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Recent advances in Mass Spectrometry: An appraisal of fundamentals and applications

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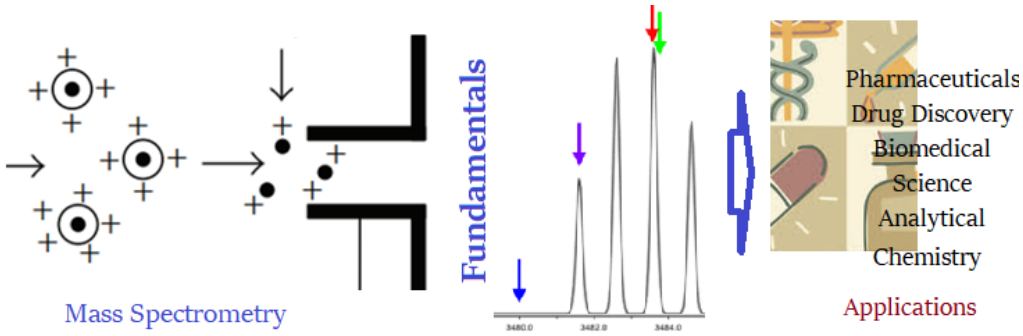
Tutorial Review



ABSTRACT

The application of mass spectrometry (MS) for structural elucidation in life sciences and bioanalytical approaches has become progressively significant. The recent advancements in MS have led to remarkable improvements in mass resolving power, mass accuracy, isotopic abundance accuracy, and accurate mass multiple-stage MS (n) capability, resulting in superior accuracy and precision in identifying and quantifying biomolecules. Furthermore, the advances in hardware and informatics tools for MS have paved the way for more efficient and accurate data acquisition and processing methods. In this review, we will elaborate on these improvements and summarize techniques such as ion mobility spectrometry (IMS), high-resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS), and data-independent acquisition (DIA) methods. The integration of MS with other analytical techniques such as chromatography, nuclear magnetic resonance (NMR), and X-ray crystallography has further improved the structural analysis of biomolecules. Overall, the ongoing progress in MS technology is anticipated to broaden its applications and influence in several fields

Keywords: Mass Spectrometry, Analyzers, FT-ICR MS, Ionization Methods, Analytical Methods

INTRODUCTION

Mass Spectrometry (MS) is an analytic destructive method used to weigh up molecular weight and provide molecular structure data. It is a highly specific technique, applied in various pharmaceutical fields (absorption of drugs, drug designing, pharmacokinetics, combinatorial chemistry, etc.) in clinical studies (doping, drug abuse, hemoglobin analysis, toxicology, neonatal screening, etc.), biotechnology (biochemical genetics, analysis of hormones, proteins, etc.), geology (composition of oil, protection of the environment (contamination of food, quality of water) and endocrinology. MS plays an important role in the evolvement of various techniques for analysis and apprehending complicated as well as natural organic molecules. Its capability ranges from basic molecule detection to advanced structural interpretation of high-resolution data produced from orbitrap instruments or FT-ICR.[1]

The technique which studies elemental analysis of biomolecules, mainly proteins and molecular functioning like binding of the ligand is MS. In the drug discovery process, it is employed to characterize various vital regents. In high-throughput screening, numerous MS-rooted techniques have come up, but these could not replace the conventional fluorometric and radiometric techniques. In the process of lead discovery, methods like frontal affinity chromatography-MS, pulsed-ultrafiltration MS, size-exclusion chromatography-MS, and affinity capillary electrophoresis-MS are being proposed. Furthermore, MS has become crucial method for biomarker assay that could trace action of drugs and progression of disease.[2]

The mass spectrometer has an inlet system that sends the gaseous sample to the vacuum chamber where ions are generated. Here, the neutral sample is ionized and excited, which induces fragmentation. The study of such fragments gives information on the structure of molecules. In the mass analyzer, each fragment is

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distinguished by the mass-to-charge ratio (m/z). These ions are separated and detected in an ion detector chamber. Subsequently, the signal is sent to the data system. The prominent peaks in the mass spectrum are those emerging from primary fragmentations and secondary fragmentations can be used for spectrum analysis. The components of the mass spectrometer are displayed in Figure 1.[3] Nowadays, as mass resolution and technologies have enhanced, no prior sample preparation or analysis is required.

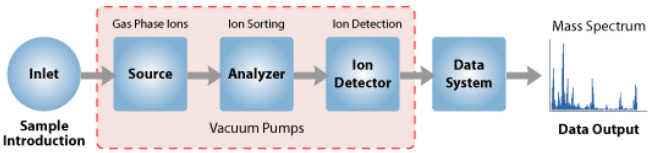

Figure 1: Components of the mass spectrometer; *Taken from* [3], *licensed under CC-BY-SA 3.0.*

Ionization methods:

In mass spectrometers, the charged analytes are detected only in gaseous form. Therefore, an ionization technique/source must be employed which converts the liquid sample to its ionized gaseous phase and fragmentation of ions takes place. The ionization of molecules can be both in negative or positive ion mode, depending on their stability.

Chemical impact: In this method, the reagent gas (ammonia, hydrogen, nitrous oxide, and isopropane) is initially ionized by the electron source method and afterward reacts with molecules of analytes to generate analyte ions.[4] And when ions are produced at atmospheric pressure using powered nitrogen, this method is known as atmospheric pressure chemical ionization (APCI).

