Vol. 16, No. 4, 2025
ISSN 2233-4203/ e-ISSN 2093-8950
REVIEW www.msletters.org | Mass Spectrometry Letters

Emerging Mass Spectrometry Technologies in Phytochemical Analysis

Chirag Saroha[1], Ishita Bisaria[1], Mani Singh[2], and Annie Gupta[1]*

[1]*Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh-201313, India*
[2]*Rajiv academy for pharmacy, Mathura, AKTU University, Lucknow, Uttar Pradesh-281122, India*

Received September 2, 2025, Revised October 29, 2025, Accepted October 30, 2025
First published on the web December 31, 2025; DOI: 10.5478/MSL.2025.16.4.95

Abstract : Mass spectrometry (MS) has been the basis of phytochemical study, yet the area is dramatically transitioning to high-throughput workflows that can disentangle the extreme complexity of biological systems. The present review deals with the shift that occurs Mass Spectrometry Technologies and their integration, which is leading to a system-level phytochemical analysis. We discuss cutting-edge concepts with emphasis on ambient ionization methods (for example, LAESI-MS, DESI-MSI, PS-MS) that by direct *in-situ* access of metabolites from the plant facilitate fast spatially resolved analysis with critical spatial resolution and minimum sample preparation. The transition to advanced hyphenation is a main idea. The coupling of chromatographic methods (LC, GC) with Ion Mobility Spectrometry (IMS) and high-resolution MS (HRMS) creates a separation space that can solve the problem of isobaric along with isomeric resolution, which is the main problem in natural product extracts. Besides that, we are acknowledging the emergence of methods of interpretation of data, such as Parallel Covariance Two-Dimensional MS (pC-2DMS) and the joining of MS with NMR spectroscopy and AI-based bioinformatics tools (like molecular networking) that are necessary to make more rapid the process of unknown compound annotation and establish a connection with the chemical structures and the biosynthetic pathways. The problems of matrix effects and accessibility are still there, yet the future of phytochemical MS is marked by miniaturization, portability, and advanced data processing, all of these facilitating a rapid pace of discovery of new bioactive molecules and leading to the dynamic understanding of plant metabolism.

Keywords : Phytochemistry; Mass Spectrometry; Ambient Ionization; Ion Mobility Spectrometry; Metabolomics; Hyphenation

Introduction

Mass spectrometry (MS) has constantly been a major analytical instrument in phytochemistry that has made it possible for scientists to dig into the incredible chemical variety of the plant kingdom with the most exquisite level of exactness and detectability.[1] Basically, MS carries its function by ionizing molecules to create charged particles that are later separated and detected as per their mass-to-charge (m/z) ratio.[2] Even though it's a conceptually simple process, this method allows for in-depth qualitative and quantitative analyses of complex plant extracts.[3] Just like that, MS shows even higher analytic potential when combined with chromatographic methods like gas chromatography (GC) and liquid chromatography (LC). These combined systems GC–MS and LC–MS are now at the forefront of natural product research, being that they provide the improved selectivity, specificity, and ability of pre-separation of complex phytochemical mixtures before mass detection.[4] This kind of hybrids makes it possible to perform a very sensitive and accurate profiling of various plant-derived compounds such as alkaloids, flavonoids, terpenes, and phenolic acids without any problem.[5] However, even though conventional MS-based workflows are highly productive, there are still some limitations, especially when the structural complexity and matrix interferences characteristic of plant materials are involved. Traditional methods often have difficulties in completely separating isomeric compounds, require a lot of sample preparation, and show diminished performance in highly heterogeneous samples.[6] The disadvantages have been the main issue for the continuous development of new and improved MS technologies that address the bottlenecks of traditional methods. For this reason, this review focuses on the recent developments and the application of MS in phytochemical identification that advances more than ten times the sensitivity of the conventional one, as well as the orthogonal separation techniques which do not combine a single chromatographic method to separate the complex mixture. The paper points out the scientific foundations of the most advanced and pioneering/

*Reprint requests to Annie Gupta
https://orcid.org/0000-0003-2624-2115
E-mail: anniegupta0601@gmail.com

progressive/explorative methods like IMS, LAESI, PS-MS, DESI-MS, and several other extremely innovative and unprecedented unanticipated MS-based platforms that among others have impacted the revolution of plant metabolomics drastically.[7] Besides the principles of their work, this discussion also depicts the way of coupling chromatographic and ambient ionization systems and the expansion of their role in automated instrument based phytochemical characterization and herbal drug discovery. Ultimately, the goal of this review is to inform about the latest MS methodological advancements and their actual impact on natural products research.

Emerging and Ambient Mass Spectrometry Technologies

Phytochemical analysis is frequently affected by the complex nature the plant extract matrix of which results in such problems as ion suppression and failure to separate structural isomers. To overcome such drawbacks, next-generation mass spectrometry (MS) platforms have been introduced. These innovative and ambient ionization methods allow fast, on-site, direct analysis of unmodified plant materials, thus opening the possibilities for further spatial resolution and improved structural specificity. The featured sections provide a description of these newly developed MS technologies that are radically changing the field of phytochemical profiling and natural product discovery.

Matrix-Assisted Laser Desorption/Ionization MS (MALDI-MS)

Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS) is a soft ionization technique, in which the analytes are co-crystallized with a laser-absorbing matrix and ionized by a pulsed UV laser.[1] This method keeps molecular ions in their entirety (usually only one charge) with minimal fragmentation; hence it is possible to trace large and unstable molecules. In the analysis of phytochemical substances, the role of MALDI-MS has been growing, as it is used for the profiling of extremely complex plant matrices, the mapping of metabolite distribution without the in-situ extraction, and the identification of metabolic heterogeneity among tissues.[2] The recent progress has brought about a considerable extension of MALDI imaging, which allows for the detection of many metabolites (alkaloids, flavonoids, phenolics, terpenoids, saccharides, etc.) from different parts of a plant, thus enabling the solving of spatial metabolomics puzzles which were not possible before.[3]

Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS) works through a soft ionization technique where analyte molecules are co-crystallized with an energy-absorbing organic matrix on a conductive plate. In this process, a pulsed UV laser irradiates the surface, the matrix takes up, and desorbs energy, simultaneously transferring charge to the analyte, minimal fragmentation, and hence intact molecular ions are formed.[4] The same principle is a perfect match for phytochemicals with various polarities and molecular weights, which are alkaloids, flavonoids, terpenoids, glycosides, and lignans. The development of matrix construction, for example, using the nanostructured materials or the reactive matrices (like TiO_2 or gold nanoparticles), has brought about the increase of ion yield and the decrease of the background noise that has led to the easier detection of low mass phytoconstituents.[5] In general, MALDI is linked to the instruments of time-of-flight (TOF) or hybrid (MALDI-QTOF, MALDI-Orbitrap), giving high resolution and mass accuracy which are appropriate for complicated plant extracts.[6] Additionally, the step of sample preparation, which is well suited like matrix sublimation under controlled conditions and homogeneous crystal deposition assures reproducibility and spatial integrity, mainly in the imaging applications. These improvements have been combined to increase the analytical sensitivity, the spatial resolution, and the mass range of plant metabolomics and secondary metabolite profiling by MALDI-MS.[7]

