

Mass spectrometry-based metabolomics for studying gut microbiota-derived metabolites in biological samples

Ya-Ting Lin ^{a,b,1}, Huai-Hsuan Chiu ^{c,1}, Chih-Ning Cheng ^{a,b}, Ching-Hua Kuo ^{a,b,*}

^a School of Pharmacy, National Taiwan University, Taipei, Taiwan

^b The Metabolomics Core Laboratory, Center of Genomic and Precision Medicine, National Taiwan University, Taipei, Taiwan

^c Department of Medical Research, National Taiwan University Hospital, Taipei, Taiwan

Abstract

Gut microbiota produces a wide range of metabolites and plays a critical role in maintaining host health. Dysregulation of these metabolites can influence host metabolism through systemic circulation, contributing to the development of various diseases, including immunological, neurological and cancer-related disorders. Chromatography coupled with mass spectrometry (MS) has emerged as a powerful analytical approach, offering high sensitivity and resolution for studying gut microbiota-related metabolites. This review provides a comprehensive overview of chromatographic MS-based methods applied to the study of the gut microbial metabolome. We summarize strategies for sample collection, storage, and preparation of commonly analyzed sample types, including feces, plasma/serum, urine, and tissue samples. In addition, we included the main chromatographic MS-based approaches, as well as data analysis techniques, for investigating the gut microbial metabolome. The characteristics and utility of liquid chromatographic-mass spectrometry (LC-MS), gas chromatographic-mass spectrometry (GC-MS), and capillary electrophoresis-mass spectrometry (CE-MS) were discussed in the context of providing broader coverage of gut microbiota-derived metabolites with diverse physicochemical properties. Finally, we summarize recent studies that have employed chromatographic MS-based approaches to investigate gut microbiota-related disease. Through the integration of appropriate sample handling and advanced analytical strategies, a deeper understanding of host–microbiota interactions and their roles in health and disease can be achieved.

Keywords: Biological samples, Chromatography, Gut microbiota, Mass spectrometry, Metabolomics

1. Introduction

The gut microbiota constitutes a highly diverse and metabolically active community that has emerged as a key regulator of host physiology, health, and disease. Increasing evidence indicates that gut microbes act as a virtual metabolic organ, generating a wide profile of bioactive metabolites that profoundly influence host physiology. These metabolites participate in signaling networks that regulate energy balance, gut homeostasis, immune function, and neuroendocrine communication.

Importantly, they can be absorbed through the intestinal epithelium and circulate systemically to affect distal organs, including the liver and brain (Fig. 1). Disruptions in these microbial metabolic profiles, often referred to as dysbiosis, have been implicated many diseases such as obesity, type 2 diabetes, inflammatory bowel disease, cardiovascular disorders, and neurodegenerative conditions [1].

Metabolomics—the comprehensive analysis of small-molecule metabolites in biological systems—has emerged as a powerful approach for studying gut microbiota–host interactions. Advances in mass

Received 2 October 2025; accepted 14 October 2025.
Available online 31 March 2026

* Corresponding author at: School of Pharmacy, College of Medicine, National Taiwan University, Rm. 418, 4F, No. 33, Linsen S. Rd., Zhongzheng Dist., Taipei, Taiwan.
E-mail address: kuoch@ntu.edu.tw (C.-H. Kuo).

¹ These authors contributed equally to the work.

<https://doi.org/10.38212/2224-6614.3568>

2224-6614/© 2026 Taiwan Food and Drug Administration. This is an open access article under the CC-BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

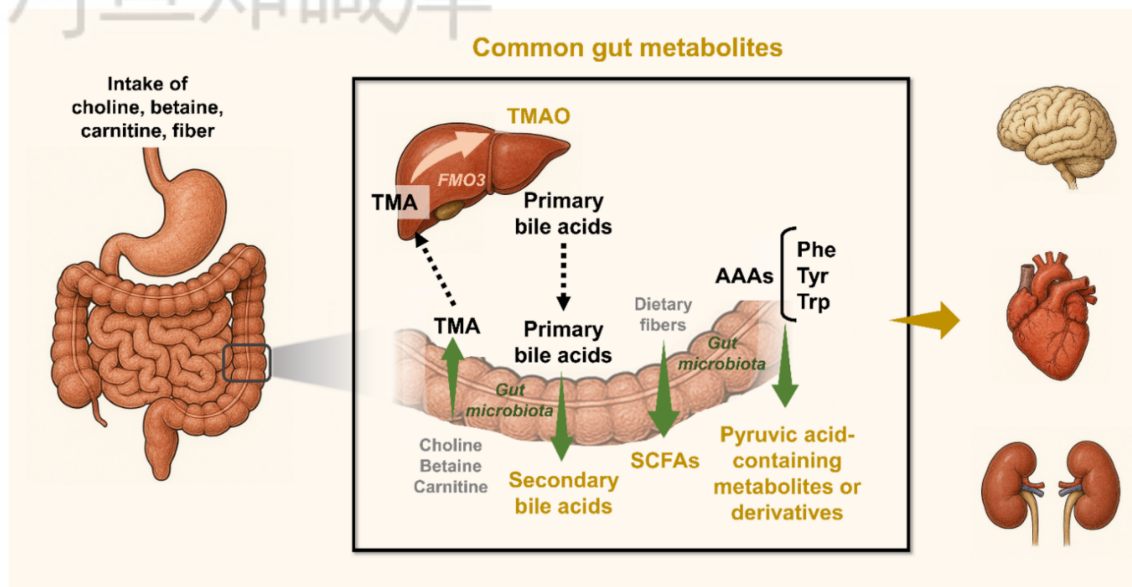


Fig. 1. Common gut microbiota-derived metabolites include trimethylamine (TMA), trimethylamine-N-oxide (TMAO), bile acids, short-chain fatty acids (SCFAs), aromatic amino acid metabolites (AAA metabolites). FMO3: flavin containing monooxygenase 3, Phe: phenylalanine, Trp: tryptophan, Tyr: tyrosine [125].

spectrometry (MS), particularly when coupled with chromatography, have enabled profiling of thousands of metabolites across diverse sample types. Chromatographic MS-based metabolomics become one of the most widely used tools for studying metabolites associated with microbial activity and disease. Recent studies have highlighted several major classes of gut microbiota-derived metabolites, and dysregulation of these metabolites may affect the host's health status. A brief summary of the most widely discussed gut-derived metabolites is provided below.

1.1. Short-chain fatty acids (SCFAs)

SCFAs, including acetic acid, propionic acid, butyric acid, and valeric acid, are produced by bacterial fermentation of dietary fiber. They serve as energy substrates for colonocytes, support gut barrier integrity, and regulate host lipid and glucose metabolism. Dysregulated SCFA production has been implicated in neurological disorders, obesity, cardiovascular disease, inflammatory bowel disease, and colorectal cancer [2].

1.2. Volatile organic compounds (VOCs)

Gut microbes produce a diverse range of VOCs, including alcohols, aldehydes, ketones, sulfur-containing compounds, and aromatic derivatives. VOCs are largely generated from microbial

fermentation of polysaccharides and amino acids, and they are detectable in feces, urine, exhaled breath, and sweat. Emerging evidence suggests that VOC profile may serve as noninvasive surrogate biomarkers for gastrointestinal disorders such as inflammatory bowel disease and infectious diarrhea [3].

1.3. Bile acid (BA) metabolites

BAs and their derivatives are amphipathic end product of cholesterol metabolism. Primary BAs (e.g., cholic acid and chenodeoxycholic acid) are synthesized by hepatocytes, whereas secondary BAs (e.g., deoxycholic acid and lithocholic acid) are produced through microbial deconjugation and transformation. BA receptors such as farnesoid X receptor (FXR) and Takeda G-protein receptor 5 (TGR5) regulate lipid metabolism, glucose homeostasis, and intestinal inflammation. Perturbations in BA metabolism have been associated with nonalcoholic fatty liver disease (NAFLD), obesity, inflammatory bowel disease, and cancer [4].

1.4. Amino acid-derived metabolites

Microbial metabolism of dietary and host amino acids generates a wide range of bioactive products, including aromatic amino acids (e.g., phenylalanine, tryptophan, and tyrosine) and polyamines [5]. Dysregulated aromatic amino acid metabolism has

been linked to Parkinson's disease and insulin resistance [6,7]. Moreover, microbial conversion of these aromatic amino acids produces indole derivatives (e.g., indole-3-lactic acid, indole-3-acetic acid, and indole-3-propionic acid), which influence epithelial proliferation and immune homeostasis. Polyamines, derived from decarboxylation of amino acids such as arginine, histidine, and lysine by the gut microbiota, also play an essential role in immune regulation and intestinal physiology [8,9].

1.5. Trimethylamine (TMA) and TMAO

Gut microbes convert dietary choline and carnitine into trimethylamine (TMA), which is subsequently oxidized in the liver to trimethylamine N-oxide (TMAO). Elevated plasma TMAO levels are strongly associated with atherosclerosis and cardiovascular events. Targeting microbial pathways involved in TMAO production is being explored as a potential therapeutic and preventive strategy for cardiovascular disease [10,11].

1.6. Other emerging metabolites

Recent metabolomics studies have revealed additional microbiota-derived metabolites such as sphingolipids, and conjugated fatty acids. Although many remain poorly characterized, these compounds are increasingly recognized as important mediators of host-microbe interactions [12,13].

Compared with metagenomics and transcriptomics, which primarily reveal microbial diversity and gene expression, metabolomics provides direct functional evidence of host-microbe interactions. By linking microbial taxa and genes to specific metabolites, metabolomics advances our understanding from descriptive associations to mechanistic insights. MS coupled with chromatography—encompassing gas chromatography mass spectrometry (GC-MS), liquid chromatography mass spectrometry (LC-MS), and capillary electrophoresis mass spectrometry (CE-MS)—offers high sensitivity and broad coverage for profiling the gut microbial metabolites, which exhibits chemical diversity. Despite these advances, standardized sample collection and processing protocols, appropriate chromatographic MS platforms, and more robust computational tools are still required to ensure unbiased results.

This review addresses critical aspects of sample collection, preparation, and storage to preserve the native metabolomic state. It also covers analytical considerations in chromatographic MS-based metabolomics and their applications in characterizing

gut microbiota-derived metabolites, followed by an overview of tools for metabolomics data analysis. Fig. 2 summarizes the workflow of gut microbiota-derived metabolomics using chromatographic MS-based methodologies. By understanding the critical considerations in these methods for measuring gut microbiota-derived metabolites, further studies can be conducted to enhance their translational potential in precision medicine and therapeutic development.