Electron Impact: An electron beam (70eV) strikes the neutral molecules to generate positively charged ions or fragments.[5]

Field Ionization: In this method, analyte ionization takes place by a very strong electric field, which is generally produced by a sharp electrode at a high electric potential. When field ionization is employed in desorption mode, the technique is known as field desorption ionization.

Atmospheric Pressure Photoionization: In this technique, UV light is passed through the gaseous molecules and the photons ejected are utilized to ionize vaporized sample.[6]

Electrospray Ionization (ESI): In this method, the analyte is ionized utilizing an electrospray. The solution is exposed to high voltage to produce an aerosol. Based on the structural properties of the analyte, multiply charged fragment ions were produced[7] as shown in Figure 2. Nowadays, nano-ESI or micro-ESI methods are gaining more popularity due to their efficiency in terms of flow rate, and ionization.

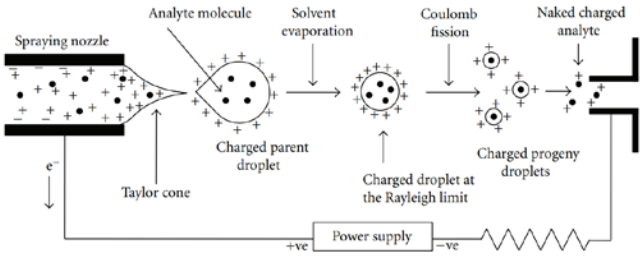

Figure 2: Schematic diagram showing ESI procedure; *Taken from* [7], *licensed under CC-BY 3.0.*

Fast Atom Bombardment: Highly current particles (Cs, Xe, or Ar) are bombarded on the analyte molecules. Due to the high energy (4-10 KeV) of the accelerated particles, they produce a speedy beam of analyte ions.

Inductively Coupled Plasma–Mass Spectrometry (ICP-MS): In this technique, the ionization takes place at a high temperature (8000k), when plasma in the nebulized solvent is introduced to the analyte sample.[8]

Direct Analysis in Real Time: In this method, the vibrational excited state of the molecule or the electronic excited state of the atom of the sample interacts with the atmospheric gases. The ions are generated by electrical discharge. This method helps in direct detection of metabolites and drug agents in urine, saliva, blood, etc.

Matrix-Assisted Laser Desorption/Ionization (MALDI) and related techniques: In MALDI, a laser beam (nitrogen laser) is used to evaporate and ionize the analyte mixture as shown in Figure 3. This method is mainly used for biomolecules like sugars, peptides, and proteins.[9] Surface-Enhanced Laser Desorption/Ionization is another method, where a light-absorbing matrix is used to ionize the analyte mixture.[10] Furthermore, Desorption Electrospray Ionization (DESI) is a novel ionization method that works in ambient conditions. It enables high throughput analysis and thus is suitable for biological samples, and metabolomics.[11]

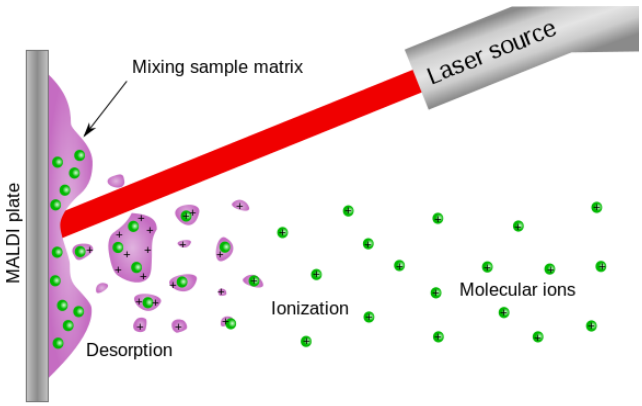

Figure 3: Schematic of MALDI process; *Taken from* [12], *licensed under CC-BY-SA 3.0.*

Mass Analyzers:

After ionization of the analyte sample, the ionized ion beam should pass through the mass analyzer where the ions are separated and analyzed according to their mass/charge ratio. Various types of mass analyzers are differentiated based on resolution, mass accuracy, analysis speed, % of ions analyzed, and mass range limit.

Orbitrap: Using this method, the ionized ions are trapped in the electrostatic field of the analyzer and then transferred to the detector for detection. This technique is very effective in detecting and quantifying positional isomers. It possesses high accuracy, resolution, and sensitivity.[13]

Time-of-Flight (TOF): These analyzers employ an electric field to accelerate ions. It comprises a flight tube where ionized ions with different m/z values are segregated based on the time taken to travel from the source to the detector as shown in Figure 4. It possesses high accuracy, unlimited m/z range, and high sensitivity.[14]

Figure 4: Schematic description of TOF-MS process; *Taken from* [15], *licensed under CC-BY-SA 4.0.*

Ion Trap: The analyzer filled with helium, comprises of two ring and two end electrodes. The pulses in ions gate direct the ions to the trap. In this analyzer, all the ionized ions are trapped and allowed to oscillate at a certain potential energy. According to the m/z value of these ions, they are ejected by a hole and reach the detector[16].

Quadrupole: It has four parallel rods which are devised in a square shape. The segregation of ions in this method is not according to the kinetic energies of the ions, moreover, it is related to the relative movement of ions in the electric field. These analyzers possess high accuracy, great sensitivity, and limited m/z range.

