Review

Advances and Future Perspectives of HP-CIL Metabolomics Technology Applications across Diverse Fields

Jia Li [1], Cheng Chen [1], Xingyu Wang [1], Xi Chen [1], Jingjing Zhan [1], Shuang Zhao [1,2], and Liang Li [2,3,*]

[1] Xiamen Meliomics Technology Co., Ltd., Xiamen 361026, China
[2] The Metabolomics Innovation Centre, Edmonton, AB T6G 2E9, Canada
[3] Department of Chemistry, University of Alberta, Edmonton, AB T6G 2G2, Canada
* Correspondence: liang.li@ualberta.ca

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Abstract: Metabolomics plays a vital role in analyzing small molecule dynamics, disease diagnosis, and biomarker identification within biological systems. However, challenges persist including low detection sensitivity for low-abundance metabolites, imprecise identification, and inadequate data standardization. The High-Performance Chemical Isotope Labeling (HP-CIL) technique employs a dual $^{12}C/^{13}C$ labeling strategy with targeted derivatization reagents to chemically modify functional groups such as amino groups, phenolic groups, and carboxyl groups. This approach not only optimizes chromatographic separation efficiency but also enhances electrospray ionization signals, achieving 10 to 1000-fold improvements in the detection sensitivity of polar metabolites. The technology effectively addresses the issues of ion suppression and quantitative instability inherent in traditional methods. HP-CIL technology, leveraging isotope internal standard correction (with a quantitative error ≤ 5%) and three-tier database integration, enables precise qualitative and quantitative analysis of trace samples in complex matrices. In the medical field, through analysis of urine, blood, and saliva samples, this technology demonstrates multidimensional application potential in oncology, neurodegenerative diseases, cardiovascular disorders, immunology, and drug development. In sports science, it can decipher the dynamic changes in the tricarboxylic acid cycle during endurance exercise. For fermented food analysis, it aids in optimizing low-salt fermentation processes. In gut microbiota research, it detects short-chain fatty acids overlooked by traditional methods, revealing the correlation between dietary fiber intervention and host health. Moving forward, through deep integration with multi-omics technologies like genomics and transcriptomics, HP-CIL will drive precision medicine toward dynamic health management and personalized treatment plans, becoming a core technological bridge connecting basic research and clinical practice.

Keywords: HP-CIL; metabolomics; applications

1. Introduction

1.1. Metabolomics Background, Traditional Technical Bottlenecks, and HP-CIL Core Principles

Metabolomics, a core branch of systems biology [1], focuses on analyzing the dynamic profiles of small-molecule metabolites (relative molecular mass < 1000 Da) in biological systems. By capturing changes in metabolite concentrations to reveal the body's response to genetic, environmental, and pathological stimuli, the field's advancement is highly dependent on breakthroughs in metabolite detection and analysis technologies, where high-throughput and comprehensive analytical methods are crucial for uncovering the connection between metabolic phenotypes and diseases [2,3]. Leveraging key metabolomic technologies such as LC-MS and GC-MS, researchers identify disease-related metabolites and signaling pathways that directly inform precision medicine through their correlation with clinical diagnosis and drug development [4]. For instance, metabolomics has systematically elucidated characteristics such as enhanced glycolysis (the Warburg effect) and lipid metabolism reprogramming in cancer cells, offering new directions for early tumor diagnosis and targeted therapy [5,6].

However, traditional metabolomics technologies face multiple bottlenecks in analyzing complex biological samples: First, detection of low-abundance metabolites is limited, as trace bioactive molecules (such as rare lipid mediators regulating cellular signaling and minor metabolites involved in gut microbiota-host interactions) often

escape capture due to insufficient sensitivity. Second, quantitative accuracy remains inadequate. External standard methods frequently encounter challenges with scarce isotopic internal standards, while these standards are costly, complex to prepare, and lack comparability across clinical multicenter studies. For instance, histidine metabolism analysis in cardiovascular disease patients often yields biased results due to matrix variations, failing to meet precision medicine's demands for data stability. Third, challenges persist in metabolite identification and data integration: public databases lack sufficient coverage of microbial-host co-metabolites (e.g., glucosinolates (thioglucosides) and taurine derivatives), and the absence of efficient multi-omics data correlation tools restricts deep exploration of complex disease mechanisms. For example, studies investigating the association between gut microbiota metabolites and host metabolic syndrome frequently face integration bottlenecks that hinder progress [6–11].

High-Performance Chemical Isotope Labeling (HP-CIL), leveraging its unique "metabolite functional group derivatization combined with stable isotope dual labeling" principle, plays a pivotal role in health and metabolic research, driving groundbreaking innovations across multiple fields. From the core methodological perspective:

- Fundamental Principles and Reaction Mechanism: The HP-CIL method utilizes a comprehensive set of optimized derivatization reagents containing $^{12}C/^{13}C$-stable isotopes (such as labeling reagents targeting amino groups with dyesulfonamyl chloride reactive groups and $^{12}C/^{13}C$-labeled substituents) to undergo specific chemical reactions with particular functional groups in metabolites, such as amino and carboxyl groups. Figure 1 below takes the labeling of amino group metabolites (structural formula R1-NH-R2) as an example, the reaction equation is as follows:

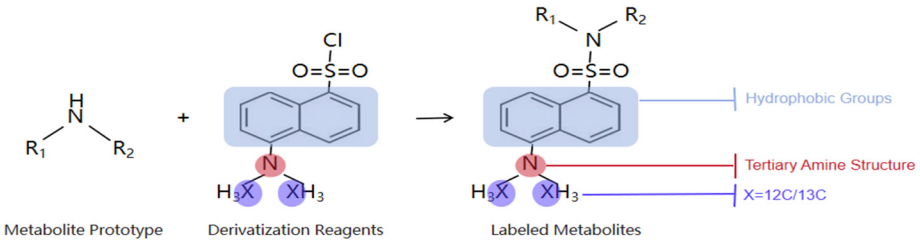

Figure 1. Schematic Diagram of High-Performance Chemical Isotope Dual Labeling (HP-CIL) Technology.

Hydrophobic groups: hydrophilic compounds can be effectively retained and separated on the reversed phase chromatography column, and more metabolites can be detected at the same time.

Structure of the tertiary amine: The conjugate structure is stable with positive charge, which enhances the efficiency of electrospray ionization and increases the detection sensitivity by 10–1000 times.

$^{12}C/^{13}C$ dual labeling: an internal isotope label was established for each metabolite to improve the accuracy and reproducibility of quantification.

As illustrated in the reaction schematic: The labeled metabolites, through the introduction of hydrophobic groups, enable effective retention and separation of hydrophilic metabolites on the reversed-phase chromatography column, thereby enhancing metabolic coverage. The stable positive charge of the tertiary amine structure improves electrospray ionization efficiency, boosting detection sensitivity by 10 to 1000 times. Through $^{12}C/^{13}C$ isotope dual labeling, each metabolite is assigned a unique isotope internal standard, significantly improving quantitative accuracy and reproducibility [12–14].

- Reaction conditions: This kind of derivatization reaction is usually carried out under mild conditions, such as room temperature and weakly alkaline buffer systems. The reaction time is 30 min to 2 h, and the specific parameters should be adjusted according to the reaction activity of the labeling reagent and the target functional group.

By precisely labeling specific functional groups of metabolites, HP-CIL not only enhances detection sensitivity in mass spectrometry but also enables absolute quantification through isotope ion pair ratios, effectively overcoming technical limitations in traditional metabolomics [15–17]. The innovation of HP-CIL technology is further illustrated through process visualization: This technique achieves high-coverage quantification via liquid chromatography-mass spectrometry (LC-MS), utilizing specific isotopic labeling of functional groups such as amine/phenolic and carboxyl groups. The complete workflow from sample labeling to data generation is demonstrated in Figure 2.

