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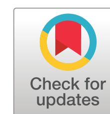

Review on analytical technologies and applications in metabolomics

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Key words: Metabolomics, Sample preparation, Analytical methods, Application of metabolomics

Abstract: Over the past decade, the swift advancement of metabolomics can be credited to significant progress in technologies such as mass spectrometry, nuclear magnetic resonance, and multivariate statistics. Currently, metabolomics garners widespread application across diverse fields including drug research and development, early disease detection, toxicology, food and nutrition science, biology, prescription, and chinmedomics, among others. Metabolomics serves as an effective characterization technique, offering insights into physiological process alterations *in vivo*. These changes may result from various exogenous factors like environmental conditions, stress, medications, as well as endogenous elements including genetic and protein-based influences. The potential scientific outcomes gleaned from these insights have catalyzed the formulation of innovative methods, poised to further broaden the scope of this domain. Today, metabolomics has evolved into a valuable and widely accepted instrument in the life sciences. However, comprehensive reviews focusing on the sample preparation and analytical methodologies employed in metabolomics within the life sciences are surprisingly scant. This review aims to fill that gap, providing an overview of current trends and recent advancements in metabolomics. Particular emphasis is placed on sample preparation, sophisticated analytical techniques, and their applications in life science research.

Introduction

Metabolomics, along with proteomics, transcriptomics, and genomics that constitutes the foundation of systems biology, have generated interest in metabolism in biology and medicine, especially in the areas of biomarker discovery. Metabolites, as important indicators of pathological or physiological status, could provide more information to identify biomarkers of disease, so that it could help to understand their progression and occurrence [1,2].

Metabolomics studies capture global biochemical events by analyzing a large number of small molecules in biological fluids, organs, tissues, or cells and then apply informatics techniques to define metabolomics characteristics, thereby mapping disease risk to metabolic pathways [3,4]. Typical metabolomics studies follow a similar workflow as depicted in Fig. 1, including sampling, sample preparation and extraction, analysis, listing statistically relevant candidate peaks, visualize variable data, extract differential peaks, establish a series of putative metabolites, and identify metabolites [5,6]. The widespread use of metabolomics has been achieved in drug discovery, systems biology, disease detection, drug research, nutrition and food science, toxicology and others [7–9]. In this review, the latest advancements and current trends in metabolomics were discussed in this article, with a focus on sample preparation and advanced analytical methods, applications in life science.

Sample preparation

In recent years, there has been remarkable progress in sample processing techniques within the field of metabolomics, with technologies like Microfluidic Technology at the forefront. Microfluidic Technology boasts numerous advantages in sample handling. It efficiently processes minute quantities of biological samples, ensuring precise control over fluid dynamics and enabling high-throughput analysis of multiple samples concurrently. Its automated features reduce human intervention and the likelihood of errors, simultaneously minimizing the risk of contamination, resulting in expedited and cost-effective analyses. The multifunctionality of this technology allows for the seamless integration of diverse processing steps, providing robust support for metabolomics research. When monitoring cellular responses to disturbances such as medication and disease, metabolic profiles of tissues and biofluids provide a panoramic