MALDI-MS represents one of the most pivotal tools for phytochemical investigations as it enables the identification, localization, and quantification of a multiplicity of secondary metabolites' classes directly in a plant tissue.[8] The use of MALDI imaging mass spectrometry (MALDI-IMS) has opened a broad way in mapping the ground distribution of these plant metabolites.[9] Besides, the researchers could see the compartmentalization of these metabolites at cellular and tissue levels by these methods. For instance, the technique totally unveiled the root of *Isatis tinctoria* by detecting the localization of indole alkaloids, glucosinolates, and phenylpropanoids in the xylem and cortex regions, among other metabolites.[10] The finding that flavonoid aglycones and glycosides have different spatial biosynthesis locations was done with the help of Phytochemical imaging of *Scutellaria baicalensis* roots by which the localization of two groups was achieved. Using MALDI-TOF MSI technology, caffeoylquinic acids, saccharides, and flavonoids have been found in the epidermal and cortical zones of *Arctium lappa* roots.[11] Besides, mass spectrometry (MS) has become a versatile and efficient tool for high throughput dereplication, and metabolite fingerprinting of plant extracts without using chromatographic separation methods. A new concept, plant tissue microarray (PTMA)-MALDI, has just been raised allowing the simultaneous analysis of over 1,000 different tissue samples every day, thereby largely shortening the time for phenotyping of plant secondary metabolism and natural product discovery.[12]

Laser Ablation Electrospray Ionization Mass Spectrometry (LAESI-MS)

Laser Ablation Electrospray Ionization Mass Spectrometry (LAESI-MS) refers to an ambient ionization technique

with great potential, designed to carry out direct chemical analysis of fresh, hydrated biological samples at atmospheric pressure with no or very little pretreatment.[13] In a traditional Mass Spectrometry (MS) approach, usually, the compounds are extracted, purified, or derivatized before analysis, which can change the native composition of plant metabolites. Whereas LAESI-MS, by direct sampling of tissues, eliminates these requirements and is, therefore, very compatible with phytochemical research, where it is essential to maintain the natural spatial and chemical integrity of metabolites. The technique uses a mid-infrared (IR) laser, generally at 2940 nm wavelength, which is the vibrational absorption band of water molecules.[14] As for most biological tissues, water content is likewise, in the case of plants. So, the laser selectively introduces water molecules with energetically absorbed photons, micro-explosions occur, and the sample is ablated. The removed molecules are ionized in an electrospray plume, and then they are drawn into the mass spectrometer for detection. It is worth mentioning here that the technique has the uniqueness of in situ and real-time chemical analysis, and thus spatial metabolite imaging. Further, single-cell resolution has even been reported.[15]

Laser ablation electrospray ionization mass spectrometry (LAESI–MS) on a large scale involves a series of physical and chemical operations, where a laser collects the tissue and ions are introduced by the plume that is formed due to the ablation. The process of defining analytical performance which includes sensitivity, spatial resolution, and metabolite coverage is a function of each operation in the workflow. The foundation of LAESI is the tissue-laser interaction identifying the mid-infrared 2.94 μm laser irradiating the sample surface.[16] Water molecules absorb light at this point; thus, the O–H bonds in water molecules produce rapid heating and the localized micro-explosions that release cytoplasmic droplets into the gas phase. The ablation scenario is limited to the water content of the tissue, thus leaves and fruits are perfect samples for water rich. The easy energy handover to water makes sure the metabolite desorption is achieved without changing the original chemical structures of the metabolites. Immediately after the procedure, a vaporized cloud comprising water, nanoscale droplets along with some dissolved analytes hover over the tissue. The characteristics of the plume are highly dependent on the experimental parameters such as the laser fluence and the spot diameter. A smaller focal spot can enhance spatial resolution and allow one to perform metabolic profiling at the subcellular level.[17] Tissue heterogeneity that also includes such features as trichomes and waxy cuticles, can impact the plume content because of changing the hydration level and analyte loading. The neutral plume is made to undergo ionization electro ionization ESI. Generally, the solvent mixture is methanol–water or acetonitrile–water along with formic acid. This mixture is given under high voltage of 2–5 kV from a nano spray tip. When the ablated neutrals meet the charged solvent stream, desolvation and charge transfer take place which leads to the formation of ions. Efficiency of ionization can be affected by the composition of the solvent. Protic solvents are good at making polar metabolites while acidic additives help in protonation of alkaloids. This simultaneous ablation–ionization step increases the transfer of the analyte and keeps the labile molecular groups intact. The formed ions can be then injected into high resolution mass analyzers such as Q-TOF, Orbitrap or FT-ICR for mass measurement and MS/MS fragmentation. LAESI's analytical capacities have been stretched far and wide due to the developments in technology. The fiber optic LAESI (f-LAESI) setting, employing optical fibers of the smallest size, permits one to aim the laser straight at individual cells. Depth-profiling modes along with serial ablation can uncover metabolite distributions in the interiors of plant tissues and hence offer 3D chemical mapping. Microscopy-guided LAESI is another tool that has the combined advantages of visual targeting and ablation for achieving selectivity in the analysis of certain areas of tissue or cells. The energy of the ions in hybrid LAESI–IMS systems can now be sorted by their mobility, in other words, as well as being separated according to their mass-to-charge ratio, which is the normal operation of a mass spectrometer. Ion mobility spectrometry thus adds an orthogonal separation dimension that not only permits direct identification of the isomers but also the conformers on the surface of untreated tissue. All in all, these technology improvements have brought LAESI–MS right at the front of versatile analytical platform choices that offer high resolution in situ metabolomic investigations from plant tissues.[18]

Laser ablation electrospray ionization mass spectrometry (LAESI-MS) allows for the direct examination of freshly harvested plant materials with a high-water content under normal conditions and thus keeps the native chemical environment intact. This technology is a "white" method because it involves a minimal amount of sample preparation. Therefore, the method is very efficient for the study of secondary metabolism, the dynamics of metabolites, as well as their distribution in space. One of the main advantages of the method is the visualization of the spatial metabolite which allows direct chemical images to be obtained from the investigated objects thus making it possible to recognize the localization of different compounds such as the localization of anthocyanins in orchid petals, the local - tissue-specific - accumulation of flavonoids, terpenes, and alkaloids in leaves, roots, and fruits, respectively.[19] Flavonoids are found most of the time in the epidermal tissues of a plant where they protect the latter from the UV radiation while alkaloids are found in the root cortex regions of the roots of the plants and they are the chemical defense that the plants use against herbivores. LAESI-MS is a method that can also be used as an advantage in the research of plant-pathogen and plant-microbe interactions. Here, small localized metabolic changes at infection sites can be

