REVIEW ARTICLE

Untargeted Metabolomics using Ultra-High Performance LC-MS: Applications in Systems Biology

Badri Sireesha[1], Saravanan Ravindran[2], R. Karthikeyan[3]

[1]Department of Pharmaceutical Analysis, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India, [2]Department of Pharmaceutics, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India, [3]Department of Pharmacognosy, School of Pharmacy, Sri Balaji Vidyapeeth, Puducherry, India

Abstract

Background: Untargeted metabolomics using ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS) enables global, hypothesis-free exploration of metabolic networks, advancing systems biology and complex disease studies. **Methodology:** The high resolution, sensitivity, and separation efficiency of UHPLC-MS allow identification and quantification of diverse small molecules in multiple biological matrices. Applications extend to precision medicine, microbiome studies, agriculture, and environmental sciences. **Results and Analysis:** This approach has been instrumental in biomarker discovery, drug development, and understanding plant–microbe and ecosystem interactions. Challenges include data complexity, unknown compound identification, and multi-omics integration. Advances in machine learning and computational tools have enhanced interpretability and opened real-time dynamic metabolomics for flux monitoring. **Conclusion:** UHPLC-MS-based untargeted metabolomics is reshaping systems biology, enabling deconvolution of intricate biological processes and supporting future advances in personalized medicine, environmental sustainability, and health research.

Key words: Biomarker discovery, multi-omics integration, systems biology, ultra-high-performance liquid chromatography-mass spectrometry, untargeted metabolomics

INTRODUCTION

Metabolomics is the analysis of small molecules in biological systems, that is, Integration of mass spectrometry (MS) and ultra-high-performance liquid chromatography (UHPLC) is one of the best-performing metabolomic strategies. Due to its extreme sensitivity, high resolution, and immunity to the presence of complex biological material, this combination has significant appeal for applications of metabolomics detection and measurement. It is possible to use UHPLC to isolate these compounds on the basis of physicochemical properties, while MS is able to identify and quantify a multitude of molecules with respect to mass determination of metabolite-derived mass-to-charge ratios.[1] UHPLC is a very efficient high-performance liquid chromatography (HPLC) technique that can be carried out under elevated pressure to increase the efficiency of separation and reduce analysis times. This significantly expands its utility over metabolomics, in which high resolution and speed of metabolites of interest with the sample matrix complexity can be desired. UHPLC is far too broad to be applied to metablomic studies in environmental science, agriculture, pharmaceutical, and human health domains, due to tool's capacity to differentiate between aqueous and apolar compounds over the concentration range,[2,3] as well as the maximum and absolute sensitivity and specificity that can be achieved by the combination of UHPLC and MS to discover and quantiate metabolites.[4] Low-abundance metabolite identification is one of the most appealing aspects. Even more so, considering the aim of the search for low-frequency biomarkers or to detect clear metabolic changes that may predict early pathologies, environmental stress, or disease. Simultaneous determination

Address for correspondence:
Saravanan Ravindran,
Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai-73, Tamil Nadu, India.
Phone: 91-9700784826. E-mail: rssrmcp@gmail.com

Received: 09-02-2026
Revised: 15-03-2026
Accepted: 24-03-2026

of both high and low concentrations of analytes in a sample by UHPLC-MS [Figure 1]. In addition, the combination of UHPLC with MS makes high-throughput analysis achievable by clustering of a large number of samples within a limited time frame. Overall, this tool is highly useful for throughput-based analysis of ecological switches or whole-genome screens in which metabolic changes in diseases, for example, diabetes disease model or cancer model, are identified. Further, it allows complex biological samples, such as tissues, blood plasma, urine, and environmental matrices to be characterized, which potentially contain a wide array of metabolites in size from small molecules to large and complex molecules.[5] The range of metabolites that can be detected is broader and reflects only one part of the untapped potential of UHPLC-MS. On the other hand, it is of very great interest to also report on the occurrence of particular metabolites in different biological systems. The metabolic changes in human diseases, such as diabetes and cancer, as previously enhanced by UHPLC-MS in human health.[6] It has also been of central importance in drug discovery through helping identify metabolic pathways and, subsequently, potential biomarkers, which can be leveraged to inform drug therapy. UHPLC-MS [Figure 2] has been applied in the agro-food sector to some extent to the profiling of environmental contaminants, to enhance crop productivity, and to the understanding of plant responses to stress (e.g., Its application in environmental science is, if anything, not less significant since it allows scientists to track the implications of climate change on the ecosystem, environmental pollution and so on.[7] Untargeted metabolomics is one of the main applications of UHPLC-MS in the area of metabolomics, where the goal is to find all metabolites in a given sample, before the identification of its components. This is especially important when the research is focused on discovery, in which the aim is to detect new biomarkers or metabolites that are unreported so far. Untargeted metabolomics has changed our understanding of drug response, host-pathogen interactions, and disease mechanisms by offering quantitative information on the metabolome landscape.[8]

WORKING PRINCIPLES OF UHPLC-MS IN UNTARGETED METABOLOMICS

For in-depth characterization of metabolites in an organismal system, untargeted metabolomics using UHPLC-MS requires a step-wise reconstitution, which includes sample preparation, metabolite separation, detection, and data analysis. The principle underlying UHPLC and MS (both of which provide extraordinary separation, sensitivity, and specificity in the analysis of complex biological matrices) is used in the present approach.[9] To stabilize the metabolites and reduce macromolecule interference, such as that of the proteins, the procedure starts with the sample pretreatment. Protein precipitation, liquid-liquid, and solid-phase extraction are methods that are used to extract biological matrices (tissue extracts, plasma, or urine). Removing contaminants, concentrating the target analytes, both of which have a higher sensibility response of the metabolites. The UHPLC system (i.e., the core device for physicochemical fractionation of derivatives in accordance with the polarity and hydrophobicity nature of the derivative) is presented in the formed sample.[10] In comparison with traditional HPLC, because UHPLC is performed at high pressure (up to 15,000 psi) and utilizes smaller stationary-phase particles, UHPLC could offer better resolution and speed increase. It is the choice of column chemistry that is tailored, for example, hydrophilic interaction liquid chromatography (HILIC) and reverse-phase, depending on which target metabolite classes are desired. For the subsequent MS analysis, which can overcome the high complexity of the metabolite mixture and ion suppression, this separation procedure is very important. MS is employed for the identification and quantification of metabolites after chromatographic fractionation. The eluted species are ionized and electrophoretically charged by the MS system and the m/Z of the species are subsequently measured with precision.[11] The most commonly used ionization technique is electrospray ionization (ESI) for the mixture analysis of metabolites. Higher resolution mass analyzers, powered by instruments with high accuracy on mass determination and structural presentation, such as time-of-flight (TOF),

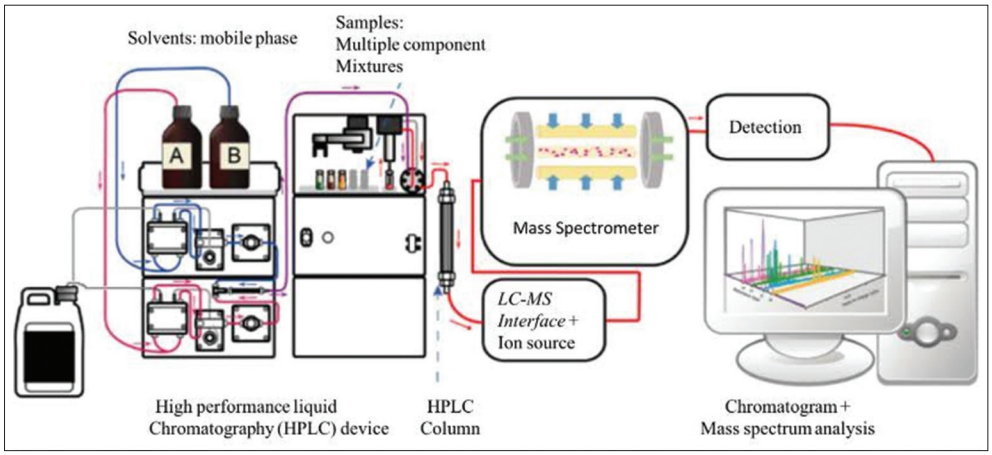

Figure 1: Workflow of ultra-high-performance liquid chromatography

Figure 2: Ultra-high performance liquid chromatography

orbitrap, or quadrupole systems, are used at the end to perform the analysis of the ionized metabolites. Guaranteed to enhance the identification accuracy, the decomposition and reconstruction of the structures of metabolites are widely employed within the Mixture of Tandetron MS (MS). Data analysis and data processing are the last steps of the process, which is not always straightforward due to the enormous amount of data generated.[12] Unadjusted raw data is processed using computer applications and software programs, which involve peak detection, alignment, and normalization. Then, the identification of metabolites is suggested to compare the features of spectra (m/z, retention time, and fragmentations) to the database and to the libraries, respectively, as the references. Significant metabolic changes are detected and biological relevance is deduced through the use of advanced statistical and machine learning (ML) techniques.[13] The UHPLC-MS method, being a key tool in untargeted (i.e., whole-metabolomic) metabolomics for systems biology studies, is due to its high-resolution viewpoint of the metabolome, which is, in general, a strong and generally reproducible method.

