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Utilization of Mass Spectrometry for the Comprehensive Metabolite Profiling of Potential Medicinal plants: A Review Article

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Abstract Mass spectrometry (MS), which provides unparalleled sensitivity, precision, and versatility for identifying and characterizing complex chemical compounds, has transformed metabolite profiling in medicinal plant research. This paper examines how MS can be used to profile primary and secondary metabolites that are essential to the pharmacological characteristics of medicinal plants, such as sugars, amino acids, alkaloids, flavonoids, terpenoids, and phenolics. Even in complex plant matrices, MS techniques like high-resolution mass spectrometry (HRMS) and tandem mass spectrometry (MS/MS) offer detailed structural elucidation of bioactive compounds. Our capacity to understand the metabolic complexity of medicinal plants has been greatly improved by the combination of targeted and untargeted metabolomics techniques. While untargeted metabolomics makes it easier to find new chemicals and identify metabolic pathways, targeted metabolomics concentrates on quantifying existing metabolites with particular therapeutic value. The resolution and dependability of metabolite detection are further improved by the application of sophisticated separation techniques, such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Through standardization and authentication procedures, MS contributes significantly to the quality, safety, and effectiveness of medicinal plant products beyond metabolite identification. MS-based metabolomics helps the creation of evidence-based plant-derived medications and verifies traditional medicinal claims by bridging the gap between traditional knowledge and contemporary analytical research. Additionally, MS applications support the sustainable use of plant resources by extending to ecological and conservation studies. The developments, difficulties, and potential paths in MS-based metabolomics for medicinal plants are highlighted in this study. It highlights how MS can be a key component of medication development and discovery, with a focus on phytochemical research, the identification of bioactive compounds, and therapeutic standardization. Researchers can increase the scientific basis of traditional medicine and promote innovation in the creation of plant-based therapies by utilizing MS technology to open up new avenues for natural product research.

Keywords Mass Spectrometry, Metabolite Profiling, Medicinal Plants, HRMS, MS/MS, Phytochemical Analysis, Metabolomics, Bioactive Compounds

1. Introduction

People have been looking for natural ways to manage their illnesses since the beginning of time. The use of medicinal herbs was originally instinctive, much like with animals [1]. Everything was based on experience because, at the time, neither the causes of the ailments nor the types of plants that could be used to treat them were sufficiently understood. With time, the rationale for the purpose of particular therapeutic plants to cure particular ailments emerged; as a result, the progressive use of therapeutic herbs moved away from the empirical structure and toward explicative facts [2]. According to estimates from the WHO (World Health Organization), 80% of people on the earth still treat a range of illnesses with medicinal plants. Traditional medicine continues to be used even in the modern day as people search for less expensive, safer, and less adverse effect-producing alternatives [3]. Plants have a complex metabolic network that produces a variety of metabolites in a time- and space-defined way, including terpenoids, alkaloids, and polyphenols. Many of these metabolites have significant applications as medicinal molecules in addition to their critical roles in signal transduction, defense responses, and plant disease resistance. Mass spectrometry is the most widely utilized analytical technology enabling quantitative plant metabolomics investigations [4].

1.1. Historical Use of Medicinal Plant

It is quite challenging to pinpoint the precise moment when plants are used as drugs. There is evidence that plants were first used as medicines some 60,000 years ago [5]. Everything was empirical at the time because there was not enough knowledge about the causes of the sickness, effective plants for treating it, or how to employ them for that purpose. As the rationale behind the use of some medicinal plants to cure specific illnesses became clear, the empirical framework was gradually abandoned and the use of medicinal plants was reduced to the facts. The first documented record of using medicinal herbs to make medications was discovered on a Sumerian clay slab from Nagpur that dates back around 5000 years [6]. Some writings claim that the Egyptians and Chinese were among the first people to use plants as medicine, having done so for over 27 centuries BC [7]. Hippocrates, the father of Greek medicine, and Aristotle, his student, both employed medicinal plants to treat illnesses, demonstrating that the ancient Greeks were aware of the therapeutic benefits of some plants. The School of Medicinal Plants was then established by the Greek scientist Theophrastus. Then, in a series of scientific investigations on medicinal plants, Pedanius Dioscorides (who lived in the first century A.D.) described 600 therapeutic medicinal plants in his encyclopedia De Materia Medica. Dioscorides was a physician and surgeon from 75 to 45 BC [8].