detected. For example, local decreases in α-tomatine upon *Cladosporium fulvum* infection of tomato leaves that is, the turning off the host's defense by the pathogen, is only one among many cases by which the researchers have found the occurring of the synthesis of flavonoids, phenolic acids and phytoalexins at intruding microbial sites.[20] That is because bulk extraction, a method that averages metabolite signals, can hardly offer these LAESI site-specific biochemical variations thus, plant defense dynamics can be easily grasped by LAESI. Also, fiber-based LAESI (f-LAESI) potentially may make single-cell analysis under the microscope guidance possible so the authors can demonstrate the extreme metabolic heterogeneity, for instance, they show that secretory trichomes and the neighboring epidermal cells separate alkaloid and terpenoid profiles thus cellular specialization in terms of metabolic diversity is highlighted. Depth profiling enables three-dimensional metabolite mapping by sequentially analyzing tissue layers thus visualizing vertical gradients of cuticular wax or defensive metabolites in trichomes. Since the sample is almost ready for analysis, the technique can be used to compare cultivars, different developmental stages, and the effects of the environment, which in turn has confirmed medicinal plant research as one of the most important fields for this technique in detecting flavonoid and alkaloid distribution hence authentication as well as adulteration detection can be supported. Also, the LAESI-MS can follow the secondary metabolite changes as the source of abiotic stress becomes apparent, for example, an increase of phenolic antioxidants during oxidative stress or a localized deposition of flavonoids under UV exposure thus, this technique is very suitable for ecological and field-based studies.[14] The combination of LAESI and high-resolution mass analyzers together with the use of ion mobility spectrometry (IMS) improves mass accuracy, resolution, as well as the differentiation of structural isomers thereby making it possible to profile complex mixtures directly and building of metabolite libraries for untargeted phytochemical research is enabled.[21] In addition, LAESI is capable of detecting very low concentrations of metabolites found only in a particular piece of tissue, or at a certain stage of development, or even in just one single cell, which makes it possible to discover natural products that are the most likely to be missed during the bulk extraction and are the isolation and bioactivity testing. However, the performance of LAESI-MS is still subject to limitations such as ion suppression in complex matrices and tissue hydration dependence. Nevertheless, the accompanying developments, i.e. the introduction of an improved laser optic and the integration of IMS are continuously enabling LAESI-MS to perform better and ultimately making it an effective analytical toolbox for in situ plant metabolomics, taxonomy, stress physiology and natural product research.

Paper Spray Mass Spectrometry (PS-MS)

Paper Spray Mass Spectrometry (PS-MS) is one of the ambient ionization techniques, its fame being due to its facility, rapidity, and capability to analyze complex biological samples with almost no preparation.[22] Forensic and clinical settings were its first markets, and, after that, it has extended its borders very fast, with the help of the natural product studies, mainly for plant-derived phytochemical research. In contrast to the traditional ionization techniques still reliant on chromatographic separation and long sample extraction steps, PS-MS direct analysis of crude extracts, plant tissues, or even dried biofluids deposited on paper substrates is feasible.[23] The concept of PS-MS embodies the use of a piece of triangular porous paper with a small sample applied to it. When a high voltage and a spray solvent are delivered, ions can be seen directly from the paper tip through such processes as electrospray, carbonization, and plasma discharge, with one common feature, which is the ionization of the sample.[24] This simple ionization method turns PS-MS into a very powerful tool for quick phytochemical screening, quality control of herbal preparation, and on-site metabolite profiling, where the time factor and minimal setup/condition are of the essence.

Paper spray mass spectrometry (PS–MS) is a newly developed technique based on the concept that porous paper is used as a sample holder and an ionization medium. Generally, a triangular piece of cellulose-based paper such as Whatman filter paper is cut to create a sharp tip that can produce a stable spray plume. Materials from plants can also be directly placed on paper in different forms, for example, crude extracts, mashed tissues, or powdered herbs. Thanks to such versatility, there is no need for chromatographic separation, which, in turn, allows fast analysis of phytochemically complex plant matrices. Once the sample is applied, a few microliters of spray solvent are put near the paper base. The solvent passes along the paper's porous fibers while dissolving the analytes and carrying them to the tip of the paper. Efficient extraction and ionization largely depend on the choice of solvent.[25] One may choose a methanol–water mixture for phenolic compounds and flavonoids, an acetonitrile-based system for the enhancement of alkaloid and terpenoid ionization, and the likes to promote protonation, thereby increasing signal intensity for alkaloids and glycosides. The effect of every phytochemical group on the respective solvent can be adjusted further to reach the best performance. It is, basically, electrospray ionization (ESI) in conventional terms but the ions are generated directly from the paper substrate without using tubing or pumps. When paper base is supplied with a high voltage (3–5 kV), the area with wetted fibers is electrified thus a charged spray plume is created at the tip. The condition of ion production and the efficiency of ionization depend significantly on the paper's geometry, solvent flow rate, and the applied voltage. The gas-phase ions then are moved by the instrument to where they are identified and their structures are established. PS–MS is a method that can be hugely improved by using high-resolu-

tion mass spectrometers like Orbitrap, FT–ICR, and Q–TOF. As there is no chromatographic step in the process, analyzers carry out a very accurate mass measurement and provide sufficient fragmentation details to allow one to differentiate overlapping species of phytochemicals. PS–MS is a tool that, albeit very simple in construction, yields good analytical performance by allowing thus stable ion currents and consistent molecular fingerprints directly from raw herbal samples. Because of this, it can be regarded as an instrument that is very useful both in the rapid screening of samples and in the identification of metabolites. Besides the present developments, there are still some improvements in the fields of paper spray-mass spectrometry (PS–MS) and consequently in the scope of their analytical applications. The modification of paper substrates with nanoparticles, polymers, or ionization-enhancing reagents is one such example. The later incorporation of ion mobility spectrometry in tandem with PS-MS (PS–IMS–MS) allowed another dimension of separation which made the resolution of co-eluting isomeric metabolites possible even though the mass data alone could not differentiate them. On-paper derivatization is also a strong method that emerged by applying pre-deposited reagents to modify analytes chemically during ionization, this enlarges the phytochemical space and facilitates the detection of difficult to ionize compounds.[26]

Due to its low cost and ability to handle crude samples, Paper Spray Mass Spectrometry (PS-MS) is rapidly developing into a versatile method of phytochemical analysis.[27] As a contrast to chromatography, only a few milligrams of the material are required. The material is applied directly to a paper substrate, which allows for the fast screening of alkaloids, flavonoids, terpenoids, and phenolic acids. One can identify the presence of atropine and scopolamine, flavonoids like quercetin, kaempferol, and rutin in herbal matrices in very short time intervals.[28] The PS-MS metabolite fingerprints are very detailed and useful for herbal authentication and quality assurance. The alkaloid profiles of Datura and Atropa can be used for differentiating the two species, as well as for confirming the identity of the tested samples against pharmacopeial standards without the need for extraction or filtration. The sample spotting method is very easy and thus it can be applied by industrial and regulatory work. This method also makes it possible to perform leaf, fruit, or root in situ profiling by direct pressing on paper. The main advantage here is that anthocyanins, catechins, and flavonoids can be detected without the use of solvent.[23] The imprint-based analysis not only allows monitoring of metabolic changes during growth or processing but also maintains spatial chemical information. Possibility of generating the species-specific chemical patterns that eliminate herbal misidentification, and adulteration makes PS-MS one of the most important tools in the field of chemotaxonomy.[26] It has also resulted in a significant contribution in the field of plant physiology. The method detects fast metabolic changes due to drought, salt, or UV stress, for example, an increase of phenolic and flavonoid levels, which in turn helps ecological and agricultural monitoring. High-resolution instruments like Orbitrap or Q-TOF can be used together with PS-MS to obtain mass and fragmentation data of complex molecules with high accuracy, thus there is no need for long sample preparation, plus, it can be an excellent bridging tool for field and laboratory workflows. The use of PS-MS does not replace the conventional methods; rather, it complements them while providing faster and more accessible options. Direct tissue analysis in natural-product discovery is a method that facilitates the localization of the rare or stage-specific metabolites just the way it was intended to be, for later isolation and bioactivity testing.[29] The new technologies are revolutionizing the field of PS-MS, creating new opportunities for advanced metabolite discovery.

Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI)

Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) is one of the revolutionary ambient ionization techniques, created to identify the samples in their original form with minimal or no sample preparation.[30] Since the first instance, DESI has turned out to be a powerful tool for surface chemical analysis that has been increasingly used in the research of phytochemistry and the natural product area.[31] In contrast to standard ionization techniques, which necessitate extraction and chromatographic separation, DESI-MS allows for the direct chemical profiling of solid surfaces, plant tissues, and crude extracts; thus, both the spatial context and chemical diversity of metabolites are maintained.[32] This method is done by aiming at a charged electrospray plume at a surface. As the droplets of the solvent hit the surface, they desorb the analytes, which are then ionized and conveyed to the mass spectrometer. DESI-MS is a multimodal instrument as it combines the advantages of gentle ionization (like electrospray ionization) with the possibility to investigate the intact samples at ambient conditions.[33] As a result, it has become very important in the analysis of plant secondary metabolites, metabolite imaging of plants, and the fast screening of herbal formulations.

Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) is a system that works in ambient conditions and does not require vacuum. The method consists of three main steps: charged droplet generation, analyte desorption, and ionization.[34] Typically, charged microdroplets from a standard electrospray source are made using methanol-water or acetonitrile-water mixtures with formic acid and a 3–5 kV field. These droplets are positioned onto the sample surface at an angle of 50^{o}–70^{o}, in this situation a very efficient way to extract small molecule metabolites from both the surface and epidermal layer without tissue integrity being compromised.[35] The primary droplets are thus

"splashing" and secondary microdroplets are generated in which phytochemicals are already solubilized. Such a "splash mechanism" makes the short way from the solid to the gas phase very easy for the analytes. Part of ionization can be protonation, deprotonation, or adduct formation, and after the solvent has been completely removed, the ions are sent to a mass spectrometer. Here, the enactment is gentle, and the phytochemicals are kept intact. Therefore, the tandem MS performance of such fragile phytochemicals as glycosides, flavonoids, alkaloids, and terpenoids is also possible. DESI-MS coupled with high-resolution mass spectrometry such as Orbitrap, Q-TOF, and FT-ICR could provide precise mass detection, along with the fragmentation patterns of the ions, which is very helpful to distinguish isobaric or close terms of metabolites in complex herbal matrices.[36] One of the most important modifications of DESI widening its application in the phytochemical field is the imaging DESI that allows mapping the distribution of metabolites in intact tissue. In the reactive DESI solubilizing and derivatizing agents are used to accelerate the ionization of the target group of metabolites. In Nano-DESI the solvent flow can be extremely low, so it is suitable for single-cell or trichome analysis, thus, the resolution of plant metabolomics at the micro-scale can be improved considerably.[37] Besides that, the use of ion mobility spectrometry (DESI-IMS) as a hyphenation technique introduces a different separation parameter, which helps to differentiate isomeric phytochemicals directly in the sample without the need for any pre-separation.[38] With the use of gentle ionization, spatial mapping, and adaptive configurations, the DESI-MS method is still the best non-destructive technique for revealing chemical diversity and the spatial organization of plant metabolites.

Desorption Electrospray Ionization Mass Spectrometry (DESI-MS) allows the ambient, on-site analysis of plant metabolites directly from the intact tissues, thus maintaining the spatial and chemical data.[39] Metabolite imaging is one of its great uses, where DESI-MSI visualizes metabolite distributions that, in turn, indicate the localization of the compounds in the whole plant tissues. In this reference, the localization of nicotine and nornicotine in the xylem and phloem of tobacco leaves has been demonstrated, and in addition to that, the distribution of flavonoids and limonoids, the metabolites that significantly contribute to the color, defense, and signaling of the plant, in the peel of citrus, have been shown.[40] Furthermore, DESI-MS beyond imaging can be considered a handy tool that allows direct and rapid screening of herbal materials, and without any prior preparation. Based on unique alkaloid surface profiles, the identification of *Datura stramonium* and *Atropa belladonna* from fraudulent or substituted samples, which can be found in powders or capsules, even after processing, is quite feasible.[41,42] This also indicates that the analysis time can be shortened drastically as well as the therapeutic potential can be maintained. One more area where DESI-MSI helps the progress of the field is in the studies of stress physiology by tracing the biochemical changes of a plant "in situ". It simultaneously spots alkaloids that were depleted locally and phenolic compounds that accumulated in leaves infected by pathogens and records osmolyte and flavonoid levels that increase with drought and salinity stress, giving a comprehensive picture of the plant's arsenal of defenses. On top of that, DESI-MS is a great aid to research that focuses on the surface metabolites of organisms that are made up of the cuticular waxes, acyl sugars, fatty acids, and trichome secretions and are the main carriers of herbivore resistance and water retention in tomato and tobacco with the impact that it can be ecological and agricultural research.[43] In the chemotaxonomy field, DESI-MS is a powerful tool that can separate those species that are very similar to each other metabolically and genetically by providing unique metabolite fingerprints and, hence, it can be used for the authentication of medicinal plants and the prevention of misidentification. Moreover, its imaging potential can dramatically speed up the discovery of natural products since it is possible to locate rare and organ-specific alkaloids and glycosylated flavonoids right on the tissues, thus paving the way for their extraction and subsequent bioactivity evaluation.[44] It is worth mentioning that DESI-MS coupled with Orbitrap or FT-ICR can perform high-precision mass and structure elucidation, whereas, in addition, the Ion Mobility Spectrometry integration (DESI-IMS) is able to separate isomeric metabolites such as caffeoylquinic acid derivatives. Despite facing issues such as ion suppression and low depth of penetration, the development of nano DESI, reactive DESI, and DESI-IMS is steadily filling out the gaps in the capabilities of DESI. What is more, spatial resolution, gentle ionization, and surface specificity are the attributes that combined make DESI-MS a powerful and ground-breaking instrument that is at the forefront of modern plant metabolomics as it connects metabolite localization, plant defense, and phytochemical discovery.