COMPARISON BETWEEN UNTARGETED AND TARGETED METABOLOMICS

In the realm of metabolite studies, untargeted and targeted metabolomics are two different but synergistic methods that are suitable for a particular research question and a given method. Unscheduled metabolomics, as an *ad hoc*, exploratory methodology with the goal of profiling the full complement metabolome, is done without a priori knowledge of any metabolite in particular. This type of objective is especially suited for the mapping of intricate biochemical pathways, the discovery of new biomarkers, and the identification of new metabolic disturbances.[14] In untargeted approaches, as in UHPLC-MS, the method is a critical factor because the method enables to detect a wide variety of compounds at high sensitivity and resolution. However, the encompassing feature of untargeted metabolomics has its limitations, including difficult data analysis, the challenge of labeling new metabolites, and inconsistent results among platforms and experiments.[15] In contrast, targeted metabolomics is directed toward the specific and only their identification, namely, a predefined set of metabolites, which are explicitly known to be relevant to the problem of interest. In this hypothesis-driven approach, calibrated analytical methods already in use with MS are employed for specificity and accuracy. Targeted metabolomics is well-suited for application in biomarker validation, analysis of aspects of certain metabolic pathways, and clinical diagnosis, etc. It provides higher reliability, repeatability, and a lower limit of detection for the target molecules, but it only analyzes a small number of metabolites rather than untargeted. Coverage of metabolites is one of the key differences. Therefore, untargeted metabolomics, offering a global picture of the metabolome, has the potential to be achieved by detecting hundreds (and thousands) of metabolites from each of numerous chemical families. Due to the instrument's capabilities and the lack of a "finished" reference library, the work has a restricted scope, but this is inherent.[16] In contrast, targeted metabolomics is a more specific approach, usually encompassing several hundred metabolites, although lower sensitive, and lower quantification limits as well, with a higher quantification precision. The intricacy of data analysis is another difference. Target identification in untargeted metabolomics, which involves the use of sophisticated computational algorithms to match peaks, extract features, and analyze the statistical value, remains a significant challenge. On the other hand, recognized analytes enable data reduction in directed metabolomics, which can be the spared time and effort of data mining and annotation.[17] Both strategies have different uses. Of the systems biology approaches, untargeted metabolomics is a study where a robust, detailed mechanistic explanation of metabolic perturbation is a cornerstone. In cases where accurate quantification of certain metabolites is a need for clinical, biomarker validation, and hypothesis testing, targeted metabolomics is preferred. The scope for exploring the unknown metabolic landscapes lies with untargeted approaches, and the specificity and reproducibility of targeted approaches allow for high specificity shown on Table 1.[18] In the sense that these methodologies are complementary to drive metabolomics discovery and validation, discovery and validation can be processed in a vast number of biological settings.[19]

TECHNOLOGICAL ADVANCES IN UHPLC-MS FOR UNTARGETED METABOLOMICS

Untargeted metabolomics has been revolutionized by UHPLC coupled with MS, allowing sensitive and well-resolved analysis of complex biological specimens. Because of the technical improvements brought to the UHPLC and MS with time, the ability of UHPLC and MS in the identification, detection, and quantification of large amounts of metabolites, even

Table 1: Comparison between targeted and untargeted metabolomics[20-23]

S. No.	Aspect	Targeted metabolomics	Untargeted metabolomics
1	Definition	Focuses on quantifying a predefined set of known metabolites	Aims to detect and analyze all possible metabolites in a sample without prior selection
2	Objective	Measures specific biomarkers or metabolic pathways for hypothesis-driven research	Provides a global, unbiased metabolite profile for exploratory research
3	Coverage	Limited to selected metabolites, providing high specificity and accuracy	Broad coverage, detecting thousands of known and unknown metabolites
4	Sensitivity and quantification	Highly sensitive and enables absolute quantification using internal standards	Semi-quantitative due to data complexity, requiring extensive statistical analysis
5	UHPLC workflow	Utilizes optimized chromatographic conditions tailored for selected metabolites	Requires broad-spectrum chromatographic methods to capture diverse metabolite classes
6	UHPLC columns	Uses specific stationary phases (e.g., C18, HILIC) to enhance the separation of known metabolites	Employs versatile columns to maximize coverage across a wide range of polarities
7	Mass spectrometry analysis	Typically employs triple quadrupole MS (QQQ) for targeted detection and quantification	Utilizes high-resolution mass spectrometers (e.g., Orbitrap, TOF) for accurate mass identification.
8	Data acquisition	Operates in multiple reaction monitoring or selected reaction monitoring mode	Uses data-dependent or data-independent acquisition modes for broad metabolite detection
9	Data processing	Straightforward; targeted compounds are identified using predefined mass transitions	Complex; requires peak alignment, normalization, and statistical modeling to interpret results.
10	Annotation and identification	Well-characterized metabolites with known retention times and fragmentation patterns	Many unknowns requiring spectral libraries, databases, and computational tools for identification
11	Reproducibility and standardization	High reproducibility due to controlled experimental conditions and reference standards	Lower reproducibility due to sample variability, instrument drift, and large data volume
12	Biological insights	Provides quantitative data on known pathways, helping validate mechanistic hypotheses	Offers novel insights, discovering new biomarkers and metabolic pathways
13	Application in disease research	Used for clinical diagnostics, monitoring metabolic changes, and validating biomarkers	Ideal for exploratory studies, biomarker discovery, and understanding disease mechanisms
14	Use in drug development	Assesses drug metabolism, pharmacokinetics, and toxicological profiling of known metabolites	Identifies unexpected metabolic effects, potential off-target effects, and novel drug interactions
15	Challenges	Limited scope, requires prior knowledge, and may miss unexpected metabolic changes	Complex data interpretation, high computational demand, and challenges in metabolite annotation

UHPLC: Ultra-high-performance liquid chromatography, HILIC: Hydrophilic interaction liquid chromatography, TOF: Time-of-flight

that of low concentrations has been enhanced. UHPLC-MS has, however, become an important reagent within modern metabolomics research due to the advances presented above that have addressed critical challenges of increasing metabolite resolution, data acquisition rate, and annotation accuracy.