2. Sample collection and storage

In gut microbial metabolomics research, feces, plasma/serum, urine, and tissue are among the most frequently employed sample types. The subsequent section presents comprehensive details regarding their collection and storage conditions. In addition, key considerations regarding the handling of these samples for the analysis of gut microbiota-derived metabolites are summarized in Table 1.

2.1. Feces

In the study of the complex interactions between the host and gut microbiota, the non-invasive nature and ease of collection make fecal samples highly advantageous for gut microbiome metabolomics research. Feces are rich in diverse metabolites produced jointly by the host and gut microbiota, providing direct and representative chemical evidence for research. As such, they have been widely used to investigate the mechanisms of various diseases, including liver diseases [14–16], obesity [17], intestinal inflammation [18,19], and cancer [20,21].

To prevent microbial activity from affecting metabolite measurements, proper storage conditions after sample collection are essential. Rapid low-temperature storage post-collection is particularly important. If fecal samples require transport, they should be maintained at 4 °C or on ice to preserve metabolomic integrity [22]. In addition to temperature, some protocols recommend placing samples quickly into an anaerobic environment, considering the gut's anaerobic conditions, to further minimize microbial metabolism and maintain the sample's original composition [22–24].

When immediate extraction and analysis are not feasible, two pre-processing storage strategies can be applied. The first involves storing crude feces, which refers to untreated solid fecal samples: these can be temporarily stored at 4 °C, while long-term storage is recommended at –80 °C, avoiding repeated freeze-thaw cycles [22]. The second

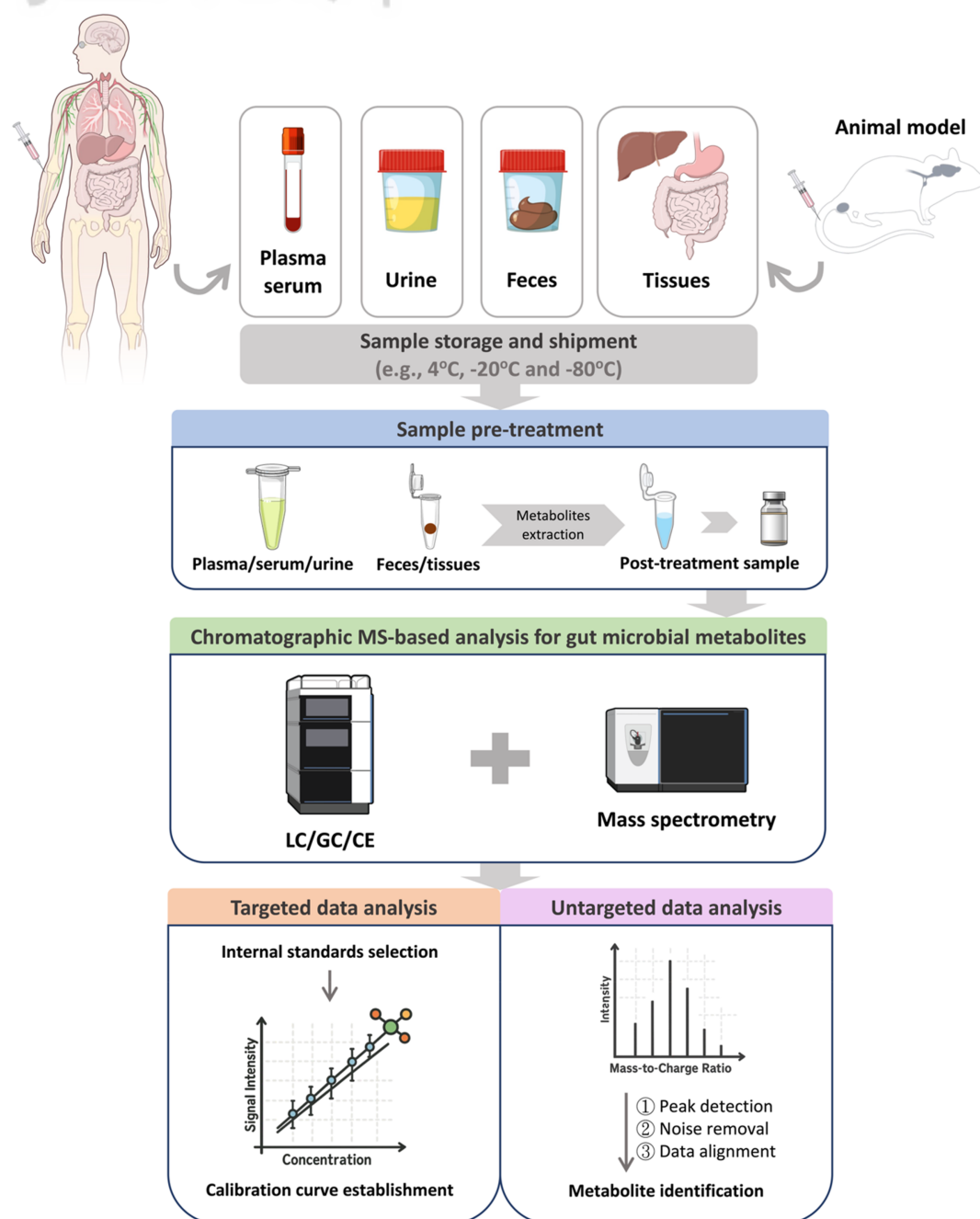


Fig. 2. Workflow of chromatographic MS-based analysis of gut microbiota-derived metabolites. A schematic overview of the experimental workflow from samples collection, storage and processing to chromatographic separation, mass spectrometry analysis, and data processing.

strategy involves preparing and storing fecal water samples, which refers to isolating water-soluble analytes from crude feces for analysis. Preparation usually involves mixing fecal samples with physiological saline, phosphate buffered saline (PBS), or deionized water at a defined ratio, followed by repeated centrifugation or ultrafiltration to remove solids and cellular debris. Previous work has

suggested that using larger amounts of homogenized crude feces for fecal water preparation (e.g., 15 g vs. 350 mg) yields more representative metabolite profiles, although the optimal smaller sample size remains unclear [22]. Fecal water should ideally be prepared within 24 h after fecal collection; otherwise, crude feces should be stored at 4 °C until processing. Once prepared, fecal water

Table 1. Sample type, pre-processing, storage, and key considerations for sample handling for gut microbiota-associated metabolites analysis.

Sample Type	Pre-processing/Storage	Key Considerations
Feces	- Crude feces: short-term 4 °C, long-term –80 °C; avoid repeated freeze–thaw - Fecal water: prepare within 24 h by mixing with PBS, saline, or deionized water; centrifuge/ultrafiltrate; store –20 °C short-term, –80 °C long-term	- Minimize microbial metabolism by rapid freezing or anaerobic handling - Transport on ice or at 4 °C
Plasma/Serum	- Centrifuge at 4 °C as soon as possible after collection to separate plasma/serum - Store at –80 °C; avoid repeated freeze–thaw	- Plasma preferred over serum to minimize metabolite release from platelets - Choice of anticoagulant (heparin, EDTA) may affect MS signal - Minimize delay between collection and processing
Urine	- Centrifuge at 1000–3000 g for ≥5 min at 4 °C to remove debris - Optional: add preservatives (e.g., 10 mM sodium azide, 200 mM boric acid) - Store at –80 °C; avoid repeated freeze–thaw	- Prevent bacterial growth and metabolite degradation
Tissue	- Snap-freeze immediately in liquid nitrogen - Store at –80 °C; avoid repeated freeze–thaw	- Handle quickly to preserve metabolites

samples are best stored at –20 °C and analyzed within 24 h, with –80 °C recommended for long-term preservation [22]. Metabolomic results from fecal water has been reported to be more stable than those from crude feces, making it a preferable sample type for preserving metabolite integrity [22]. However, because fecal water cannot capture non-water-soluble or intracellular metabolites, the choice between crude feces and fecal water should ultimately be guided by research objectives and target metabolites.

2.2. Plasma/serum

Although blood specimens do not provide a direct focus on intestinal metabolite changes as fecal samples do, plasma or serum samples, owing to their systemic circulation characteristics, can reflect gut microbiota-derived metabolites that have been absorbed and subsequently exert systemic effects on the host. Blood is therefore one of the most widely used sample types in metabolomics. Numerous studies have employed blood samples to investigate the association between gut microbial metabolites and diseases such as neurodegenerative diseases [25,26], diabetes [27], cardiovascular disorders [28], and depression [29]. In addition, previous research has demonstrated that gut microbiota can significantly influence the levels of amino acids and indole derivatives in blood [30], further highlighting the importance of blood specimens in studying how gut microbiota regulate systemic metabolism, drug metabolism, and disease risk.

The handling and storage of blood samples for microbiota-related metabolomic analysis generally follow standard metabolomics procedures, and a

number of standardized protocols and reviews are already available [31–33]. Nevertheless, the stability of metabolites in blood remains a critical issue, as pre-analytical factors such as delays in centrifugation and fluctuations in storage temperature can markedly affect metabolite profiles. To minimize these effects, blood samples should be stored and centrifuged at 4 °C as soon as possible after collection to separate plasma or serum, thereby reducing metabolite alterations caused by cell lysis. In clinical settings, however, immediate centrifugation is often not feasible due to operational constraints. Under such circumstances, careful control of storage conditions prior to processing becomes particularly important. Once processed, plasma or serum samples should be stored immediately at –80 °C to maintain metabolite stability. Prolonged exposure to room temperature and repeated freeze–thaw cycles should be strictly avoided to ensure data accuracy and reproducibility. Another key pre-analytical consideration is the choice between plasma and serum. Plasma is often preferred in metabolomic studies, since platelet activation during clotting can release a large number of metabolites and mask or alter biologically relevant signals [34]. For plasma collection, heparin or EDTA tubes are commonly recommended [35,36]. However, both anticoagulants may introduce distinct signal interferences in MS [37], and the choice should therefore be tailored to the metabolites of interest. For animal models, the preparation and storage follows the same principles as applied to clinical samples, while a wider range of blood collection routes are allowed, including tail vein and jugular vein sampling, with cardiac puncture being mainly reserved for terminal experiments [38].

2.3. Urine

Urine samples have also been used in previous studies of gut microbiota-related metabolomics. Although it does not directly reflect the intestinal environment like fecal samples, a large proportion of small molecules metabolized by gut microbiota and subsequently absorbed by the host are ultimately excreted via the kidneys [39,40]. Therefore, urine can provide important information on how the host processes these gut-derived metabolites. In particular, urine has recently been recognized as a sample type that can more specifically reveal the influence of diet and lifestyle on the host [41], making it valuable for assessing the effects of gut microbiota on host health and for identifying disease biomarkers. Urinary metabolomics has increasingly been applied in studies related to gut microbiota, including those focused on diet [42], cardiovascular metabolism-related diseases [43], and gut dysbiosis induced by antibiotics [39,44].