1.2. Medicinal Herbs and Traditional Medicine

Traditional medicinal plants are defined by the World Health Organization (WHO) as natural plant materials that are utilized locally or regionally to cure illnesses, either with or without industrial processing [9]. Due to their natural origin and relatively low risk of adverse effects, traditional herbal treatments have been used for thousands of years in both developed and developing nations [10]. The history of herbal medicine aligns closely with the early development of medical practices. In Asia, Africa, and Latin America, a variety of traditional medicines are extensively used to treat fundamental medical conditions. This practice, commonly referred to as complementary or alternative medicine (CAM), is also expanding rapidly in developed nations. The National Institutes of Health (NIH) in the United States defines CAM as health systems, practices, and products that are not currently considered part of conventional medicine. Among the globally recognized traditional medical systems, Indian traditional medicine (Ayurveda) and Traditional Chinese Medicine (TCM) are the most widely practiced. In Western nations, "Oriental medicine" generally refers to Chinese, Japanese, and Korean remedies, particularly those adopted by Korean immigrants. As illustrated in Figure 1, Indian traditional medicine primarily consists of Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homeopathy (AYUSH). Ayurveda, which dates back over 5,000 years, is based on balancing bodily doshas (Vata, Pitta, and Kapha) using herbal formulations, diet, and lifestyle modifications. Siddha and Unani systems also emphasize the use of medicinal plants for therapeutic purposes. With an increasing global interest in holistic healing approaches, these traditional medical systems continue to gain scientific and clinical recognition [11].

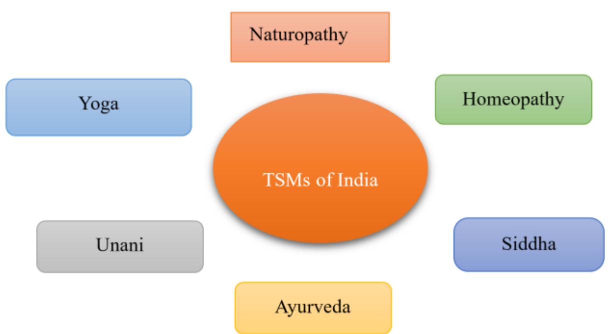

Figure 1. Traditional Medicine System of India

1.3. Phytoconstituents Present in Plant and Their Role in Disease Treatment

1.3.1. Flavonoids

These polyphenolic chemicals exhibit a multitude of biological activities, including immune regulation, the prevention of cardiovascular and cerebrovascular illnesses, cancer prevention and resistance, bacterial and viral resistance, anti-aging, pain relief, and hemostasis [12]. Example: Quercetin: Natural flavonoids like quercetin are widely distributed in nearly all consumable fruits and vegetables. It offers significant therapeutic promise for the management and defense against a range of chronic illnesses, such as cancer, neurological and cardiovascular diseases [13].

1.3.2. Alkaloids

Alkaloids are plant-derived secondary metabolites that possess single nitrogen atom in a ring that is heterocyclic. Alkaloids are mostly found in plants and have a range of genetic properties, including antiviral, anti-inflammatory, anticancer, and antibacterial properties [14]. Example: Morphine: Morphine used effectively for pain relief in humans [15].

1.3.3. Terpenes

Terpenes are the largest family of secondary metabolites; they are made up of five carbon molecules, isoprene units containing hydrocarbons, which are produced during isoprenoid biosynthesis. Terpenoids are phytopharmaceuticals with greater therapeutic action that are derived from isoprenoid chemicals found in plants. Terpenoids are important in the treatment of viral infections [14]. Example: Artemisinin: Derived from *Artemisia annua,* used as an antimalarial drug.

1.3.4. Glycosides

The presence of a sugar moiety linked to a non-sugar aglycone identifies substances as organic. These chemicals exhibit a range of biological characteristics that have been utilized for medicinal purposes. Cardiovascular, antimicrobial, anticancer, anti-inflammatory, and neurodegenerative diseases are among their functions. Example: Cardiac Glycosides: Such as digoxin from *Digitalis purpurea*, used to treat heart conditions [16].

Various classes of phytochemicals play crucial roles in disease treatment. A summary of key phytoconstituents, their sources, and therapeutic uses is presented in Table 1.

1.4. Need for Metabolite Profiling

A potent analytical technique that provides previously unheard-of insights into the intricate metabolic networks and biochemical components of plant extracts is metabolomic profiling. Researchers may now analyze the complex metabolite profiles seen in plant tissues by using state-of-the-art technologies and bioinformatics techniques. This comprehensive approach explores the wider interactions between these metabolites within the plant's biochemical environment, going beyond the simple identification of individual molecules. A comprehensive study of the chemical components as well as the underlying physiological and ecological roles they play is made possible by the incorporation of metabolomics into plant studies. Additionally, it creates new opportunities for examining these chemicals' bioactivity and illuminating their possible uses in biotechnology, agriculture, and medicine [17].