Enhanced resolution columns and pressure tolerance

It is one of the fundamental advances within UHPLC-MS that is an area currently focused on developing columns with increased pressure tolerance that enable sub-2-micron particle columns to be used. This high resolution columns offer a larger separation efficiency, resulting in the enhanced peak resolution and also improved capacity for metabolomic identification of structurally similar metabolites.[24] The higher pressure stability has led to more efficient chromatographic separations, which have been realized with shorter sample analysis times without having to sacrifice accuracy. Therefore, peak overlap has been even reduced further, which is of particular importance for metabolomics studies because the number of compounds with structural relationships is

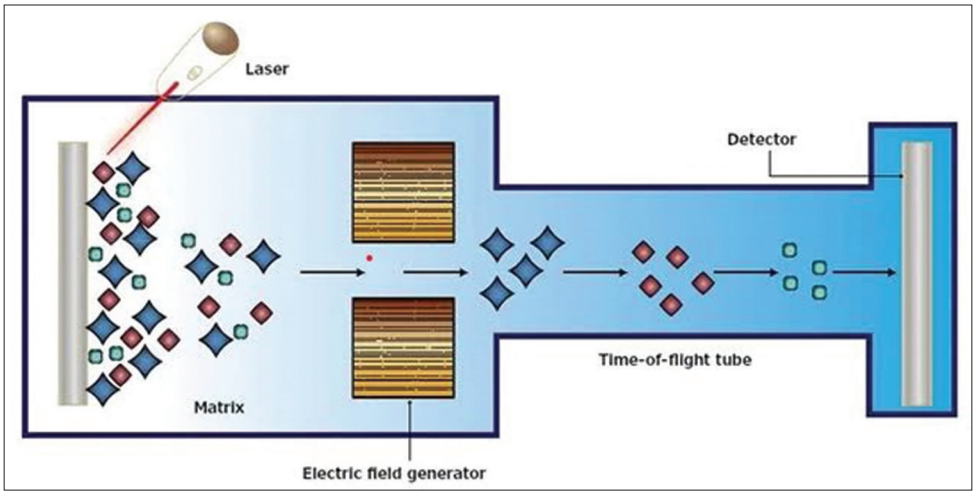

Figure 3: Time of flight mass spectrometry

relatively large. In addition, metabolite quantification can be made more accurate in very complex biological matrices by using higher resolution peaks, thanks to the high complexity of metabolite quantification.[25]

Advanced column chemistries for improved coverage of metabolite

The increase in the number of metabolites that could be detected has been increasingly critical through the invention of high-sensitivity stationary phases. In parallel, due to the increased detection sensitivity coming from the new column chemistries (e.g., HILIC, pH-HILIC) to polar metabolites, the old liquid chromatography (RP-LC) is still a usual technique for non-polar and semi-polar metabolites.[26] One of the major problems for metabolomics has been low retention of the polar molecule, but HILIC columns solved the problem by offering polar metabolite separation efficiency, which translates to an improved resolution. Research on amino acids, sugars, and some small organic acids, previously intractable to quantitation by RP-LC, has been advanced considerably by this development. Columns with mixed-mode (i.e., multiple retention mode) retention are also under study with a view to improving metabolite retention strength.[27]

High-throughput features in large-scale experiments

Due to the exponential growth of throughput from automation of UHPLC-MS workflows, samples with high throughput can now be analyzed efficiently. In particular, improvements in rapid gradient elution, multiplexing, and multi-injection protocols have made it possible to compensate for data loss, and simultaneously increase the sample processing rate.[28] When hundreds of samples need to be screened rapidly for clinical biomarker discovery, environmental monitoring, and population-based studies, high-throughput metabolomics is a workhorse. Other than the introduction of automation, human error has been reduced and therefore, the results have

Figure 4: Sequential window acquisition of all theoretical mass spectra

been much more accurate and reproducible. Specifically, software-driven gradient optimization and the development of improved auto-sampler technology have all enabled retention time repeatability, thereby increasing confidence in high-throughput metabolomics.[29]

Mass analyzers with high resolution for enhanced sensitivity and specificity

Metabolite detection and categorization have been significantly improved by the introduction of high-resolution mass spectrometers, such as orbitrap and TOF analyzers. Since then, it is, but it is dependent on the instrument's high mass accuracy, defining the advantage of an isomeric compound versus isotopic compound.[30] Specifically, orbitrap MS, and in particular, has modified metabolism and the latter is capable of publishing high-resolution mass spectra with sub-ppm resolution that allows accurate metabolite identification. To the contrary, the acquisition rates of TOF mass spectrometers are incredibly high, making them ideal for high-throughput experiments [Figure 3]. The capacity of these devices to make extremely small mass changes has provided the foundation of a solution to structural inconsistencies and precise properties characterization of biological material at a complex scale.[31]

Employing state-of-the-art ionization methods

A broader set of metabolites can be measured by increasing the analysis range in UHPLC-MS, since with a broader range of metabolites available to the analysis, it provides a complete analysis from polar to semi-polar metabolites, and thus, the most widely used ionization method in metabolomics is ESI.[32] Nevertheless, the sensitivity and degree of ionization of UHPLC-MS are also recently enhanced by the new applications of air pressure chemical ionization and matrix-assisted laser desorption ionization (MALDI). These methods are particularly useful for non-polar metabolites- a class of metabolites that are rather hard to ionize by ESI (electrospray ionization). Thanks to the pooling of ionization interfaces (e.g., co-MALDI and nano-ESI merged), one may now obtain a broader range of metabolites in a single chromatographic separation (i.e., the chromatographic run).[33] This advance has been particularly meaningful in steroid metabolomics and lipidomics, both fields in which low-abundance metabolites are identified at very high concentration.

Using tandem MS (MS) to clarify structures

The structural characterization of metabolites has been improved with recent advancements in tandem MS (MS/MS).[34] Due to their high fragmentation specificity, advanced fragmentation strategies, such as electron transfer dissociation and higher-energy collision dissociation have also further enhanced the metabolite identification accuracy. These methods are able to specifically target metabolite structure detection in complex biological matrices. By enabling extensive MS/MS analysis over a large mass range, the employment of data-independent acquisition, for example, sequential window acquisition of all theoretical mass spectra has thus exacted [Figure 4] even more metabolite coverage.[35] Hence, undetectable metabolites by conventional methods have been found to be a consequence.

Dynamic and real-time metabolomics for time-dependent study

In a time correlated manner, the temporal resolution of metabolic flux changes in an intact system can be available to researchers thanks to recent technologies facilitated research efforts (real-time and dynamic metabolomics). For the 1st time, real-time monitoring of metabolic fluxes has been available – as a result of direct-infusion MS and fast scan detection – throughout longitudinal metabolomic studies.[36] These advances are of considerable significance for our understanding of dynamic biological processes, such as circadian rhythm control, stress response, and disease progression. Furthermore, real-time metabolomics-based analysis has been employed in toxicology and pharmacokinetics for uncovering drug metabolism and effects of xenobiotics on a metabolic network.[37]

Integrated computational strategies to support data interpretation

By accelerating data analysis as well as metabolome identification, UHPLC-MS with advanced computer workflows has revolutionized untargeted metabolomics. Data from metabolomics has been deconvolved to understanding through the development of massively-scale reference libraries, open-source software, and cloud-based tools.[38] To extract valuable biological insights from massive datasets, ML techniques have been utilized more and more for peak alignment, feature selection, and route analysis. In addition, network-based methods have been used to metabolomics data combined with the other omics layers and they have allowed a more convincing characterization of the metabolic interactions.[39] These computational advances have significantly improved the quality of metabolite identification, and the robustness of metabolomics studies.

Portability and miniaturization for on-site metabolomics

On-site metabolomics is now possible for point-of-care diagnostics and environmental monitoring because of recent developments in the miniaturization of UHPLC-MS equipment. Portable UHPLC-MS systems have been set up for "on-the-go" analysis of biological and environmental specimens outside the lab at an accelerated rate.[40] Real-time metabolic profiling is supported by such portable devices and holds potential utility for bedside medical diagnosis, food safety applications, and environmental fieldwork. The analytical power and mobility of such devices have allowed untargeted metabolomics to be available to a wider range of clinical and scientific uses.[41]

ML AND COMPUTATIONAL ANALYTICS OF UNTARGETED METABOLOMICS DATA

Untargeted metabolomics production of large contiguous data sets presents a challenge that requires the use of cutting-edge computational processing and ML techniques to obtain timely data analysis. To extract biologically meaningful representations from raw data, such technologies are critical for the preprocessing and feature extraction, statistical analysis, metabolite identification, and biological interpretation.[41] Data pre-processing involving denoising, peak identification, retention time alignment, and normalization achieved in the context of the absence of a reference (or cross-sample comparability), by keeping in view the data quality. Since these tools provide automated workflows for the complexity of UHPLC-MS data, such as XCMS, MZmine, and OpenMS, the tools are generally used for these tasks.[42] Interestingly, the discrete metabolic peaks are disambiguated by means of feature extraction and, although there are technological discrepancies, synchronization ensures reproducibility of the