Partial Covariance Two-Dimensional Mass Spectrometry (pC-2DMS)

Parallel Covariance Two-Dimensional Mass Spectrometry (pC-2DMS) is an innovative method that, in essence, recreates the functions of conventional tandem mass spectrometry by generating two-dimensional correlation maps of precursor and fragment ions.[45] In standard MS/MS, only one precursor ion is usually selected and then broken down. However, pC-2DMS applies a covariance statistical approach across multiple spectra to detect ion–ion correlations that happen concurrently. Consequently, it becomes possible to follow the source–fragment connections in such complicated mixtures; hence, the serial ion selection limitations are not there anymore. Phytochemical work is an area where plant extracts can be so metabolically diverse that even with the largest dynamic range, there are thousands of

structurally different metabolites. In this situation, the use of pC-2DMS would likely result in more thorough coverage and better-defined structures. As it more thoroughly captures precursor–fragment connections, thus, it can reveal the existence of minor constituents, isomers, and novel compounds that are also difficult to differentiate by regular methods.[46]

Parallel Covariance Two-Dimensional Mass Spectrometry (pC-2DMS) innovates fragmentation analysis mostly by changing the ion ensemble acquisition method from serial to ensemble. Contrary to conventional MS/MS, which selects only one precursor at a time, pC-2DMS allows one to record multiple ions as ensembles from both precursors and their wide-range fragments at the same time. Due to this parallel collection, not only can the amount of data be collected much faster, but also the representative view of complex mixtures is obtained. Essentially, pC-2DMS utilizes covariance analysis—one of the statistical methods, where the intensity fluctuations of ions that are across the scans are compared.[47] When a signal of a fragment ion varies with that of the precursor in a consistent manner, a covariance link is confirmed, showing precursor–fragment relationships without the need for explicit ion isolation. Moreover, this method can be effectively applied to phytochemical and biological samples in which low-abundance components that co-elute may become hidden due to conventional selection. Based on the precursor and fragment *m/z* values, two-dimensional covariance maps have been plotted, which are visual indicators of fragmentation fingerprints with very high degrees of details. In contrast with conventional 1D spectra, the 2D plots are giving connecting bonds not disclosed by the molecular and structural subtleties detection method, thereby speeding up the identification of the isomeric or low-intensity metabolites. Since all precursor–fragment relationships are recorded in a single experiment, pC-2DMS does not suffer from the serial bias of typical MS/MS workflows and offers better sensitivity towards rare metabolites. Isomeric species of similar masses, that are thus difficult to separate, can be further characterized relying on distinct covariance patterns that allow the separation of complex natural product mixtures.[47] Putting pC-2DMS together with e.g. FT-ICR or Orbitrap analyzers, which have high resolution, can guarantee accurate covariance mapping, and combining pC-2DMS with Ion Mobility Spectrometry (IMS) can further increase the capability of the instrument to differentiate structures. Thus, pC-2DMS, IMS, and high-resolution MS as a combination, offer the most profound insights in phytochemical architecture.[48] pC-2DMS accomplishes a metamorphic platform for metabolomics and complex phytochemical characterization by combining the benefits of ensemble acquisition, statistical correlation mapping, and two-dimensional visualization, which are comprehensive, sensitive, and isomer-resolving, respectively.[49]

Parallel covariance two-dimensional mass spectrometry (pC-2DMS) has completely changed phytochemical and metabolomics investigations. This is because the method allows one to do a fingerprinting of the metabolites in their entirety without the need for precursor isolation.[50] The method captures simultaneously fragment–precursor pairs over a very wide mass range, thus providing a full chemical profile of the plant extracts. Since the technique parameters encompass the whole chemical space, it ensures the detection of both the most abundant metabolites such as flavonoids and the least abundant compounds like minor alkaloid derivatives, which is very important, as the metabolites present in very low quantities are quite often the ones that have a significant impact on the biological and pharmacological activity of organisms. The great thing about pC-2DMS is that it is very powerful for the separation of isomers and glycosides of that kind, which are difficult to differentiate by using conventional MS/MS.[51] One can identify thus different coexisting fragmentation networks very clearly by using a correlation of fragment–precursor ion intensities via covariance analysis, thus obtaining a perfect separation of isomers such as caffeoylquinic acids and glycosylated flavonoids.[52] This in turn broadens up the range of molecular characterization possibilities and at least the chemical diversity of plant secondary metabolites can be unveiled to a greater extent. Moreover, the technique is very beneficial to chemotaxonomy as well as herbal authentication.[53] Small chemical differences are brought to light by covariance fingerprints, making it possible to distinguish closely related plant species and to detect the presence of adulteration in herbal materials in a reliable way. Since this dual sensitivity to both major and minor metabolites can support quality control as well as species verification in the herbal industry, it cannot help but become an indispensable tool there.[54] Naturally, in addition to identification, the method also opens to the discovery of novel natural products by means of the detection of fragment–precursor relationships that were not present in already established spectral libraries. Very often, it is the recognition of these unprecedented fingerprints that leads to the uncovering of new alkaloids, terpenoids, or glycosides, thus providing initial steps for separation and bioactivity studies. In addition, comparative metabolomics with the use of pC-2DMS can reveal changes of metabolic pathways during the development or because of different environmental conditions, for example, changed correlations of phenolic fragments under drought stress, thus offering an understanding of stress physiology and ecological adaptations.[55] The integration of pC-2DMS with chromatographic separation or ion mobility spectrometry (IMS) is a further step that goes in the direction of extending its resolving power by adding temporal and structural dimensions. This approach has several advantages such as, among others, less spectral congestion and the possibility of reliable identification of the isomeric families like withanolides, terpenes, and complex alkaloids. To summarize, pC-2DMS offers unbiased and

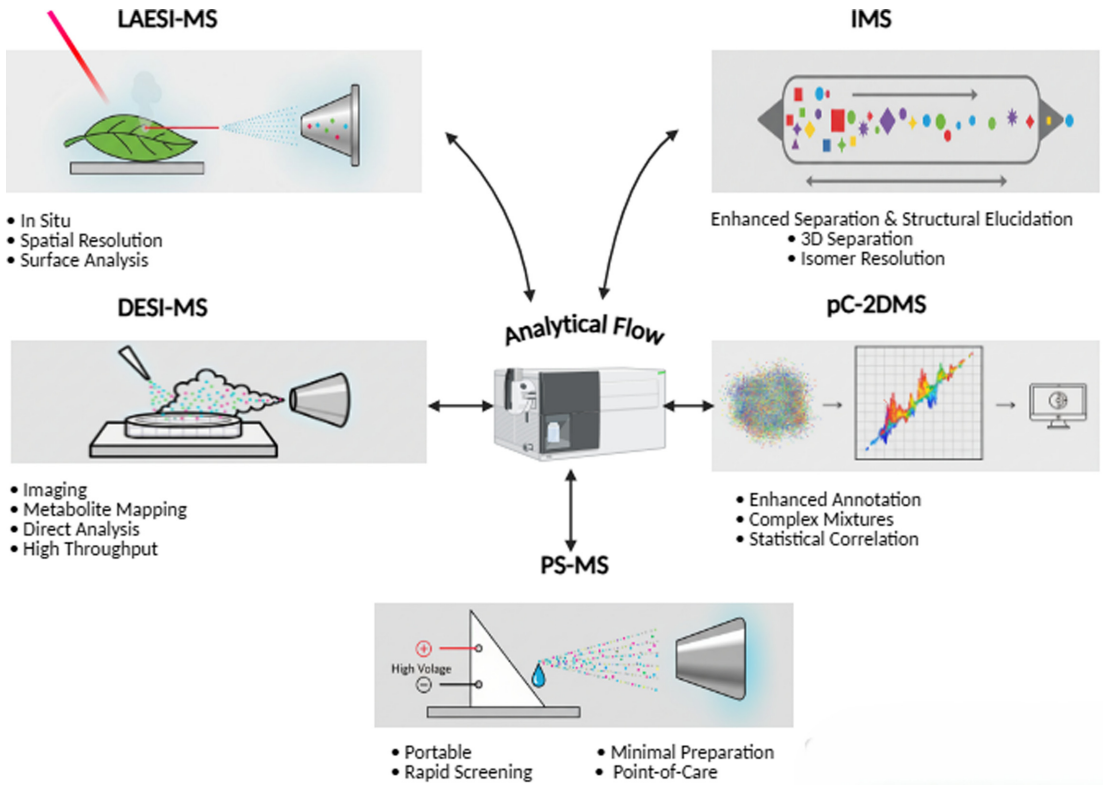

Figure 1. Overview of Emerging and Multidimensional Mass Spectrometry Technologies for Phytochemical Analysis.