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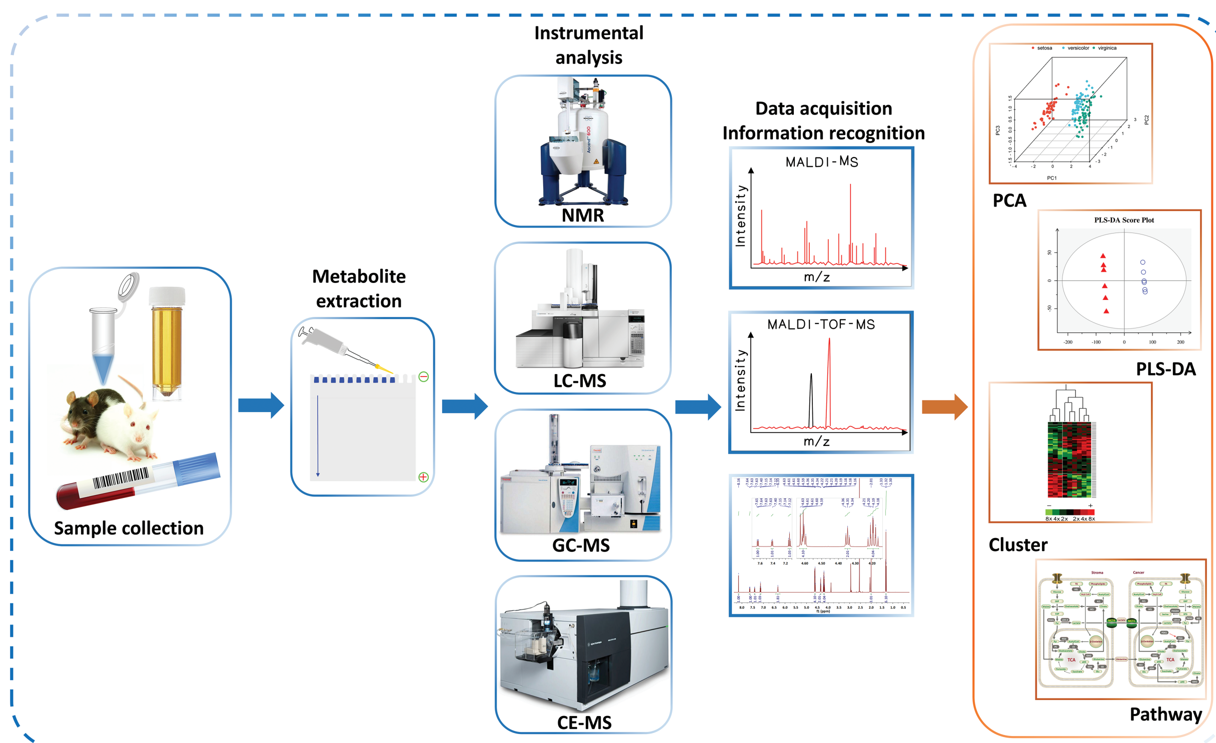

FIGURE 1. Typical metabolomics workflow.

view of changes in endogenous metabolite abundance. Metabolomics, with its myriad advantages, serves as a potent diagnostic tool, employing a range of biological fluids like urine, blood, plasma, saliva, synovial fluid, cerebrospinal fluid, semen, and tissue for analyses.

Urine

Urine, a byproduct of metabolic fluids excreted from the kidneys, flowed through the ureter to the bladder. During urination, urine is quickly excreted from the urethra. Cellular metabolism produces many metabolites that need to be removed from the bloodstream. Micturition is the main way to release water-soluble metabolites from the body.

These metabolites can be detected by urine analysis. Urine collection is a convenient biological sampling strategy, which possesses the advantages of providing time-averaged patterns of metabolites and capturing metabolites of exogenous compounds. Solid debris in urine should be removed by centrifugation at 10000~13000×g at 4°C for 5 to 10 min [10,11]. Fractions of the urine supernatants, filtered with 0.20–0.22 μm filter membrane, should be stored at −80°C [12,13]. In rodent urine, a quadruple volume of methanol is added to remove Macropeptide components and precipitatable proteins, which are freeze to −20°C for 20 min, and then centrifuged [2,14]. In nitrogen flow, the supernatant containing metabolites is evaporated to dry. Usually, the supernatant of methanol is freeze-dried for nuclear magnetic resonance (NMR) spectroscopic analysis [15,16], and then the freeze dried supernatants are dispersed in $CDCl_3$, D_2O or CD_3OD [17]. While dried materials are dissolved in 0.1% formic acid or water for liquid chromatography-mass spectrometry (LC-MS) analysis [18].

Blood and other biological fluids

Blood samples are the most common sampling biological fluids, which can provide metabolic information at the time of sampling. There are large amounts of phospholipids and proteins in blood and other biological fluids [19,20]. The fluids could be extracted by the Bligh–Dyer method to separate and obtain lipid and water-soluble metabolite fractions, with butylated hydroxyl to luene to prevent oxidation. In most cases, add methanol/deuterated chloroform (*v*/*v*, 1:1) to blood samples (serum, plasma, whole blood). After vortexing for 10–15 s, supernatants are collected after series of centrifugation process. The supernatants could be dried under nitrogen or be frozen immediately to store until analysis [21]. Additionally, for more expeditious sample processing, the samples of plasma and serum could be filtered, although the result will lead to the loss of hydrophobic metabolites [22,23]. Precious blood samples, mixed with the internal chemical shift reference material and a saline solution in D_2O, can be analyzed by NMR directly so that the samples could be re-used for other assays or experiments [16,24].