Due to its non-invasive nature and accessibility, urine collection is relatively easy and allows repeated sampling at multiple time points. However, sample quality must still be carefully considered. Midstream urine is generally recommended, and samples should be centrifuged immediately at 1000–3000 g for at least 5 mins at 4 °C to remove cells and debris [45]. To prevent bacterial growth and maintain metabolite stability, preservatives such as sodium azide at a final concentration of 10 mM or boric acid at 200 mM can be added to the samples [46,47]. For long-term storage, urine samples should be maintained at –80 °C to ensure metabolite stability, and repeated freeze–thaw cycles should be avoided as they may lead to metabolite degradation or concentration changes, which can severely affect the accuracy of subsequent analyses.

2.4. Tissue

Tissue samples in animal models can provide deeper insights into related physiological mechanisms when supported by well-designed experiments. Recent studies have utilized a variety of tissue specimens including mouse liver, intestinal tissues [48–50], cerebral cortical brain tissues [51], heart tissue [52,53], and others—to investigate the systemic impact of gut-microbiota-derived metabolites. Analyzing these diverse tissues allows researchers to assess how microbial metabolites influence distant organ function, providing insights from studies on multiple gut-organ axes, such as the gut-brain and

gut–heart axes. Similarly, the gut and skin microbiome communicate through inflammatory pathways and oxidative stress, highlighting a functional gut–skin axis [54]. Profiling gut microbiota-derived metabolites in various tissue types, including skin, serves as a valuable approach to capture tissue-specific microbial signatures and to illustrate how microbial metabolites may impact different organs. This approach highlights the importance of analyzing diverse tissues for a comprehensive understanding of gut microbiota–host relationships.

Preparing intestinal tissue requires careful handling, typically through sequential washing and mucosal scraping to minimize contamination from luminal contents [55]. Even with these precautions, a key limitation remains: distinguishing host-derived metabolites from microbial ones can be challenging. While physical contamination from luminal contents can be mitigated by careful tissue processing, accurately attributing metabolites to microbial versus host origin requires additional approaches, such as using germ-free mice or metabolic tracing. Special attention should be paid to the handling of tissue samples, which should be snap-frozen in liquid nitrogen immediately after collection and stored at –80 °C.

3. Sample preparation and extraction

In addition to collection and storage, appropriate preparation and extraction strategies must be adapted to the characteristics of both specimens and analytes. The following sections outline considerations for feces, plasma/serum, urine, and tissue samples.

3.1. Feces

Although a universally accepted standardized protocol for fecal sample pre-processing is currently lacking, homogenization is widely recognized as a critical step in fecal sample handling. Proper homogenization ensures that collected subsamples are representative, which is essential for both crude feces and fecal water analysis. It also enhances metabolite extraction efficiency in subsequent analyses [56].

Metabolomic analysis of fecal samples is generally performed using a fixed sample weight. Due to the unique characteristics of crude feces, samples can be weighed either as wet weight or, after lyophilization, as dry weight. Reported sample wet weights range widely, from 150 to 1000 mg [57–60]. To minimize the influence of variable water content on sample weight and solvent ratios during

extraction, many studies prefer using lyophilized feces, which improves matrix homogeneity and significantly enhances data reliability [57,61]. However, lyophilization may increase the risk of volatile compound loss, such as SCFAs [62], although some studies suggest that lyophilization does not significantly affect SCFA measurements. Despite this potential risk, lyophilization remains commonly adopted to improve sample matrix consistency and facilitate standardized processing, ultimately enhancing the accuracy and comparability of analytical results [63,64].

The ratio of solvent to fecal sample varies depending on the sample type. For wet feces, reported sample-to-solvent ratios range from 1:2 to 1:10 (w/v) [64–67], while for lyophilized feces, higher ratios are typically applied, ranging from 1:10 to 1:40 (w/v) [67–69]. This is mainly because the absence of water leads to higher metabolite concentrations, requiring more solvent to achieve sufficient extraction, proper dilution, and prevention of signal saturation. Although reference ranges exist in the literature, ratios should be optimized according to the target metabolites, instrument sensitivity, and specific experimental requirements. Extraction solvent selection should likewise be tailored to the target analytes, as fecal samples contain metabolites of varying polarity. For polar small molecules, commonly used solvents include water, methanol, acetonitrile, isopropanol, buffer solution, or their mixtures [70]. For more lipophilic metabolites, liquid–liquid extraction (LLE) is generally applied, using solvents such as chloroform/methanol mixtures or methyl tert-butyl ether (MTBE) to extract into the organic phase [71,72].

For highly volatile metabolites such as SCFAs, sample preparation and extraction should be performed at low temperatures with minimal air exposure to inhibit ongoing microbial metabolism and reduce volatilization [58,73–75]. SCFA extraction typically involves acidifying the samples to convert salts into free acids, followed by LLE to enhance recovery and reduce potential column damage in GC-MS [76,77]. To enhance analytical sensitivity, targeted compounds subjected to GC-MS analysis are often derivatized prior to measurement. Silylation, which produces trimethylsilyl (TMS) derivatives, is one of the most widely used derivatization methods [78]. Common reagents include N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N-methyl-N-(tri-methylsilyl)-trifluoroacetamide (MSTFA) [79]. This reaction can modify a broad range of functional groups, including hydroxyl, carboxyl, amino, and thiol groups. However, it is sensitive to moisture and may lead to

higher sample loss during preparation. Esterification is another frequently employed strategy, particularly effective for carboxyl-containing metabolites [77]. Compared with silylation, esterification is generally milder and carries a lower risk of sample loss, although its applicability is more limited to specific functional groups.

3.2. Plasma/serum

To extract common gut microbiota-derived metabolites from blood samples, which are predominantly polar, acetonitrile, methanol, or their mixtures with water are commonly used. These solvents effectively dissolve water-soluble or highly hydrophilic metabolites. After adding organic solvents, the samples are typically vigorously mixed and then centrifuged at high speed and low temperature (e.g., 4 °C) to remove precipitated proteins. Lipid LLE methods can also be used to extract other non-polar metabolites [80,81]. For SCFAs, which are commonly investigated metabolites, derivatization is generally necessary before LC-MS analysis because of their low blood concentrations and high volatility [82].

3.3. Urine

Common pre-treatment methods for metabolomic analysis of urine samples involve dilution followed by direct injection. The dilution factor should be adjusted based on the concentration of target analytes to ensure measurements fall within the linear range of the assay [83,84]. For pH-sensitive metabolites or when LLE is required, the urine pH may need to be adjusted accordingly [85]. In addition to these pre-analytical considerations, it is important to account for the inherent variability of urine metabolite concentrations, which can be influenced by dietary intake, hydration status, and genetic factors. To reduce such variability, metabolite concentrations are commonly normalized to creatinine levels or urine osmolality [86,87]. However, in patients with kidney disease, creatinine may not be an optimal normalization factor, as its concentration can be significantly affected by the pathology. In such cases, post-analytical data normalization has been recommended to account for urine concentration differences [88].

For specific gut microbiota-derived metabolites, such as SCFAs and BAs in urine, additional specialized processing is often necessary due to their low concentrations. SCFA extraction is similar to that in blood samples, requiring an acidic environment and derivatization to enhance detection

sensitivity [89]. In contrast to plasma samples, where BA are predominantly detected as glycine- or taurine-conjugated species, urinary BAs largely exist in sulfated forms, with free parent compounds occurring only at trace levels [90,91]. Although sulfated BAs constitute the major form in urine, their absolute concentrations are relatively low, and the urine matrix is complex. Therefore, these metabolites typically require enrichment and purification, most commonly through solid-phase extraction (SPE) or drying and reconstitution, prior to LC-MS analysis to ensure adequate sensitivity [90–92].

3.4. Tissue

Tissue samples generally require pulverization or homogenization either prior to or during the extraction process, followed by solvent-based extraction [93,94]. In some protocols, the extraction step is further assisted by tools such as ultrasonic sonication or small steel ball to improve extraction efficiency [95,96]. While mechanical homogenization and sonication improve metabolite recovery, care should be taken to minimize excessive heat generation and sample exposure, as volatile and labile microbiota-derived metabolites—such as trimethylamine (TMA) and SCFAs—may be lost or degraded during tissue processing. Subsequent normalization of metabolite signal intensities across different samples—such as by using equal tissue weight—should be carefully considered and optimized based on the nature of the experiment and study design [96,97].

4. Chromatographic MS-based metabolomics for studying microbiota-derived metabolites

MS techniques, such as single quadrupole mass spectrometry (SQ-MS), triple quadrupole mass spectrometry (TQ-MS), time of light mass spectrometry (TOF-MS), and Orbitrap mass spectrometry (Orbitrap-MS), have been widely applied in microbiota-related metabolomics analyses. MS is typically coupled with GC, LC, or CE to achieve analyte separation, thereby enabling accurate qualitative and quantitative measurements. The selection of an MS instrument mainly depends on the purpose of the study, whether targeted or untargeted.

TQ-MS employs three quadrupole stages in series, providing highly selective and sensitive detection. This configuration is widely used for quantitative analysis, particularly in multiple reaction monitoring (MRM) mode for targeted

metabolomic analysis. This is a powerful approach for the quantitative analysis of predefined microbiota-derived metabolites, such as SCFAs, BAs, amino acid-derivatives, and TMAO. Such targeted strategies are particularly valuable in hypothesis-driven studies, where precise quantification of selected metabolites is required to assess metabolic alterations, validate biomarker candidates, or monitor therapeutic interventions.

In contrast, TOF-MS and Orbitrap-MS are high-resolution mass spectrometry (HRMS) platforms that measure the mass-to-charge ratio (m/z) of ions with high accuracy and resolving power. These instruments enable precise determination of molecular formulas and identification of complex compounds in untargeted metabolomic analyses. Untargeted strategies aim to detect and profile as many metabolites as possible in a biological sample without prior knowledge of their identities. This approach enables the discovery of novel or unexpected compounds, provides global metabolic fingerprints, and facilitates comparative analyses across disease conditions to identify associated metabolites and underlying metabolic mechanisms.

Given the chemical diversity of microbiota-derived metabolites, integrating chromatographic separation with MS platforms is essential for comprehensive analysis. Each platform offers distinct advantages for analyzing different classes of metabolites and describes in the following sections. Fig. 3 summarizes the chromatographic MS-based techniques applied in microbiota-related metabolomics.