Table 1. Comparative Overview of Emerging and Multidimensional Mass Spectrometry Technologies for Phytochemical Analysis.

Feature/ Technique	MALDI-MS	LAESI-MS	PS-MS	DESI-MSI	pC-2DMS
Principle	Soft ionization; UV laser on analyte + matrix	Laser ablation + ESI in open air	ESI from paper triangle	Solvent spray on surface, desorbs ions	Statistical correlation of MS signals over time/ space
Primary Use in Phyto-chemical	Spatial metabolomics (MSI); Direct profiling; High throughput dereplication	Rapid in situ analysis, direct tissue profiling	Rapid screening, portable, field analysis	Metabolite mapping (spatial distribution) in tissues	Enhanced untargeted annotation, complex mixture deconvolution
Sample Type	Solid tissue sections; Dried extracts; PTMA	Intact plant tissues (leaf, stem, root)	Crude extracts, plant sap, biofluids on paper	Intact plant tissues, thin sections	Complex MS data from LC-MS, IMS-MS experiments
Key Advantage	Minimal fragmentation; Good for large /unstable molecules; Imaging capability	Minimal prep, high throughput, in situ, minimal damage	Portable, fast, low-cost, minimal sample volume	Visualize spatial distribution without extraction	Resolves co-eluting compounds, improves data quality
Key Limitation	Matrix interference (low mass); Variable ion yield; non-polar challenges	Potential for matrix effects, limited spatial resolution (vs. MSI)	Limited sample volume, potential for carry-over	Sensitivity can vary, matrix effects, sample preparation for sections	Computationally intensive, requires specific software/expertise
Integration	Typically, TOF or Hybrid (Q-Tof, Orbitrap)	Often standalone or integrated with robotic sampling	Standalone, portable	High-resolution MS imaging	Post-processing for LC-MS or LC-IMS-MS data
References	8	56	57	58	59

structure-rich data sets for the identification, authentication, and discovery of plant metabolites. Although it requires complex instrumentation and data processing, still its power to unveil the hidden relationships talking at a molecular level makes it a revolutionary tool in natural product research and metabolomics (Figure 1) and in (Table 1) there is comparative overview of emerging and multidimensional mass spectrometry technologies for phytochemical analysis.

Multidimensional and Integrated MS Workflows

In-depth analysis of complicated phytochemical mixtures requires the use of analytical methods, which are beyond the capacity of a single mass spectrometry (MS) unit. The combined processes are necessary to deal with such deficiencies as ion suppression, deciphering the structure, and providing the coverage of metabolites that are complete. With the aid of MS along with proper separation, structural, and computational techniques, scientists may construct the strong, large-scale, fast platforms that are necessary for current phytochemical studies.

Conventional Chromatographic-MS Integration (GC-MS and LC-MS)

One of the main technical bases for combined studies in plant chemistry is the use chromatographic methods together with mass spectrometry (Hyphenated MS). These systems are essential on their own, as they allow both the separation of compounds that are not fully resolved from the same sample thus reducing co-eluting compounds before they reach the MS instrument, hence lessening matrix effects and improving signal quality.[60]

Gas Chromatography-Mass Spectrometry (GC-MS)

Gas Chromatography-Mass Spectrometry (GC-MS) is considered one of the most acknowledged and in-depth methods used in the phytochemical field, particularly for the analysis of volatile and semi-volatile compounds. Highly efficient separation is provided by GC, while MS offers a sensitive and selective detection to the extracted analytes. The compounds in the GC column are separated based on their volatility and their interactions with the stationary phase before they are delivered to the mass spectrometer for ionization and identification. GC-MS is mainly utilized to identify volatile metabolites such as terpenes, essential oils, and low-molecular-weight alkaloids. The current integration is still supported by the spectral libraries that are very well recognized, which allow co-identification to be faster and more accurate.[61]

Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid Chromatography–Mass Spectrometry (LC-MS) represents one of the best platforms for handling non-volatile, thermally unstable, and polar phytochemicals. Compared to GC-MS it is a method with no derivatization of such compounds which makes it a more agile tool for metabolite profiling. LC is fully responsible for the separating of the components, while MS contributes with very sensitive detection and, in addition, with structural information. This combination is extensively used for the identification of phenols, flavonoids, saponins, glycosides, and other secondary metabolites that are deemed to have medicinal properties. The uniqueness of LC-MS is due to the large number of ionization sources (e.g., Electrospray Ionization, ESI and Atmospheric Pressure Chemical Ionization, APCI) and analyzers (e.g., Quadrupole, Ion Trap, and Time-of-Flight, TOF). Such features allow users to adjust this instrument so it can fit different groups of compounds. Moreover, LC-MS is a platform for multi-metabolite, therefore, it can be exploited in phytochemical studies of both targeted and untargeted ones [6]. One of the main features of these chromatographic platforms is the integration of High-Resolution Mass Spectrometry (HRMS) and Tandem Mass Spectrometry (MS/MS). HRMS offers very accurate measurements of the exact mass-to-charge (*m/z*) ratios with very minimal mass error (usually less than 5 ppm). Such perfect accuracy is very important in the case of herbal extracts, as these extracts are the main source of numerous structurally related metabolites. HRMS apparatus like Orbitrap and Q-TOF (Quadrupole-Time-of-Flight) possess the resolving power of over 50,000 FWHM, which allows the different compounds to be separated even in very complex mixtures. In the same manner, tandem MS (MS/MS or MS^n) raises the selectivity in combination with the specific fragmentation pattern output that is used for dependable structural elucidation and for telling apart very similar compounds.[62]

Advanced Hyphenation: Ion Mobility Spectrometry (IMS)

Ion mobility spectrometry (IMS) coupling, on the other hand, is one of the most significant advances in multidimensional separation, allowing a supplementary separation dimension based on the ions' size, shape, and charge. IMS is rarely solely utilized; however, its capacity is achieved when combined with MS as LC–IMS–MS or GC–IMS–MS. While regular MS only measures mass-to-charge ratio, IMS adds separation by identifying differences in the size and shape of ions. The method is very effective as it detects ions moving through a neutral buffer gas under an electric field, giving a drift time that is associated with the ions' effective collision cross-section (CCS). This instrument is very helpful in the identification process, as it allows differentiating isomeric and isobaric metabolites, which are usually indistinguishable by normal MS alone. Thus, IMS becomes a very good separation tool in complex plant extracts, as it shapes multidimensional datasets (retention time, drift time, CCS, and *m/z* ratios) that make unequivocal assignment easier. There are several different types, such as Drift Tube (DTIMS), Traveling Wave (TWIMS), and Trapped IMS (TIMS), which in combination with par-

allel acquisition techniques like PASEF, are very fast in mobility-resolved MS/MS. This feature is the whole point of natural products research that IMS, by far, is the most convincing in such cases when it manages to separate isomer pairs in those phytochemicals classes that are most critical. To illustrate, it separates O-glycosylated from C-glycosylated flavonoids, resolves close pairs like orientin/isoorientin, characterizes positional isomers of caffeoylquinic acids (CQAs), and separates catechin epimers. Such separation not only is free from ion suppression but also allows for quantitative accuracy of extracts to be enhanced. Additionally, by providing experimentally measured CCS values, it assists in the rapid construction of extensive spectral libraries for confident metabolite annotation and quality control.[63]