Whole tissues

Tissue is a cellular framework between cells and organs, consisting of many similar cells and interstitial cells. *In vivo*, the concentration of metabolites in body fluid reflects the overall effect *in vivo*. However, in the same tissue, different positions and different types of cells are metabolized simultaneously. In animal and human models, biopsy or tissue samples are more and more become the hot spot of research. When collecting tissue samples, it should be quickly frozen in −196°C liquid nitrogen until extracted to prevent further metabolism [25,26].

Normally, samples are cleaned with cold saline to reduce the interference of blood metabolites in the detection of tissue metabolites. In extracting tissue metabolites, frozen tissue needs to be ground into powder in the pestle and freezed with liquid nitrogen. It can also be used for liquid nitrogen impact crushing and the use of French Press (for plant tissue). The frozen powder tissue was then extracted by cold methanol (4 v/g) or 0.1% perchloric acid to precipitate and inactivate the enzyme [27], and the precipitated protein was removed by centrifugation. Add chloroform and water to remove lipids [28,29]. Perchloric acid was removed from the extract by potassium hydroxide neutralization. Because the solubility of potassium perchlorate in water should be very low, it can be removed by centrifugation [30].

Cells

For adherent cells growing on culture plates or suspension cells, Pasteur pipettes connected to vacuum lines are an effective way to remove the medium quickly [31,32]. After removing the culture medium, the cells should be washed with isotonic saline and rotated for a few seconds. The cells were then extracted from methanol freeze to dry ice temperature (−43°C). If no immediate extraction is needed, it should be kept in liquid nitrogen [33]. The preparation of cell samples must prevent further metabolism. During the analysis, cells were removed from the culture plate with a cell scraper [34,35]. The mixture was centrifuged (4000×g) for 30 min and the supernatant was sucked out. Methanol is evaporated in nitrogen flow. The specific operation methods need to be partly adjusted according to the experimental cells and experimental types.

In the future, sample processing in metabolomics is set to undergo significant transformations. These will focus on refining precision and bolstering efficiency. A key development will be the enhanced automation in sample preparation processes, ensuring consistent outcomes, minimizing human errors, and thus bolstering the reproducibility of findings across various studies. It's anticipated that the field will align more closely with other 'omics' technologies, necessitating innovative preparation techniques to safeguard the integrity of diverse molecules under study. Furthermore, cutting-edge preservation methodologies, encompassing emerging cryopreservation techniques and novel chemical stabilizers, might drastically extend sample shelf life without compromising their quality.

Targeted and untargeted analysis

Targeted metabolomics enables researchers to investigate specific compounds or compound groups within metabolites [36–38]. In scenarios with limited known data, untargeted analyses are more appropriate. These analyses are intricate due to the vast number of metabolites present, ranging from hundreds in prokaryotes to thousands in humans [39–41]. Nonetheless, untargeted analysis is instrumental in preliminary research, granting a wealth of information about the sample.

At present, metabolites can be analyzed by gas chromatography-mass spectrometry (GC-MS) or LC-MS to study gene-induced metabolic disorders [42,43]. In clinical practice, the targeting analysis of certain known compounds is usually used to determine the occurrence and development of the disease. Modern mass spectrometry can track hundreds of compounds or even more in one analysis [44,45]. When metabolic changes in the disease are not clear, researchers should adopt a more open analytical method, non-targeted metabolomics studies, for non-discriminatory analysis. This method can provide comprehensive information about metabolic changes [46].

Instrumentation in Metabolomics Research

Currently, NMR spectroscopy and mass spectrometry, either used singly or in combination, dominate the field of metabolomics research. Both NMR and MS offer distinct advantages and challenges in metabolite analysis, as summarized in Table 1. Although NMR is more stable and repeatable, can provide more structural information, its instrument cost is high, the sensitivity is low. When using NMR analysis, many low metabolites are not easy to detect, and then affect the analysis results [47,48]. Although mass spectrometry has higher sensitivity, the results of mass spectrometry are related to the composition and proportion of mobile phase and the structure of the analyte. When using an electrospray ion source, the ionization efficiency of different molecules is different, resulting in different analytical results. At present, MS is still widely used in metabolomics research [20,49,50].