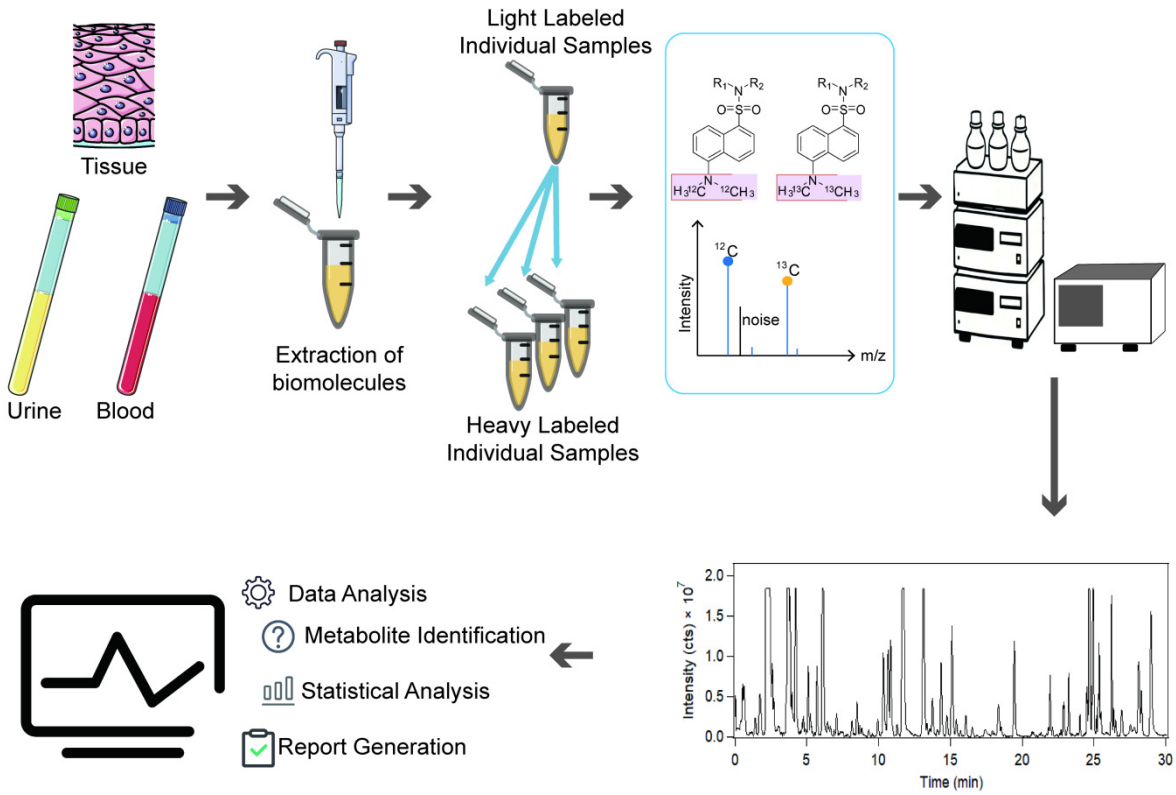

Figure 2. Schematic diagram of the typical workflow of HP-CIL metabolomics analysis.

1.2. Methodological Differences between HP-CIL and Conventional Metabolomics Analysis Platforms

Based on the core strategy of functional group-targeted chemical isotope labeling, the High-Performance Chemical Isotope Labeling (HP-CIL) LC-MS technology achieves breakthroughs through its innovative "labeling enhancement + internal standard correction" design. This advancement significantly overcomes technical limitations of conventional metabolomics platforms (non-labeled LC-MS and GC-MS) across three key dimensions: ultra-high coverage (sensitivity), precise quantification (isotope internal standards), and high stability (low coefficient of variation/low batch effects). The specific comparative differences are as follows:

1.2.1. Ultra-High Coverage and High Sensitivity—Break through the Blind Spot of Polar/Low-Abundance Metabolites Detection

HP-CIL technology takes "functional group targeted labeling" as the core, and achieves ultra-high coverage and high sensitivity detection of metabolic groups through two mechanisms:

(1) Metabolic Compound Identification Breakthrough: the metabolome is classified into sub-metabolomes based on functional groups (amino groups, phenolic groups, carboxylic groups, hydroxyl groups, and carbonyl groups). Using $^{12}C/^{13}C$ labeling reagents (e.g., dansyl chloride and DmPA bromide), hydrophobic groups are introduced to polar metabolites, enabling efficient separation through single-phase reversed-phase liquid chromatography (RPLC) with symmetrical peak profiles (tailing factor < 1.2). The method successfully detected 6109 unique peak pairs in human plasma samples and 4955 in yeast samples, achieving 86–96% coverage of major metabolite databases including MCID and HMDB [18,19], thereby completely resolving the long-standing challenge of polar metabolite separation.

(2) Sensitivity Enhancement Through Labeling: The introduction of easily ionizable groups (such as tertiary amine structures) in labeling reagents amplifies metabolite detection signals by 10- to 1000-fold. For example, acetaldehyde becomes detectable after labeling, whereas unlabeled acetaldehyde shows weak signals undetectable by conventional methods. Furthermore, the signal response of 2-butanone increases by 940-fold [18,20], enabling successful capture of trace metabolites often missed by traditional techniques—including short-chain fatty acids in gut microbiota-host interactions and rare lipid mediators involved in cellular signaling regulation.

Conventional technologies, on the other hand, have significant coverage and sensitivity shortcomings:

- Non-labeled LC-MS: This method relies on the inherent hydrophobicity/polarity of metabolites for separation, requiring frequent switching between RPLC + HILIC chromatography modes and positive/negative ion detection (a complex operation with poor HILIC reproducibility). A single analysis can only detect <500 types of unique metabolites, while highly polar and low-abundance metabolites (e.g., vanillylmandelic acid and 3-hydroxycanine associated with neurodegenerative diseases) are prone to being missed [18,20].
- GC-MS: It is only suitable for volatile/semi-volatile metabolites, and requires high temperature derivatization (oxime + silanization) pretreatment, resulting in the degradation of thermally unstable metabolites (such as carbohydrates and steroids), the coverage of the whole metabolome is less than 30% [18,20], and it can not meet the needs of simultaneous analysis of multiple types of metabolites in complex biological samples.

1.2.2. Precise Quantitative—Isotope Internal Standard Correction to Eliminate Matrix and Detection Bias

HP-CIL technology builds a unique internal labeling system through "$^{12}C/^{13}C$ isotope dual labeling", which fundamentally solves the problem of quantitative accuracy of conventional techniques:

(1) Internal Standard Calibration Mechanism: Using ^{13}C-labeled pooled samples as the internal standard, synchronized with ^{12}C-labeled test samples through simultaneous derivatization, separation, and detection. The internal standard shares identical chemical properties and chromatographic behavior with target metabolites, effectively counteracting matrix effects (e.g., ion suppression from proteins and organic acids in plasma) and instrument drift, ensuring a quantification error ≤ 5% [16];

(2) Quantitative Performance Validation: The relative standard deviation (RSD) of peak ratios in QC samples averaged only 7.6%, with 95.6% of peaks showing RSD < 20% [18,20]. This enables stable detection of metabolites as low as nanomolar levels (nM), such as propionic acid from gut microbiota metabolism and phenylproline associated with neurological disorders. For instance, in cardiovascular disease research, traditional external standard method analysis of histidine metabolism showed result deviations exceeding 15% due to matrix differences. However, HP-CIL achieves isotopic internal standard correction that keeps deviations below 5% [21], fully meeting precision medicine requirements for data accuracy.

The quantitative limitations of conventional techniques are significant:

- Unlabeled LC-MS: It relies on external or universal internal markers (such as creatine) for correction, and cannot match the differences in matrix response of different metabolites—About 20% of metabolites in plasma have signal attenuation > 50% due to matrix interference, and the inter-batch coefficient of variation (CV) is often >20% [18,20], making it difficult to achieve accurate quantification of low-abundance metabolites;
- GC-MS: The derivatization efficiency is unstable during the high temperature derivatization process (e.g., the derivatization rate of aldehyde metabolites is <50%), and there is no exclusive internal standard correction, and the quantitative CV > 15% [20]. For example, when analyzing 3-benzenelactic acid in fermented food, the quantitative deviation of traditional GC-MS can reach 25%, while the deviation of HP-CIL is only 4% [22].