Table 1. Role of Phytoconstituents in Disease Treatment

Sr.no	Phytoconstituents	Example	Role	Reference
1	Flavonoids	Quercetin, Catechins, Anthocyanins, Apigenin	Anticancer, Antioxidant, anti-inflammatory, Antiviral	[12,13]
2	Alkaloids	Morphine, Ephedrine, Vincristine, Reserpine, Quinine	Analgesic, anti-asthmatic, antihypertensive, antipyretic, antihyperglycemic	[14,15]
3	Terpenes	Artemisinin	Antibacterial and Antifungal	[14]
4	Glycosides	Cardiac Glycosides, Cyanogenic Glycosides	Antitumor, Antidiabetic, Antithrombotic	[16]

2. Techniques for Extraction, Isolation and Identification of Metabolites

2.1. Extraction

Extraction is a key separation technique used in pharmaceutical and natural product industries to isolate bioactive compounds. It involves separating components into two phases based on solubility: the raffinate phase (solvent-rich) and the extract phase (solute-rich). The efficiency of this process depends on the solvent system, ensuring optimal solute transfer. Figure 2 illustrates the extraction mechanism and phase separation [18].

To maximize their potential applications and get the appropriate qualities, bioactive components must be extracted from natural sources. In order to effectively separate and concentrate these important elements derived from their sources, a number of sophisticated extraction methods have been developed over traditional approaches [19].

The various extraction methods are illustrated in Figure 3.

2.1.1. Decoction

This process works well for extracting components that are heat-stable and water-soluble. Typically, the botanical specimen is either powdered or fragmented into tiny bits before being cooked with water during the decoction process. The crude medication, known as yavakuta (little pieces), is put into earthen pots or tinned copper containers with clay on the outside in the Ayurvedic procedure, also known as kwatha. The pot is heated over a fire and water is added. A plant: water ratio of 1:4 was utilized for soft materials, a ratio of 1:8 for moderately hard drugs, and a ratio of 1:16 for extremely hard drugs. After that, the mixture is heated over a low heat until it reduces to 1/4 of the starting volume for easy drugs and 1/8 of the starting volume for moderate or extremely harsh drugs. Upon cooling and removing the extract through straining, the filtrate is collected. It operates on the same idea as maceration [20].

2.1.2. Maceration

The long extraction time and low extraction efficiency of this extremely basic extraction process are its drawbacks. The extraction of thermolabile components could be accomplished with it.

2.1.3. Percolation

Compared to maceration, percolation is a continuous process where new solvent is continuously added to replace the saturated solvent, making it more efficient.

2.1.4. Soxhlet Extraction

The process of Soxhlet extraction, which constantly extracts the herb with fresh solvent by using the principles of reflux and siphoning, combines the advantages of reflux extraction with percolation. The Soxhlet extraction is a high-efficiency method as opposed to maceration or percolation, automated continuous extraction technique that uses less solvent and takes less time. Thermal deterioration will be more likely due to the elevated temperature and extended retrieval period in the Soxhlet extraction [21].

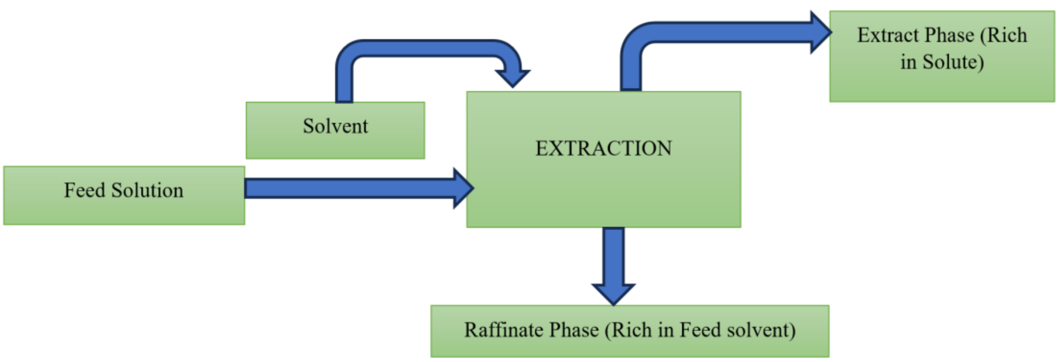

Figure 2. Block Diagram of the Extraction Process, showing phase separation into raffinate (solvent-rich) and extract (solute-rich)