Nuclear magnetic resonance spectroscopy

NMR analysis can detect molecules with nonzero magnetic moments, such as ^{1}H, ^{13}C, ^{14}N, and ^{31}P. Metabolites usually contain hydrogen atoms, and the abundance of ^{1}H atoms (99.98%) is very high, nuclear magnetic signal is relatively

TABLE 1

Pros and Cons of the currently used metabolomics methods

	Mass-spectrometry based methods	NMR-based methods
Objective	Yes	Yes
Reproducibility	Good	Good
Resolution	Fair	Good
Sensitivity	Selective	Fair
Unbiased detection	No	Yes
Holistic	No	Yes
Molecular information	Fair	Rich
Sample preparation	Extensive	Little or no
Pre-knowledge requirements	Yes	No
In vivo/*in situ*	Almost impossible	Yes
Throughput	High	High
Recurrent expenditure	High	Low
Labour intensiveness	Fair	Low

strong, so metabolomics often test and analyze the hydrogen spectrum. In NMR, each metabolite has its own unique spectrum, and each absorption peak represents the chemical and magnetic environment of each proton [51,52]. In the metabolomics samples, mixed samples of different metabolites produce complex NMR spectra.

Currently, the 800 MHz NMR spectrometer is more advanced in metabolomics research. The greater the magnetic field intensity, the higher the resolution. In order to obtain better resolution, improve the magnetic field intensity as much as possible. Compared with earlier instruments, today's NMR spectrometers can automatically load and unload metabolite extracts [52,53]. Many instruments also use cryo probe and micro probe to increase the sensitivity of the instrument. The analysis of metabolites is based on the pulse sequence and the nuclear magnetic resonance phenomenon of hydrogen atoms [53–55].

NMR, as a non-destructive analytical technique, can be used to analyze the samples after NMR spectrum analysis, and then to analyze GC- or LC-MS. The fine structure of metabolites can be observed by NMR analysis, which provides valuable information for the discovery of new compounds [56,57]. In addition, magic angle NMR can directly observe metabolite signals in tissues without preliminary extraction, thus reducing the errors caused by the pretreatment process. It is also suitable for *in vivo* analysis [58,59].

In the analysis, the amount of sample required for nuclear magnetic resonance analysis is higher than that of mass spectrometry. NMR analysis of humoral metabolites requires at least 0.5 milliliters of urine, serum and plasma. If it is solid, it needs 0.5 g tissue or feces [60,61]. If analyze the cells cultured *in vitro*, it need at least 10 million cells [62]. If a small sample is used, a large number of low abundance metabolites will be lost. A small diameter NMR tube can be used when using less NMR samples. The use of heparin is superior to EDTA or citrate in the analysis of plasma because heparin interferes less with the hydrogen spectrum [63]. In metabolomics, many databases can be used to identify metabolites in samples when using NMR for targeted and non-targeted analysis [64]. Now NMR spectroscopy has developed a new pulse procedure for analyzing lipids [51,65].

The magic angle spinning (MAS) technique has gained traction for intact cell or tissue analysis. By rapidly rotating the sample at an angle of 54.7 degrees (5 kHz) within the instrument, MAS mitigates resonance broadening and information loss stemming from short relaxation times and sample heterogeneity [66–68]. This method has been used to analyze the whole cell of the microalgae Thalassiosirapseudonana, the disease biomarkers of red abalone and to determine the mode of action of biochemical substances, to analyze the metabolite concentration changes of Saccharomyces cerevisiae mutants [67,68], and to determine the functional association between unknown functional genes and known genes [68].

Mass spectrometry

Mass spectrometry is based on the separation of metabolites through certain methods, such as liquid phase, gas phase, capillary electrophoresis as depicted in Fig. 2, the sample of metabolites are separated, and then into the mass spectrometer, ionized by ion source, analyzed, resulting in a spectrum.

Gas chromatography-mass spectrometry: Gas chromatography is usually employed to analyze volatile components. Metabolomics originally used gas chromatography to analyze volatile components in plants [34,69,70]. As a result of the development of gas chromatography and mass spectrometry technology, it is commonly used gas chromatography and mass spectrometry analysis of common biological samples, such as urine, serum, plasma, bacteria, cells and so on [71–73]. If the sample is a volatile compound, it can be injected by a headspace sampler. Headspace sampling only measures gas molecules captured in the top air. In most cases, metabolites in biological samples need to be extracted first, then derived, and then entered the gas chromatograph [74,75]. Most metabolites contain polar functional groups and are not volatile or heat-labile at separation temperature. Therefore, derivatization is necessary before gas chromatography.