4.1. GC-MS

Highly volatile and thermally stable metabolites (e.g., SCFAs and VOCs) can be directly analyzed by GC-MS without additional derivatization. For non-volatile and thermally unstable metabolites, chemical derivatization is a complementary strategy to enhance volatility for GC-MS analysis [98]. Moreover, electron ionization (EI) used at 70eV is the standard ionization technique used in GC-MS to generate MS spectral libraries. This approach generates highly specific and reproducible ion fragmentation patterns, providing a significant advantage by enabling direct metabolite identification through established commercial spectral database (e.g., NIST or FiehnLib). Overall, GC-MS coupled with EI offers robust, reproducible, and database-supported analysis, making it particularly powerful for profiling microbiota-derived metabolites. Its standardized fragmentation patterns and well-established spectral databases further support

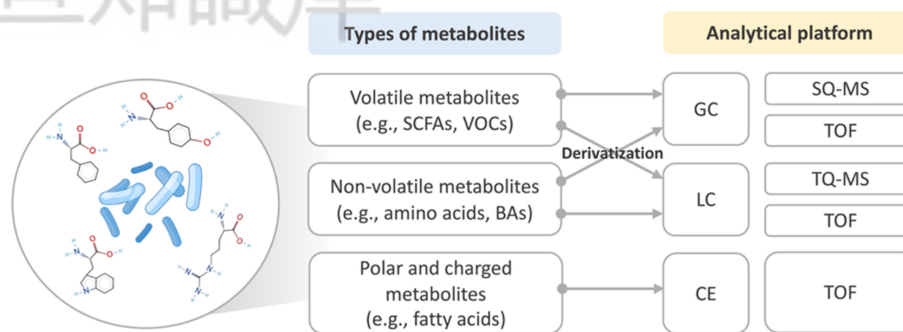


Fig. 3. Chromatographic MS-based platforms for the analysis of different classes of gut microbial metabolites.

biomarker discovery and diagnostic applications. The next section highlights targeted and untargeted metabolomics approaches applied GC-MS in gut microbiota-related disease studies.

4.1.1. Targeted metabolome in microbiota-related disease studies

SCFAs are the most important gut microbiota-derived metabolites, commonly analyzed by GC-MS without prior derivatization. For example, Baert et al. investigated changes in SCFA concentrations in fecal samples from patients with Parkinson's disease (PD) using GC-SQ-MS to investigate the effect of dietary fiber supplementation on fecal butyric acid levels, which has been proposed as an important SCFA in reducing PD symptoms [99]. They observed that the administration of butyrogenic fibers to PD patients stimulated butyric acid production, which was associated with alleviation of PD symptoms. This study provided preliminary evidence for an association between dietary fiber intake and improvement of PD symptoms [100]. Similarly, Su et al. applied GC-SQ-MS to measure SCFA profiles in both fecal and hippocampus samples from a rat model of chronic cerebral hypoperfusion. They reported decreased fecal acetic acid and propionic acid, as well as reduced acetic acid levels in the hippocampus. Importantly, fecal microbiota transplantation and SCFA supplementation alleviated cognitive impairment induced by chronic cerebral hypoperfusion [101]. These studies support the utility of GC-MS as a robust tool for SCFA analysis.

4.1.2. Untargeted metabolome in microbiota-related disease studies

Volatile metabolites can be directly analyzed by GC-MS. For instance, Hassani et al. applied GC-TOF-MS to measure fecal volatile organic compounds (VOCs) in infants diagnosed with necrotizing

enterocolitis (NEC). This study characterized 14 unique VOCs that could serve as predictive biomarkers for the early diagnosis of NEC [102]. Similarly, Maqoud et al. used GC-TOF-MS to analyze fecal and urinary VOC profiles in obese individuals. They reported significantly increased urinary levels of m-cymen-8-ol and 1,3,5-undecatriene, along with decreased levels of carvone, p-cresol, and nonane compared with healthy controls, providing evidence of metabolic dysregulation associated with increased body mass index (BMI) [103]. To analyze non-volatile metabolites, chemical derivatization is required prior GC-MS analysis. Jo et al. used MSTFA as a derivatization reagent to expand metabolites coverage while used GC-SQ-MS to study the gut microbial metabolome in high-fat diet induced obese mice. They observed enrichment of specific bacterial taxa, accompanied by upregulated serum phenylalanine and tyrosine biosynthesis [104]. Since derivatization in GC-MS increases methodological complexity, recent studies primarily apply GC-MS to volatile compounds without derivatization.

4.2. LC-MS

LC-MS is a versatile analytical platform capable of detecting a wide range of microbiota-derived metabolites, including both hydrophilic and hydrophobic compounds (e.g., amino acids or bile acids). Chromatographic separation can be achieved using either normal- or reverse-phase columns. In addition, simple sample preparation procedures enable high-throughput analysis. Owing to the extensive chemical diversity of gut microbiota-derived metabolites, recent studies continue to develop new strategies to enhance analytical performance and broaden metabolite coverage. For metabolites that are highly volatile, poorly retained on reverse-phase columns, or present isobaric forms, novel analytical approaches

have also been developed to overcome these challenges.

Although C18 columns are typically applied to analyze most metabolites, such as BAs and amino acids, various LC columns have been employed to improve the separation of different classes of gut microbiota-derived metabolites. Hermeling et al. established a rapid 6.5-min LC-TQ-MS method using a biphenyl column with a basic pH mobile phase, enabling separation of BA isomers and quantification of 27 BA species. This method was successfully applied to mouse samples, including plasma, bile, and liver, for BA concentration measurement [105]. Wang et al. developed a polarity-switching MRM-MS method that identified 118 amino acid-conjugated BA isomers and epimers, and for the first time reported 14 types of amino acid conjugations with host BAs from mouse and human feces [106]. Zheng et al. demonstrated that using a pentafluorophenyl (PFP) column in LC-Orbitrap-MS enables broad coverage of gut microbiota-derived metabolites. Applying this platform in germ-free mice, they successfully identified 282 previously unreported metabolites, providing a comprehensive strategy for advancing our understanding of gut microbiota-host metabolic interactions [107].

Besides addressing chromatography and MS challenges in facilitating the analysis of gut microbiota-derived metabolites, chemical derivatization is an effective strategy to expand metabolite coverage, improve retention on reversed-phase columns, and enhance sensitivity. Volatile metabolites such as SCFAs are difficult to analyze directly by LC-MS. Moreover, their low concentrations in plasma make detection by GC-MS challenging [108]. Our group developed a derivatization method using 3-nitrophenylhydrazine (3-NPH) as a derivatization reagent for the simultaneous quantification of 22 metabolites, including SCFAs, BAs, and tryptophan, in plasma from patients with cardiovascular disease. We further employed $^{13}\text{C}_6$ -3NPH-labeled standards to generate one-to-one internal standards, allowing accurate quantification of these metabolites, which are measured within 14 min of total run time on a reverse-phase column [82]. To identify gut microbial-derived metabolites at low abundant, Wang et al. applied N,N-dimethylethylenediamine (DMED) as a derivatization reagent to enhance sensitivity and analytical coverage, successfully detecting 261 carboxylated metabolites and characterizing a novel class of phenylacetylated BA conjugates from mouse feces [109]. P-cresol, a by-product of tyrosine, is typically present at trace levels in biological systems and organs. To improve

its detection, Fabregat-Safont et al. demonstrated 1,2-dimethylimidazole-5-sulfonyl chloride as a derivatization reagent to improve the sensitivity of p-cresol measurement and successfully quantified p-cresol in mice brain [110]. These studies provide different derivatization strategies to overcome the limitations of LC-MS in facilitating gut microbial metabolomics research. Based on these advances, the following section focuses on targeted and untargeted LC-MS-based metabolomics approaches in the context of gut microbiota-related disease studies.

4.2.1. Targeted metabolome in microbiota-related disease studies

BA metabolites, amino acid-derived metabolites, and SCFAs are among the most frequently studied gut microbiota-derived metabolites. Moreover, TMAO, a metabolite derived from TMA, is also importantly associated with several disease. Collectively, these metabolites have become popular targets for LC-MS analysis in recent studies. Silva et al. employed LC-TQ-MS to investigate the association between gut microbiota-derived metabolites, such as SCFAs and indole-3-propionic acid, and dietary intake in women with polycystic ovary syndrome (PCOS). They used 3NPH as a derivatization reagent for SCFA analysis. Their results showed that women with PCOS had lower serum indole-3-propionic acid levels, along with higher fecal acetic acid and propionic acid, compared with healthy controls. These findings support the notion that women with PCOS display distinct microbiota-related metabolic outcomes [111]. Cheng et al. applied LC-TQ-MS to evaluate the association of trimethylamine N-oxide (TMAO) and its related metabolites in participants with chronic kidney disease (CKD). They observed that higher plasma TMAO levels were associated with an increased risk of reduced estimated glomerular filtration rate (eGFR) and CKD progression. Their study proposed TMAO may become a biomarker and a potential therapeutic target for mitigating the progression of CKD [112]. Chang et al. used LC-TQ-MS to analyze plasma amino acid levels and explore their association with autism spectrum disorder (ASD) in children. They found that lower ornithine levels and higher valine levels were associated with an increased risk of ASD [113]. Moreover, our group combined LC-TQ-MS and GC-TOF-MS to analyze SCFAs in plasma and feces from PD patients with different stages. Plasma samples were further derivatized with 3NPH for LC-TQ-MS analysis. Interesting, we observed distinct trends in SCFA concentrations between

fecal and plasma samples, with lower fecal SCFAs but increased plasma SCFA levels in PD patients, which correlated with specific gut microbiota. These results provide preliminary evidence that the leaky gut phenomenon links SCFAs to the circulatory system in patients with severe PD. Paired analysis of plasma and feces can provide a more systematic understanding of how gut-derived metabolites affect human health [114].