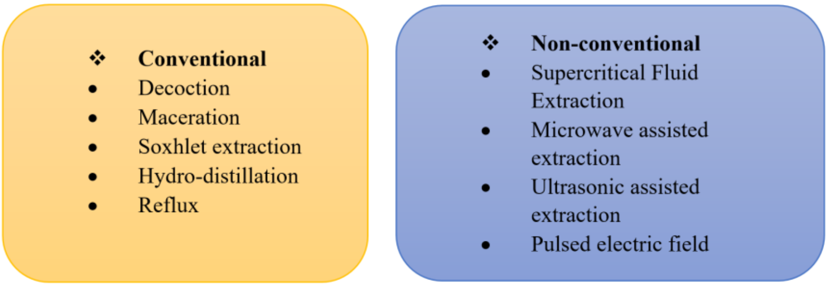

Figure 3. Methods of extraction

2.2. Isolation

2.2.1. Column Chromatography

The most efficient method for separating unrefined plant extracts into their constituent parts in pure form is column chromatography. This is a preparative chromatographic technique where the extracts are loaded onto the surface of the stationary phase (silica gel), which is crammed into a column, and the mobile phase (eluent) is then run via the column. The natural products in the mixture are carried by the mobile phase at varying rates according to their connections for the stationary and mobile phases. The mobile phase can be collected with separated components [22].

2.2.2. Thin Layer Chromatography (TLC)

In natural product research, TLC is the most widely used planar chromatographic technique. This method is the simplest and least expensive, and it can be used for isolation, analysis, and parameter setting in column chromatography. Typically, the mobile phase is made up of less polar organic solvents, whereas the stationary phase is made up of more polar materials like alumina or silica. Normal phase chromatography is the term used to describe this circumstance. As an alternative, reverse phase TLC is accessible, where the mobile phase is a polar solvent such as alcohol, water, or silica bound through an alkyl bond, making it not as polar as the phase that is stationary [23].

2.2.3. High Performance Liquid Chromatography (HPLC)

It is a durable, adaptable, and often used method for isolating natural compounds. Especially in biological, pharmaceutical, food, environmental, and industrial samples, HPLC is an analytical method for separating and determining both organic and inorganic solutes. A detector needs to be chosen before any compound may be identified using HPLC. The selection of the stationary phase and mobile phase primarily determines the extent or degree of separation. A polar liquid phase, usually a combination of water and another solvent, and a non-polar solid phase, such as C18, are used in modern HPLC. Analytes must be eluted via a column at high pressures of up to 400 bars in order for them to transit via a detector array of diodes (DAD). To assist in the identification of the analytes, a DAD examines their absorption spectra. For the identification of substances that cannot be evaporated or that break down at high temperatures, high-performance liquid chromatography (HPLC) is a valuable adjunct to gas chromatography [24].

2.2.4. High Performance Thin Layer Chromatography (HPTLC)

Planar chromatography is used to separate natural substances on high-performance layers while detecting and gathering data. These top-performing layers consist of plates that have been coated beforehand with a 150–200-micron layer thickness and a sorbent with particle sizes between 5 and 7 microns. The plate efficiency and separation nature increase as a result of the layer thickness and decrease of particle size [25].

2.2.5. Isolation Using Flash Chromatography

40-63 micron (230-400 mesh) spherical silica gel, C-18; 60Å pore size, pH range of 6.5-7.5, was used for flash column chromatography in glass columns intended for FCC. The silica gel was provided by Sorbent Technologies (Atlanta, GA, USA). C-18 silica was used to manually dry pack glass flash columns. A volume of the first combination of solvents was forced through the following the column, silica had been filled to capacity and put on the apparatus in order to exclude air and wet and acclimate the column. The sample was loaded into a solid sample loading cartridge between Teflon discs after being previously dried and triturated three times with column silica. Before elution, the sample-loaded cartridge was positioned above the column head. Test tubes were used to gather fractions and were graded according to the volume and number of the fraction [26].

2.3. Identification Techniques

2.3.1. Spectroscopic Methods

a. Nuclear Magnetic Resonance (NMR): An analytical chemistry method for figuring out a sample's composition, purity, and molecular structure is nuclear magnetic resonance (NMR) spectroscopy. It is employed in quality assurance and research. Component structure can be better understood thanks to NMR, which is also the most effective method for obtaining structural information. It is common practice to analyze all organic substances using 1 H NMR and 13 C NMR spectroscopies3. Utilized successfully in plant and mammalian biochemistry, nuclear magnetic resonance is a non-destructive, multinuclear, multiparametric, and frequently non-invasive technology [27].

b. Infrared Spectroscopy (IR): Analyses both qualitative and quantitative can be used to investigate infrared spectroscopy. With tiny levels, this approach yields information about the compound makeup of complex samples. Chemometric methods can be used to solve the information's complexity [28].

c. Gas chromatography (GC): The stationary phase in this approach is a column that is inserted into the apparatus and holds a liquid stationary phase that is adsorbed on the inactive solid's surface. "Gas-liquid" chromatography is what GC is. Its carrier phase is made up of gases like N2 or He. An inactive gas known as the mobile phase is forced through a column at high pressure. After being vaporized, the sample for analysis moves into the phase of gaseous mobility. On the firm support, the sample's constituents are between the periods of mobility and stationary. Gas chromatography is an incredibly effective method for the highly precise separation of very small compounds. It is a straightforward, versatile, highly sensitive, and quickly applied technique. Very little analyte separation is accomplished with its help [29].