In recent years, many papers have reported the use of derivatization to analyze metabolites by gas chromatography-mass spectrometry, such as the use of oxime reagent derivatization, then methylsilylation, analysis of carbohydrates, organic acids, amino acids and so on [76–78]. Metabolites containing hydroxyl, amine, amide, phosphate and mercaptan groups can be derived by alkylmethylsilane method. Recently, many metabolomics studies of plant metabolites or urine samples have adopted GC-MS analysis techniques of methylsilylation derivatization or oximation and methylsilylation derivatization [34,76,79]. GC-MS, as a high-throughput metabolomics analysis method, can be used to quantify a large number of metabolites in a single assay [72,80,81]. This method has obvious advantages in the determination of small metabolites (<400 Da), but it is not reliable in the determination of thermolabile compounds [82–84].

As a mature analytical technology, GC-MS has been widely applied in metabolomics. GC-MS can be applied to urine screening, disease research, microbial research, clinical

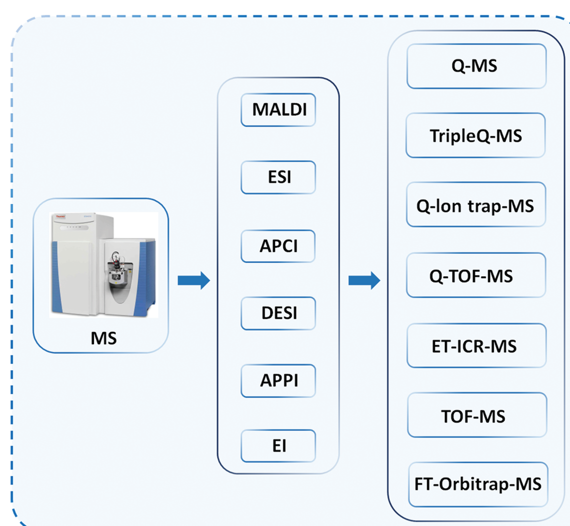

FIGURE 2. Various configurations of mass spectrometers.

research, etc. [85–87]. The metabolites and volatile substances in plant cells were analyzed by GC-MS to study the effects of gene and environmental changes on metabolism [50,88]. Multidimensional analysis (e.g., GC×GC-TOF-MS) will enhance the specificity and sensitivity of the analysis, and will make GC-MS more widely used in metabolomics research [80,89].

Liquid chromatography-mass spectrometry: Liquid chromatography mass spectrometry is used to separate metabolites in samples by high performance liquid chromatography. Then, mass spectrometry was analyzed by electrospray ionization or ionization of atmospheric chemical ion sources. The transfer of charged ions from the liquid phase to the gas phase in the LC-MS analysis requires no derivatization. ESI ion sources were used to analyze peptides and proteins, carboxylates, nitrogen compounds and amines [38,90,91]. For neutral molecular metabolites, atmospheric pressure chemical ionization can be used to enhance the formation of $[M+H]^+$ or $[M-H]^+$ or complex molecular ions [92–94]. In metabolomics analysis, LC-MS can be used to quantitative analyze metabolites suspected to be known compounds. This method is usually performed on triple quadrupole or Qtrap mass spectrometer and is widely used to analyze known biochemical molecules and drugs.

In recent years, LC-MS has been widely applied in metabolomics for clinical research [95,96]. In this way, biomarkers and drugs of many diseases in animals and humans are found to interfere with diseases. Using screening technology, some characteristic metabolites will be identified, followed by target analysis. At present, LC-NMR-MS technology is being explored in metabolomics to help identify metabolites [97,98].

Capillary electrophoresis-mass spectrometry: Capillary electrophoresis (CE) has been used in metabolomics analysis for a long time, and it is very effective in separating hydrophilic compounds [99,100]. Compared with liquid chromatography, capillary electrophoresis can be analyzed with a lower sample size (1–20 nl) [101,102]. Because of the technical problems of CE-ESI interface, the application of CE-MS in metabolomics has been greatly restricted.