FT-ICR: Once the ions are ionized, they are directed to the trapping cell which is having uniform magnetic field strength. The m/z value is inversely related to the angular frequency of the analyzer. FT-ICR offers high accuracy, sensitivity, and resolution.[17]

Detectors:

The detectors detect the ionized ions so that valuable mass spectra can be generated from them. A Faraday cup is a traditional detector, which produces secondary electrons when ions strike its surface. The generated electrons induce an electric current to flow till these electrons are recollected. An electron multiplier is another detector, made up of dynodes series. At times, these dynodes comprise of scintillator which releases photons that are identified by a photomultiplier tube. Due to the sensitivity of this technique, metastable ions are very effectively studied by it. Furthermore, high vacuum and complex elements utilized in MS are subjected to damage, unwanted constituents, and contamination of the sample. And therefore, MS hyphenation is needed with separation methods. All the hyphenation methods, which are equilateral to MS, work as free constituents of the system, whereas MS is bound constituent whose outcome is the act of the former one.

Main components of obtaining Mass Spectra:

Creation of spectra:

As the sample molecules enter into a mass spectrophotometer, they encounter an energy source. The energy released from the source (electron impact tungsten filament) removes a single electron from the sample molecule. Methanol, for example

$e^- + CH_3OH \rightarrow [CH_3OH]^{\cdot+} + 2e^-$

The detector is sensitive to positively charged molecules and not to any radical or neutral molecules. The detector transforms the molecule into an electrical signal and the integrator translates these signal peaks to a bar graph. There are more bars than just the mass of a sample molecule. And these other peaks are credited to the cleavage of bonds in the original sample molecule. The main fragments for methanol are:

$[CH_3OH]^{\cdot+} \rightarrow CH_3O^+ + H\cdot$

$[CH_3OH]^{\cdot+} \rightarrow CH_2O^+ + H_2$

$[CH_3OH]^{\cdot+} \rightarrow CH_3^+ + OH\cdot$

In any mass spectrum, the two most vital peaks are the molecular ion peak and the base peak. The base peak is the largest in the spectrum. In the case of methane, the base peak corresponds to the CH_3O^+ fragment (m/z 31). The other peaks in the spectrum are referenced as the percentage of base peak and referred to as relative abundance. And the normalization of peak heights helps in the recognition of fragmentation patterns and hence analyte identification[18,19]. The molecular ion peak signifies an analyte molecule that has not undergone fragmentation. It is referred to as $M^{\cdot+}$ ion. The molecular ion peak in the case of methanol is caused by $[CH_3OH]\ \cdot+$ ion (m/z 32).

Identifying molecular ion peak:

The molecular ion peak in the spectrum should be the most abundant peak, but it is not the case for a majority of compounds like alcohols, nitrogen, highly branched compounds, esters, and carboxylic acids. The fragment peaks mustn't be accidentally identified as molecular ion peaks so that the misidentification of an analyte can be avoided. Obtaining the chemical ionization spectrum can aid in accurately identifying the molecular ion.

Apart from this, other rules can help in finding out the potential masses of molecular ions. One valuable tool is the "nitrogen rule". This rule specifies that if the molecular ion possesses an odd mass, then it must own the odd number of nitrogen whereas a molecular ion having an even mass must contain the even number of nitrogen or must lack them. Since a majority of organic compounds that are analyzed either contain one or zero nitrogen atoms and so, the rule practically states that the odd molecular ion is ascribed to single nitrogen and the even molecular ion indicates that the sample lacks nitrogen[20]. This rule is only applicable to compounds that contain nitrogen, carbon, hydrogen, halogens, oxygen, Sulphur, and a few other infrequent elements.

Recognizing Analytes using Isotopic Ratios:

Isotopes have the same chemical properties but they differ in mass. All elements possess several natural-state isotopes[21–23]. Since a majority of elements own two or more isotopes, a ratio of these isotopes acts as a powerful tool in deriving the composition of unknown samples. Some isotopes are so eminent that they are easily observed with the quadrupole mass spectrophotometer having unit resolution.

Table 1: The natural isotopes of most common elements confronted in organic chemistry[24,25]

Element	Isotope	Relative abundance	Isotope	Relative abundance
Hydrogen	^{1}H	100	^{2}H	0.0151
Carbon	^{12}C	100	^{13}C	1.112
Nitrogen	^{14}N	100	^{15}N	0.37
Fluorine	^{19}F	100		
Silica	^{28}Si	100	^{29}Si	5.10
Phosphorous	^{31}P	100		
Chlorine	^{35}Cl	100	^{37}Cl	31.98
Bromine	^{79}Br	100	^{81}Br	97.28
Iodine	^{127}I	100		
Oxygen	^{16}O	100	^{17}O	0.04

Abundances are calculated by allocating the 100 values to an eminent isotope.

Fragmentation:

The goal of explicating mass spectra is identifying the structure of a molecular ion by examining fragments of the actual molecule[26]. The size and frequency of the fragments depend on the bond energy and structure of the sample molecule. These fragments are observed by the interaction of energy emitted from the source. This energy removes a single electron; however, the excess energy is allocated over numerous degrees of freedom. And when the sample molecule comes back to its ground state through relaxation, a molecular ion is created as shown in Figure 5. Other times this energy beats the fragmentation's activation energy and is released by breaking the bonds.