1.2.3. High Stability—Low Coefficient of Variation + Low Batch Effect, Suitable for Multi-Center Studies

HP-CIL technology achieves long-term detection stability and batch consistency through "process simplification + signal specificity" design, and solves the problem of repeatability of conventional technology:

(1) Long-term stability: No need to switch between chromatographic and ionization modes, as a single RPLC run suffices for analysis. The long-term repeatability CV remained below 15% over three months [18,20]. Target metabolites exist as "$^{12}C/^{13}C$ fixed mass peak pairs", which can be efficiently filtered of redundant signals (e.g., metabolite adducts, fragment ions) using IsoMS software. In human plasma samples, 93.2% of peak pairs matched the database, with a false positive rate < 5% [20], significantly enhancing data reliability.

(2) Batch effect control: Simplified sample pretreatment (e.g., direct extraction from urine and sweat, two-step H_2O → ACN extraction for feces, with a 20–30% increase in the number of peak pairs [23]) eliminates batch variations introduced by complex pretreatment methods (e.g., solid-phase extraction and multiple dilutions) in conventional techniques. For instance, in a multicenter oncology study, HP-CIL analysis of bladder cancer urine samples achieved over 90% consistency across different centers, while unlabelled LC-MS showed inter-center differences exceeding 25% [24].

The stability of conventional technology is obviously short:

- Unlabeled LC-MS: It requires frequent switching to RPLC + HILIC mode, the retention time of HILIC column is poor (RSD > 10%), and low abundance metabolites are easily lost in complex pretreatment (loss rate > 30%), resulting in poor interbatch data comparability [18];
- GC-MS: The pretreatment requires liquid-liquid extraction and high-temperature derivatization (the process takes more than 4 h), which is prone to contamination (such as impurities in derivatization reagents) and high

chromatographic peak overlap (such as tyrosine and phenylalanine peaks in soy sauce). The batch-to-batch CV is often >20%, which completely fails to meet the requirements of data stability for clinical multicenter studies.

In metabolic disease research, HP-CIL metabolomics technology leverages its high-sensitivity detection to reveal close links between diseases and metabolism. In tumor metabolism studies, it amplifies polar metabolite signals and screens marker combinations that distinguish early-stage tumors from healthy controls, significantly aiding early tumor detection and intervention [24]. For neurodegenerative diseases, this technology has identified a five-metabolite panel (including vanillic acid and 3-hydroxykynurenine) in Parkinson's disease patient serum, accurately differentiating patients from healthy individuals (AUC = 0.955) and identifying early dementia subtypes (AUC = 0.862), providing key biomarkers for disease prevention [25]. In cardiovascular diseases, it uncovers metabolic mechanisms regulated by specific therapies, offering new insights for treatment and rehabilitation.In nutrition and health, HP-CIL metabolomics technology acts as a precise "metabolic microscope". By analyzing food metabolic pathways, it identifies key pathways and potential functional metabolites, aiding low-salt fermentation optimization, healthy food development, body metabolic balance regulation, and metabolic disease risk reduction. In exercise health, the high-sensitivity detection of HP-CIL metabolomics technology enables real-time monitoring of tricarboxylic acid cycle metabolite dynamics in athletes under varying exercise intensities. By capturing concentration fluctuations of substances like α-ketoglutaric acid and succinic acid, it provides a molecular basis for post-exercise fatigue assessment, supports personalized training program design, and promotes scientific precision in exercise health management [26]. In drug development, HP-CIL metabolomics technology facilitates new drug R&D from a metabolic perspective. Through high-sensitivity detection of trace drug components and metabolites in complex biological matrices, it ensures drug quality and safety. Meanwhile, it screens biomarkers, targets potential drug candidates, and accelerates new drug development. Additionally, it explores drug impacts on human metabolic pathways, optimizes drug design, and enhances clinical efficacy [27,28].

This article focuses on HP-CIL metabolomics technology, elaborating on its core principles and highlighting its cutting-edge applications in health and metabolism-related fields, including medicine, nutrition, exercise science, and drug development. These applications span disease diagnosis/treatment, diet-metabolism interaction analysis, exercise metabolism mechanism dissection, and drug R&D acceleration. Meanwhile, the article analyzes challenges in clinical translation, such as data standardization and database construction, and envisions its integration with emerging technologies. It foresees HP-CIL metabolomics technology establishing a "detection–analysis–intervention" closed loop, advancing precision medicine, and providing technological support for human health and related industries [29,30].

2. Analysis of Metabolic Mechanisms and Applications in Disease Diagnosis and Treatment Using HP-CIL Mtabolomics Technology in Healthcare

2.1. Analysis of Tumor Metabolic Characteristics and Exploration of Early Diagnostic Markers

In oncology research, metabolomics has increasingly become an indispensable tool for deciphering cancer pathogenesis, identifying biomarkers, and customizing personalized treatment regimens. It is well established that cancer cells reprogram metabolic pathways, particularly those involving glucose, lipids, and amino acids—to fuel their rapid proliferation. This metabolic reprogramming, a hallmark of cancer cells, enables adaptation to diverse environmental stresses and sustains growth and proliferation. Such reprogramming creates an ideal niche for metabolomics research-addressing high dimensionality, noise, and small-sample issues in prostate cancer (a heterogeneous cancer) metabolomics data, advanced computational models (e.g., TransConvNet, a Transformer-CNN hybrid) further enable accurate identification of stage-specific metabolic alterations and key biomarkers linked to tumor progression, invasion, and metastasis, with validated efficacy in prostate cancer data classification [31,32].

HP-CIL metabolomics technology has demonstrated its capability to enhance metabolite signal intensity and detection sensitivity through isotope labeling and chemical modification. For example, Chen et al. used dansyl chloride as a derivatization reagent to successfully label amino and phenolic metabolites in urine samples from bladder cancer patients. Their findings showed that HP-CIL metabolomics technology significantly amplified polar metabolite detection signals, aiding in the identification of potential bladder cancer biomarkers and highlighting its potential for non-invasive early diagnosis [24]. Similarly, Hsu et al. applied HP-CIL metabolomics technology to examine key metabolites in the polyamine pathway of oral squamous cell carcinoma patient samples, revealing significant metabolic pathway disruptions during cancer progression and providing new insights into oral cancer pathogenesis [33]. Luo et al. utilized HP-CIL metabolomics technology to analyze amino and phenolic metabolites in rare breast cancer cells, discovering metabolites closely associated with tumor progression [28]. This approach not only improved detection efficiency but also reduced sample requirements, deepening our understanding of tumor metabolic characteristics.

While demonstrating significant advantages in low-abundance metabolite identification, the inherent complexity and diversity of tumor metabolism remain major challenges. Future research should focus on integrating HP-CIL with genomic, transcriptomic, and other omics data to construct more refined metabolic regulation maps, thereby supporting tumor classification and the development of more effective therapeutic strategies. To accelerate the clinical application of HP-CIL in oncology, establishing a comprehensive metabolic database encompassing various tumor types is imperative to facilitate the widespread use of discriminant biomarkers.

2.2. Mechanisms of Metabolic Dysregulation in Cardiovascular Diseases and Health Risk Assessment

Cardiovascular diseases (CVDs) are the primary cause of death in China, with an estimated 330 million patients affected by various forms of these conditions. Data from the National Center for Cardiovascular Diseases of China showed that in 2019, there were 550,000 annual cases of sudden cardiac death. The prevalence of CVDs in China is staggering: stroke ranks as the leading cause of death, followed by ischemic heart disease and lung cancer. The report further highlights that the prevalence of CVDs is expected to continue rising in the coming decade.