data. Metabolite annotation is a critical, but laborious task, in untargeted metabolomics. By use of masses-to-charge ratios as well as fragmentation patterns, computational systems, such as METLIN, HMDB, and GNPS, provide spectrum libraries for metabolite-matches-identification. However, due to the limited database, it is still very challenging to annotate uncharacterized compounds.[43] As an effort to enhance the detection sensitivity of novel metabolites, recent *in silico* strategies (SIRIUS and MS-DIAL) are largely based on fragmentation models and prediction algorithms. Statistics and ML have transformed the interpretation of untargeted metabolomics. Part-whole methods involving multivariable pattern recognition (principal component partial least squares discriminant analysis) have been applied for discriminating groups. Identification of biomarkers/metabolic signature is optimized by employing ML methods through which classification, clustering, and prediction can be achieved.[44] For example, such algorithms include random forests, support vector machines, and neural networks. These approaches are particularly of value for the analysis of high-dimensional, high-resolution, high-throughput data, as well as for the discovery of low-level metabolic alterations. Network-based analysis of the metabolomics-based data and other omics data layers give a systems-level description of the biological process. Incorporating metabolites are into biochemical pathways and functional networks, applications (e.g., Cytoscape and MetaboAnalyst) are available that are able to visualize and, for analysis, to rule the pathway. Whole-genome, whole-transcriptome, and whole-metabolomic multi-omics analyses are increasingly being made and accessible, due to ML integrated with metabolomics, and with proteomics and transcriptomics, and genomics. Artificial intelligence (AI) and deep learning are two emerging computational tools rapidly transforming untargeted metabolomics.[45,46] These approaches are highly applicable to complex data structures, to automated feature extraction and annotation, and for the prediction of metabolites. At last, the metabolomics community can unite to make data and software available for sharing through the provision of computing power by the advent and popularization of cloud-based infrastructure and open-source software. Conclusions ML and computational approaches have been indispensable contributions to untargeted metabolomics by overcoming the data analysis and interpretation challenges and leading to metabolomics becoming a valuable tool in precision medicine, systems biology, and other areas.[47]

UNTARGETED METABOLOMICS APPLICATION FOR HUMAN HEALTH, DISEASE, AND ENVIRONMENT USING UHPLC-MS

Untargeted metabolomics applied to environmental, health, and disease sciences. The resolution of extremely complex small molecule profiling (i.e., "drug-discovery landscapes" in biological and environmental systems has been revolutionized by UHPLC-MS. Of all the domains in which agriculture, environmental sciences, disease, and human health are concerned; this particularly effective methodology is capable of contributing to all of them extensively. Due to the potential that untargeted metabolomics might use to identify a whole spectrum of metabolites, a priori knowledge is not required; it can serve as a powerful probe to identify new biomarkers, unravel the disease mechanisms, as well as facilitate the toxicological screening and drug discovery.[48] Further, in the field of agriculture and ecology, UHPLC-MS supports a more accurate understanding of ecosystem functions, stress reactions, and plant-microbe interactions.

USES IN HUMAN ILLNESS AND HEALTH

Identification of biomarkers, or measurement (assessment) indicators for physiological or pathological conditions, is called biomarker and metabolomics is responsible for finding new biomarkers for a huge number of diseases.[49] Metabolomics

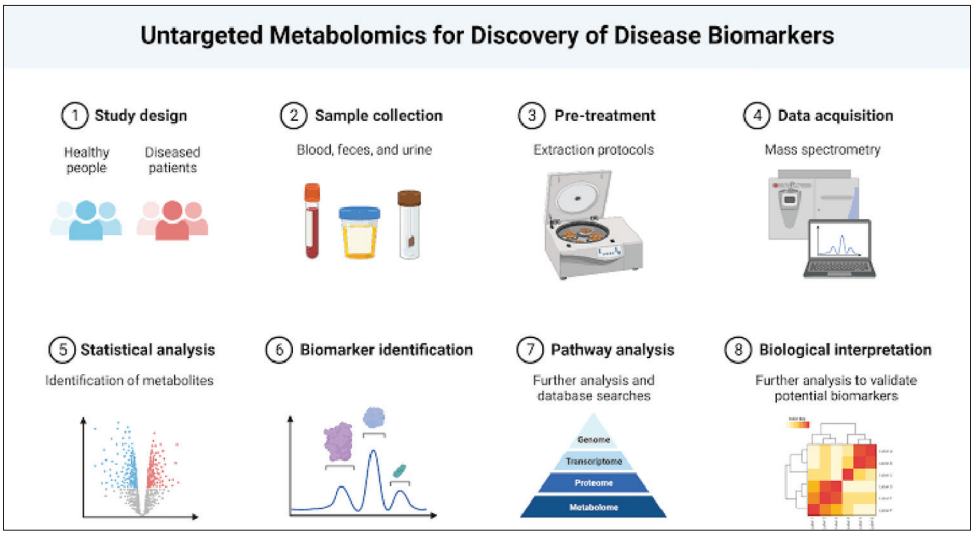

Figure 5: Untargeted metabolomics for discovery of disease biomarkers

offers an unobvious representation of metabolic changes, which are related to disease course, in contrast to genomics and proteomics, which mainly target genetic and protein level changes, respectively. Metabolomics, based on UHPLC-MS, allows objective identification of metabolic changes in biofluids (e.g., blood, urine, saliva, or cerebrospinal fluid [CSF]) and has been utilized to show pancreatic abnormalities.[50] Untargeted metabolomics has been used by cancer investigators to make novel discoveries regarding associated biochemical patterns with tumorigenesis and metastasis. Energy metabolism, lipid metabolism, and amino acid metabolism are also known to be dysregulated in tumors, such as breast, lung, and colorectal cancers (CRC), etc. Particularly in the neurodegenerative disease, both Parkinson's and Alzheimer's disease, in which metabolic impairment of neurotransmitter systems has been shown, metabolomics-based biomarker discovery has played an indispensable role.[51] Metabolic alterations related to oxidative stress markers, energy metabolism, and lipids have been unraveled through metabolomics in cardiovascular diseases (CVD). The identification of CVD related metabolites, for example, trimethylamine-N-oxide, has provided novel treatment and prevention possibilities. In particular, metabolomic profiling using UHPLC-MS has become an irreplaceable method to detect metabolic perturbations in the initial phase of primary metabolic disorders, such as diabetes, and early diagnosis and personalized therapy are made possible.[52]

CLARIFICATION OF THE DISEASE MECHANISM

Untargeted metabolomics is of significant value for the elucidation of the pathology at the molecular level and not of potential value for biomarker discovery. Researchers can determine metabolic pathways that are abnormal in pathological states, by studying the metabolome in different disease states. For example, the Warburg effect is a phenomenon in which cancer cells select for glycolysis over oxidative phosphorylation even when oxygen is present.[53] Untargeted metabolomics has shed light on this phenomenon. Alterations of glucose, lactate, and amino acid metabolism have been shown to be associated with this metabolic adaptation, and these alterations directly influence the survival and proliferation capabilities of the tumor. Across UHPLC-MS, metabolic vulnerabilities in cancer cells have been found, which contributed to the design of personalized therapeutics disrupting the essential metabolic pathways.[54] Across an investigation of metabolic alterations during infection, metabolomics has led to the identification of host-pathogen interactions in infectious diseases. For example, modifications in lipid metabolism have been reported in tuberculosis, which can result in altered immunological response and disease outcome. Across the recognition of metabolic alterations related to inflammation and immune activations, metabolomics has also been applied to describe viral infections, for example, COVID-19. Untargeted metabolomics has also been widely used in neurological diseases, such as schizophrenia and Alzheimer's disease.[55] Dysregulation of lipid metabolism, neurotransmitter metabolism, and oxidant markers has been useful in understanding disease pathophysiology. Provided with an understanding of these metabolic changes, the development of new treatment strategies focusing on metabolic pathways to slow disease progression has been facilitated. Unsupervised metabolomics also has been widely used for investigations of neurological diseases, such as schizophrenia and Alzheimer's disease. Changes in lipid, neurotransmitter, and oxidative stress indicators have yielded important clues about the pathogenesis of disease.[56] The development of new treatment approaches involving targeted manipulation of metabolic pathways toward slowing disease progression has been facilitated by the understanding of such metabolic derangements [Figure 5].