Stevenson's rule states that if two fragments are competitive to produce a cation, then the fragment having low ionization energy will be frequently formed. The fragmentation can proceed via two pathways, either heterolytic or homolytic cleavage. In heterolytic cleavage, the pair of electrons move to the charged site by double-headed arrow giving a radical and a cation. Whereas fragmentation from the hemolytic cleavage results from the movement of single electrons. And these fragmentation patterns are generally the result of functional groups in the compound. The bonds that usually break are α and β bonds. The β bond is frequently broken as heteroatom's non-bonding electrons allow for resonance forms which stabilize the cation.

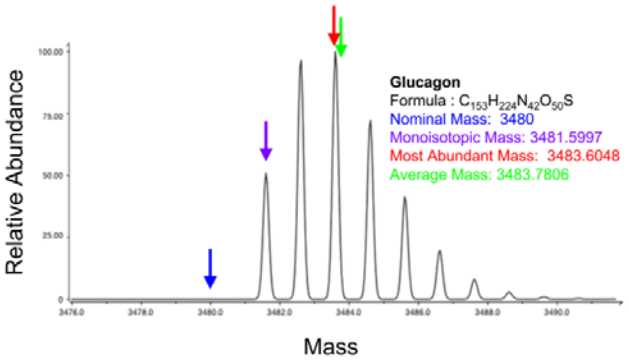

Figure 5: Molecular ion distribution of Polypeptide glucagon; *Taken from*[27]*, licensed under CC-BY-SA-3.0*

Rearrangements:

If the molecular ion has even molecular weight, then usually even peaks were created from the rearrangement. In contrast, if a molecule has an odd molecular weight, then peaks will be odd. Since the low-energy transitions assist in stabilizing the products, these rearrangements are favored. Rearrangements are responsible for eminent peaks in the spectrum such as loss of water from alcohol or McLafferty rearrangement.

After surveying common compounds in chemistry and their respective spectra some conclusions about fractionation patterns were drawn[28]:

i. The molecular ions of aldehydes, ethers, carboxylic acids, and nitrogen-containing molecules like nitriles and amides can be very light or potentially absent. And that of branched compounds and alcohols is almost undetected.
ii. Increasing the size of the compound or branching of the alkyl portion will reduce the intensity of the peak.
iii. Cyclic structures, aromatic groups, and elements of unsaturation elevate the intensity of the peak.
iv. Resonance-stabilized cations are promoted as they help in the delocalization of positive charge all through the molecule.
v. The cleavage of bonds at substituted carbon atoms is favored which produces the most stable cation.
vi. Generally, the longest chain is removed because a greater number of carbon atoms permits the delocalization of radicals.

Resolution and Mass Accuracy:

Current Fourier transform (FT) and TOF mass analyzers deliver quantitative performance and mass resolution at a speed well under 1 sample/second[29]. The equipment capable of high mass resolution power, dynamic range, and mass accuracy is the requirement of MALDI MSI. Moreover, in MALDI MSI analysis, FT-ICR MS is reported to give the maximum mass spectral performance. This helps in the elucidation of molecular features which are otherwise not clear on a low-resolution analyzer. In addition to that, increasing the magnetic field increases the performance of FT-ICR MS, for instance, with enhancement in magnetic field, dynamic range and mass accuracy increase quadratically while mass resolving power

shows linear improvement. In a 21T MALDI FT-ICR MSI experiment, rms mass measurement accuracy of less than 100 ppb was observed along with a resolution of molecular features as minor as 1.79 mDa. [30,31].

It is very important to know the difference between mass resolution and resolving power; while the former is a function of mass being measured and ion width, the latter is fixed through the mass range on a TOF apparatus. Reduction in sensitivity is observed upon an increase in the mass resolving power [32]. Only when molecular weights become significant, the need for higher mass resolution is felt. In addition to that, the spectral acquisition rate is compromised when the resolution is very high that is 100,000 or more than that. For instance, FT-ICR MS delivers matchless mass accuracy and resolving power provided adequate time is given so that the requisite degree of information can be acquired.[33] Presently, high-performance liquid chromatography (HPLC) together with triple quadrupole mass spectrometers (QqQ-MS) is used to carry out multi-analyte methods. They have high precision and selectivity but consume a lot of time, detect only analytes included in the MS-acquisition method and data cannot be analyzed retrospectively.[34,35] A full scan MS detection should be highly sensitive, and highly selective for each of the compounds in the mixture, should confirm the identity of the compound, and should accurately quantify analytes. For meeting these requirements, one would need mass spectrometers that have high mass accuracy and high mass resolving power.[36]

In ToF-Secondary Ion MS (SIMS), a cluster ion beam used as the sputtering ion source can effectively achieve the depth profiling of organic and amino acid films without inflicting any damage to the ions.[37–42] Various proteins and peptides having a molecular weight of up to 15 kDa that were not detected by atomic ion beams based TOF-SIMS were easily detected by argon gas cluster ion beams (Ar-GCIB).[43] Ar-GCIB has been reported to have low mass accuracy and sensitivity and loss of space and mass resolution. For the preservation of mass accuracy, external mass calibration can be used.[44] In addition to that, ‘delayed extraction’, a technique in which an initial pulse is applied on the ions to account for the velocity distributions can help to maintain high spatial and mass resolution.[45,46] The problem with delayed extraction is that it makes mass calibration difficult leading to low mass accuracy. Many alternatives to Ar have been proposed like the CO_2 cluster ion beam which is much more stable than the Ar cluster ion beam and thus improves the image resolution by a factor of two.[47–50] Moreover, the isobaric mass tag technology helps in the improvement of precision and helps in the simultaneous comparison of multiple protein samples.[51,52]

Isotopic abundance accuracy:

The isotope-ratio mass spectrometer (IRMS) is an efficient tool to measure isotopic abundance ratios[53] as shown in Figure 6. Stable isotope analysis is used to measure the natural differences in the abundance of stable isotopes of the same element.[54] IRMS is of two types: dual inlet IRMS [55] and continuous flow IRMS.[56] The latter is more convenient and has higher sample throughput than the former but its precision is ten-fold lesser.