4.2.2. Untargeted metabolome in microbiota-related disease studies

LC-combined with high resolution mass spectrometry provides high mass accuracy, resolution, and scan speed, enabling comprehensive and reliable non-targeted metabolomics for the discovery of novel metabolites associated with gut microbiota-related diseases. Wang et al. used LC-Orbitrap-MS to profile serum gut microbiota-derived metabolites in patients with autoimmune liver disease, including primary biliary cholangitis (PBC), autoimmune hepatitis (AIH), and their overlap syndrome (OS). In addition to reduced gut microbial diversity in OS compared with PBC and AIH, serum pentadecanoic acid and aminoimidazole carboxamide ribonucleotide were distinctly identified in OS patients. Moreover, L-tyrosine levels were negatively correlated with aspartate aminotransferase (AST) activity, and the metabolomic signature improved the differential diagnosis of these liver disease [115]. Yang et al. employed LC-TOF-MS to investigate the role of high fat diet (HFD) in increasing colorectal cancer (CRC) risk in a mouse model. Elevated fecal lysophosphatidic acid was identified as a gut microbiota-derived metabolite promoting CRC development under HFD condition. This study demonstrated that HFD may increase CRC risk through altered gut microbiota and host metabolism. Gan et al. applied LC-Orbitrap-MS to investigate gut microbial metabolites associated with chronic apical periodontitis (CAP) contribute to nonalcoholic fatty liver disease (NAFLD). Their results showed elevated fecal D-galactosamine and reduced 16-hydroxyhexadecanoic acid and 3-methylindole in CAP patients. Given the potential role of CAP in promoting NAFLD through gut microbial metabolism, this study provides new insights into the CAP and NAFLD connection [116].

Overall, LC-MS approach provides broad metabolite coverage and high throughput potential, enabling comprehensive and sensitive simultaneous profiling of different classes of microbiota-derived metabolites. Although volatile

metabolites are difficult to analyze by LC-MS, chemical derivatization strategies are effective in addressing this issue. Therefore, LC-MS has become a primary analytical platform in recent studies among chromatographic MS-based approaches for unraveling host–microbiome interactions in human health.

4.3. CE-MS

CE-MS offers advantages for analyzing highly polar and charged microbiota-derived metabolites (e.g., amino acids, organic acids, and fatty acids), providing exceptional separation efficiency based on electrophoretic mobility. This makes it particularly suitable for resolving isomeric or structurally similar compounds that are difficult to analyze by LC-MS. Despite these advantages, few studies have employed CE-MS approaches in gut microbiota-related metabolomic research. This may be because CE-MS has notable disadvantages compared with other chromatographic MS-based techniques, such as low sensitivity and limited robustness. Although CE-MS is not considered a primary approach for analyzing gut microbiota-derived metabolites, it retains advantages for the measurement of specific metabolites in recent studies. In the following section, we summarize targeted and untargeted metabolomics studies that have applied CE-MS in gut microbiota-related disease research.

4.3.1. Targeted and untargeted metabolome in microbiota-related disease studies

The CE-MS approach has advantages in analyzing polar and charged metabolites and has been applied in recent studies. Barker-Tejeda et al. combined CE-TOF-MS and GC-TOF-MS to comprehensively profile different classes of gut microbiota-derived metabolites in infants and their elderly individuals. First, they applied GC-TOF-MS for large-scale metabolomic analysis. Second, they used targeted CE-TOF-MS with multisection injection, which enables multiple sample analyses in a single run and offers advantages for high-throughput analysis. By further combining CE-MS, they were able to detect metabolites that could not be measured by GC-MS alone. They found that fecal SCFAs and branched-chain fatty acids were associated with shifts in bacterial community composition, providing novel insights into microbiota–metabolite interactions relevant to child health [117]. Kure et al. used CE-TOF-MS to investigate gut microbiota-related metabolic profile changes in prostate cancer patients receiving androgen deprivation therapy (ADT).

Significant alterations were observed in fecal lactic acid, butyric acid, 2-hydroxyisobutyrate, and S-adenosylmethionine, suggesting that ADT may exert adverse effects through metabolic modulation [118]. These studies demonstrated that CE-MS offers advantages for analyzing specific polar and charged metabolites.

Together, these studies highlight the utility of targeted and untargeted MS-based approaches for linking metabolic alterations to gut microbiota-related disorders, underscoring that modulation of microbiota-derived metabolites may provide promising therapeutic opportunities in precision medicine. Table 2 summarizes recent applications of chromatography-MS-based metabolomics in elucidating associations between microbial metabolites and microbiota-related diseases.

5. Data analysis

In targeted metabolomics, the data analysis workflow is relatively straightforward. A calibration curve is first generated, and the ratio of analyte to internal standard responses in samples is plotted against the curve to determine concentrations. One of the critical issues in targeted quantification is the selection of internal standards. For example, although many studies have reported quantification of BAs, not all of them used a one-to-one stable isotope-labeled internal standard [92,119]. Instead, targets were often divided into groups, with a representative internal standard selected for each class [92]. In such cases, the efficiency of matrix effect correction must be carefully considered to ensure accurate quantification. Alternatively, some studies have introduced a derivatization step to overcome the limitation of unavailable one-to-one stable isotope-labeled standards [82]. This approach not only enables the generation of isotopically labeled derivatives that can serve as one-to-one internal standards, but also enhances detection sensitivity.

In untargeted metabolomics, the general workflow typically includes data alignment, noise removal, peak detection, missing value imputation, and statistical analysis. Open-source software such as XCMS [120], MS-DIAL [121], and MZmine [122] are commonly used for these steps. For metabolites derived from gut microbiota, many compounds may be novel and lack authentic standards. In such cases, *in silico* fragmentation analysis (e.g., using SIRIUS) and spectral similarity-based molecular networking (e.g., via the GNPS platform) can facilitate structural annotation and identification of unknown metabolites [123,124].

Table 2. Selected MS-based metabolomics investigations of gut microbiota-associated diseases.

Metabolites	Model	Sample type	Analytical platform	Quantification	Disease	Identified metabolites	Ref.
Targeted analysis							
SCFAs	Human	Feces	GC-SQ-MS	Yes	Parkinson's disease	Butyric acid	[100]
SCFAs	Human	Serum	GC-SQ-MS	Yes	Obesity	Acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, and valeric acid	[126]
SCFAs	Rat	Feces	GC-SQ-MS	Yes	Cognitive dysfunction	Acetic acid and propionic acid	[101]
SCFAs and tryptophan metabolites	Human	Hippocampus Serum Feces	LC-TQ-MS GC-SQ-MS		Cirrhosis Hepatic encephalopathy	Valeric acid, melatonin, 5-HTOL, indole-3-acetyl-alanine	[127]
SCFAs and 5-hydroxytryptamine	Human	Serum Feces	LC-TQ-MS GC-SQ-MS		Prenatal depression	Propionic acid, 5-hydroxytryptamine	[128]
Aromatic amino acids	Human	Plasma	LC-TQ-MS	Yes	Parkinson's disease	Phenylacetylglutamine, p-cresol sulfate, p-cresol glucuronide, indoxyl sulfate	[6]
SCFAs and indole-3-propionic acid	Human	Plasma Feces	LC-TQ-MS	Yes	Polycystic ovary syndrome	Indole-3-propionic acid, acetic acid, propionic acid	[111]

(continued on next page)

Table 2. (continued)

Metabolites	Model	Sample type	Analytical platform	Quantification	Disease	Identified metabolites	Ref.
Amino acids	Human	Serum	LC-TQ-MS		Autism spectrum disorder	Ornithine, valine	[113]
Lipids	Rat	Plasma	LC-TQ-MS	Yes	Intestinal ischemia-reperfusion	Linoleic acid, 10-hydroxy-cis-12-octadecenoic acid	[129]
		Mesenteric lymph					
TMAO, TMA, choline, carnitine, g-butyrobetaine	Human	Plasma	LC-TQ-MS	Yes	Chronic kidney disease	TMAO	[112]
Nicotinamide	Human	Feces	CE-TOF-MS		Ulcerative Colitis	Nicotinamide	[130]
Untargeted analysis							
	Human	Feces	GC-TOF-MS		Necrotizing enterocolitis	14 unique VOCs	[102]
	Human	Feces	GC-TOF-MS		Obesity	m-cymen-8-ol, 1,3,5-Undecatriene, p-cresol, carvone, p-cresol, nonane	[103]
		Urine					
	Mice	Colonic epithelium	LC-TOF-MS		Colorectal cancer	Taurodeoxycholic acid	[131]
	Human	Peripheral blood	LC-TOF-MS		Obesity	Indole-3-acetic acid	[132]
	Mice	Feces	LC-TOF-MS		Colorectal cancer	Lysophosphatidic acid	[133]
	Mice	Brain heart infusion	LC-TOF-MS		Colorectal cancer	Indole-3-lactic acid	[134]
	Human	Feces	LC-Orbitrap-MS		Postmenopausal women with osteoporosis	Levulinic acid, N-Acetylneuraminic acid, L-pipecolic acid	[135]
	Human	Serum	LC-Orbitrap-MS		Autoimmune overlap syndrome	Pentadecanoic acid, aminoimidazole carboxamide ribonucleotide, L-Tyrosine	[115]
	Mice	Feces	LC-Orbitrap-MS		Chronic apical periodontitis and NAFLD	D-galactosamine, 16-hydroxyhexadecanoic acid, 3-methylindole	[116]
	Human	Feces	CE-TOF-MS		Microbiota of newborns	SCFAs, branched-chain fatty acids	[117]
			GC-TOF-MS		environmental exposure		
	Human	Feces	CE-TOF-MS		Prostate cancer	Lactate, butyrate, 2-hydroxyisobutyrate, S-adenosylmethionine	[118]
	Mouse	Feces	CE-TOF-MS		Sepsis	Valine, glycine, betaine, TMA, isovaleric acid, butyric acid, propionic acid	[136]

6. Conclusions

Gut microbiota-associated metabolites have emerged as crucial mediators in health and disease, underscoring their potential as diagnostic markers and therapeutic targets. This review has provided an overview of essential aspects in gut microbiota metabolomics, ranging from sample collection and preparation to instrumental and data analysis. Advances in metabolomics technologies and analytical methods have enabled more comprehensive profiling of microbiota-associated metabolites. Ongoing progress in this field will deepen our understanding of host–microbiota interactions and may facilitate the development of novel therapeutic strategies and drug discovery.

Acknowledgement

This study was supported by the National Science and Technology Council (NSTC 113-2113-M-002-010-MY3 and NSTC 114-2735-B-001-001). We gratefully acknowledge the National Taiwan University (NTU) School of Pharmacy Endowment Fund in support of the Platform for Clinical Mass Spectrometry and NMR Structure Elucidation.