DEVELOPMENT OF DRUGS AND TOXICOLOGY

Untargeted metabolomics that offers a global view of drug metabolism, efficacy, and potential toxicity has played important roles in toxicology and drug discovery. The development of new or existing medicinal drug pharmacokinetics and pharmacodynamics can be enhanced by researchers' analysis of drug-induced metabolic alterations by UHPLC-MS. In the early drug development process, metabolomics is used to find good candidates of drugs with desirable metabolic properties.[57] From the point of view of scientists, the efficient transformation of a compound into toxic metabolites can be determined by tracking cell lines, animal models, or human subjects' cell biological pathways toward the candidate pharmaceutics. With regard to cancer, this method has proven to be of high value as treatment regimens are countered by observations of metabolic response to anticancer agents. Untargeted metabolomics is also very beneficial for toxicology research. Although metabolomics provides an objective identification of metabolic aberrations induced by xenobiotics, conventional toxicological approaches are based on defined biomarkers. For instance, UHPLC-MS is able to identify metabolic shifts due to industrial chemicals, drugs, and environmental pollutants.[58] It has been used in research of adverse effects of air pollutants, heavy metals, and pesticides. Metabolism is also being used more and more in personalized medicine to adapt drug regimens in each patient in a personalized fashion. Clinicians can diagnose therapeutic response and toxicity based on a patient's metabolic response to drug treatment, in turn enabling the development of more efficient therapeutic strategies.[59]

APPLICATIONS IN AGRICULTURE AND THE ENVIRONMENT

New understandings of plant-microbe interactions—which are essential for plant growth, health, and stress tolerance—have been made possible by untargeted metabolomics. Several metabolites produced by plants have an impact on the microbial communities of the rhizosphere, the root-associated microbial

community. Identification of compounds involved in signaling between plants and beneficial microorganisms, including mycorrhizal fungi and nitrogen-fixing bacteria, is made possible using UHPLC-MS.[60] For example, it has been demonstrated that the flavonoids released by leguminous plants function as signaling chemicals that promote symbiotic relationships with bacteria that fix nitrogen. Researchers have discovered a new bioactive molecule regulating microbial activity and uptake of nutrient through using UHPLC-MS to detect root exudates. Development of biofertilizers and environmentally benign agricultural practices, which decrease chemical fertilizer use, depends on this knowledge.[61] Moreover, plant-pathogen interactions have been studied in untargeted metabolomics, which has unveiled plant defense pathways. In response to microbial infection, plants synthesize secondary metabolites (terpenoids, phenolics, and alkaloids), etc. Disease-immune crop species have been genetically engineered by the discovery of these defense metabolites, which is facilitated through UHPLC-MS of plant metabolism. Plant metabolism is also heavily regulated by environmental stresses (contaminant, salinity, and drought). Researchers' use of UHPLC-MS-based metabolomics to better elucidate and thereby detailed characterization of the dynamic nature of onset and convergence of metabolic responses to abiotic stress may now become more significant.[62] For example, metabolomics has revealed alterations in hormone signaling, antioxidant metabolism, and osmolyte production in plants exposed to drought stress. Higher stress tolerance has been associated with the accumulation of metabolites, such as proline, polyamine, and flavonoid. Thus, the understanding of such information for breeding crops with stress tolerance for their survival under extreme conditions is of great significance.[63] Metabolomics is also applied in ecosystem monitoring and environmental health assessments beyond plant studies. Soil and water metabolomics have been used to assess pollution levels, microbial diversity, and ecosystem resilience. Except for pollutants that modify microbial metabolism and disrupt ecological balance, for example, pesticides and heavy metals, which end-members can be covered by UHPLC-MS, metabolomics has also been applied to climate change research in an attempt to understand the responses of terrestrial and marine animal metabolisms to changing environmental conditions and warming temperatures.[64] Methods in this approach provide important insights into the effect of global change impacts on ecosystem functioning and biodiversity.

CASE STUDIES OF UNTARGETED METABOLOMICS IN SYSTEMS BIOLOGY EMPLOYING ULTRA-HIGH-PERFORMANCE LCD-MS

Case study 1: Type 2 diabetes (T2D) biomarker discovery

Finding biomarkers for long-term conditions, such as T2D is a noteworthy use of untargeted metabolomics. Researchers profiled plasma samples from healthy controls and T2D patients using UHPLC-MS. The study identified specific metabolic markers linked to T2Ds, such as changed levels of bile acids, lipid metabolites, and branched-chain amino acids. These indicators offered fresh perspectives on the underlying processes of the illness, including inflammation and abnormal energy metabolism. Furthermore, new metabolites that had not previously been connected to T2D might be identified thanks to UHPLC-MS. This finding illustrated how the technique might reveal unforeseen metabolic alterations in addition to targeted techniques. The results have potential for creating individualized treatment plans and diagnostic instruments.[65]

Case study 2: Cancer progression metabolomic profiling

Understanding the metabolism and growth of tumors is one area of cancer research where UHPLC-MS has proven invaluable. Untargeted metabolomics was used in a study on CRC to compare tumor tissues with nearby non-tumor tissues. The Warburg effect, a defining feature of cancer metabolism, was supported by UHPLC-MS analysis, which showed increased levels of metabolites involved in glycolysis, such as lactate and pyruvate. The study found alterations in nucleotide biosynthesis and amino acid metabolism in addition to well-known metabolic pathways, underscoring the function of metabolic reprogramming in promoting fast tumor development. These discoveries revealed possible metabolic targets for therapeutic intervention and offered a clearer knowledge of the course of CRC.[66]

Case study 3: Plants' reaction to environmental S stress

Untargeted metabolomics has been used in agricultural systems to investigate how plants react to environmental stresses, such as salt and drought. A research that examined the metabolic alterations in rice plants under salt stress using UHPLC-MS. Significant changes were seen in secondary metabolites, such as flavonoids and phenolic acids, as well as osmoprotective metabolites, such as proline and glycine betaine. These results indicated possible targets for creating crop types that are resistant to stress, in addition to offering insights into the metabolic processes underpinning stress tolerance. Researchers were able to investigate intricate plant-microbe interactions and pinpoint important metabolic pathways involved in stress adaptation by utilizing UHPLC-MS.[67]

Case study 4: Interactions between the microbiome and metabolome

The research of microbiome-metabolome interactions has been made easier by UHPLC-MS, which has illuminated the function of gut microorganisms in both health and illness. Fecal samples from mice given various diets were analyzed

using untargeted metabolomics in a research examining the effects of nutrition on the gut flora and its metabolic output. The findings showed that certain microbial taxa had an impact on the synthesis of metabolites, including derivatives of bile acids and short-chain fatty acids. These metabolites were connected to immunological response and host metabolic control. Novel microbial-derived chemicals that contribute to host health were identified thanks to UHPLC-MS, opening up new treatment options for inflammatory and metabolic disorders.[68]

Case study 5: Understanding neurodegenerative diseases via metabolomic analysis

Investigating the metabolic underpinnings of neurodegenerative illnesses, such as Parkinson's and Alzheimer's has been made possible in large part by UHPLC-MS. Untargeted metabolomics was used in a study to find metabolic alterations unique to Alzheimer's disease, utilizing CSF samples from people with the condition. Sphingolipids and phospholipids, which are essential for signaling and the integrity of neuronal membranes, were found to have changed amounts. In addition, the study found disruption in oxidative stress and energy metabolism pathways, which may serve as indicators for early diagnosis and treatment targets. The identification of these minute but crucial metabolic changes was made possible by the excellent resolution and sensitivity of UHPLC-MS.[69]

Case study 6: Toxicology and drug metabolism

Drug development has used untargeted metabolomics to investigate drug metabolism and possible toxicological consequences. In one example study, metabolites in preclinical animals were profiled using UHPLC-MS for a potential anticancer drug. Drug metabolites were found through the research, along with off-target effects linked to adverse responses, such as changes in the metabolism of lipids and amino acids. This method gave researchers a thorough understanding of the drug's metabolic effects, allowing them to maximize both its efficacy and safety. In addition, UHPLC-MS revealed novel metabolic pathways that the medication impacted, which improved our knowledge of how it works.[70,71]