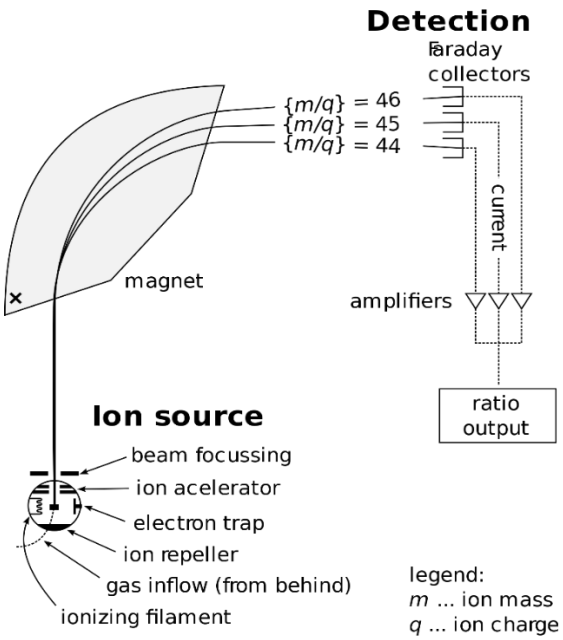

Figure 6: Schematic of an isotope-ratio mass spectrometer for measuring CO_2. *Taken from* [57]*, licensed under the public domain.*

Mostly these are magnetic sector type and are superior to the quadrupole type as it gives high-quality 'peak shapes' and helps in the multiple-collector analysis leading to very high precision and accuracy in isotope-ratio analysis. For instance, in an investigation, it was reported that for the determination of specific and unique elemental composition, high resolution and mass accuracy alone are not enough. Fast-scanning TOF mass spectrometers combined with LC would significantly gain if isotopic abundances are used as an orthogonal filter as it is cheaper than FT-ICR mass spectrometers. Compared to ion traps and quadruple mass spectrometers, these provide precise masses and precise isotopic abundance together.

There are many applications of isotopic ratio analysis, for instance, the isotopic ratio outlier analysis (IROA) method coupled with highly accurate mass GC-MS is used for the identification of unknown metabolites from artifacts.[58–60] In another application, laser absorption spectroscopy (LAS) was used to develop a near-infrared methane sensor for analyzing carbon isotopic- abundance analysis and is very promising for multi-parameter analysis.[61] For the calibration of a Multicollector-ICPMS or Thermal ionization MS (TIMS), the mixtures that are near to the isotopic composition of natural potassium are fit.[62] In a recent study, a technique that combined ‘laser ablation molecular isotopic spectrometry’ boosted by ‘isotopic dilution’ and ‘molecular laser-induced fluorescence’ (ID-LAMIS-MLIF), was utilized for exact quantitative analysis of boron in aqueous solution.[63] Another effective mass analyzer is charge detection mass spectrometry (CDMS) and it allows calculation of ion’s charge and mass simultaneously. It provides mass spectra with high resolution[64] and is highly potent to analyze hypersensitive protein assemblies like adeno-related viruses and ribosomes.[65]

Accurate mass multiple-stage MS (n) capability:

There have been many exemplary developments in increasing the capability of the accuracy of mass multiple-stage MS. For instance, the design of a "basket in a basket" technique which is founded on the principle of 'multistage accurate mass spectrometric (MAMS)' was designed. The demonstration of this technique was done by procuring a unique elemental composition of a compound up to 5 stages of MAMS. Also, tandem MS is not enough for elucidating the structure of unknown compounds because there is a lot of uncertainty in defining the elemental compositions of fragments.

The use of MAMS in combinatorial drug discovery is very useful as the built-in chemical information from the synthesis can act as limitations. Application of nano electrospray ionization technique helps in characterizing small quantities of compounds.[66]

APPLICATIONS OF MS:

Pharmaceutical Applications:

Phytochemical analysis: Mass spectroscopy is widely used in the phytochemical analysis because of its capability to measure and identify metabolites having low molecular weight at low concentration ranges i.e. below nanogram per milliliter[67]. Therefore, it is contemplated as a trace analysis methodology. Various analyte separation techniques such as capillary electrophoresis, high-performance liquid chromatography, and gas chromatography are associated with mass spectroscopy for concurrent determination and separation of analytes called high-performance liquid chromatography (HPLC)-MS, capillary electrophoresis (CE)-MS and gas chromatography (GC)–MS respectively.[68] Also, temperature-sensitive analytes with high molecular weight can be efficaciously analyzed by HPLC associated with atmospheric pressure ionization-mass spectrometer (API-MS).[69] Some of the latest research articles highlighting the applications of MS are listed in Table 2