References

- [1] Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. *Science* 2012;336:1262–7.
- [2] Mukhopadhyay I, Louis P. Gut microbiota-derived short-chain fatty acids and their role in human health and disease. *Nat Rev Microbiol* 2025;23:635–51.
- [3] Sagar NM, Cree IA, Covington JA, Arasradnam RP. The interplay of the gut microbiome, bile acids, and volatile organic compounds. *Gastroenterol Res Pract* 2015;2015:398585.
- [4] Collins SL, Stine JG, Bisanz JE, Okafor CD, Patterson AD. Bile acids and the gut microbiota: metabolic interactions and impacts on disease. *Nat Rev Microbiol* 2023;21:236–47.
- [5] Chen Y, Fang JY. The role of colonic microbiota amino acid metabolism in gut health regulation. *Cell Insight* 2025;4:100227.
- [6] Chen SJ, Wu YJ, Chen CC, Wu YW, Liou JM, Wu MS, et al. Plasma metabolites of aromatic amino acids associate with clinical severity and gut microbiota of Parkinson's disease. *NPJ Parkinson's Dis* 2023;9:165.
- [7] Wiklund P, Zhang X, Pekkala S, Autio R, Kong L, Yang Y, et al. Insulin resistance is associated with altered amino acid metabolism and adipose tissue dysfunction in normoglycemic women. *Sci Rep* 2016;6:24540.
- [8] Milovic V. Polyamines in the gut lumen: bioavailability and biodistribution. *Eur J Gastroenterol Hepatol* 2001;13:1021–5.
- [9] Kitada Y, Muramatsu K, Toju H, Kibe R, Benno Y, Kurihara S, et al. Bioactive polyamine production by a novel hybrid system comprising multiple indigenous gut bacterial strategies. *Sci Adv* 2018;4:eaat0062.
- [10] Roncal C, Martínez-Aguilar E, Orbe J, Ravassa S, Fernández-Montero A, Saenz-Pipaon G, et al. Trimethylamine-N-oxide (TMAO) predicts cardiovascular mortality in peripheral artery disease. *Sci Rep* 2019;9:15580.
- [11] Canyelles M, Borràs C, Rotllan N, Tondo M, Escolà-Gil JC, Blanco-Vaca F. Gut microbiota-derived TMAO: a causal factor promoting atherosclerotic cardiovascular disease? *Int J Mol Sci* 2023;24.
- [12] Brown EM, Ke X, Hitchcock D, Jeanfavre S, Avila-Pacheco J, Nakata T, et al. Bacteroides-derived sphingolipids are critical for maintaining intestinal homeostasis and symbiosis. *Cell Host Microbe* 2019;25:668–80.e7.
- [13] Brown EM, Clardy J, Xavier RJ. Gut microbiome lipid metabolism and its impact on host physiology. *Cell Host Microbe* 2023;31:173–86.
- [14] Raman M, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, et al. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol H* 2013;11:868–75.e3.
- [15] Carr RM, Li Y, Chau L, Friedman ES, Lee JJ, Adorini L, et al. An integrated analysis of fecal microbiome and metabolomic features distinguish non-cirrhotic NASH from healthy control populations. *Hepatology* 2023;78:1843–57.
- [16] Huang HJ, Zhang AY, Cao HC, Lu HF, Wang BH, Xie Q, et al. Metabolomic analyses of faeces reveals malabsorption in cirrhotic patients. *Digest Liver Dis* 2013;45:677–82.
- [17] Salazar N, Ponce-Alonso M, Garriga M, Sánchez-Carrillo S, Hernández-Barranco AM, Redruello B, et al. Fecal metabolome and bacterial composition in severe obesity: impact of diet and bariatric surgery. *Gut Microbes* 2022;14.
- [18] Robinson AM, Gondalia SV, Karpe AV, Eri R, Beale DJ, Morrison PD, et al. Fecal microbiota and metabolome in a mouse model of spontaneous chronic colitis: relevance to human inflammatory bowel disease. *Inflamm Bowel Dis* 2016;22:2767–87.
- [19] Jagt JZ, Struys EA, Ayada I, Bakkali A, Jansen EEW, Claesen J, et al. Fecal amino acid analysis in newly diagnosed pediatric inflammatory bowel disease: a multicenter case-control study. *Inflamm Bowel Dis* 2022;28:755–63.
- [20] Clos-García M, García K, Alonso C, Iruarizaga-Lejarreta M, D'Amato M, Crespo A, et al. Integrative analysis of fecal metagenomics and metabolomics in colorectal cancer. *Cancers (Basel)* 2020;12.
- [21] Sun Y, Zhang X, Hang D, Lau HC, Du J, Liu C, et al. Integrative plasma and fecal metabolomics identify functional metabolites in adenoma-colorectal cancer progression and as early diagnostic biomarkers. *Cancer Cell* 2024;42:1386–13400 e8.
- [22] Gratton J, Phetcharaburanin J, Mullish BH, Williams HRT, Thursz M, Nicholson JK, et al. Optimized sample handling strategy for metabolic profiling of human feces. *Anal Chem* 2016;88:4661–8.
- [23] Armstrong CW, McGregor NR, Lewis DP, Butt HL, Gooley PR. The association of fecal microbiota and fecal, blood serum and urine metabolites in myalgic encephalomyelitis/chronic fatigue syndrome. *Metabolomics* 2017;13.
- [24] Yen S, McDonald JAK, Schroeter K, Oliphant K, Sokolenko S, Blondeel EJM, et al. Metabolomic analysis of human fecal microbiota: a comparison of feces-derived communities and defined mixed communities. *J Proteome Res* 2015;14:1472–82.
- [25] MahmoudianDehkordi S, Arnold M, Nho K, Ahmad S, Jia W, Xie GX, et al. Altered bile acid profile associates with cognitive impairment in Alzheimer's disease-An emerging role for gut microbiome. *Alzheimer's Dement* 2019;15:76–92.
- [26] Shao YP, Li TB, Liu ZY, Wang XL, Xu XJ, Li S, et al. Comprehensive metabolic profiling of Parkinson's disease by liquid chromatography-mass spectrometry. *Mol Neurodegener* 2021;16.
- [27] Wang Z, Peters BA, Yu B, Grove ML, Wang T, Xue XN, et al. Gut microbiota and blood metabolites related to fiber intake and type 2 diabetes. *Circ Res* 2024;134:842–54.

- [28] Dong CR, Yang YN, Wang YH, Hu XM, Wang QC, Gao F, et al. Gut microbiota combined with metabolites reveals unique features of acute myocardial infarction patients different from stable coronary artery disease. *J Adv Res* 2023;46:101–12.
- [29] Amin N, Liu J, Bonnechere B, MahmoudianDehkordi S, Arnold M, Batra R, et al. Interplay of metabolome and gut microbiome in individuals with major depressive disorder vs control individuals. *Jama Psychiat* 2023;80:597–609.
- [30] 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. *P Natl Acad Sci USA* 2009;106:3698–703.
- [31] Dunn WB, Broadhurst D, Begley P, Zelena E, Francis-McIntyre S, Anderson N, et al. Procedures for large-scale metabolic profiling of serum and plasma using gas chromatography and liquid chromatography coupled to mass spectrometry. *Nat Protoc* 2011;6:1060–83.
- [32] Chen YH, Xu J, Zhang RP, Abliz Z. Methods used to increase the comprehensive coverage of urinary and plasma metabolomes by MS. *Bioanalysis* 2016;8:981–97.
- [33] Lepoittevin M, Blancart-Remaury Q, Kerforne T, Pellerin L, Hauet T, Thuillier R. Comparison between 5 extractions methods in either plasma or serum to determine the optimal extraction and matrix combination for human metabolomics. *Cell Mol Biol Lett* 2023;28.
- [34] Hagn G, Meier-Menches SM, Plessl-Walder G, Mitra G, Mohr T, Preindl K, et al. Plasma instead of serum avoids critical confounding of clinical metabolomics studies by platelets. *J Proteome Res* 2024;23:3064–75.
- [35] Barri T, Dragsted LO. UPLC-ESI-QTOF/MS and multivariate data analysis for blood plasma and serum metabolomics: effect of experimental artefacts and anticoagulant. *Anal Chim Acta* 2013;768:118–28.
- [36] Yin PY, Peter A, Franken H, Zhao XJ, Neukamm SS, Rosenbaum L, et al. Preanalytical aspects and sample quality assessment in metabolomics studies of human blood. *Clin Chem* 2013;59:833–45.
- [37] Thachil A, Wang L, Mandal R, Wishart D, Blydt-Hansen T. An overview of pre-analytical factors impacting metabolomics analyses of blood samples. *Metabolites* 2024;14.
- [38] Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother* 2010;1:87–93.
- [39] Obrenovich ME, Tima M, Polinkovsky A, Zhang RL, Emancipator SN, Donskey CJ. Targeted metabolomics analysis identifies intestinal microbiota-derived urinary biomarkers of colonization resistance in antibiotic-treated mice. *Antimicrob Agents Ch* 2017;61.
- [40] Zheng SJ, Zhou LA, Hoene M, Peter A, Birkenfeld AL, Weigert C, et al. A new biomarker profiling strategy for gut microbiome research: valid association of metabolites to metabolism of microbiota detected by non-targeted metabolomics in human urine. *Metabolites* 2023;13.
- [41] Wu JQ, Gao YH. Physiological conditions can be reflected in human urine proteome and metabolome. *Expert Rev Proteomic* 2015;12:623–36.
- [42] Jain A, Li XH, Chen WN. An untargeted fecal and urine metabolomics analysis of the interplay between the gut microbiome, diet and human metabolism in Indian and Chinese adults. *Sci Rep-Uk* 2019;9.
- [43] Li M, Shu XB, Xu HC, Zhang CL, Yang LL, Zhang L, et al. Integrative analysis of metabolome and gut microbiota in diet-induced hyperlipidemic rats treated with berberine compounds. *J Transl Med* 2016;14.
- [44] Yu M, Jia HM, Zhou C, Yang Y, Sun LL, Zou ZM. Urinary and fecal metabolomics study of the protective effect of Chaihu-Shu-Gan-San on antibiotic-induced gut microbiota dysbiosis in rats. *Sci Rep-Uk* 2017;7.
- [45] Chen MX, Wang SY, Kuo CH, Tsai IL. Metabolome analysis for investigating host-gut microbiota interactions. *J Formos Med Assoc* 2019;118:S10–22.
- [46] Thongboonkerd V, Saetun P. Bacterial overgrowth affects urinary proteome analysis: recommendation for centrifugation, temperature, duration, and the use of preservatives during sample collection. *J Proteome Res* 2007;6:4173–81.
- [47] Roux A, Thevenot EA, Seguin F, Olivier MF, Junot C. Impact of collection conditions on the metabolite content of human urine samples as analyzed by liquid chromatography coupled to mass spectrometry and nuclear magnetic resonance spectroscopy. *Metabolomics* 2015;11:1095–105.
- [48] Cai HY, Wen ZG, Meng K, Yang PL. Metabolomic signatures for liver tissue and cecum contents in high-fat diet-induced obese mice based on UHPLC-Q-TOF/MS. *Nutr Metab* 2021;18.
- [49] Wang JL, Han X, Li JX, Shi R, Liu LL, Wang K, et al. Differential analysis of intestinal microbiota and metabolites in mice with dextran sulfate sodium-induced colitis. *World J Gastroentero* 2022;28:6109–30.
- [50] Zarei I, Koistinen VM, Kokla M, Klävis A, Babu AF, Lehtonen M, et al. Tissue-wide metabolomics reveals wide impact of gut microbiota on mice metabolite composition. *Sci Rep-Uk* 2022;12.
- [51] Lai YJ, Liu CW, Yang YF, Hsiao YC, Ru HY, Lu K. High-coverage metabolomics uncovers microbiota-driven biochemical landscape of interorgan transport and gut-brain communication in mice. *Nat Commun* 2021;12.
- [52] Wang YC, Koay YC, Pan C, Zhou ZQ, Tang WL, Wilcox J, et al. Indole-3-propionic acid protects against heart failure with preserved ejection fraction. *Circ Res* 2024;134:371–89.
- [53] Makrecka-Kuka M, Volska K, Antone U, Vilskersts R, Grinberga S, Bandere D, et al. Trimethylamine N-oxide impairs pyruvate and fatty acid oxidation in cardiac mitochondria. *Toxicol Lett* 2017;267:32–8.
- [54] Ferrara F, Valacchi G. Role of microbiota in the GUT-SKIN AXIS responses to outdoor stressors. *Free Radical Bio Med* 2024;225:894–909.
- [55] Bell HN, Das NK, Shah YM. Protocol for isolation and analysis of small volatile microbiome metabolites from human or mouse samples. *STAR Protoc* 2022;3:101311.
- [56] Carrillo KT, Nam SL, de la Mata AP, de Bruin OM, Doukhanine E, Harynuk J. Optimization of fecal sample homogenization for untargeted metabolomics. *Metabolomics* 2023;19.
- [57] Ng JSY, Ryan U, Trengove RD, Maker GL. Development of an untargeted metabolomics method for the analysis of human faecal samples using-infected samples. *Mol Biochem Parasit* 2012;185:145–50.
- [58] Hsu YL, Chen CC, Lin YT, Wu WK, Chang LC, Lai CH, et al. Evaluation and optimization of sample handling methods for quantification of short-chain fatty acids in human fecal samples by GC-MS. *J Proteome Res* 2019;18:1948–57.
- [59] Cesbron N, Royer AL, Guitton Y, Sydor A, Le Bizec B, Dervilly-Pinel G. Optimization of fecal sample preparation for untargeted LC-HRMS based metabolomics. *Metabolomics* 2017;13.
- [60] Hosseinkhani F, Dubbelman AC, Karu N, Harms AC, Hankemeier T. Towards standards for human fecal sample preparation in targeted and untargeted LC-HRMS studies. *Metabolites* 2021;11.
- [61] Karu N, Deng L, Slae M, Guo AC, Sajed T, Huynh H, et al. A review on human fecal metabolomics: methods, applications and the human fecal metabolome database. *Anal Chim Acta* 2018;1030:1–24.
- [62] Saric J, Wang Y, Li J, Coen M, Utzinger J, Marchesi JR, et al. Species variation in the fecal metabolome gives insight into differential gastrointestinal function. *J Proteome Res* 2008;7:352–60.
- [63] Moosmang S, Pitscheider M, Sturm S, Seger C, Tilg H, Halabalaki M, et al. Metabolomic analysis-Addressing NMR and LC-MS related problems in human feces sample preparation. *Clin Chim Acta* 2019;489:169–76.