CHALLENGES AND FUTURE DIRECTIONS IN UHPLC-MS FOR METABOLOMICS

Untargeted metabolomics by UHPLC-MS is known to be associated with a plethora of challenges due to the biological nature of samples, technical and interpretation of data are difficult. Extraction, isolation, and identification of tens of thousands of metabolites, present in biological specimens (i.e., blood, urine, and tissues) are difficult due to the extremely diverse chemical properties and different concentrations of metabolites.[72] Certain component classes (e.g., very polar or hydrophobic metabolites) are commonly under-represented in the standard UHPLC-MS analysis and the metabolite chemical diversity is often time-consuming and requires dedicated methods. Furthermore, the extreme feature dimensionality of untargeted datasets, the limited scope of the current spectrum library/reference database, as well as the narrow width of spectrum libraries/reference databases, still create significant challenges for quality metabolite annotation and identification.[73] The complexity of this approach is also augmented by the inclusion of uncharacterized compounds, structural mimics, and collision adducts, resulting in *in vitro* (biologically) interpretation and a higher number of unannotated features. A subsceptible aspect is the analytical reproducibility, that is, the reproducibility from test to test that is inconsistent as a result of differences in sample preparation, instrument performance, and data processing.[74] Furthermore, the low-level compounds may be hidden and then desensitize the sensitivity through the phenomenon of ion suppression due to the co-eluting metabolites or matrix-associated compounds. Computational hurdles are also due to the enormous amount of data produced by untargeted metabolomics and, as such, due to the demand for sophisticated statistical and computational tools to perform data normalization, peak alignment, and feature selection.[75] Computational frameworks that are at least robust to multi-omics-analyses and combine metabolomics data with the other omics layers (i.e., the proteomics and the genome), and ideally even with a higher complexity level are required. The most crucial operational expense and technical capability for UHPLC-MS operation are characterized by IVs (e.g., lack of access to high-resolution mass spectrometry and accurate calibration). Although work is underway on a few of these challenges with the introduction of spectrum repositories, ML, and ion mobility spectrometry, the community continues to struggle with the need for prioritized standard protocols that will enable data sharing and reproducibility. Challenges must be overcome to fulfill the vast potential of UHPLC-MS-based off-target untargeted metabolomics, as well as overcome the wide potential of UHPLC-MS-based off-target untargeted metabolomics for systems biology, medicine, and ecology.[76]

Integrative metabolomics-based analysis, including multi-omics platforms (e.g., transcriptomics, proteomics, or genomes), is an iterative core process of continuous progress. By linking metabolic reprogramming to genetic and proteomic regulation, this integrative strategy will give a unifying insight into biological function and ultimately lead to a more precise understanding of disease etiologies and adaptation to the environment.[77] Real-time and dynamic metabolomics continues to be one of the fastest growing areas of research in which therapeutic UHPLC-MS can enable *in situ* quantification of metabolic fluxes and in time-dependent alterations in living organisms. Single-cell metabolomics, *in vivo* metabolic discovery, exploration of metabolic responses at resolutions that have never previously been within reach of this technology, will provide a trajecto-dynamic picture of

biological function.[78] New computational tools are urgently required to cope with the complexity of metabolomics data, and with the advent of ML and AI, new applications for metabolomics may arise. Powerful databases and spectrum libraries will boost methanol identification, although this will be accomplished by technologies that will automate a number of data processing steps (peak identification, metabolite annotation, and pathway identification).[79] Predictive models based on AI will also facilitate the discovery of novel biomarkers and the characterization of metabolites not yet known. Besides, the domain is also poised to increase its scope toward precision medicine in developing patient-specific treatment scheme for diverse diseases, including cancer, neurology, and metabolic disorders, which are intimately linked to the patient's individual metabolic profile.[80] Metabonomic-driven pharmacological therapies will further evolve in terms of their usefulness, thanks to improvements in high-resolution technology and data mining tools. At last, the UHPLC-MS will be expanded to the new field in agricultural and environmental systems to deal with the question of crops resistance, the situation of the ecosystems, and the climate adaptation. *In situ* analysis of metabolomics will become feasible with improved instrumentation, namely, desk-top, miniaturized UHPLC-MS, and overall will play an important role in the acceptance of metabolomic research.[81] The fact that all these advances give UHPLC-MS-based untargeted metabolomics the power potential to further insights into the biological complexity and set the agenda for its current relevance in agriculture, health, and the environment has provided us with the opportunity to develop and present the one here.

CONCLUSION

Due to the development of UHPLC-MS, untargeted metabolomics has become an extremely powerful method for comprehensive discovery of metabolites in biological systems. That it can also be used for screening of a pannopticon of chemical entities a priori, that is, without prior knowledge has allowed data for the investigation of complex metabolic networks, the identification of novel biomarkers, and the discovery of physiological and pathophysiological processes in a broad array of contexts, that is, systems biologist, environmentalist, precision physician. Nevertheless, this overall range is also tied to some restrictions requiring a perpetual methodological/technical development. Metablom detection, isolation, and measurement are challenging but have been substantially improved with the advent of UHPLC coupled with high-resolution MS. Advances in technology, for example, including ion mobility spectrometry, even more sophisticated ionization methods, and miniaturization, have enabled a tenfold expansion of the scope of untargeted metabolomics. The current computational tools and ML methods used to streamline the data pre-processing process, to extract features, as well as to label metabolites, are summarized with these developments, and are discussed at the interpretive stage of untargeted experiments. However, there remain important problems despite these advances, such as accurate identification of novel compounds, reproducibility among measurements, and attribution of metabolomics observations to the other layers of omics. Standardized methodologies, further cataloguing of the spectrum library and ML for data interpretation are the pillars for next-generation untargeted metabolomics. Due to the high efficiency of computational pipelines, multi-omics integration could provide a systems-level insight for understanding the underlying mechanisms of biological processes and, subsequently, could be used to monitor the environment appropriately and optimize the treatment rendering. In addition, the measurement of *in vivo* temporal metabolic alteration by the use of dynamic flux analysis and dynamic real-time metabolomics has now become possible because of these emerging fields that have the potential to revolutionize the field in the near future. Conclusion -Broadly, untargeted metabolomics using UHPLC-MS is an evolving concept with great potential to change the way biology and medicine are understood. While there are still some obstacles, research has continued toward the aim of making it increasingly accurate, robust, and useful, while advances in computation and technology progress. Untargeted metabolomics represents an attractive strategy for understanding biological systems because of its strengths in addressing metabolite discovery limitations, metabolome integration, and metabolome normalization. All of the following have the potential to make a contribution toward ecological sustainability, health, and agriculture. As a result of its interdisciplinary nature and potential to provide a new biochemical perspective, the importance laid in tackling global challenges and scientific breakthroughs is discussed in this paper.

AUTHOR'S CONTRIBUTIONS

Saravanan Ravindran: Contributed to conceptualization, literature synthesis, and initial drafting. Performed formal analysis of included studies and critical evaluation, resource curation, and data validation. R Karthikeyan: Supervised the research design and finalized the manuscript. Badri Sireesha: Reviewed, edited, and refined the intellectual content. All authors approved the final version and agreed to accountability for the work.

ACKNOWLEDGMENT

Declared none.

REFERENCES

1. Silva E, Graça JP, Porto C, Prado RM, Nunes E, Marcelino-Guimarães FC, *et al*. Untargeted metabolomics analysis by UHPLC-MS/MS of soybean plant in a compatible response to 'Phakopsorapachyrhizi' infection.