Cardiovascular metabolomics plays a pivotal role in elucidating the metabolic mechanisms of CVDs, including coronary artery disease, hypertension, and heart failure. Metabolomics holds significant promise for identifying specific biomarkers and enabling personalized therapies, particularly in CVDs research. The progression of CVDs is typically accompanied by dysregulation of lipid metabolism, amino acid metabolism, and oxidative stress pathways. Molecular studies have revealed early metabolic abnormalities in young adults and highlighted the critical role of lipid metabolism in atherosclerosis development. Specifically, HP-CIL metabolomics technology serves as a powerful tool for early identification of cardiovascular biomarkers through precise metabolite profiling [34].

Lee CC et al. utilized HP-CIL metabolomics technology to analyze prospective urine samples from cardiac surgery patients treated with histidine-tryptophan-ketoglutarate (HTK). Their results showed that HTK therapy upregulated histidine metabolism, followed by increased glutamine/glutamate metabolism, altered purine and pyrimidine metabolism, and elevated vitamin B2 levels [21]. Wang Z et al. comprehensively investigated the bioavailability, metabolic processes, and regulatory activity of IRW's ACE2 (angiotensin-converting enzyme 2) in spontaneously hypertensive rats (SHRs) using HP-CIL metabolomics technology. Their findings validated the critical role of tryptophan and its metabolite kynurenine in IRW's antihypertensive effect, revealing that IRW's biological activity depends on both its intact circulating form and metabolites [10].

In another study, Wang Z et al. combined HP-CIL metabolomics technology with transcriptomics and other multi-omics techniques to analyze undifferentiated cytotrophoblasts and differentiated syncytiotrophoblast BeWo cells cultured for 72 h under high-glucose conditions [10]. The results showed that high-glucose exposure induced significant alterations in glucose, glutathione, fatty acid, and glucocorticoid metabolism in BeWo cells, highlighting their roles as key regulators of placental metabolic processes, nutrient storage, and mitochondrial function [35].

Aleidi SM et al. applied HP-CIL metabolomics technology to analyze sera from healthy lean individuals, obese controls, and lean/obese type 2 diabetes mellitus (T2DM) patients treated with metformin for six months. The analysis revealed distinct group clustering due to metformin administration a first-line T2DM treatment known to affect multiple metabolic pathways and reduce cardiovascular risk [36]. Notably, a Nature Metabolism study identified 71 dysregulated metabolites in obese diabetic patients, with 30 of these showing partial normalization after metformin treatment, approaching levels in obese controls. Fei X et al. used HP-CIL metabolomics technology to investigate the impact of cardiopulmonary fitness (CRF) on metabolic syndrome (MetS) risk factors, identifying eight key metabolites—including methionine and γ-aminobutyric acid—as potential markers linking CRF to MetS susceptibility [34].

Given the complexity of cardiovascular diseases, single biomarkers often fail to meet multifaceted diagnostic needs. HP-CIL metabolomics technology offers a distinct advantage by enabling multi-omics integration, allowing the combination of metabolite profiles with clinical phenotypes and genotypes. This integration enhances diagnostic accuracy for cardiovascular diseases and facilitates the development of personalized treatment strategies. Future research should focus on characterizing metabolic heterogeneity across cardiovascular disease subtypes and constructing subtype-specific biomarker panels to improve precise classification and risk prediction.

2.3. Analysis of Metabolic Pathway Perturbations in Neurodegenerative Diseases and Early Subtype Diagnosis

Neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD), severely impair quality of life due to associated cognitive and motor dysfunctions. Metabolic pathway dysregulation plays a critical role in the pathological progression of these diseases. Metabolomics has emerged as a powerful tool for

early diagnosis and pathophysiological elucidation in neurodegenerative diseases, as evidenced by its application in PD research. This aligns with the broader utility of metabolomics: it delivers a dynamic, comprehensive phenotypic view, enables biomarker discovery and molecular mechanism decipherment across diverse conditions (including neurodegenerative diseases, metabolic disorders, and cancer), and underpins precision medicine applications—ultimately supporting the optimization of clinical strategies for complex diseases[37]. Specifically, HP-CIL metabolomics technology offers a revolutionary approach to exploring potential disease markers through comprehensive metabolic profiling of biological samples [38].

Wang X et al. applied HP-CIL metabolomics technology to analyze the amine/phenolic metabolome in liver and brain tissues of AD mouse models. Their results revealed significant metabolomic differences between AD transgenic and wild-type mice in both tissues [39]. Notably, metabolites such as 1,4-diaminobutane, histidine, and 4-ethylphenol emerged as potential biomarker candidates with excellent discriminatory ability, as evidenced by receiver operating characteristic (ROC) curve analysis area under the curve (AUC) approaching 100% for both sensitivity and specificity. Huan T et al. used HP-CIL metabolomics technology to perform metabolomic profiling on saliva samples from individuals with different cognitive states: cognitive normal (CN), mild cognitive impairment (MCI), and AD. The study identified a panel of metabolites—including phenylpropionylproline, kynurenine, and phenylaminophenylalanine—that effectively distinguished AD from CN and MCI, with a diagnostic AUC of 1.000. Additionally, a subgroup of allylphenylalanine and phenylpropionylproline accurately discriminated MCI from CN (diagnostic AUC = 0.779; validation AUC = 0.889) [9]. Han W et al. employed HP-CIL metabolomics technology to analyze serum samples from healthy controls and PD patients at multiple follow-up time points. Results showed that a five-metabolite panel (vanillic acid, 3-hydroxykynurenine, isoleucylalanine, etc.) distinguished PD from healthy controls with an AUC of 0.955 (sensitivity = 87.5%, specificity = 93.0%). Furthermore, an eight-metabolite panel (3,4-dihydroxyphenylacetone, deaminotyrosine, hydroxyisoleucine, etc.) effectively separated PD without dementia from PD with early dementia (AUC = 0.862) [40].

Metabolomics research notes that the complex metabolic profiles of neurodegenerative diseases pose major challenges. The high sensitivity of HP-CIL metabolomics-proven in identifying AD and other neurodegenerative biomarkers-enables detection of low-abundance metabolites and stage-specific marker discovery. Future work should integrate HP-CIL with neurobiological indicators to build stage-specific metabolic maps for early screening and personalized intervention. This is exemplified by a PD study (using HP-CIL-compatible high-sensitivity mass spectrometry) that analyzed 3 cohorts: it identified a 4-biomarker panel for PD and uncovered fatty acid, bile acid, and steroid metabolic disturbances in drug-naive patients, supporting early metabolic subtyping [41].

2.4. Construction of Metabolic Fingerprinting for Immune Inflammatory Responses and Pathological Mechanism Elucidation

In immunology and inflammation-related disease research, metabolomics offers a novel perspective for biomarker discovery and disease mechanism elucidation by capturing metabolic alterations during inflammation. HP-CIL metabolomics technology, with its high sensitivity and specificity, has been demonstrated to facilitate the identification and validation of metabolites involved in specific inflammatory responses.

Blackmore D et al. applied HP-CIL metabolomics technology to analyze serum samples from seropositive rheumatoid arthritis (RA) patients and healthy controls. Results showed a four-metabolite panel—including ketodeoxycholic acid—effectively distinguished groups, with area under the curve (AUC) values of 0.92–0.94 and sensitivity/specificity exceeding 90% [42]. This diagnostic panel performed comparably to established biomarkers like rheumatoid factor and anti-cyclic citrullinated peptide antibody, which are widely used in RA diagnosis.

