according to their m/z values. The Orbitrap can also receive ions produced in the HCD collision cell, an octopole device where precursor ions can undergo higher energy fragmentation. The Orbitrap does not suffer from the low mass cutoff issue that isencountered in an LTQ and is thus suitable for detection of low molecular weight reporter ions and immonium ions.

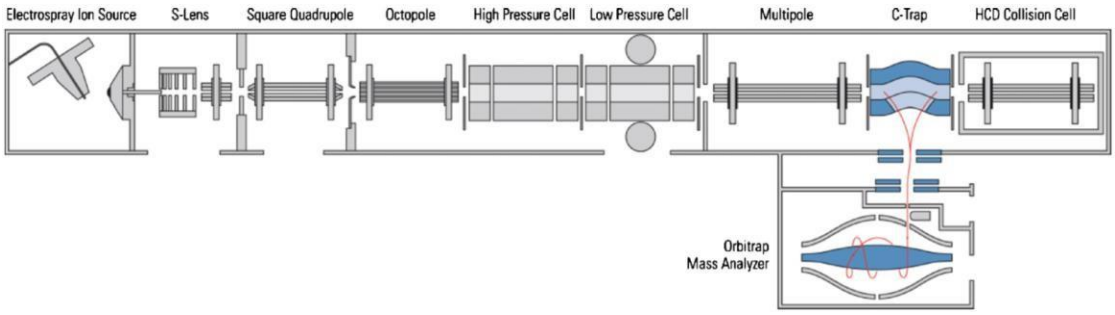

Figure 7 Schematic of an LTQ-Orbitrap Velos mass spectrometer, adapted from reference [10]

4 Tandem Mass Spectrometry

Tandem mass spectrometry, also known as MS/MS or MS2, is a powerful analytical technique that involves two or more stages of mass analysis with a fragmentation step in between.

In tandem MS, molecules are first ionized and separated based on their mass-to-charge ratio (m/z) in the first mass analyzer (MS1). Ions of a specific m/z are then selected and fragmented, typically by collision-induced dissociation (CID) or other fragmentation methods. The resulting fragment ions are then analyzed in the second mass analyzer (MS2) based on their m/z ratios [29,30].

4.1 MS3 and MSn Spectrometry

MSn refers to performing multiple stages of mass spectrometry fragmentation, where n represents the number of fragmentation stages. This allows for in-depth structural characterization by fragmenting precursor ions and then further fragmenting the resulting product ions.MS3 is a specific case of MSn, where the precursor ion is fragmented (MS2) and then one of the resulting product ions is selected and fragmented again (MS3). This provides additional structural information compared to just MS2.

Common tandem MS instruments include triple quadrupole (QqQ), quadrupole-time-of-flight (Q-TOF), ion trap, and hybrid instruments like quadrupole-Orbitrap. These instruments combine different types of mass analyzers and fragmentation techniques [29,30].

Advantages:

High sensitivity and specificity: The combination of mass analysis and fragmentation increases the ability to detect and identify low-abundance analytes in complex matrices.

Structural information: Fragmentation patterns provide valuable structural information for compound identification and characterization.

Quantitative capabilities: Tandem MS enables accurate quantification using stable isotope-labeled standards and techniques like multiple reaction monitoring (MRM).

Applications

Proteomics: Tandem MS is extensively used for protein identification, characterization of post-translational modifications (PTMs), and quantitative proteomics through techniques like selected reaction monitoring (SRM) and parallel reaction monitoring (PRM) [31]. Techniques like electron transfer dissociation (ETD) in MS3 can preserve labile post-translational modifications while providing backbone fragmentation.

Metabolite profile and identification: MS/MS enables the structural elucidation and quantification of metabolites in complex biological samples [30].

Biomarker discovery: Tandem MS is crucial for identifying and validating potential biomarkers for various diseases, including cancer, metabolic disorders, and infectious diseases [31].

Clinical diagnostics: MS/MS is increasingly used in clinical laboratories for newborn screening, therapeutic drug monitoring, endocrinology, and other diagnostic applications [31].

4.2 Fragmentation method in Tandem mass spectrometry

Tandem mass spectrometry (MS/MS) employs various fragmentation methods to induce the dissociation of precursor ions into product ions, providing valuable structural information for compound identification and characterization. The choice of fragmentation method depends on factors such as the type of analyte, the desired structural information, and the instrumentation available [32-36].

4.2.1 Collision-Induced Dissociation (CID)

CID is the most widely used fragmentation technique in MS/MS. It involves accelerating the precursor ions and colliding them with an inert gas (e.g., argon, nitrogen) in a collision cell or ion trap. The collisions transfer kinetic energy to the precursor ions, causing them to vibrate and eventually dissociate into smaller product ions.