In the analysis of metabolites in serum and cerebrospinal fluid samples, these fluids should be ultrafiltered to avoid protein precipitation in capillaries [103,104]. In addition, water can be used to dilute plasma, cerebrospinal fluid and urine at a ratio of 1:1 to improve the analytical results. In urine analysis, 9 nl samples can analyze more than 3500 characteristics. Up to now, the sheathless fluid interface of CE-MS has been widely used in the study of cationic metabolites, such as drug beta-glucosidate non-aqueous CE-MS method. CE-MS with no sheath fluid interface has broad application prospects in metabonomics research.

CE-MS, as an effective method for analyzing charged metabolites, can be used to detect metabolites containing hydroxyl, amino, phosphate and carboxyl groups [105,106]. The advantage of CE-MS is that it requires less samples. In metabolomics, CE-MS was used to analyze the metabolites of Bacillus subtilis, and 1692 peaks were obtained. At present, CE-MS analysis of metabolites is widely used in environmental, biomedical, clinical medicine, plant metabolism and so on [100,107].

Atmospheric pressure scanning microprobe matrix-assisted laser desorption ionization mass spectrometry imaging: Spatial Metabolomics is a method based on mass spectrometry imaging technology for the analysis of samples in metabolomics. It allows for the extension of information about the types and quantities of metabolites in a sample from the realm of metabolomics to the spatial dimension, enabling the analysis of differential changes in biological metabolism processes in terms of qualitative, quantitative, and spatial aspects [108]. Atmospheric pressure scanning microprobe matrix-assisted laser desorption ionization mass spectrometry imaging (AP-SMALDI-MSI) is employed for direct scanning and imaging of biological samples in operating environments ranging from atmospheric to intermediate pressure. This technique uses matrix-assisted laser desorption ionization (MALDI) to measure the mass-to-charge ratio (m/z) of ions with a mass spectrometer and utilizes specialized mass spectrometry imaging software to generate molecular images. This technology not only enables the untargeted detection of the composition and relative abundance of metabolites in samples but also provides information on the specific spatial distribution of metabolites within the sample. As a result, it holds significant promise for applications in various fields, including clinical medicine, molecular biology, botany, and zoology [109].

With the rapid advancement of metabolomics, specialized analytical instrument technologies for detecting small molecules within biological systems have also made significant progress. Presently, these instruments offer higher sensitivity, resolution, and accuracy. In the future, we anticipate further miniaturization of these instruments and their integration with artificial intelligence and machine learning technologies to enhance the efficiency of data processing and interpretation. Simultaneously, the development of multi-omics analysis platforms will provide support for a more comprehensive understanding of biological systems. In summary, the prospects for metabolomics analytical instruments are promising, and technological advancements will continue to drive our understanding of the metabolic pathways behind health and disease.

Applications

Metabolomics, with its powerful phenotyping ability, has emerged as an invaluable tool in understanding life systems and influencing health determinants [110–112]. With the continuous development of metabolomics research technology, many clinical researchers and drug researchers have provided new ideas and methods [113–115]. Currently, metabolomics is commonly used in disease research, drug development, Chinmedomics, nutrition and gut microflora research [110,116,117]. This evolving technology is mainly applied as follows.

Chinmedomics

Chinese medicine is an ancient practice of the Chinese people to prevent and cure diseases from ancient times to the present [118,119]. With the development of modern science and

medicine, as an effective treatment method, millions of people in the world still use traditional Chinese medicine. Chinmedomics, which integrates serum pharmacochemistry with metabolomics, uses modern science and technology to clarify the scientific value of traditional Chinese medicine, and uses a unique method combined with metabolomics and chemometrics to clarify the major changes of metabolites in the body under the condition of comprehensive symptoms and to analyze the effective components of Chinese medicine in treating diseases [120–123]. Given the multi-target, multi-component treatment approach of traditional Chinese medicine and the holistic focus of metabolomics, Chinmedomics represents a harmonious integration of both domains. By discovering biomarkers unique to specific Traditional Chinese Medicine syndromes, it offers a modern scientific interpretation of ancient TCM theories [121,124–126].