2.3.2. Coupled Technique

a. HPLC-MS (High Performance Liquid Chromatography-Mass Spectrometry): First and second phases drug metabolites are identified and determined by using tandem mass spectrometry (MS/MS) methods in conjunction with HPLC. Selecting appropriate preparation of samples techniques is the first step before an HPLC-MS bioanalysis. By adding other detectors in between the HPLC and MS devices, useful analytical data can be added to the MS results. As supporting evidence for metabolite identification, the relationship between the structural alterations brought on by metabolic processes and matching variations in retention behavior in reversed-phase systems is examined [30].

3. Overview of Mass Spectrometry Techniques

3.1. Basic Principle of Mass Spectrometry

The most precise technique for figuring out a compound's molecular mass and elemental makeup is mass spectroscopy. This method involves launching a powerful electron beam at molecules. Ionization and fragmentation of the molecules occur, with some of the fragments containing positive ions. Every type of ion has a unique mass-to-charge ratio, or m/e ratio (value) [31]. The block diagram of the mass spectrometry process is illustrated in Figure 4, demonstrating the key steps involved in ionization, fragmentation, and analysis.

3.2. Modules of MS Instruments

I. Ion source
II. Mass analyzer
III. Detector

A source of ions that has the capacity to move ions from solution into the gas phase during electrospray ionization, or change molecules in the gas phase sample into ions. The ions are sorted by mass using electromagnetic fields in a mass analyzer. A detector that offers information for determining the amounts of every ion that are present by measuring the value of an indicator quantity. The method can be applied to both qualitative and quantitative data. These consist: recognizing unidentified substances, figuring out a molecule's isotopic elemental makeup, and deducing a compound's structure from fragmentation observations [32].

3.2.1. Ionization Methods

a. Electron Spray Ionization Method: For liquid samples, this hard ionization technique is frequently employed. In this instance, the liquid samples undergo degradation into droplets due to exposure to high voltage, air pressure, and continuous gas movement. Subsequently, these droplets are heated to a high temperature while under vacuum pressure, accelerating the gas flow that releases the ions from the droplets as it approaches the mass analyzer. These days, desorption electrospray ionization, static nanospray, picospray, and nanospray ionization techniques all exploit this fundamental idea with increased sensitivity [33].

b. Electron Impact Ionization Method: It works well with vaporous specimens and has an easy-to-use interface with gas chromatography. A filament-produced electron beam is used to blast the gas sample once it is inserted into the entrance. When the expelled ions (radical ions) reach the mass analyzer, they are electrically neutral. This kind of ionization is hard since the ions are entirely broken up [34].

c. Matrix-Assisted Laser Desorption Ionization (MALDI): The instance is combined utilizing a matrix in the MALDI process, which transfers a proton into the sample to generate a neutral ion while also absorbing low-energy laser radiation. The neutral ions that are produced proceed to enter the mass spectrometer. Imaging mass spectrometry uses MALDI in conjunction with time-of-flight mass spectrometers [35].

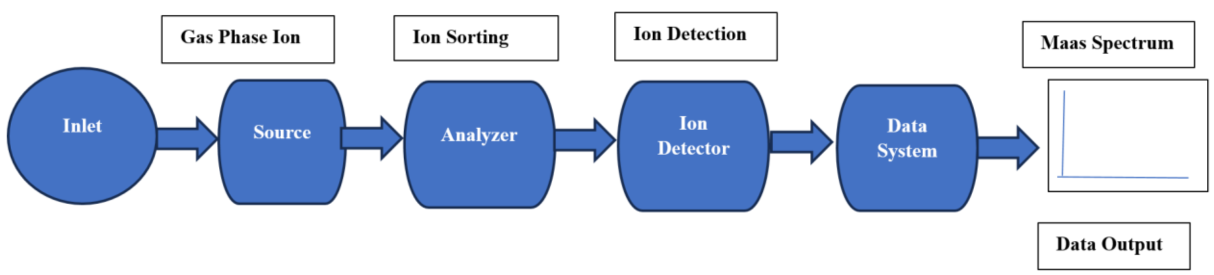

Figure 4. Block Diagram of Mass Spectrometry, illustrating the ionization, fragmentation, and separation of ions based on mass-to-charge ratio (m/e value)