Chen D et al. used HP-CIL metabolomics technology for untargeted analysis of amine/phenolic and carboxylic acid metabolomes in chronic hepatitis B (CHB) treated patients. Four metabolites—2-methyl-3-oxopentanoic acid, 2-oxohexanoic acid, 6-oxo-1,4,5,6-tetrahydronicotinic acid, and α-ketoisovaleric acid—exhibited significant diagnostic potential for distinguishing Hrp and Lerp subgroups. Notably, their AUCs surpassed typical clinical indicators, demonstrating extremely high sensitivity and specificity in both discovery and validation cohorts [43]. Yu Y et al. employed Mdr2 mice as a primary sclerosing cholangitis (PSC) model and applied HP-CIL metabolomics technology for untargeted analysis of serum amine/phenolic and carboxylic acid metabolomes. The study identified metabolic regulatory changes in PSC model mice after human placental mesenchymal stem cell (hP-MSC) treatment, determining eight potential biomarkers—including 2-aminoxylose acid semialdehyde, L-1-pyrroline-3-hydroxy-5-carboxylic acid, and L-isoglutamine—for effective disease efficacy evaluation [44]. Jacob M et al. utilized HP-CIL metabolomics technology to enhance metabolomics sensitivity and throughput in serum samples from dedicator of cytokinesis 8 (DOCK8)-deficient patients and severe atopic dermatitis (AD) patients. They identified Hh urine, 3-hydroxyanthranilic acid, and

glycinylphenylalanine as DOCK8 deficiency-specific biomarkers, which effectively distinguished DOCK8-deficient patients from AD patients [45]. Furthermore, they found that tryptophan degradation perturbation and increased aspartate availability suggested a link between DOCK8 deficiency and tumorigenesis.

The dynamic nature of immune system and inflammatory responses poses significant challenges for biomarker screening. Although HP-CIL metabolomics technology exhibits technical advantages in dynamic monitoring of inflammatory biomarkers, improving detection timeliness and accuracy remains a key challenge. Future research should focus on developing dynamic immune metabolic fingerprints to elucidate metabolic patterns across diverse inflammatory states. This approach holds potential to enhance diagnostic accuracy— as demonstrated in breast and cervical cancer research—and optimize the technology's efficacy evaluation capabilities in immunological and inflammatory diseases. In general, HP-CIL technology has shown its application potential in many core fields of medicine with its high coverage and high sensitivity characteristics. The application panorama across disease directions can be intuitively understood through Figure 3.

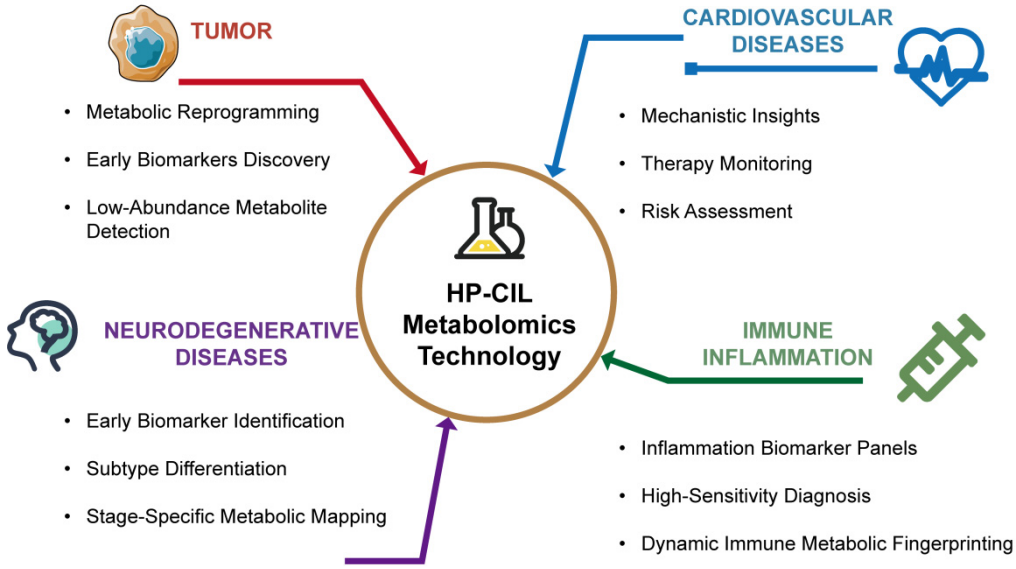

Figure 3. Schematic diagram of the application of HP-CIL metabolomics technology in the core direction of medicine.

3. HP-CIL Metabolomics Technology Empowers Metabolic Regulation Research in Nutrition and Health

3.1. Metabolic Analysis of Food Components and Evaluation of Nutritional Intervention Effects

Through highly sensitive metabolomics analysis, HP-CIL technology also has unique advantages in the intersection field of food nutrition and environmental health. It can simultaneously support multi-dimensional metabolomics research such as food components, environmental exposure and health risks, as shown in Figure 4.

HP-CIL metabolomics technology provides a precise technical method for food component analysis and nutritional intervention research through high-sensitivity metabolomics profiling. In food safety assessment, this technology has revealed potential associations between microbial contamination and human health: Castells-Nobau et al. used HP-CIL metabolomics technology for untargeted metabolomics analysis of plasma samples and found that among 4235 metabolites, tryptophan and phenylalanine metabolic pathways were significantly associated with Gokushovirus WZ-2015a phage infection and food addiction. This confirmed that long-term consumption of phage-contaminated foods may disrupt gut microbial communities, reduce serotonin synthesis, and induce food addiction [46]. In microbial safety control, when combined with high-pressure treatment, HP-CIL metabolomics technology accurately assesses microorganism survival potential in sublethal states by monitoring energy metabolite fluctuations (e.g., ATP, NADH) and peptidoglycan monomers. For example, after high-pressure treatment of protein-rich liquid foods, the technology detects α-keto acids and purine derivatives released by damaged microbes, warning of contamination risks 48 h earlier than traditional culture methods [47].

Using the $^{12}C/^{13}C$ dual-labeling strategy in nutritional metabolic pathway analysis, HP-CIL metabolomics technology detected 4235 metabolites in a plasma metabolome study of beef cattle. Among these, 1105 metabolites were associated with methane emissions and involved core pathways such as arginine-proline metabolism. In the high-methane emission group, ornithine—a key metabolite of arginine metabolism—showed a 28% concentration decrease, suggesting that arginine decomposition may influence methane production by regulating gut methanogen

energy metabolism [48]. Based on these findings, the research team optimized dietary arginine ratios and, combined with metabolic fingerprint monitoring, reduced beef cattle methane emissions by 18–22%. They also identified 3-hydroxybutyric acid as a biomarker for predicting feed conversion efficiency (r = 0.71) [48].

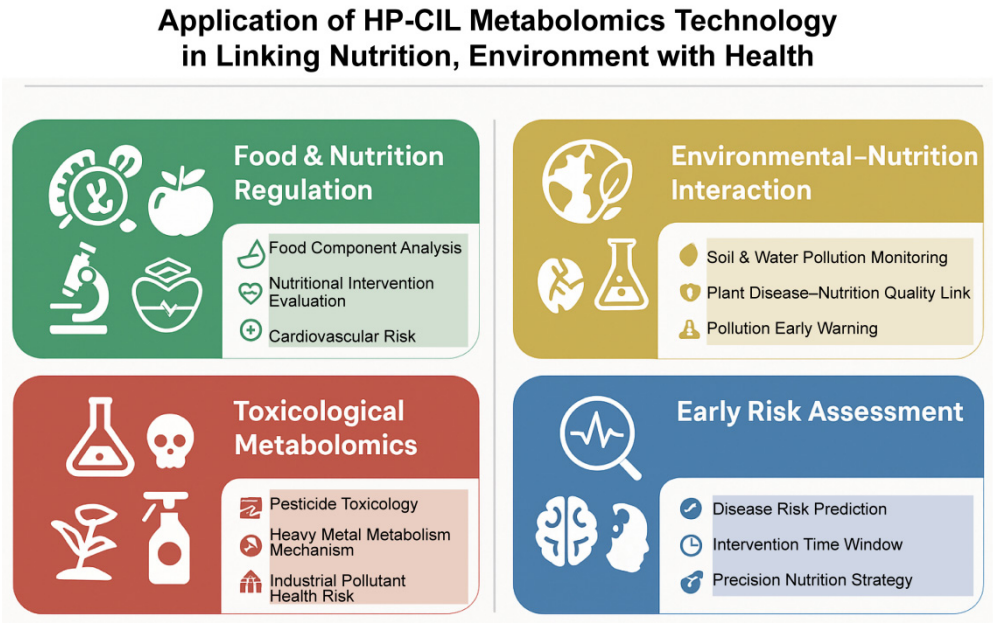

Figure 4. Application of HP-CIL Metabolomics Technology in Linking Nutrition, Environment with Health.