Integration with Spectroscopic and Computational Techniques

Although MS is highly sensitive and efficient in generating fragmentation-based hints, it sometimes does not give unambiguous structural aspects. The major improvements of MS in plant chemical studies are mostly utilized when it is integrated with other techniques. These amalgamations not only allow for more efficient separation of complex mixtures but also provide several advantages such as easier structural identification, broader metabolite coverage, and confirmation of results through cross-validation.[64]

Integration with Nuclear Magnetic Resonance (NMR)

Consequently, the part that Nuclear Magnetic Resonance (NMR) spectroscopy plays is crucial to the entire process of the structural characterization. In fact, NMR is extremely instrumental in determining stereochemistry and other aspects such as the conformity and the glycosidic linkages that mass spectrometry (MS) cannot achieve. Generally, NMR is used as a complement to the identification of new substances, while MS is mainly referred to as a tool for quick screening and dereplication. One of the many hyphenated methods, such as LC-MS-NMR, which facilitates an arduous step without the need for further separation, identification, and structural elucidation, thus saving both time and the amount of the material.[65]

Integration with Other Spectroscopic Methods

Spectroscopic methods, for instance, IR and UV–visible spectroscopy, act as a bridge to MS results by offering more detailed features of the functional groups and chromophores. IR supports MS-based structural suggestions by confirming functional groups through their vibrational frequencies, whereas UV–Vis delivers the typical absorption for flavonoids, polyphenols, and other conjugated metabolites. Researchers become more precise in their identifications if they combine spectroscopic and MS results. Integrated systems like LC–UV–MS and LC–IR–MS are particularly successful in the profiling of polyphenolic-rich herbal extracts.[64]

Integration with Computational and Bioinformatics Tools

The integration of computational and bioinformatics along with phytochemical MS is another aspect that can change the entire field of phytochemical MS. Untargeted MS workflows create tremendously large datasets that have to be processed effectively using algorithms and machine learning methods. One such platform is GNPS (Global Natural Products Social Molecular Networking), which is an acronym referring to the community of natural product researchers who employ spectral networking for their MS/MS data, i.e., they compare their datasets with the spectra found in libraries for annotations. Apart from this, the combination of MS with cheminformatics and molecular docking not only links chemical data with the predictions of biological activity but also makes the journey from phytochemistry to pharmacology shorter. In this way, through computational MS integration, MS, which is just an analytical method, becomes a systems-level tool for understanding plant metabolism.[65]

Future Integrated Strategies

Further methods that were not possible even a few years ago allow MS to be opened at the cellular and subcellular levels, and this is what modern-day means. Just to give an example, capillary electrophoresis–mass spectrometry (CE–MS) and microfluidic chip-based separations might concentrate on the spatially resolved analysis of metabolites in single cells, certain tissues, and organelles. These techniques offer plant metabolomics the chance to discover the existence of cell-type-specific metabolites, which as a result indicate the functional specialization of the new kind of complex tissue. The methods are very effective in the case of rare metabolites that are localized in the specialized structures of the like glandular trichomes or resin ducts.[66]

Persistent Challenges and Limitations in Advanced Phytochemical Mass Spectrometry

Though high-resolution and ambient ionization mass spectrometry (MS) have come a long way, the phytochemical analysis is still struggling with various challenges and limitations, which cannot be overlooked (Figure 2). The difficulty caused by the matrix complexity of plant extracts is still the main challenge because ion suppression brought about by abundant co-eluting compounds even in elaborate workflows hinders qualitative and quantitative interpretation. The problem has been primarily solved using advanced hyphenation techniques such as LC-IMS-MS; as they provide orthogonal separation, they allow one to see the issue from a different angle.[67] However, matrix effects continue as the main challenge, especially in the case of very complicated crude extracts and ambient ionization methods (DESI, LAESI) that have been used for tissues directly. Besides that, the problem of structural and stereoisomers (e.g., flavonoid glycosides, caffeoylquinic acid isomers) is

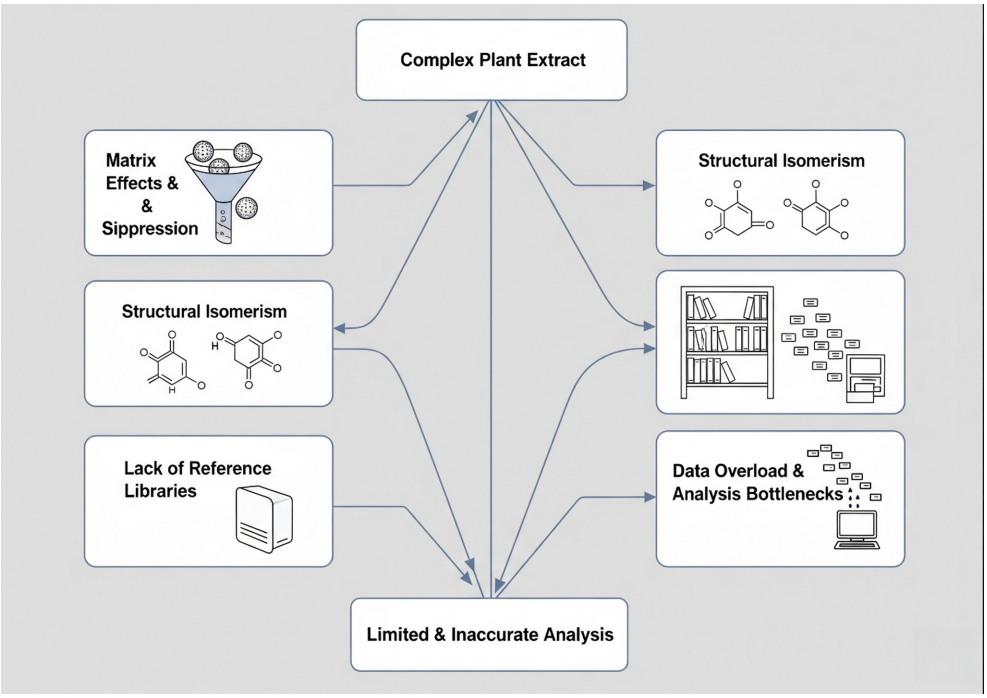

Figure 2. A conceptual framework illustrating how inherent plant complexity and post-acquisition bottlenecks limit the accuracy and throughput of advanced mass spectrometry workflows.