3.2.2. Mass Analyzers

a. Quadrupole: It is a less expensive and more widely used mass analyzer. Ions are separated by the quadrupole mass analyzer using oscillations in an electric field. With energy-spraying ionization, which generates ions whose mass/charge ratio is less than 300 m/z, it works well. It is simple to connect this mass analyzer to both liquid and gas type chromatograph [36,37].

b. Time-of-Flight (TOF): This mass spectrometer creates ions by subjecting the sample to short electron or photon pulses produced by a laser. The ions are sorted according to how long it takes them to get to the detector. This kind of analyzer doesn't have a magnetic field. It takes longer for the ions having a large ratio of m/z to arrive at the detector. Similar to mass spectrometry using laser desorption and plasma desorption, it is a straightforward and easy-to-use technology that works with ions that have generated on the surface. The accuracy of the ionization area and time is provided by the pulsed nature of TOF. Result is increased in TOF by using reflectrons. The optical components known as reflectrons enable ions to flow via a mirror. Compared to ions with low energy, those having a lot of kinetic energy strike the reflector first and travel a greater distance before reaching the detector. For good resolution, an electron multiplier detector is utilized [38,39,40,41].

3.2.3. Detection

Electron Multiplier Detectors

a. Liquid Chromatography -Mass Spectroscopy (LC-MS): LC coupled with MS reduces sample complexity and enables metabolite separation before detection, making metabolite identification and quantitation easier [42]. Additionally referred to as high performance liquid chromatography-mass spectrometry (HPLC-MS), LC-MS, it is a hybrid method that was created in the 1980s. It's an analytical method that blends chromatographic division with high resolution and excellent mass spectral detection [43]. Elements composition and sample structure clarification are improved by LC-MS mass spectrometry. In LC-MS, mass spectrometer aids in identifying a sample's essential components and providing structural clarification. LC-MS has several different clinical applications, and because it is more frequently used in clinical laboratories for LC separations and can analyze a wider spectrum of natural atoms than GC-MS, it is a more widely applicable technology. Analyzing biochemical, inorganic, and natural compounds—which are typically present in complex materials with biological and environmental origins—is possible with this bicycle technique. Thus, LC-MS is applicable in a wide range of sectors, such as biotechnology, food processing, pharmaceutical, agricultural, cosmetic, and environmental monitoring industries [44].

Applications

In Pharmacokinetics: Phanit Songvut et al. [45] performed a highly sensitive and selective liquid chromatography triple quadrupole tandem mass spectrometry for pharmacokinetics that allowed for the quantitative identification of the main diterpenoids in plasma and urine.

Drug development: Pengfei Song et al. [46] described how the selection and advancement of one or two "best" drugs is facilitated by the use of very sensitive and selective liquid chromatography tandem mass spectrometry (LC/MS/MS) assays during early in vitro and in vivo testing. The successful development of medications that target the central nervous system (CNS) depends on the characterization of CNS pharmacokinetics (PK), even though this is usually done during secondary or tertiary screening.

Molecular Weight (MW) Determination: Jennifer L Lippens et al. **[**47**]** developed a 5-minute denaturing LC-MS technique that allows for medium-to high-throughput analysis by accurately determining the molecular weight of membrane proteins and bispecific antibodies. This technique produced dependable findings for hydrophobic proteins in a broad size range by successfully identifying unanticipated changes and sequence clipping.

Structural Determination: Ravi Tiwari et al. [48] separated, identified, and described acebutolol degradation products (DPs) under stress settings advised by the ICH using LC and LC-MS. Acebutolol and DP-I were shown to be carcinogenic, whereas acebutolol and DP-IV were found to be hepatotoxic, according to in-silico toxicity experiments.