4.2.2 Electron Transfer Dissociation (ETD)

ETD is a fragmentation method particularly useful for analyzing larger biomolecules like proteins and peptides. It involves transferring an electron from a radical anion to the multiply charged precursor cation, inducing fragmentation along the backbone while preserving labile post-translational modifications.

4.2.3 Electron Capture Dissociation (ECD)

Similar to ETD, ECD is a fragmentation technique used for analyzing large biomolecules. It involves capturing low-energy electrons by multiply charged precursor cations, leading to dissociation along the backbone while retaining labile modifications.

4.2.4 Higher-energy Collisional Dissociation (HCD)

HCD is a beam-type CID method used in Orbitrap mass analyzers. It employs higher collision energies than traditional CID, resulting in more extensive fragmentation and improved detection of low-mass product ions.

4.2.5 Photodissociation

In photodissociation, precursor ions are fragmented by absorbing photons from a laser or infrared source. Techniques like infrared multiphoton dissociation (IRMPD) and ultraviolet photodissociation (UVPD) are used for various applications, including the analysis of biomolecules and small molecules.

4.2.6 Surface-Induced Dissociation (SID)

SID involves colliding precursor ions with a solid surface, leading to fragmentation. It is particularly useful for analyzing large biomolecules and can provide complementary structural information to other fragmentation methods.
CID is the most widely used due to its simplicity and compatibility with various mass analyzers, while other methods like ETD and ECD are preferred for preserving labile modifications in biomolecules.

5 Data Interpretation

Mass-to-Charge Ratio (m/z): The x-axis of the mass spectrum represents the mass-to-charge ratio (m/z) of the ions. This ratio is the mass of the ion divided by its charge. The mass is measured in atomic mass units (amu).
Relative Abundance: The y-axis of the mass spectrum typically represents the relative abundance of ions. The area under each peak is proportional to the number of ions producing that peak.
Base Peak: Identify the tallest peak in the spectrum. This is called the base peak and represents the most abundant ion fragment. It's usually assigned a relative abundance of 100%.
Fragment Peaks: Analyze the smaller peaks to identify fragments of the original molecule. Each peak corresponds to a fragment formed when the molecule breaks apart due to the energy of the electrons.

5.1 Mass definitions

Monoisotopic mass: The monoisotopic mass of a molecule refers to the mass calculated using the masses of the most abundant isotope of each element present in the molecule. This is the most accurately defined molecular mass and is preferred if a measurement of it can be determined.
^{1}H=1.007825, ^{12}C=12.000000, ^{16}O=15.994915, etc.

Average mass: The average mass, also known as the average atomic mass or atomic weight, is a weighted average of the masses of all the isotopes of an element. It takes into account the natural abundance of each isotope. This is the common chemical molecular weight that is used for stoichiometric calculations (H=1.0080, C=12.011, O=15.994, etc.).

Nominal mass: The mass of the most abundant isotope of each atom in a compound is used to compute its nominal mass, which is represented by an integer. For example, since the most abundant isotope of hydrogen is 1 and that of oxygen is 16, the nominal mass of water is (1+1+16=) 18 Da [38].

Mass accuracy

Mass Accuracy or Mass Measurement Error (MA) is the difference between the experimental mass (Mexp) and the theoretical value (Mtheo), calculated from elemental composition [36].

In absolute term, $MA = M_{exp} - M_{theo,}$ in Da or milli Da

In relative term, $MA= (M_{exp} - M_{theo}) / M_{theo}$, unit less (ppm for high resolution MS)

6 Conclusion

Mass spectrometry has evolved as a vital analytical tool, transforming a wide range of academic and industrial applications. Its ability to provide extensive information about the chemical composition, structure, and dynamics of compounds has made it a vital resource for scientists and researchers all around the world. The versatility of mass spectrometry comes from its broad spectrum of ionization techniques, mass analyzers, and fragmentation procedures, which allow for the examination of a wide range of samples, from small molecules to large macromolecules. Instrumentation advancements, such as high-resolution mass analyzers like the Orbitrap and FT-ICR, have considerably improved mass accuracy. Mass spectrometry has several uses, including proteomics, metabolomics, environmental analysis, forensics, and pharmaceutical research. In proteomics, mass spectrometry has become the foundation for protein identification, quantification, and characterization of post-translational changes, which is driving our understanding of cellular processes and disease pathways. Metabolomics depends primarily on mass spectrometry to profile and identify metabolites, throw light on metabolic pathways, and develop biomarkers. As technology advances, mass spectrometry will definitely play a more important role in unraveling the intricacies of biological systems, clarifying molecular structures, and propelling scientific discoveries across disciplines.

Declaration of Competing Interest

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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