only partially solved with today's technology. Often, these related phytochemicals have similar precursor masses and fragmentation patterns. Although Ion Mobility Spectrometry (IMS) and pC-2DMS signify great progress in isomer resolution, correct and confident identification still often relies on supporting methods such as Nuclear Magnetic Resonance (NMR).[68] It is one of the main bottlenecks in exact metabolite annotation that depend on NMR besides MS techniques. The issue of cost, data processing, and reproducibility of advanced MS platforms is one of the largest sets of challenges. Extremely powerful machines such as Orbitraps, FT-ICR, and hybrid IMS-MS are very expensive and require specialists with high expertise, which makes them not very accessible, especially in places where there are limited resources. On the other hand, data quality and experiment reproducibility remain problematic, especially in untargeted approaches. MS experiments produce very large datasets that require a lot of computer power to process. What is even more, there are certain persistent bottlenecks that happen because the libraries of reference spectrum for plant compounds are not complete.[69] The reason being that databases like METLIN and MassBank do not encompass the entire vast structural diversity of plant metabolites; thereby, researchers quite often come across signals that they cannot confidently annotate. Along with this, the MS method's sensitivity to very minor changes in experimental conditions (e.g., solvent composition, ionization conditions) also contributes to data variability emphasizing the requirement for a strict quality control regime. Moreover, limitations continue to exist for spatiotemporal resolution. Even though imaging MS methods (MALDI-MSI and DESI-MSI) allow metabolite distribution mapping, their spatial resolution, especially for single cells, is still normally not up to par with that of advanced chromatography. Another important point is that the presently used methods are mainly indicative of plant metabolism as static and not as a source of dynamic changes over time. The progress of real-time, in vivo MS-based monitoring, as well as the accomplishment of the actual single-cell resolution, remain the last frontiers and their limitations are still one of the main obstacles to the understanding of phytochemicals' dynamic physiological and ecological roles.[70]

Future Perspectives and Emerging Trends in Phytochemical Mass Spectrometry

Mass Spectrometry (MS) is a prominent example of a technology that has effectively developed into the most powerful tool in the field of phytochemical research, yet the future is still more magnificent for the use of MS. Such a scenario is possible by ongoing progress and upgrading in instrumentation, data analysis, and workflows wherein phytochemical MS is to transition from basic detection to more extensive, high-throughput, and functionally insightful platforms. These changes will have an impact on the way researchers comprehend the occurrence of secondary

metabolites in plants, their interaction with the environment and, potential use in medicine.

Next-Generation Hybrid Instrumentation

Without a doubt, the future growth is tied to the rise of high-resolution and multidimensional platforms. The prime example in this regard is the advent of new high-resolution devices such as Orbitrap and Fourier Transform Ion Cyclotron Resonance (FT-ICR), which have achieved the feat of separating thousands of metabolites in a single run. Consequently, these future changes will, among other things, bring about shortened acquisition times, increased ion transmission efficiency, as well as the dynamic range that will allow much deeper exploration of the low-abundance plant metabolites. Such extension would become especially valuable in the identification of trace alkaloids, rare glycosides, and the unstable intermediates in biosynthetic pathways that have been overlooked by the traditional methods. Furthermore, the evolution of hybrid and multidimensional MS methods with excellent performance is expected, where instruments like LC–IMS–MS/MS or GC–IMS–MS can combine chromatographic separation, ion mobility, high-resolution detection, and two-dimensional mapping into one single workflow. These multidimensional systems will unlock the potential for features like more secure annotation, enhanced isomer discrimination, and greater structural elucidation of plant metabolomes, which are mostly associated with complex classes like terpenoids and flavonoid glycosides.[71]

Expanding In Situ, Portable, and Dynamic Analysis

Along with the enhancements in instruments, new ionization procedures such as Desorption Electrospray Ionization (DESI), Laser Ablation Electrospray Ionization (LAESI), and Paper Spray MS (PS-MS) are changing the perspective on plant metabolites for the scientists. These ambient ionization techniques provide the ability to analyze direct tissue, leaf, or single cell; thus, spatial and temporal information of metabolite presence and changes can be achieved. This not only will bring about the realization of metabolism monitoring in real-time, but plant physiological studies and natural product discovery will also be made much faster than they have been so far. On top of this, miniaturization and the coming of portable MS devices are another leap forward. The simplification of ionization sources and the application of solid detectors in field-deployable instruments will eliminate the need for laboratory workflows, thus making it possible to analyze plant materials on-site. This can be a very valuable capability in biodiversity research, field studies, and herbal quality control as the data can be collected in situ, thus, phytochemical research can be easily accessed beyond the lab. In addition, spatial and temporal resolutions for metabolite analyses will also be enhanced. Scientists will increasingly employ the very sensitive and high-resolution imaging MS techniques like MALDI-MSI, DESI-MSI, and LAESI-MSI to pinpoint metabolites even at cellular and subcellular levels. The combination of these technologies for real-time data acquisition will be ideal for the monitoring of metabolic events during plant growth, stress adaptation and microbial interactions; thus, such events can have a profound impact on the ecological and physiological contexts of phytochemicals.[72]

Computational Intelligence and Omics Integration

An equally important point is that MS-based omics platform integration, such as genomics, transcriptomics, and proteomics, which will unravel the levels of secondary metabolism systems. The reason is that research in the field of metabolite data, gene expression, and protein interaction networks can uncover the new biosynthetic pathways, show gene-metabolite connections, and thus target metabolic engineering for higher yields of bioactive compounds. On that point, the combination of mass spectrometry-driven metabolomics with genome exploration is going to be the breakthrough in getting access to new opportunities for natural products discovery that are the writing of silent or minimally studied biosynthetic gene clusters. Simultaneously, the other key move will be the growth of spectral databases alongside the adoption of artificial intelligence (AI) annotation. For decades, phytochemical MS has suffered from the challenge of incomplete spectral libraries for plant metabolites. The main point will be community-driven projects aimed at creating fully comprehensive repositories. Moreover, scientists are coming up with deep learning models which can forecast fragmentation patterns, identify unknown metabolites, and suggest chemical classes right from the raw data. These AI-driven methods will really reduce the amount of manual annotation work that can be a bottleneck in the process of the identification of known and novel compounds.[73]

Conclusion

Mass spectrometry (MS) is still the main analytical platform across the globe for phytochemical analysis, with no competition for the sensitivity and depth it offers when characterizing plants' chemical diversity. The field's major revolution has been changing the mode of operation from single independent detection to multidimensional and integrated workflows. The main feature of these new technologies is their capability to make on-site, fast, and high-throughput analysis along with giving the structural context, which is beyond the reach of normal extraction-based methods. Still, a few issues such as matrix effects, the resolution of structural isomers, and the limitations of spectral libraries have been holding back advancements in the field. The best course of action will be deeper integration of different techniques which will allow for a combination of each advantage. For example, integrating chromatographic

separation with Ion Mobility Spectrometry (IMS) can be used as an orthogonal method to separate isomers that are hard to isolate while coupling with NMR spectroscopy can provide structural information. The future of MS in phytochemical studies will be determined by factors such as accessibility, intelligence, and dynamics. This technology will become more widespread when portable MS devices are developed along with the use of AI, which will automate annotation and help in completing the database thus overcoming the data bottleneck. The main goal has now shifted to making real-time, in vivo monitoring, and single-cell resolution possible so that plant metabolism can be seen in its ecological and physiological context, thereby natural product discovery and systems biology research becoming faster.

Acknowledgements

We would like to thank Amity Institute of Pharmacy, Amity University, Noida, for providing the opportunity and resources for writing the review.

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