Analytical Technique	Sample Source	Analytes	Ref.
HPLC-ESI-MS	Leontopodium species (Asteraceae)	Fatty acids, diterpenes, sucrose, sesquiterpene	[70]
GC × GC–MS	Pelargonium graveolens essential oil	Limonene, α-Pinene, myrcene, citronellal, geraniol	[71]
GC–MS	Momordica charantia methanolic fruit extract	Vitamin E, 1-pentadecyne, Gentisic acid,	[3]
GC–MS	Extracts of Aerva lanata	5,14-di (N-butyl)-octadecane, (R)-(+)-ç-Valerolactone, 2-propynoic acid, 9-octadecenoic acid	[72]
GC–MS	Azolla microphylla	5,14-di (N-butyl)-octadecane, (R)-(+)-ç-Valerolactone, 2-propynoic acid, 9-octadecenoic acid	
UHPLC-ESI-MS	Rhizopus microsporus var. oryzae questioned soya bean seedlings	Isoflavonoids and prenylated isoflavonoids like glycitein, daidzein, genistein	[73]
HPLC-ESI-MS/ MS	Radix astragali	Formononetin, calycosin, formononetin-7-O-glycoside, calycosin-7-O-β-D-glycoside	[74]
HPLC–MS/MS	Glycyrrhiza uralensis Fisch. extract	Licuraside, ononin, liquorice saponin G2, glycycoumarin, glycyrrhizic acid, liquiritin,	[75]
LC/MS/MS	Dried plums	Hydroxycinnamics, including glycosides, acids, and esters; hydroxybenzoic acids and a flavonoid	[76]
UHPLC–MS	The oot extract of Licorice in 70% ethanol, ethyl acetate, and ethanol	Prenylated flavonoids	[77]

Pharmaceutical analysis: Mass spectroscopy appeared as a strong tool for several operations in the pharmaceutical field majorly in drug discoveries due to its speed, high sensitivity, versatility, and selectivity. It is widely used for the identification of impurities in samples. Similarly, LC-MS is used for the multidimensional detection of impurities in drug development is described. For peptide drugs for example ion trap mass spectrometer along with electrospray ionization is employed[78]. Likewise, it can also be utilized for identifying the purity of active pharmaceutical ingredients (API), such as MK-0969, M3 antagonist; MK-0677, cathepsin K inhibitor, API-A, and growth hormone secretagogue.[79] A protocol was described for quantitative and qualitative analysis of pharmaceutical compounds using MALDI-TOF MS[80]. MALDI-MS imaging also emerged as a helpful tool in the analysis of pharmaceutical formulations.[81,82] FT-ICR is another evolving technique used in pharmaceutical industry which provide more data per measurement. It is used for chemical fingerprinting, de-replicating, and elemental structure verification of natural compounds, like antibiotics.[83]

Forensic applications: In forensic research, a sample is used in minute quantity, and therefore, high sensitivity is needed for analysis. Both GC-MS and LC–MS emerged as indispensable tools in the forensic domain. The use of MS is becoming remarkable because of increased demand to investigate the use of illegal drugs by analyzing body tissues and fluids. Some of the drugs are lysergic acid diethylamide (LSD), opiates, marihuana, amphetamines, and cocaine. However, cases of death or murders

due to drug overdose and poisoning are also the main targets for these analyses. LC thermospray tandem mass spectrometric technique was invented for quantitative analysis of drugs having hypnotic, tranquilizing, and sedative properties, that is diphenylbutylpiperidine, benzodiazepine, thioxanthene, butyrophenone and methadone in whole blood.[84]

BIOMEDICAL APPLICATIONS

Structure elucidation: Mass spectroscopy is useful in the structure elucidation of various compounds. Mass spectrum in the form of a bar graph is interpreted by using various peaks such as molecular ion peaks, base peaks, isotopic peaks, etc. Fragmentation pattern is also a very important component that aids in the qualitative analysis of numerous compounds. From the Beynon table, prediction of possible elemental composition or arrangement of particular mass and determination of the molecular formula can be done. Some examples from the literature that support the application of mass spectroscopy are:

a) The structure of apigenin and flavonoid monoglycosides, isolated from the shoot of lupin (Lupinus luteus L.), was interpreted by using electron ionization (EI)-MS and liquid secondary ion (LSI)-MS.
b) The examination of sulfated heparin viz glycosaminoglycan oligosaccharides was done by employing tandem MS/MS utilizing quadrupole ion-trap mass spectrometer.[85]
c) A complete analysis method was invented for galacto-oligosaccharide mixtures using β-galactosidase based on ion-mobility spectrometry-tandem MS with electrospray ionization.[86]
d) The characterization of a prebiotic galacto-oligosaccharide mixture with an ion-trap mass spectrometer associated with high-performance anion-exchange chromatography (HPAEC) was done using electrospray ionization in combination with NMR.[87]
e) The characterization of polyisobutylenes by several MS techniques such as tandem MS with ESI-quadruple ion-trap (QIT) and MALDI-TOF was done. The primary structure was induced by multistage mass spectrometric scanning, and the presence of particular functional groups and differentiation of isomeric functional groups was confirmed.[88]

Peptide and protein sequence and structure analysis: Mass spectroscopy is very useful in the analysis of amino acids sequence and structure of peptides and proteins. Firstly, peptides are converted to amino alcohols which are evaporative. And these amino alcohol derivatives are analyzed in a mass spectrometer that aids sequence analysis. Sequencing of underivatized peptides by fast atom bombardment MS is also employed. Novel techniques like tandem MS and MALDI are also in trend. Some examples to support the present application are:

a) Analysis of peptide sequence was done using gas-phase ion/ion chemistry along with tandem MS. And for the characterization of the primary structure of proteins, quadrupole linear ion trap was combined with chemical ionization and electrospray ionization.[89]
b) The initial complex structure of RNA polymerase II transcription was examined by cross-linking with mass spectroscopy. It was used as an analysis tool for multi-protein complexes.[90]
c) Selective detection and sequencing of serine-, tyrosine-, and threonine-phosphopeptides was done by ES-MS employing a quadrupole mass spectrometer.
d) An amino acid sequence of peptides from apolipoprotein-B was discovered by tandem MS. Here, a triple quadrupole mass spectrometer with LSI-MS was utilized.