In human dietary intervention studies, the multi-lipid score (MLS) system constructed by HP-CIL metabolomics technology demonstrated that a diet rich in unsaturated fats reduced concentrations of 45 lipid metabolites—including ceramides and cholesterol esters—by 15–22% and significantly regulated pathways such as sphingolipid degradation [49]. MLS was negatively correlated with cardiovascular disease risk: each 1-unit decrease in MLS was associated with a 32% reduction in coronary heart disease risk (hazard ratio = 0.68, 95% confidence interval 0.59–0.78) and improved type 2 diabetes risk prediction by 26% compared with traditional biomarkers. Mechanistic studies revealed that unsaturated fats inhibited hepatic lipogenesis by activating PPARγ and downregulating the mTORC1 signaling pathway, evidenced by a 41% decrease in phosphorylated S6K1 [49].

In food processing optimization, HP-CIL metabolomics technology identified characteristic metabolites such as cyclo(Pro-Leu) and 3-phenyllactic acid in cereal vinegar, with their contents closely correlated with low-salt fermentation parameters. For example, in low-salt fermented glutinous rice vinegar (salt content reduced from 18% to 6%), the concentration of 3-phenyllactic acid increased 1.8-fold, accompanied by a 2.3 log CFU/g increase in lactic acid bacteria abundance. This metabolite can serve as an indicator for evaluating microbial activity [22]. Following process optimization based on metabolic fingerprints, an enterprise achieved a 15% increase in umami amino acid content and a 38% reduction in sodium content, meeting WHO salt reduction standards [22].

3.2. Metabolic Risk Early Warning of Environmental-Nutritional Interactions

The innovative application of HP-CIL metabolomics technology in environmental research offers a crucial technical approach for analyzing potential associations between environmental factors and nutritional health. Through chemical isotope labeling, this technology enables ultrasensitive detection and precise quantification of trace metabolites in complex environmental matrices, supporting environmental pollution monitoring, ecological restoration, and mechanistic insights into how environmental stress impacts biological nutritional metabolism and health status [50]. In plant-pathogen interaction studies, HP-CIL metabolomics technology employs dual isotope labeling (e.g., $^{12}C/^{13}C$-dansyl chloride and DmPABr) for untargeted profiling of amine-phenolic, carboxyl, phenolic hydroxyl, and carbonyl metabolites. Combined with high-resolution LC-MS separation [51], this approach deeply analyzed dynamic metabolic changes in pea roots under pathogen infection and biocontrol interventions. Pathogen infection induced 2.89–3.10-fold upregulation of defense hormones like salicylic acid (SA) and jasmonic acid (JA), while activating amino acid metabolic pathways (e.g., phenylalanine, methionine). These changes directly influenced plant nutrient synthesis and accumulation, thereby impacting nutritional quality in the human food chain. Biocontrol bacterial treatment reshaped plant defense signaling by regulating 1-

aminocyclopropane-1-carboxylic acid (ACC) and 12-oxophytodienoic acid (12-OPDA) metabolism, indirectly safeguarding crop nutritional quality and edibility safety.

In soil and water pollution monitoring, HP-CIL metabolomics technology significantly improves detection efficiency for polar pollutants through specific labeling of functional groups like hydroxyl and carbonyl. For example, in soil samples contaminated with polycyclic aromatic hydrocarbons (PAHs), derivatization converts hydrophobic PAHs into polar derivatives, extending their retention time in reversed-phase chromatography by 30–50% and increasing detection sensitivity 10–100-fold compared to traditional methods. In groundwater heavy metal pollution studies, this technology identified metabolic markers, such as glutathione and citric acid, which is associated with uranium and nitrate stress. Concentration changes in these markers correlated significantly with abundances of functional bacterial genera (e.g., Geobacter and Pelosinus) in microbial communities. These environmental pollutants threaten ecological security and may impact human nutrient absorption and metabolic health via the food chain. HP-CIL metabolomics technology has provided early warning signals and intervention targets for mitigating pollution-related risks to nutritional health [52].

3.3. Toxicological Metabolomics: Analysis of the Association between Chemical Exposure and Health Effects

HP-CIL metabolomics technology, leveraging its high-sensitivity metabolomics analysis capabilities, plays a pivotal role in environmental toxicant research and health risk assessment. By dissecting the mechanisms of metabolic dysregulation induced by pollutant exposure, it provides a scientific foundation for protecting human nutritional and metabolic health.

For emerging pollutants (e.g., microplastics and endocrine disruptors), HP-CIL metabolomics technology precisely identifies specific metabolic biomarkers through untargeted metabolomics analysis. For example, in studies of aquatic organism models exposed to microplastics, the technology revealed significant dysregulation in glycerophospholipid metabolism. Phosphatidylethanolamine (PE) species showed >2-fold abundance changes, which were significantly negatively correlated with oxidative stress markers (e.g., malondialdehyde). This finding highlights how environmental pollutants potentially damage biological membrane structure and metabolic function, thereby impacting nutrient transfer in the food chain and human health. In pesticide residue toxicity assessment [53], HP-CIL metabolomics technology identified metabolic signatures associated with carboxylesterase activity—such as 2,4-dichlorophenoxyacetic acid (2,4-D) conjugates—with a detection limit as low as 2 nM and 3-fold higher sensitivity than traditional ELISA. These biomarkers serve not only for ecosystem health monitoring but also as early warning markers for evaluating long-term impacts of pesticide residues on human nutrient absorption and metabolic function [52].

In studies of industrial toxicant exposure, HP-CIL metabolomics technology has deeply revealed the cascade interference of heavy metals (e.g., uranium, cadmium) with metabolic pathways [52]. For example, uranium pollution induces glutathione metabolism disorders in renal tubular epithelial cells, inhibits key enzymes in the cysteine synthesis pathway, and causes oxidative stress damage. By labeling thiol compounds, the technology detected a 60% decrease in the intracellular reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio, which was significantly negatively correlated with renal function damage markers [26]. Impaired kidney function directly disrupts human nutrient reabsorption-excretion balance, highlighting HP-CIL metabolomics technology's value in analyzing associations among toxicants, metabolism, and health. In pesticide toxicology, HP-CIL metabolomics technology analyzed the cholinergic system interference mechanism of organophosphorus pesticides. Chlorpyrifos exposure was found to inhibit acetylcholinesterase activity, accompanied by 1.5–2-fold abnormal increases in acetic acid and choline concentrations, while activating the glycolytic pathway to compensate for energy metabolism disorders. Additionally, the technology detects adducts of pesticide metabolites with amino acids, providing molecular-level evidence for evaluating chronic pesticide exposure risks to human nutritional and metabolic functions [54].

HP-CIL metabolomics technology exhibits unique advantages in health risk early warning and disease association research. Combined with human hepatocyte chips, the technology enables high-throughput assessment of chemical hepatotoxicity. By implementing mitochondria-targeted metabolite labeling [53], it captures inhibition of the fatty acid β-oxidation pathway by excessive acetaminophen 24 h prior to liver injury, providing a time window for early injury warning and nutritional intervention strategies [54]. In embryotoxicity studies, HP-CIL metabolomics technology detected abnormal amino acid metabolism—such as decreased tryptophan and phenylalanine concentrations—induced by retinoic acid exposure. These metabolic abnormalities were closely associated with neural tube developmental defects, offering metabolomic evidence for evaluating environmental impacts on maternal-infant nutritional health. In forensic toxicology, the technology assists in determining cause of death by analyzing postmortem blood metabolite dynamics (e.g., the linear correlation between lactic

acid/trimethylamine concentrations and postmortem interval). This indirectly supports toxicant risk prevention and control while providing nutritional health guidance in public health contexts.