Functional genomics

In functional genomics, metabolomics can effectively characterize the metabolite changes caused by gene expression [42,127,128]. Through changes in metabolites, functional gene changes can be speculated, such as insertion or deletion [45,129,130]. At the same time, metabolomics can be used to determine the success of gene editing by comparing the metabolites before and after gene insertion or deletion. In recent years, researchers have also studied the function of location genes through metabolomics [130–132]. This technology has gradually formed metabologenomics, which is a new method combining biometabolites with genomics. Metabologenomics uses bioinformatics matching methods to identify biosynthetic small molecules by non-targeted metabolomics analysis of metabolites for discovering new natural products and determining gene functions [133–135].

Pharmacometabolomics

The application of metabolomics in pharmacology gives rise to a new field called "pharmacometabolomics" [136–138]. The basic tenet of pharmacometabolomics is to determine the metabolism of the drug itself and its influence on the metabolic process of the organism during treatment. It is also used to identify specific metabolic pathways that are responsible for drug-mediated outcomes. Pharmacometabolomics is applied to a wide range of research areas, including drug discovery, pharmacology, clinical pharmacology, personalized drug therapy and clinical trials [138–140]. Such information subsequently facilitates the discovery of biomarkers that are associated with pathologic conditions and play vital roles in mapping the effectiveness of drug therapies through various metabolomics approaches [141]. The identification of metabolic pathways affected by a specific drug treatment through metabolomics, as well as a subsequent analysis of genetic variations within these metabolic pathway components could help to achieve personalized medicine [138,142,143]. Profiling of drug-induced changes in metabolites or creating metabolite signatures of different disease subtypes, such as invasive or non-invasive tumors, through high-resolution targeted metabolomics tools serves an important biomarker discovery and diagnostic tool development [143–145].

Nutrimetabonomics

Nutrimetabonomics is a combination of diet and metabolomics [146–148]. It is mainly used to study the molecular relationship between nutrients and biochemical processes, to analyze the effects of food nutrients on metabolism, and to study the cross-genomic metabolite interactions between mammalian hosts and gut microflora as depicted in Fig. 3 [149–151].

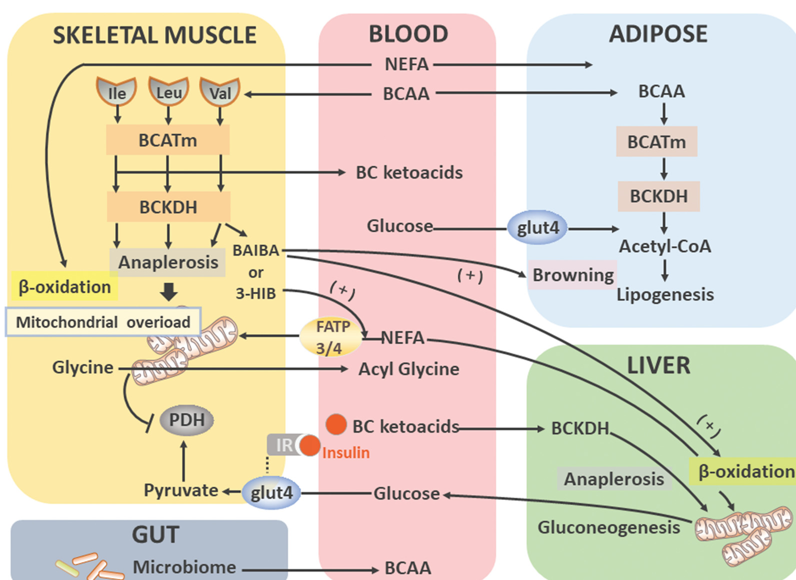

FIGURE 3. Various configurations of mass spectrometers.

Metabolites in body fluids are usually influenced by endogenous and exogenous factors, endogenous factors such as gender, age, underlying diseases, etc. Small bowel tumors are also one of the potential sources of influence on metabolites [152]. Exogenous factors are mainly diet and medicine, and the diet is divided into nutrients and non-nutrients. Metabolomics is an effective method to determine biological characteristics and metabolic fingerprints, which can detect the final impact of these factors on life [153,154].

Gut microflora

In the last decade, metabolomics has been used as an effective research method to study the interaction between gut microbiota and mammalian metabolism [155,156]. Gut microbiota constitute a microecosystem in organisms. Their metabolites interact with the metabolites of organisms and participate in physiological activities, such as immune regulation, fat metabolism, energy production, digestion, nutrient absorption and so on [157–159]. Multiple interactions between intestinal flora and host multiple metabolites can be characterized by metabolomics [160–162].