The achievement of MS in analyzing tiny molecules has propelled clinical MS to analyze proteins and peptides for diagnostic studies. Recently, a highly focused method (shotgun proteomics) has evolved to quantitatively analyze protein. This technique generally employ m/z transitions with tandem MS, dilute isotopes for normalization, and utilize enzymatic degradation of the sample.[91]

Endocrinology applications: Inappropriate specificity, the hook effect, cross variability, and anti-reagent antibodies are some of the properties of immunoassays which greatly affect their diagnostic usage. For evaluation of proteins and tiny molecules, the antibodies-related detection techniques have lowered the innate defects of immunoassays.[92] With the advancement of MS methods, the study of vitamin D is gaining interest. LC-MS/MS technique highlights the epimeric state of vitamin D, and isolates vitamin D2 from D3, which was earlier not feasible with already existing immunoassays[93]. Furthermore, to diagnose the endocrine diseases at low concentrations, MS techniques are mostly recommended rather than steroid immunoassays because of their low accuracy, and specificity. Also, to enhance the testing of endocrine functioning, the application of MS needs high technical experience, competence, and skill.[94]

Clinical studies: Implementing mass spectroscopy in the clinical field results in significant advancements. When the analyte quantity is very low, mass spectroscopy because of its higher sensitivity is employed.[95] In any disease condition, changes in excretion and body fluids products can be detected by chromatographic instruments such as gas chromatography with mass spectroscopy. Also, MALDI-MS is used to directly image and analyze pharmaceutical compounds in tissues.[96] This technique is also used to diagnose and characterize inborn errors of organic acidurias. A simplified method was invented for the clinical analysis of organic aciduria using GC–MS with a quadrupole mass spectrometer. Methylcitric acid, glutaric acid, and margaric acid were identified while analyzing the urine sample.[97] Mass spectroscopy also has applications in clinical microbiology. MALDI-TOF MS has been adapted for the identification of whole microorganisms directly from cultures in urine and blood. This method can accurately identify even those bacteria that are difficult by conventional techniques.[98]

Toxicology applications: Numerous immunoassay-dependent toxicological screening techniques are employed in various laboratories. Some clinical laboratories provide complete

screening of drugs and confirmatory analysis using GC-MS and LC-MS/MS. Screening assays has cutoff sets which offer suitable specificity, sensitivity and are qualitative in nature. But rather than making a final recognition (like hydromorphone vs morphine), these assays are used to recognize a class of compounds (like opioids). However, confirmatory analysis is quantitative in nature and is done when high sensitivity is required. Furthermore, toxic impurities are of great concern during research and drug development process.[99] For structure elucidation of these impurities, mass fingerprinting methods such as LC-Multistage MS, LC-MS/TOF, LC-Multistage-TOF, capillary electrophoresis-MS are employed.[100] And for certain isolation, mass-dependent tools are used.

Metabolites analysis: Determination of different metabolites and metabolic pathways of the drug or xenobiotics is important to assess its pharmacokinetic parameters.[101] Drug metabolic reactions are divided into two parts; functionalization reactions or Phase I and conjugation reactions or Phase II. Both these transformations include changes in molecular weight which can be correctly measured by a mass spectrometer. In structural characterization, the exchange of labile hydrogen in small organic molecules with deuterium (H/D exchange) has been widely utilized and it occurs in solution having functional groups that have labile hydrogens like -SH, −OH, -COOH, −N(R)H, and $-NH_2$. A quadrupole mass spectrometer with FAB, electron impact, APCI, thermospray, and ESI systems was used in the study. Stable isotope-labeled xenobiotics can facilitate metabolite identification and detection by MS, especially when the radio-labeled parent drug is unavailable. Custom-designed isotopic clusters emerging from a mixture of synthetic and naturally enriched isotopes can facilitate the identification and detection of metabolites. For instance, the identification and detection of ribavirin metabolites present in rats were done with the help of a stable isotope-labeled drug. Furthermore, metabolomics by MS really helped in newborn screening, leading to multiplexed assays for fatty acid oxidation, amino acids, and organic acids.[102] The LC-MS and tandem LC-MS screening techniques offer quick analysis of wide range of compounds. The methods employed to magnify throughput are pooling techniques (like simple screens, cassette dosing, etc.), automatic data processing, and quick chromatography. These MS methods are highly helpful in recognizing metabolite as they possess the capability to detect and predict the metabolites in complicated samples of plasma, urine, and bile.[103]

Drug Discovery Applications:

Drug development and discovery is a time-consuming and labor-intensive process[104]. MS technology is used throughout the drug development process and plays a vital role in advancing the production of pharmaceuticals. Due to its high selectivity and sensitivity, it is widely employed for the analysis of degradation and impurities. And when MS is associated with chromatographic separation techniques, it becomes a strong analytical tool for sample analysis and provides an assessment of pharmaceutical compounds. Drug discovery involves instant testing of compound design and requires a short duration for execution. Typically, many compounds are tested and synthesized for each discovery project till a suitable clinical compound is selected. Here, analytical chemistry ensures that every compound of interest (COI) possesses the correct structure and satisfies purity requirements.