b. Gas Chromatography–Mass Spectrometry (GC-MS): To recognize various chemicals within a test specimen, GC-MS is a technique that blends the attributes of mass spectrometry and gas-liquid chromatography [49]. This device separates mixtures of chemicals (the GC component) and molecularly identifies the constituents (the MS component). It works well for analyzing metabolites that are volatile after derivatization, have low boiling points, or are low polarity. GC-MS, or gas chromatographic-mass spectrometry, is an extremely potent analytical method [50]. Sample preparation, injection, and separation on a GC column are the first steps in a GC-MS experiment. The molecules must be directed from the GC to the mass spectrometer via an interface since the mass spectrometer needs a high vacuum system to function. The molecules exiting the column enter an ionization chamber in the most popular form of equipment, where they are subjected to a stream of intense electrons that ionizes and fragments some of the molecules. The ions resulting from fragmentation or rearrangement reactions can be combined with the molecule ion (unfragmented). In a mass analyzer, a magnetic or electric field is used to accelerate and quickly sort the ions based on the m/z. Thousands of distinct ion masses (m/z) can be sorted by the mass analyzer in a second. The current of electrons produced when the ions impact the detector is then measured by a detector to determine the ion abundance for each m/z. For GC, which produces a chromatogram showing the amount of each compound as a function of retention time, MS acts as the detector. An array of masses, which is a histogram of each ion's abundance as a work of m/z and acts as a fingerprint to identify the substance represented by a peak on the chromatogram, is the fundamental dimension of data unique to mass spectrometry [51].

Application

Academic research: S. Yogeswari et al. [52] identified bioactive chemicals utilizing GC-MS from *Monochaetia kansensis*. Significant chemicals with documented biological activities were E-15-heptadecenal, phenol (2,4-bis(1,1-dimethyl ethyl)), and hexadecane in all extracts, highlighting their potential for antioxidant and antibacterial uses.

Industrial applications: Nitesh Bhalla et al. [53] discovered a variety of bioactive chemicals using GC-MS from aqueous and methanolic extracts of Moringa leaves. The methanolic extracts demonstrated a greater ingredient diversity and a stronger capacity to scavenge free radicals (IC50: 1.83 ml/ml). These extracts have the potential to be used in food, cosmetics, and medicine as anti-inflammatory and antioxidant agents.

c. MALDI-MS: The molecular weight distribution, end-groups that cap the polymer chains, and repetition units, or monomers, are commonly used to characterize the chemical structures of polymers. Mass spectrometry (MS) cannot characterize polymers since it needs gas phase ions to function properly, while polymers are made up of long, entangled chains that are difficult to convert to gas phase ions. Conventional mass spectroscopy methods, like pyrolysis gas chromatography, were created for polymer analysis and use thermal energy to evaporate nonvolatile materials like polymers. Despite the fact that heat energy also broke down polymers into their component parts, fragmentation caused the complete chemical structure information to be lost during vaporization. A novel method has been devised to quantify this feature of the polymer specimen. The term MALDI-MS refers to this innovative technique [54].

Application: MALDI-MS is a platform with a lot of potential for clinical applications because of its versatility. It has been a successful diagnostic tool in microbiology for a long time, providing high output efficiency, economy, and throughput of data [55,56]. Additional benefits include its ability to determine molecular mass quickly and accurately over a large mass range and little to nonexistent sample preparation needed. Sample preparation, on the other hand, provides a pretreatment meant to lower sample complexity, enhance analytical performance, and increase sensitivity in order to target the intended analyte because biological samples are composed of thousands of analytes with different quantity, composition, and molecular weights. Pre-treatment methods like chromatography, extraction, electrophoresis, and isolation are frequently employed for focused analysis. Depending on the kind of analysis, MALDI-MS can be used for quantitative, semiquantitative, or qualitative applications. The sections that follow include information on MALDI-MS's clinical and research applications, MALDI variants, and possible future uses [57]. MALDI-MS enables direct tissue analysis, preserving spatial orientation while detecting endogenous and foreign chemicals with molecular specificity. Applications such as tumor tissue classification, protein alterations in the cerebellum, and medication distribution mapping in whole-body sections are made possible by this approach, which has demonstrated promise in the fields of neurology, cancer research, and pharmaceutical development [58].

Future Clinical Prospects

In addition to the both near-clinical and current clinical uses of MALDI-MS, the technique has been used in biomarker discovery studies to find new markers in a variety of disorders, including single and multimarker platform [59,60]. Future success with the sensitive and specific assays reported in this work may depend heavily on their validation and technique development. There is a lot of potential for MALDI-MS to develop into a potent method in upcoming therapeutic applications. Its applications are expanded into additional areas through the invention of methods, the optimization of current protocols, and the identification of appropriate matrices. The course for the future of MALDI-MS is supported by the highly valued advancement of MALDI imaging and its potential for pharmaceutical research and cancer biopsy analysis. Regarding the development and research of clinical diagnostics, benchtop MALDI-MS systems with mechanization, resilience, sensibility, and a reduced imprint have been introduced [61].

d. ESI-MS: With the work of Dole and associates (1960), who were successful in introducing a polystyrene polymer into the gas phase as a charged species, electrospray ionization development got underway in the late 1960s. This ionization method is surprisingly one of the easiest to comprehend. Typically, samples are dissolved in a solvent or buffer that is sprayed into the mass spectrometer. This method was further improved in the early 1980s and was connected to a quadrupole mass analyzer. Nowadays, a wide range of simple inorganic compounds that are nonvolatile and thermally labile, as well as complex biological structures, are studied qualitatively and quantitatively using ESI-MS [62].