- [64] Cheng K, Brunius C, Fristedt R, Landberg R. An LC-QToF MS based method for untargeted metabolomics of human fecal samples. *Metabolomics* 2020;16.
- [65] Lamichhane S, Yde CC, Schmedes MS, Jensen HM, Meier S, Bertram HC. Strategy for nuclear-magnetic-resonance-based metabolomics of human feces. *Anal Chem* 2015;87:5930–7.
- [66] Deda O, Chatzioannou AC, Fasoula S, Palachanis D, Raikos N, Theodoridis GA, et al. Sample preparation optimization in fecal metabolic profiling. *J Chromatogr B* 2017;1047:115–23.
- [67] Wu JF, An YP, Yao JW, Wang YL, Tang HR. An optimised sample preparation method for NMR-based faecal metabonomic analysis. *Analyst* 2010;135:1023–30.
- [68] Kelly PE, Ng HJ, Farrell G, McKirdy S, Russell RK, Hansen R, et al. An optimised monophasic faecal extraction method for LC-MS analysis and its application in gastrointestinal disease. *Metabolites* 2022;12.
- [69] Nam SL, Carrillo KT, de la Mata AP, Harynuk JJ. Untargeted metabolomic profiling of aqueous and lyophilized pooled human feces from two diet cohorts using two-dimensional gas chromatography coupled with time-of-flight mass spectrometry. *Metabolites* 2023;13.
- [70] Zhou LN, Yu D, Zheng SJ, Ouyang RZ, Wang YT, Xu GW. Gut microbiota-related metabolome analysis based on chromatography-mass spectrometry. *Trac-Trend Anal Chem* 2021;143.
- [71] Gregory KE, Bird SS, Gross VS, Marur VR, Lazarev AV, Walker WA, et al. Method development for fecal lipidomics profiling. *Anal Chem* 2013;85:1114–23.
- [72] Van Meulebroek L, De Paepe E, Vercruysse V, Pomian B, Bos S, Lapauw B, et al. Holistic lipidomics of the human gut phenotype using validated ultra-high-performance liquid chromatography coupled to hybrid orbitrap mass spectrometry. *Anal Chem* 2017;89:12502–10.
- [73] Cunningham JL, Bramstang L, Singh A, Jayarathna S, Rasmusson AJ, Moazzami A, et al. Impact of time and temperature on gut microbiota and SCFA composition in stool samples. *PLoS One* 2020;15.
- [74] Ueyama J, Oda M, Hirayama M, Sugitate K, Sakui N, Hamada R, et al. Freeze-drying enables homogeneous and stable sample preparation for determination of fecal short-chain fatty acids. *Anal Biochem* 2020;589.
- [75] Bridgman SL, Azad MB, Field CJ, Haqq AM, Becker AB, Mandhane PJ, et al. Fecal short-chain fatty acid variations by breastfeeding status in infants at 4 months: differences in relative versus absolute concentrations. *Front Nutr* 2017;4.
- [76] He LQ, Prodhon MAI, Yuan F, Yin XM, Lorkiewicz PK, Wei XL, et al. Simultaneous quantification of straight-chain and branched-chain short chain fatty acids by gas chromatography mass spectrometry. *J Chromatogr B* 2018;1092:359–67.
- [77] Czarnowski P, Mikula M, Ostrowski J, Zeber-Lubecka N. Gas chromatography-mass spectrometry-based analyses of fecal short-chain fatty acids (SCFAs): a summary review and own experience. *Biomedicines* 2024;12.
- [78] Zhang SM, Wang HB, Zhu MJ. A sensitive GC/MS detection method for analyzing microbial metabolites short chain fatty acids in fecal and serum samples. *Talanta* 2019;196:249–54.
- [79] Schummer C, Delhomme O, Appenzeller BM, Wennig R, Millet M. Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis. *Talanta* 2009;77:1473–82.
- [80] Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957;226:497–509.
- [81] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Phys* 1959;37:911–7.
- [82] Liao HY, Wang CY, Lee CH, Kao HL, Wu WK, Kuo CH. Development of an efficient and sensitive chemical derivatization-based LC-MS/MS method for quantifying gut microbiota-derived metabolites in human plasma and its application in studying cardiovascular disease. *J Proteome Res* 2021;20:3508–18.
- [83] Nelis M, Decraecker L, Boeckstaens G, Augustijns P, Cabooter D. Development of a HILIC-MS/MS method for the quantification of histamine and its main metabolites in human urine samples. *Talanta* 2020;220.
- [84] Chen GY, Liao HW, Tseng YJ, Tsai IL, Kuo CH. A matrix-induced ion suppression method to normalize concentration in urinary metabolomics studies using flow injection analysis electrospray ionization mass spectrometry. *Anal Chim Acta* 2015;864:21–9.
- [85] Fernández-Peralbo MA, de Castro MDL. Preparation of urine samples prior to targeted or untargeted metabolomics mass-spectrometry analysis. *Trac-Trend Anal Chem* 2012;41:75–85.
- [86] Ryan D, Robards K, Prenzler PD, Kendall M. Recent and potential developments in the analysis of urine: a review. *Anal Chim Acta* 2011;684:17–29.
- [87] Gyamlani GG, Bergstralh EJ, Slezak JM, Larson TS. Urinary albumin to osmolality ratio predicts 24-hour urine albumin excretion in diabetes mellitus. *Am J Kidney Dis* 2003;42:685–92.
- [88] Gagnebin Y, Tonoli D, Lescuyer P, Ponte B, de Seigneux S, Martin PY, et al. Metabolomic analysis of urine samples by UHPLC-QTOF-MS: impact of normalization strategies. *Anal Chim Acta* 2017;955:27–35.
- [89] Jauchico A, Sangaraju D, Shahidi-Latham SK. A rapid derivatization based LC-MS/MS method for quantitation of short-chain fatty acids in human plasma and urine. *Bioanalysis* 2019;11:741–53.
- [90] Bathena SPR, Mukherjee S, Olivera M, Alnouti Y. The profile of bile acids and their sulfate metabolites in human urine and serum. *J Chromatogr B* 2013;942:53–62.
- [91] Huang JG, Bathena SPR, Csanaky IL, Alnouti Y. Simultaneous characterization of bile acids and their sulfate metabolites in mouse liver, plasma, bile, and urine using LC-MS/MS. *J Pharmaceut Biomed* 2011;55:1111–9.
- [92] Sangaraju D, Shi Y, Van Parys M, Ray A, Walker A, Caminiti R, et al. Robust and comprehensive targeted metabolomics method for quantification of 50 different primary, secondary, and sulfated bile acids in multiple biological species (human, monkey, rabbit, dog, and rat) and matrices (plasma and urine) using liquid chromatography high resolution mass spectrometry (LC-HRMS) analysis. *J Am Soc Mass Spectr* 2021;32:2033–49.
- [93] Rahman AMA, Pawling J, Ryczko M, Caudy AA, Dennis JW. Targeted metabolomics in cultured cells and tissues by mass spectrometry: method development and validation. *Anal Chim Acta* 2014;845:53–61.
- [94] Cao GD, Song ZB, Hong YJ, Yang ZY, Song YY, Chen ZJ, et al. Large-scale targeted metabolomics method for metabolite profiling of human samples. *Anal Chim Acta* 2020;1125:144–51.
- [95] Yu QQ, Tian XJ, Shao LL, Li XM, Dai RT. Targeted metabolomics to reveal muscle-specific energy metabolism between bovine and during early postmortem periods. *Meat Sci* 2019;156:166–73.
- [96] Yuan M, Kremer DM, Huan H, Breitkopf SB, Ben-Sahra I, Manning BD, et al. Ex vivo and in vivo stable isotope labelling of central carbon metabolism and related pathways with analysis by LC-MS/MS. *Nat Protoc* 2019;14:313–30.
- [97] Saoi M, Percival M, Nemr C, Li A, Gibala M, Britz-McKibbin P. Characterization of the human skeletal muscle metabolome for elucidating the mechanisms of bicarbonate ingestion on strenuous interval exercise. *Anal Chem* 2019;91:4709–18.
- [98] Fiehn O. Metabolomics by gas chromatography-mass spectrometry: combined targeted and untargeted profiling. *Curr Protoc Mol Biol* 2016;114:30.4.1–32.
- [99] St Laurent R, O'Brien LM, Ahmad ST. Sodium butyrate improves locomotor impairment and early mortality in a rotenone-induced *Drosophila* model of Parkinson's disease. *Neuroscience* 2013;246:382–90.