Supercritical fluid chromatography (SFC) associated with MS offers a vast range of applications for both preparative and analytical fields[105], such as achiral and chiral separation and mass-directed fragment collection in preparative SFC[106,107]. Some researchers have evaluated more specialized methodologies for the separation of chiral compounds and structural isomers. They demonstrated that by associating ion mobility spectrometry with MS, enantiomers can be insulated in the gas phase by using a chiral modifier[108]. In another study, it was depicted that chiral identification and separation of enantiomers can be achieved by employing Capillary Electrophoresis with Electrospray Ionization MS (CESI-MS)[109].

The quantitation and identification of potential metal contamination present in APIs are crucial in drug development. ICP-MS is the technique for elemental determination, particularly for heavy metals in APIs[110]. One challenge in interpreting the structure of unknown complexes using MS is that the non-volatile buffers, which do not react to MS ionization, are often compelled to the isolation of COI. In this case, 2D-LC-MS can be utilized to overcome this problem and it improves chromatographic resolution also.[111,112]

During the formulation or development of any drug, the drug agents are put through numerous tests under certain stress settings like oxidation, reduction, basicity, acidity, light, humidity, temperature, etc. For this, LC-MS/MS or LC-MS methods are employed for elucidating the elemental structure of the degrading compounds by gaining their fragmentation pattern, molecular mass, and retention time. Moreover, a structural dataset could be created for quick recognition of the degrading compounds and to differentiate between the unstable compounds within the drug agent.[113]

Computationally studying MS:

Native MS is evidenced as a quick high-throughput technique to screen various structures[114,115] and study different pH-dependent conformational alterations,[116] mainly transmembrane β-barrels[117] and protein-logic gates.[118] The software to study native MS can be of 2 types:

1. help in deconvolution. It can be both non-commercial (iFAMS and UniDec)[119] and commercial (BioPharma Finder and Intact Mass).[120] Both these can couple with the automatic running of the sample and possess an ability to convert in a high-throughput technique.
2. help in data interpretation of ion mobility

Associating experimental with theoretical collision cross-section can compare different structures and models[121]. The most basic method is projection approximation, which is implemented in various forms like Ion Mobility Projection Approximation Calculation Tool, which can calculate collision cross-section from small-angle X-ray scattering, NMR spectroscopy, electron microscopy, and X-ray crystallography data. Another technique that works on a similar theory is the projection super approximation method. Apart from calculating collision cross-

section, it also determines size and shape effects. Next is the trajectory technique which considers the ion to be a group of atoms. It reports multiple collisions, distant interactions, and collisions of buffer gas and the ion. Based on the trajectory technique, Collidoscope is been implemented for computing collision cross-sections. It possesses advanced algorithms for quick analysis.[122] Various other tools to study the protein-complex interactions, unfolding, and stability have been developed.[123] In a study, the interaction of 16 heterodimers was studied using ion exchange chromatography integrated with native MS.[124] The development of charge detection MS is also useful in characterizing multimeric complexes.[125]

Conclusion

Mass spectrometry (MS) has played a pivotal role in advancing the field of life sciences and bio-analytical approaches. In recent years, the use of MS for structural elucidation has become increasingly important, and it has led to significant developments in the field. These developments have not only improved the accuracy and precision of biomolecule identification and quantification but also enabled the analysis of increasingly complex samples.

One of the most critical advancements in MS has been the improvement of mass resolving power, mass accuracy, isotopic abundance accuracy, and accurate mass multiple-stage MS (n) capability. With these improvements, MS has enabled researchers to achieve better accuracy and precision in identifying and quantifying biomolecules, thereby contributing to a better understanding of their structure and function. Furthermore, the development of hardware and informatics tools for MS has led to more efficient and accurate data acquisition and processing methods, which has further increased the speed and sensitivity of MS.

MS techniques, such as IMS, high-resolution FT-ICR MS, and DIA methods, have enabled the identification and characterization of complex biomolecules and their interactions in greater detail. IMS, for instance, can separate isomeric compounds, while FT-ICR MS can detect trace impurities in complex samples. DIA methods also enable the identification and quantification of low-abundance proteins, which are often missed in DDA experiments. These techniques, coupled with advancements in hardware and informatics tools, have greatly enhanced the capabilities of MS.

Moreover, the integration of MS with other analytical techniques, such as chromatography, NMR, and X-ray crystallography, has further expanded the range of applications for MS in the structural analysis of biomolecules. Such integration has enabled researchers to achieve a deeper understanding of the interactions between biomolecules and their functions.

Overall, the ongoing advancements in MS technology are expected to continue to expand its applications and impact in various fields. These advancements will enable the analysis of even more complex samples, leading to a better understanding of the structure and function of biomolecules. Additionally, the integration of MS with other analytical techniques will facilitate the characterization of biomolecular interactions and contribute to the development of new therapeutics. Thus, the future of MS in life sciences and bio-analytical approaches is promising, and it is expected to remain a valuable tool for many years to come.

Conflict of Interest

Authors declare no conflict of interest is there for publication of this article.

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