Application

The ESI-MS's application fields quickly grew from big macromolecules to small chemical and inorganic compounds following its development. These days, analyte properties can be deeply and clearly understood from the ion signals in ESI-mass spectra, which go much beyond the analyte's conventional mass and structural characteristics. Here, we'll briefly talk about a few ESI-MS applications [63].

4. MS-Based Metabolomics Studies

The discipline of metabolomics has gradually advanced from the traditional chemometrics approach, which combines statistical techniques and global metabolite profiles, to a more robust and trustworthy quantitative approach (quantitative metabolomics). The quantitative analysis of metabolites is currently receiving a lot of attention in the field. The quantitative metabolomics approach has the ability to minimize mistakes caused by a variety of factors, such as peak misalignments, matrix interferences, and background distortions. Targeted tandem MS techniques are typically used in quantitative metabolomics, where the data is highly reliable due to the combination of robust chromatography and the usage of precursor and product ions. Absolute metabolite concentrations can be determined by the use of isotope-labeled internal standards in focused quantitative techniques. One major obstacle to expanding the pool of measured metabolites, however, is the high cost or unavailability of isotope-labeled standards for many substances. One method to get around this issue is to use microbial metabolism to produce large quantities of tagged chemicals in vivo [64, 65].

5. MS Imaging

Further advancement of MS imaging is a significant corollary of improvements in ambient ionization techniques. Real-time tissue characterization of biological material, including tissue samples, in two and three dimensions has become possible thanks to MS imaging, which also holds great promise for therapeutic applications and many other fields [66]. High-quality pictures with high sensitivity and resolution are crucial tools for molecular characterization both in vivo and ex vivo, and they hold potential for use in clinical diagnostic settings. Several studies show that MS imaging can be used to diagnose conditions like brain, bladder, kidney, and prostate malignancies. DESI-MS imaging of lipids for the diagnosis of human brain tumors demonstrated comparable performance to the existing gold standard, histology, highlighting the potential of MS imaging for real-time surgical guidance [67,68].

6. Opportunities, Challenges, and Future Directions

With applications in early detection, therapy prediction, treatment monitoring, and recurrence detection, mass spectrometry presents substantial prospects in the field of metabolomics, including the identification of illness biomarkers and insights into disease mechanisms. While new fast mass spectrometry techniques allow for real-time, in situ tissue examination, chromatography advances have enhanced metabolite separation and detection. However, problems still exist, including sample preparation, matrix effects, data unpredictability, and the inability to compare studies because of different methodologies and protocols. It is important but difficult to go from relative to absolute metabolite concentration measurements; appropriate standards and calibration are needed. For clinical applications, fast MS techniques present additional calibration difficulties. The development of absolute measurement techniques, improving the reproducibility of fast MS techniques, standardizing protocols for data comparability, and growing real-time MS applications in surgical and biomedical contexts are the main areas of focus for future research [69].

7. Conclusions

Mass spectrometry (MS) has completely changed the study of metabolomics, especially when it comes to thoroughly analyzing possible therapeutic plants. In the study of plant-based chemicals, MS has become an essential instrument due to its unmatched sensitivity, accuracy, and capacity to detect a wide variety of metabolites. The ability to identify primary and secondary metabolites, including important bioactive substances like alkaloids, flavonoids, terpenoids, and phenolics, which are essential for comprehending the therapeutic qualities of plants, has been demonstrated by techniques including LC-MS, GC-MS, and MS/MS. Additionally, MS is essential in the search for new substances with potential medical uses. Researchers can quickly identify known metabolites and investigate the structural complexities of novel substances by integrating MS with bioinformatics and sophisticated metabolomic databases. This highlights the important role that MS plays in the pharmaceutical business by speeding up the medication discovery and development process. Furthermore, a variety of metabolites can be profiled thanks to MS's adaptability, irrespective of their volatility, molecular weight, or polarity. Improvements in high-resolution mass spectrometry (HRMS) have greatly increased the accuracy of metabolite identification, enabling more precise chemical fingerprinting of medicinal plants. To sum up, metabolite profiling by mass spectrometry is a fundamental component of contemporary natural product research. Along with facilitating the discovery of new bioactive substances, it also allows for a deeper understanding of plant metabolomes, opening the door for future therapeutic advances from medicinal plants.

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