- [100] Baert F, Matthys C, Maselyne J, Van Poucke C, Van Coillie E, Bergmans B, et al. Parkinson's disease patients' short chain fatty acids production capacity after in vitro fecal fiber fermentation. *NPJ Parkinson's Dis* 2021;7.
- [101] Su SH, Chen M, Wu YF, Lin Q, Wang DP, Sun J, et al. Fecal microbiota transplantation and short-chain fatty acids protected against cognitive dysfunction in a rat model of chronic cerebral hypoperfusion. *CNS Neurosci Ther* 2023;29:98–114.
- [102] El Manouni El Hassani S, Frerichs NM, Berkhout DJC, Nijssen T, Knobel HH, Weda H, et al. Longitudinal fecal microbiota and volatile metabolomics preceding necrotizing enterocolitis in preterm infants: a case-control study. *Sci Rep* 2025;15:10419.
- [103] Maqoud F, Calabrese FM, Celano G, Mallardi D, Goscilo F, D'Attoma B, et al. Role of increasing body mass index in gut barrier dysfunction, systemic inflammation, and metabolic dysregulation in obesity. *Nutrients* 2024;17.
- [104] Jo JK, Seo SH, Park SE, Kim HW, Kim EJ, Kim JS, et al. Gut microbiome and metabolome profiles associated with high-fat diet in mice. *Metabolites* 2021;11.
- [105] Hermeling S, Plagge J, Krautbauer S, Ecker J, Burkhardt R, Liebisch G. Rapid quantification of murine bile acids using liquid chromatography-tandem mass spectrometry. *Anal Bioanal Chem* 2025;417:687–96.
- [106] Wang YZ, Mei PC, Bai PR, An N, He JG, Wang J, et al. A strategy for screening and identification of new amino acid-conjugated bile acids with high coverage by liquid chromatography-mass spectrometry. *Anal Chim Acta* 2023;1239:340691.
- [107] Zheng S, Qin W, Chen T, Ouyang R, Wang X, Li Q, et al. Strategy for comprehensive detection and annotation of gut microbiota-related metabolites based on liquid chromatography-high-resolution mass spectrometry. *Anal Chem* 2024;96:2206–16.
- [108] Yao L, Davidson EA, Shaikh MW, Forsyth CB, Prenni JE, Broeckling CD. Quantitative analysis of short-chain fatty acids in human plasma and serum by GC-MS. *Anal Bioanal Chem* 2022;414:4391–9.
- [109] Wang YZ, Chen YY, Wu XZ, Bai PR, An N, Liu XL, et al. Uncovering the carboxylated metabolome in gut microbiota-host co-metabolism: a chemical derivatization-molecular networking approach. *Anal Chem* 2023;95:11550–7.
- [110] Fabregat-Safont D, Coadic L, Haro N, Montané-García M, Canaguier J, Mallaret G, et al. Improving the detectability of low-abundance p-cresol in biological matrices by chemical derivatization and LC-MS/MS determination. *Talanta* 2025;290:127770.
- [111] da Silva TR, Marchesan LB, Rampelotto PH, Longo L, de Oliveira TF, Landberg R, et al. Gut microbiota and gut-derived metabolites are altered and associated with dietary intake in women with polycystic ovary syndrome. *J Ovarian Res* 2024;17:232.
- [112] Cheng E, Hung SC, Lin TY. Association of trimethylamine N-oxide and metabolites with kidney function decline in patients with chronic kidney disease. *Clin Nutr* 2025;44:239–47.
- [113] Chang X, Zhang Y, Chen X, Li S, Mei H, Xiao H, et al. Gut microbiome and serum amino acid metabolome alterations in autism spectrum disorder. *Sci Rep* 2024;14:4037.
- [114] Chen SJ, Chen CC, Liao HY, Lin YT, Wu YW, Liou JM, et al. Association of fecal and plasma levels of short-chain fatty acids with gut microbiota and clinical severity in patients with Parkinson disease. *Neurology* 2022;98:e848–58.
- [115] Wang Q, Sun LN, Shi H, Ma XY, Gao W, Xu B, et al. Multi-omics analysis reveals gut microbiota-metabolite interactions and their association with liver function in autoimmune overlap syndrome. *World J Gastroenterol* 2025;31:106371.
- [116] Gan G, Luo Y, Zeng Y, Lin S, Lu B, Zhang R, et al. Gut microbiota dysbiosis links chronic apical periodontitis to liver fibrosis in nonalcoholic fatty liver disease: insights from a mouse model. *Int Endod J* 2024;57:1608–22.
- [117] Barker-Tejeda TC, Zubeldia-Varela E, Macías-Camero A, Alonso L, Martín-Antoniano IA, Rey-Stolle MF, et al. Comparative characterization of the infant gut microbiome and their maternal lineage by a multi-omics approach. *Nat Commun* 2024;15:3004.
- [118] Kure A, Tsukimi T, Ishii C, Aw W, Obana N, Nakato G, et al. Gut environment changes due to androgen deprivation therapy in patients with prostate cancer. *Prostate Cancer Prostatic Dis* 2023;26:323–30.
- [119] Gómez C, Stücheli S, Kratschmar DV, Bouitbir J, Odermatt A. Development and validation of a highly sensitive LC-MS/MS method for the analysis of bile acids in serum, plasma, and liver tissue samples. *Metabolites* 2020;10.
- [120] Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal Chem* 2006;78:779–87.
- [121] Tsugawa H, Cajka T, Kind T, Ma Y, Higgins B, Ikeda K, et al. MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. *Nat Methods* 2015;12:523–6.
- [122] Katajamaa M, Miettinen J, Oresic M. MZmine: toolbox for processing and visualization of mass spectrometry based molecular profile data. *Bioinformatics* 2006;22:634–6.
- [123] Ludwig M, Fleischauer M, Dührkop K, Hoffmann MA, Böcker S. De Novo molecular formula annotation and structure elucidation using SIRIUS 4. *Methods Mol Biol* 2020;2104:185–207.
- [124] Nothias LF, Petras D, Schmid R, Dührkop K, Rainer J, Sarvepalli A, et al. Feature-based molecular networking in the GNPS analysis environment. *Nat Methods* 2020;17:905–8.
- [125] Zhu Y, Shui X, Liang Z, Huang Z, Qi Y, He Y, et al. Gut microbiota metabolites as integral mediators in cardiovascular diseases (Review). *Int J Mol Med* 2020;46:936–48.
- [126] Rahman MN, Diantini A, Fattah M, Barliana MI, Wijaya A. A highly sensitive, simple, and fast gas chromatography-mass spectrometry method for the quantification of serum short-chain fatty acids and their potential features in central obesity. *Anal Bioanal Chem* 2021;413:6837–44.
- [127] Wang Q, Chen C, Zuo S, Cao K, Li H. Integrative analysis of the gut microbiota and faecal and serum short-chain fatty acids and tryptophan metabolites in patients with cirrhosis and hepatic encephalopathy. *J Transl Med* 2023;21:395.
- [128] Xie T, Fan X, Pang H, Zang T, Wu N, Liu J, et al. Association between gut microbiota and its functional metabolites with prenatal depression in women. *Neurobiol Stress* 2024;28:100592.
- [129] Nakatsutsumi K, Morishita K, Costantini TW, Adachi T, Suekane A, Suzuki K, et al. Analysis of lipid metabolites derived from gut microbiota in ischemia-reperfusion model. *J Trauma Acute Care Surg* 2024;96:542–7.
- [130] Aoyama K, Yamamura R, Katsurada T, Shimizu T, Takahashi D, Kondo E, et al. Decreased fecal nicotinamide and increased bacterial nicotinamidase gene expression in ulcerative colitis patients. *Inflamm Bowel Dis* 2025;31:2815–25.
- [131] Bai X, Wei H, Liu W, Coker OO, Gou H, Liu C, et al. Cigarette smoke promotes colorectal cancer through modulation of gut microbiota and related metabolites. *Gut* 2022;71:2439–50.
- [132] Su X, Zhang M, Qi H, Gao Y, Yang Y, Yun H, et al. Gut microbiota-derived metabolite 3-iodoacetic acid together with LPS induces IL-35(+) B cell generation. *Microbiome* 2022;10:13.
- [133] Yang J, Wei H, Zhou Y, Szeto CH, Li C, Lin Y, et al. High-fat diet promotes colorectal tumorigenesis through

- modulating gut microbiota and metabolites. *Gastroenterology* 2022;162:135–49.e2.
- [134] Sugimura N, Li Q, Chu ESH, Lau HCH, Fong W, Liu W, et al. *Lactobacillus gallinarum* modulates the gut microbiota and produces anti-cancer metabolites to protect against colorectal tumourigenesis. *Gut* 2021;71: 2011–21.
- [135] Wang H, Liu J, Wu Z, Zhao Y, Cao M, Shi B, et al. Gut microbiota signatures and fecal metabolites in post-menopausal women with osteoporosis. *Gut Pathog* 2023;15:33.
- [136] Muratsu A, Ikeda M, Shimizu K, Kameoka S, Motooka D, Nakamura S, et al. Dynamic change of fecal microbiota and metabolomics in a polymicrobial murine sepsis model. *Acute Med Surg* 2022;9:e770.