Toxicity assessment

Metabolomics plays an essential role in toxicological research by providing a detailed analysis of altered metabolic pathways that are targeted by harmful chemicals [163–165].

Toxicity assessment, through metabolomics analysis of urine or blood samples collected to detect the physiological changes and toxic damage caused by the substance [164–166]. The main advantage of using metabolomics in toxicology is that alterations in metabolism are terminal events to genetic, transcriptomic, and proteomic levels. Thus, metabolomics facilitates the understanding of direct cellular phenotypes that are induced by toxic insults. In addition, since changes in extracellular metabolites mirror the intracellular metabolic scenario, metabolomics analysis of biological fluids obtained through non-invasive or minimally invasive techniques suffices for the evaluation of target organ toxicities [166–168]. Another advantage of metabolomics is that information on a large number of metabolites could be obtained through a single measurement, an oftentimes-basic requirement for rapidly understanding the level of drug toxicity. A large-scale screening of drugs or chemical compounds can be achieved through *in vitro* metabolomics approaches [169–171].

One important use of *in vitro* metabolomics, which employs cell lines, primary cells, co-cultures in 2D or 3D format, etc., is to predict toxicological patterns of unknown chemicals in the biological system using known reference chemicals [170,172]. Such analysis starts with the comparison of a metabolic profile of a known reference chemical with its toxicological profile *in vivo*. This allows for the generation of a ‘toxicity pattern,’ which defines the metabolic profile related to a distinct toxicological endpoint [168,173,174]. Afterwards, the degree of overlap is calculated by aligning the metabolic profile of the unknown chemical with the toxicity pattern. The data are used to predict the toxicological pattern of an unknown chemical.

Disease diagnosis

Metabolomics plays a crucial role in disease diagnosis in recent years. By analyzing the types and quantities of metabolites within an individual, metabolomics provides a powerful tool for early disease detection, disease classification, monitoring treatment responses, and biomarker discovery [175,176]. Early disease diagnosis is a prominent application area. Metabolomics can detect changes in metabolites within the body, aiding in the identification of early signs of various conditions such as diabetes, cancer, and cardiovascular diseases [177]. Early diagnosis often offers better treatment opportunities and enhances the likelihood of successful outcomes. Furthermore, metabolomics contributes to disease classification and subtype differentiation. By studying metabolic differences among different subgroups, a better understanding of disease diversity and individual variations is achieved, supporting the field of precision medicine. During treatment, metabolomics is also utilized to monitor changes in disease progression and treatment effectiveness [177,178]. This helps physicians adjust treatment plans to ensure optimal patient outcomes. Metabolomics plays a pivotal role in biomarker discovery as well. By comparing metabolic differences between healthy individuals and those with specific diseases, metabolomics aids in identifying novel biomarkers. These biomarkers can be used for disease diagnosis, prognosis, and monitoring, providing essential information for medical decision-making [179,180].

In conclusion, the applications of metabolomics in disease diagnosis represent significant breakthroughs in the medical field. Metabolomics has the potential to improve disease management, enhance the quality of life for patients, and offer new opportunities for medical research.

Outlook

An important task of metabolomics is to screen reliable biomarker metabolites to detect changes in organisms. Therefore, in metabolomics analysis, a more sensitive, fast and reliable analytical tool is needed. The treatment of samples changed with the upgrading of the instrument. With the different methods of metabolomics, the sample processing methods are different. With the development of science and technology, metabolomics as an effective analytical method will be applied to more research fields. At present, NMR and MS-based metabolomics is gradually expanding its application with the development of science and technology. In the future, *in vivo* analysis and on-line analysis are the research directions of metabolomics.

Despite numerous advantages of metabolomics applied in many fields of life sciences and clinical medicines, some difficulties and concerns partly hold back its full-fledged application. Firstly, in order to apply metabolomics in biomarker and drug discovery, an accurate and highly reproducible quantification of metabolites is a prime requirement. Such need is often difficult to achieve due to complexities of analytical tools. Secondly, in order to establish an optimal drug-response, the right samples and

an adequate sample size should always be kept in check. Thirdly, since metabolomics data often involves a large number of variables with minimal sample size, selecting appropriate statistical tools must be ensured in order to avoid data misinterpretation. Further studies should widen the scope of this interesting method.

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Availability of Data and Materials: Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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