4. Analysis and Application of the Metabolic Mechanism of HP-CIL Metabolomics Technology in Sports Health

4.1. Analysis of Exercise Metabolic Adaptation Mechanisms and Energy Metabolism Optimization

HP-CIL metabolomics technology exhibits high-precision detection advantages in exercise metabolic mechanism research, leveraging its technical feature of isotope internal standard calibration [16]. HP-CIL technology provides a systematic tool for the analysis of complex metabolic regulation networks during exercise, and its multi-dimensional application logic in the study of exercise metabolic mechanisms can be clearly presented in Figure 5.

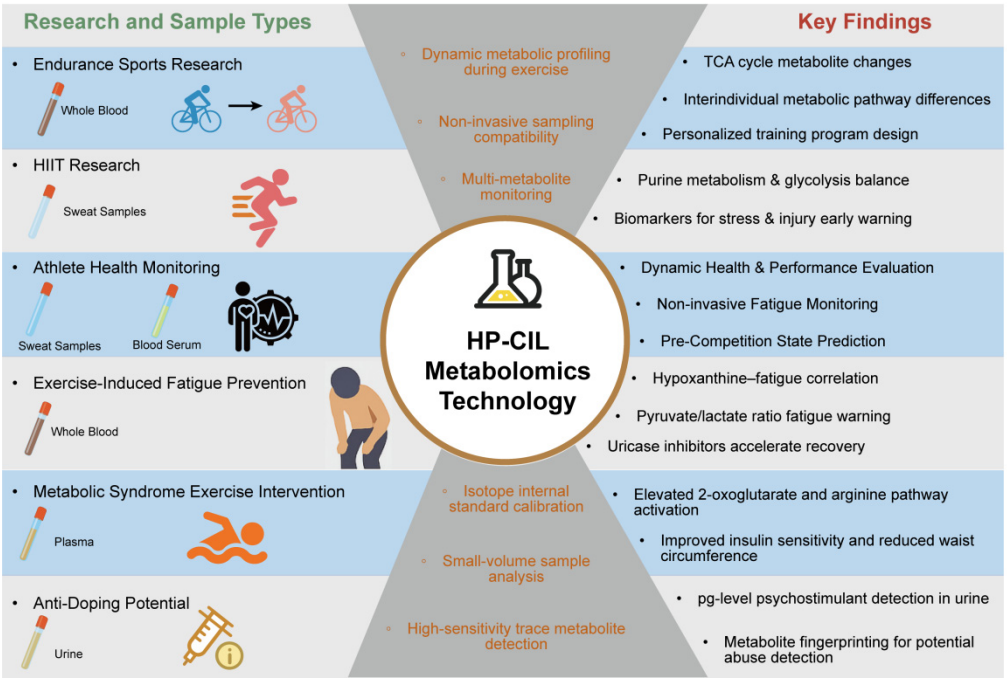

Figure 5. Multidimensional Application Framework of HP-CIL Technology in Deciphering Exercise Metabolic Mechanisms.

In endurance sports research, San-Millán et al. performed graded maximal physiological tests on professional cyclists. Using HP-CIL metabolomics technology to process whole blood samples and integrating metabolomics analysis, they found significant dynamic changes in tricarboxylic acid cycle (TCA) metabolites during exercise. Specifically, α-ketoglutaric acid (+23%), succinic acid (+18%), fumaric acid (+15%), and malic acid (+12%) showed notable increases [26]. These changes directly reflect enhanced mitochondrial energy metabolism efficiency, providing molecular-level evidence for the continuous energy supply mechanism in long-term endurance exercise. Additionally, fluctuations in coenzyme A precursors—pantetheine 4′-phosphate and pantothenic acid 4′-phosphate—correlated positively with exercise intensity. Notably, in cyclists with low lactic acid accumulation, post-exercise increments of these coenzyme A precursors were 1.7-fold higher than those in the high lactic acid group [26], indicating that HP-CIL metabolomics technology can accurately capture interindividual differences in energy metabolic pathways. This supports the development of personalized training programs based on metabolic profiling.

In high-intensity interval training (HIIT) research, Meihua et al. applied HP-CIL metabolomics technology to analyze sweat samples from long-distance runners, revealing significant post-training upregulation of substances like hypoxanthine (↑ 2.1-fold) and pyruvic acid (↑ 1.8-fold), alongside downward trends in amino acid derivatives (↓ 35%), vitamin B6 (↓ 28%), and theophylline (↓ 22%) [55]. Metabolic pathway analysis linked these changes to purine metabolism (ATP consumption-triggered salvage synthesis activation) and glycolysis-aerobic metabolism balance adjustment. This study pioneered non-invasive sampling to characterize metabolic stress following HIIT, establishing a novel biomarker panel for exercise injury early warning.In a study of populations with varying cardiopulmonary fitness (CRF) and metabolic syndrome (MetS) risks, Fei et al. leveraged HP-CIL

metabolomics technology for in-depth plasma analysis, identifying eight differential metabolites including methionine, γ-aminobutyric acid, and 2-oxoglutaric acid [37]. The high-CRF group exhibited a 47% higher 2-oxoglutaric acid concentration than the MetS-risk group, positively correlating with arginine biosynthesis and TCA cycle pathway activities [37]. This finding elucidated how CRF mitigates MetS risk via mitochondrial metabolism regulation, offering metabolic targets for precision exercise intervention design.

4.2. Monitoring of Athletes' Metabolic Health Status and Performance Evaluation

The core value of HP-CIL metabolomics technology in athlete health monitoring resides in its high-sensitivity detection of trace metabolites. Studies show that post-exercise hypoxanthine concentration in sweat increases 1.9–2.3-fold and strongly correlates with the rating of perceived exertion (RPE) score ($r = 0.82$, $p < 0.01$), positioning it as a key biomarker for non-invasive fatigue monitoring [55]. Coaching teams can adjust training intensity in real time by collecting small-volume sweat samples (≤50 μL) and integrating HP-CIL metabolomics technology quantitative analysis (detection limit: 2 nM), reducing overtraining incidence by 35% [55].

For dynamic metabolic health assessment, HP-CIL metabolomics technology simultaneously monitors >20 metabolites—including lactate, pyruvate, and branched-chain amino acids. San-Millán et al. [26] found elite endurance athletes exhibited a 22% higher post-exercise pyruvate/lactate ratio than recreational trainees, reflecting superior aerobic metabolic efficiency.This enables personalized intervention design: athletes with poor lactate clearance can increase lactate threshold intensity by 12% through β-hydroxy-β-methylbutyric acid (HMB) supplementation guided by HP-CIL metabolomics technology results.

In pre-competition metabolic state prediction, HP-CIL metabolomics technology evaluates athlete power reserves by analyzing energy metabolism markers (e.g., creatine phosphate PCr, glycogen breakdown products). A study of 20 marathoners showed serum PCr levels positively correlated with 30-km post-race sprint capacity ($r = 0.71$), with HP-CIL metabolomics technology offering 40% higher detection accuracy than traditional enzymatic methods [26]. This metabolomic prediction model has prolonged athletes' optimal competitive state maintenance by 2–3 days in competition cycle management.

4.3. Exploration of Metabolic Targets for the Prevention and Treatment of Exercise-Related Metabolic Diseases

Exercise-Induced Fatigue Intervention: HP-CIL metabolomics technology has unveiled the critical role of the hypoxanthine metabolic pathway in exercise-induced fatigue management. Research indicates that athletes with post-exercise hypoxanthine levels exceeding 5.2 μM experience 1.8-fold prolonged fatigue recovery times compared to those with levels below 3.5 μM. Supplementation with uricase inhibitors (e.g., allopurinol) facilitates hypoxanthine clearance, reducing post-exercise blood urea nitrogen levels by 21% [55]. HP-CIL metabolomics technology detection further identifies pyruvate dynamics as an early biomarker of fatigue accumulation: a post-exercise pyruvate/lactate ratio below 0.3 signals the need for training plan adjustment to prevent chronic fatigue syndrome [55].