Metabolites 2021;11:179.
2. Yang Z, Wang D, Li Y, Zhou X, Liu T, Shi C, *et al*. Untargeted metabolomics analysis of the anti-diabetic effect of Red ginseng extract in type 2 diabetes mellitus rats based on UHPLC-MS/MS. Biomed Pharmacother 2022;146:112495.
3. Beger RD, Goodacre R, Jones CM, Lippa KA, Mayboroda OA, O'Neill D, *et al*. Analysis types and quantification methods applied in UHPLC-MS metabolomics research: A tutorial. Metabolomics 2024;20:95.
4. Hou Y, He D, Ye L, Wang G, Zheng Q, Hao H. An improved detection and identification strategy for untargeted metabolomics based on UPLC-MS. J Pharm Biomed Anal 2020;191:113531.
5. Lacalle-Bergeron L, Goterris-Cerisuelo R, Beltran J, Sancho JV, Navarro-Moreno C, Martinez-Garcia F, *et al*. Untargeted metabolomics approach using UHPLC-IMS-QTOF MS for surface body samples to identify low-volatility chemosignals related to maternal care in mice. Talanta 2023;258:124389.
6. Pousinis P, Virgiliou C, Mouskeftara T, Chalvatzi S, Kroustallas F, Panteris E, *et al*. Untargeted metabolomics pilot study using UHPLC-qTOF MS profile in sows' urine reveals metabolites of bladder inflammation. Metabolites 2022;12:1186.
7. Bozza D, Barboni D, Spadafora ND, Felletti S, De Luca C, Nosengo C, *et al*. Untargeted metabolomics approaches for the characterization of cereals and their derived products by means of liquid chromatography coupled to high resolution mass spectrometry. J Chromatogr Open 2024;6:100168.
8. La Gioia D, Salviati E, Basilicata MG, Felici C, Botrugno OA, Tonon G, *et al*. Leveraging the potential of 1.0-mm i.d. columns in UHPLC-HRMS-based untargeted metabolomics. Anal Bioanal Chem 2025;417:2837-47.
9. Ahmed F, Eticha T, Hymete A, Ashenef A. Principles and applications of ultra-high-performance liquid chromatography. In: High Performance Liquid Chromatography - Recent Advances and Applications. London: InTechOpen; 2023.
10. Dong MW, Zhang K. Ultra-high-pressure liquid chromatography (UHPLC) in method development. TrAC Trends Anal Chem 2014;63:21-30.
11. Cielecka-Piontek J, Zalewski P, Jelińska A, Garbacki P. UHPLC: The greening face of liquid chromatography. Chromatographia 2013;76:1429-37.
12. Basharat R, Kotra V, Loong LY, Mathews AD, Kanakal MM, Devi CP, *et al*. A mini-review on ultra performance liquid chromatography. Oriental J Chem 2021;37:847-57.
13. Tome T, Obreza A, Časar Z. Developing an improved UHPLC method for efficient determination of european pharmacopeia process-related impurities in ropinirole hydrochloride using analytical quality by design principles. Molecules 2020;25:2691.
14. Ribbenstedt A, Ziarrusta H, Benskin JP. Development, characterization and comparisons of targeted and non-targeted metabolomics methods. PLoS One 2018;13:e0207082.
15. Zhou J, Hou D, Zou W, Wang J, Luo R, Wang M, *et al*. Comparison of widely targeted metabolomics and untargeted metabolomics of wild ophiocordyceps sinensis. Molecules 2022;27:3645.
16. Qin N, Qin M, Shi W, Kong L, Wang L, Xu G, *et al*. Investigation of pathogenesis of hyperuricemia based on untargeted and targeted metabolomics. Sci Rep 2022;12:13980.
17. Liang L, Xu J, Zhou WW, Brand E, Chen HB, Zhao ZZ. Integrating targeted and untargeted metabolomics to investigate the processing chemistry of polygoni multiflori radix. Front Pharmacol 2018;9:934.
18. Gu C, Hu X, Shan B, Wu X, Chen J. Targeted and non-targeted metabolomics uncovering the effects of Er-Miao-Wan formula on rats with hyperuricemia. J Pharm Biomed Anal 2023;226:115246.
19. Rahman S, Ul Haq F, Ali A, Khan MN, Shah SM, Adhikhari A, *et al*. Combining untargeted and targeted metabolomics approaches for the standardization of polyherbal formulations through UPLC-MS/MS. Metabolomics 2019;15:116.
20. Gorrochategui E, Jaumot J, Lacorte S, Tauler R. Data analysis strategies for targeted and untargeted LC-MS metabolomic studies: Overview and workflow. TrAC Trends Anal Chem 2016;82:425-42.
21. Chen L, Zhong F, Zhu J. Bridging targeted and untargeted mass spectrometry-based metabolomics via hybrid approaches. Metabolites 2020;10:348.
22. Schwaiger-Haber M, Stancliffe E, Arends V, Thyagarajan B, Sindelar M, Patti GJ. A workflow to perform targeted metabolomics at the untargeted scale on a triple quadrupole mass spectrometer. ACS Meas Sci Au 2021;1:35-45.
23. Yang M, Zhao Y, Qin Y, Xu R, Yang Z, Peng H. Untargeted metabolomics and targeted quantitative analysis of temporal and spatial variations in specialized metabolites accumulation in Poria cocos (Schw.) Wolf (Fushen). Front Plant Sci 2021;12:713490.
24. Plumb RS, McDonald T, Rainville PD, Hill J, Gethings LA, Johnson KA, *et al*. High-throughput UHPLC/MS/MS-based metabolic profiling using a vacuum jacketed column. Anal Chem 2021;93:10644-52.
25. Sammarco G, Dall'Asta C, Suman M. Untargeted metabolomics liquid chromatography-high resolution mass spectrometry approach for the geographical origin assessment of Italian dehydrated apples. J Mass Spectrom 2023;58:e4954.
26. Plumb RS, Gethings LA, Rainville PD, Isaac G, Trengove R, King AM, *et al*. Advances in high throughput LC/MS based metabolomics: A review. TrAC Trends Anal Chem 2023;160:116954.
27. Anderson BG, Raskind A, Habra H, Kennedy RT, Evans CR. Modifying chromatography conditions for improved unknown feature identification in untargeted

metabolomics. Anal Chem 2021;93:15840-9.
28. Stancliffe E, Schwaiger-Haber M, Sindelar M, Murphy MJ, Soerensen M, Patti GJ. An untargeted metabolomics workflow that scales to thousands of samples for population-based studies. Anal Chem 2022;94:17370-8.
29. Brunius C, Shi L, Landberg R. Large-scale untargeted LC-MS metabolomics data correction using between-batch feature alignment and cluster-based within-batch signal intensity drift correction. Metabolomics 2016;12:173.
30. Lacalle-Bergeron L, Izquierdo-Sandoval D, Sancho JV, López FJ, Hernández F, Portolés T. Chromatography hyphenated to high resolution mass spectrometry in untargeted metabolomics for investigation of food (bio) markers. TrAC Trends Anal Chem 2021;135:116161.
31. Nizioł J, Ossoliński K, Płaza-Altamer A, Kołodziej A, Ossolińska A, Ossoliński T, *et al*. Untargeted ultra-high-resolution mass spectrometry metabolomic profiling of blood serum in bladder cancer. Sci Rep 2022;12:15156.
32. Alonso A, Marsal S, Julià A. Analytical methods in untargeted metabolomics: State of the art in 2015. Front Bioeng Biotechnol 2015;3:23.
33. Tebani A, Schmitz-Afonso I, Rutledge DN, Gonzalez BJ, Bekri S, Afonso C. Optimization of a liquid chromatography ion mobility-mass spectrometry method for untargeted metabolomics using experimental design and multivariate data analysis. Anal Chim Acta 2016;913:55-62.
34. Rakusanova S, Fiehn O, Cajka T. Toward building mass spectrometry-based metabolomics and lipidomics atlases for biological and clinical research. TrAC Trends Anal Chem 2023;158:116825.
35. Mellon FA. Mass spectrometry. Principles and instrumentation. In: Caballero B, editor. Encyclopedia of Food Sciences and Nutrition. 2nd ed. India: Academic Press; 2003. p. 3739-49.
36. Cao M, Han Q, Zhang J, Zhang R, Wang J, Gu W, *et al*. An untargeted and pseudotargeted metabolomic combination approach to identify differential markers to distinguish live from dead pork meat by liquid chromatography-mass spectrometry. J Chromatogr A 2020;1610:460553.
37. Perez De Souza L, Alseekh S, Scossa F, Fernie AR. Ultra-high-performance liquid chromatography high-resolution mass spectrometry variants for metabolomics research. Nat Methods 2021;18:733-46.
38. Zhao JJ, Zhang Y, Wang XC, Wang X, Zhang Q, Lu P, *et al*. A new platform for untargeted UHPLC-HRMS data analysis to address the time-shift problem. Anal Chim Acta 2022;1193:339393.
39. Perez de Souza L, Fernie AR. Computational methods for processing and interpreting mass spectrometry-based metabolomics. Essays Biochem 2024;68:5-13.
40. Miggiels P, Wouters B, Van Westen GJ, Dubbelman AC, Hankemeier T. Novel technologies for metabolomics: More for less. TrAC Trends Anal Chem 2019;120:115323.
41. Pinu FR, Goldansaz SA, Jaine J. Translational metabolomics: Current challenges and future opportunities. Metabolites 2019;9:108.
42. Sirocchi C, Biancucci F, Donati M, Bogliolo A, Magnani M, Menotta M, *et al*. Exploring machine learning for untargeted metabolomics using molecular fingerprints. Comput Methods Programs Biomed 2024;250:108163.
43. Heinemann J. Machine learning in untargeted metabolomics experiments. Methods Mol Biol 2019;1859:287-99.
44. Russo F, Ottosson F, Van Der Hooft JJ, Ernst M. Deep learning models for LC-MS untargeted metabolomics data analysis. In: Cantone D, Pulvirenti A, editors. From Computational Logic to Computational Biology. Lecture Notes in Computer Science. Vol. 14070. Cham: Springer; 2024.
45. Sen P, Lamichhane S, Mathema VB, McGlinchey A, Dickens AM, Khoomrung S, Orešič M. Deep learning meets metabolomics: A methodological perspective. Brief Bioinform 2021;22:1531-42.
46. Petrick LM, Shomron N. AI/ML-driven advances in untargeted metabolomics and exposomics for biomedical applications. Cell Rep Phys Sci 2022;3:100978.
47. De Jonge NF, Mildau K, Meijer D, Louwen JJ, Bueschl C, Huber F, *et al*. Good practices and recommendations for using and benchmarking computational metabolomics metabolite annotation tools. Metabolomics 2022;18:103.
48. Nash WJ, Dunn WB. From mass to metabolite in human untargeted metabolomics: Recent advances in annotation of metabolites applying liquid chromatography-mass spectrometry data. TrAC Trends Anal Chem 2019;120:115324.
49. Paul KC, Zhang K, Walker DI, Sinsheimer J, Yu Y, Kusters C, *et al*. Untargeted serum metabolomics reveals novel metabolite associations and disruptions in amino acid and lipid metabolism in Parkinson's disease. Mol Neurodegener 2023;18:100.
50. Pathmasiri W, Rushing BR, McRitchie S, Choudhari M, Du X, Smirnov A, *et al*. Untargeted metabolomics reveal signatures of a healthy lifestyle. Sci Rep 2024;14:13630.
51. Gonzalez-Covarrubias V, Martínez-Martínez E, Del Bosque-Plata L. The potential of metabolomics in biomedical applications. Metabolites 2022;12:194.
52. Dalamaga M. Clinical metabolomics: Useful insights, perspectives and challenges. Metabol Open 2024;22:100290.
53. Lin C, Tian Q, Guo S, Xie D, Cai Y, Wang Z, *et al*. Metabolomics for clinical biomarker discovery and therapeutic target identification. Molecules 2024;29:2198.
54. Gao Y, Li J, Hu K, Wu Q, Wang S, Yang S, *et al*. Untargeted metabolomic profiling identifies Alzheimer's disease-specific signatures in serum and faeces impacted by tetrahydroxy stilbene glycoside through UPLC-Q-TOF/MS. Pharmacol Res Mod Chin Med 2024;10:100343.
55. Thistlethwaite LR, Li X, Burrage LC, Riehle K, Hacia JG,

Braverman N, *et al*. Clinical diagnosis of metabolic disorders using untargeted metabolomic profiling and disease-specific networks learned from profiling data. Sci Rep 2022;12:6556.
56. Goutman SA, Boss J, Guo K, Alakwaa FM, Patterson A, Kim S, *et al*. Untargeted metabolomics yields insight into ALS disease mechanisms. J Neurol Neurosurg Psychiatry 2020;91:1329-38.
57. Bowen TJ, Southam AD, Hall AR, Weber RJ, Lloyd GR, Macdonald R, *et al*. Simultaneously discovering the fate and biochemical effects of pharmaceuticals through untargeted metabolomics. Nat Commun 2023;14:4653.
58. Amante E, Alladio E, Rizzo R, Di Corcia D, Negri P, Visintin L, *et al*. Untargeted metabolomics in forensic toxicology: A new approach for the detection of fentanyl intake in urine samples. Molecules 2021;26:4990.
59. Alarcon-Barrera JC, Kostidis S, Ondo-Mendez A, Giera M. Recent advances in metabolomics analysis for early drug development. Drug Discov Today 2022;27:1763-73.
60. Cui Y, Pan D, Feng J, Zhao D, Liu M, Dong Z, *et al*. Untargeted metabolomics and soil community metagenomics analyses combined with machine learning evaluation uncover geographic differences in ginseng from different locations. J Agric Food Chem 2024;72:21922-34.
61. Bedair M, Glenn KC. Evaluation of the use of untargeted metabolomics in the safety assessment of genetically modified crops. Metabolomics 2020;16:111.
62. Marchev AS, Vasileva LV, Amirova KM, Savova MS, Balcheva-Sivenova ZP, Georgiev MI. Metabolomics and health: From nutritional crops and plant-based pharmaceuticals to profiling of human biofluids. Cell Mol Life Sci 2021;78:6487-503.
63. Withers E, Hill PW, Chadwick DR, Jones DL. Use of untargeted metabolomics for assessing soil quality and microbial function. Soil Biol Biochem 2020;143:107758.
64. Han W, Ward JL, Kong Y, Li X. Editorial: Targeted and untargeted metabolomics for the evaluation of plant metabolites in response to the environment. Front Plant Sci 2023;14:1167513.
65. Wang-Sattler R, Yu Z, Herder C, Messias AC, Floegel A, He Y, *et al*. Novel biomarkers for pre-diabetes identified by metabolomics. Mol Syst Biol 2012;8:615.
66. Denkert C, Budczies J, Weichert W, Wohlgemuth G, Scholz M, Kind T, *et al*. Metabolite profiling of human colon carcinoma - deregulation of TCA cycle and amino acid turnover. Mol Cancer 2008;7:72.
67. Wang W, Vinocur B, Shoseyov O, Altman A. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 2004;9:244-52.
68. Wikoff WR, Anfora AT, Liu J, Schultz PG, Lesley SA, Peters EC, *et al*. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A 2009;106:3698-703.
69. Miller Z, Xiong B, Roman-White S, Tasker T, Farina B, Piechowicz B, *et al*. Chemical degradation of polyacrylamide during hydraulic fracturing. Environ Sci Technol 2018;52:327-36.
70. Clayton TA, Baker D, Lindon JC, Everett JR, Nicholson JK. Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. Proc Natl Acad Sci U S A 2009;106:14728-33.
71. Trushina E, Dutta T, Persson XM, Mielke MM, Petersen RC. Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics. PLoS One 2013;8:e63644.
72. Johnson CH, Gonzalez FJ. Challenges and opportunities of metabolomics. J Cell Physiol 2012;227:2975-81.
73. Vorkas PA, Isaac G, Anwar MA, Davies AH, Want EJ, Nicholson JK, *et al*. Untargeted UPLC-MS profiling pipeline to expand tissue metabolome coverage: Application to cardiovascular disease. Anal Chem 2015;87:4184-93.
74. Matsuda F. Technical challenges in mass spectrometry-based metabolomics. Mass Spectrom (Tokyo) 2016;5:S0052.
75. Collins SL, Koo I, Peters JM, Smith PB, Patterson AD. Current challenges and recent developments in mass spectrometry-based metabolomics. Annu Rev Anal Chem (Palo Alto Calif) 2021;14:467-87.
76. Li S, Looby N, Chandran V, Kulasingam V. Challenges in the metabolomics-based biomarker validation pipeline. Metabolites 2024;14:200.
77. Schrimpe-Rutledge AC, Codreanu SG, Sherrod SD, McLean JA. Untargeted metabolomics strategies-challenges and emerging directions. J Am Soc Mass Spectrom 2016;27:1897-905.
78. Steiner D, Malachová A, Sulyok M, Krska R. Challenges and future directions in LC-MS-based multiclass method development for the quantification of food contaminants. Anal Bioanal Chem 2021;413:25-34.
79. Di Minno A, Gelzo M, Caterino M, Costanzo M, Ruoppolo M, Castaldo G. Challenges in metabolomics-based tests, biomarkers revealed by metabolomic analysis, and the promise of the application of metabolomics in precision medicine. Int J Mol Sci 2022;23:5213.
80. Yang Q, Zhang AH, Miao JH, Sun H, Han Y, Yan GL, *et al*. Metabolomics biotechnology, applications, and future trends: A systematic review. RSC Adv 2019;9:37245-57.
81. Ghafari N, Sleno L. Challenges and recent advances in quantitative mass spectrometry-based metabolomics. Anal Sci Adv 2024;5:e2400007.

Source of Support: Nil. **Conflicts of Interest:** None declared.