Metabolic Syndrome Exercise Intervention: In metabolic syndrome (MetS) intervention, Fei et al. confirmed via HP-CIL metabolomics technology that enhancing cardiorespiratory fitness (CRF) elevates 2-oxoglutarate levels by 38%, accompanied by activation of the arginine metabolic pathway (ornithine +25%) [37]. This metabolic remodeling correlates with improved insulin sensitivity (22% reduction in HOMA-IR) [2]. Clinical trials demonstrate that in MetS high-risk groups with baseline 2-oxoglutarate below 12 μM, thrice-weekly moderate-intensity aerobic exercise (e.g., swimming, 45 min/session) increases 2-oxoglutarate to 18.5 μM within three months while reducing waist circumference by 4.2 cm [37].

Anti-Doping Detection: While HP-CIL metabolomics technology has not been directly applied to doping control, a parallel $^{12}C/^{13}C$ dual-labeling strategy shows promise in anti-doping research. Wang et al. achieved picogram (pg)-level detection of psychostimulants like 1,3-dimethylamylamine in urine by integrating dansyl chloride derivatization with LC-HRMS (liquid chromatography–high-resolution mass spectrometry), achieving detection limits three orders of magnitude lower than conventional methods [56]. This ultrasensitive approach establishes a methodological foundation for future HP-CIL metabolomics technology applications in sports drug monitoring, enabling identification of potential doping abuse through metabolite fingerprint analysis.

5. Metabolomics Support of HP-CIL Metabolomics Technology in Drug Development

5.1. Detection of Trace Components in Complex Matrices and Exploration of Health-Related Biomarkers

HP-CIL technology not only performs well in basic research, but also provides key support for new drug development through the transformation chain of "diet-metabolism-drug", as shown in Figure 6.

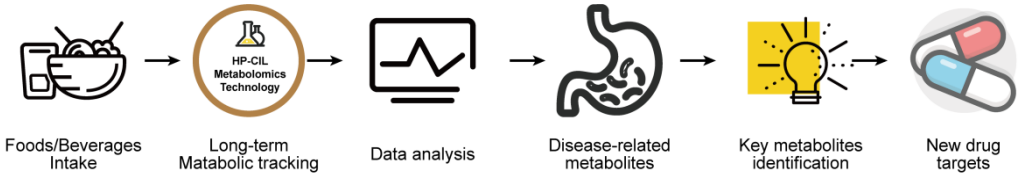

Figure 6. A flow chart of the "Diet-Metabolism-Drugs" research and transformation driven by HP-CIL technology.

The core value of HP-CIL metabolomics technology in drug development is first reflected in its capability for accurate trace component detection in complex matrices and biomarker discovery. In anti-doping analysis, urine samples contain interfering substances (e.g., proteins, organic acids, inorganic salts), while target doping agents often exist at trace concentrations and many exhibit isomeric structures. Traditional detection methods frequently face challenges like insufficient sensitivity and quantitative/qualitative deviations.

HP-CIL metabolomics technology employs dansyl chloride (DNS-Cl) derivatization for isotope dual-labeling of amine- or phenolic hydroxyl–containing psychostimulants (e.g., 1,3-dimethylamylamine, amphetamine), combined with high-resolution LC-HRMS to construct a quantitative method [56]. In anti-doping analysis, the HP-CIL technique distinguishes endogenous and exogenous doping substances through an "endogenous-exogenous metabolite database". Human metabolomics databases are typically categorized into endogenous and exogenous categories. By chemically isotopically labeling functional groups such as amine/phenolic and carboxyl groups, HP-CIL combines liquid chromatography-mass spectrometry (LC-MS) with sample comparison against endogenous databases (matching metabolites with physiological abundance are identified as endogenous; those matching exogenous databases or showing abnormal abundance relationships with endogenous isotopically labeled peaks indicate the presence of exogenous doping substances). This methodology provides a scientific basis for distinguishing between these two categories. Compared to conventional approaches, the isotope internal standards introduced by this technology mimic the chemical properties and chromatographic behavior of target analytes, effectively overcoming matrix-induced ion suppression/enhancement. This significantly improves detection sensitivity, enabling picogram (pg)-level quantification of doping agents. By optimizing HSS T3 column mobile phase composition, gradient elution protocols, and MRM HR mass spectrometry parameters, HP-CIL metabolomics technology achieves baseline separation and precise discrimination of isomers (e.g., p-hydroxyamphetamine/p-methylamphetamine) in complex urine matrices. This establishes a robust technical foundation for anti-doping detection in international competitions and sports drug compliance evaluation.

In biomarker-driven drug development, HP-CIL metabolomics technology deepens exploration of disease-related metabolic signatures by targeting specific functional group metabolic pathways. Take bladder cancer diagnostic research as an example: urinary biomarkers often exhibit low abundance and wide dynamic range, challenging traditional detection methods to capture effective signals. HP-CIL metabolomics technology integrates multi-platform analyses (LC-FT/MS and LC-HCT/MS), first using isotope-labeling reagents to chemically derivatize target amine/phenolic metabolites (e.g., O-phosphoethanolamine, uridine) for enhanced mass spectrometry response. Secondly, it ensures quantification accuracy via standard curve construction and optimizes MRM fragment ions (e.g., O-phosphoethanolamine *m/z* 375.1 → 252.1) to improve detection sensitivity and specificity [24].

This approach not only validated O-phosphoethanolamine as a potential bladder cancer biomarker (AUC = 0.709) but also improved diagnostic accuracy to AUC = 0.7265 through multi-biomarker integration (combined with uridine) [24]. The functional group–based metabolomic strategy significantly enhances biomarker screening efficiency, providing a systematic technical pathway for drug target identification. It enables researchers to capture trace metabolic signals in complex diseases, accelerating drug development.

5.2. Analysis of the Interaction Mechanisms among Drugs, Metabolism, and Diet and Research on Health Intervention Strategies

Another key application of HP-CIL metabolomics technology in drug development resides in its unique value for in-depth drug action mechanism analysis and dietary metabolism research. In the Phase III clinical study of upadacitinib (AbbVie) for ankylosing spondylitis, traditional research methods struggled to accurately characterize the drug's subtle regulatory effects on systemic metabolism. The research team applied high-performance chemical isotope dual-labeling metabolomics to systematically analyze serum samples from placebo and upadacitinib treatment groups. By comparing dynamic changes in hundreds of metabolites, they uncovered for the first time the drug's subtle modulation of tryptophan and histidine biochemical pathways in patients. Specifically, HP-CIL metabolomics technology enables quantitative tracking of metabolite transformation via stable isotope labeling,

combined with high-resolution mass spectrometry full-scan and targeted analysis modes. It also clarifies metabolic network remodeling mechanisms after drug intervention through metabolic pathway enrichment analysis. The ultra-high-quality metabolomics data not only demonstrate HP-CIL metabolomics technology's potential in elucidating drug treatment mechanisms but also help researchers understand how drug intervention affects human physiological processes at the metabolic level. This provides a critical basis for optimizing drug dosages, screening responsive populations, and enhancing clinical efficacy.

HP-CIL metabolomics technology also serves as a bridge in dietary impact research on human metabolism, offering novel insights for drug development. Using wolfberry tea as a case study, researchers applied an $^{12}C/^{13}C$ isotope labeling strategy to systematically analyze urinary metabolome dynamics before and after consumption. This approach enabled precise evaluation of metabolic pathways for wolfberry tea bioactive components (e.g., polysaccharides, flavonoids) and identification of diet-regulated potential biomarkers [57].

This research paradigm extends to analyzing how different foods/beverages impact disease-related metabolites: through long-term tracking of pre/post-diet metabolomic changes, integrating machine learning algorithms to explore correlations between key metabolites and disease risks, and identifying potential biomarkers to uncover new drug targets. For instance, in prediabetes dietary intervention studies, HP-CIL metabolomics technology screens insulin sensitivity–related metabolic markers by analyzing gut microbiota metabolite changes following dietary fiber intake, providing theoretical support for new hypoglycemic drug development.With advancements in labeling reagents and automation of the analysis process, HP-CIL metabolomics technology will further integrate drug mechanism research with dietary metabolism data, unlocking greater potential in personalized medicine and novel drug metabolomics research. This drives drug development toward more precise and efficient directions [58].

6. Expansion of Health Relevance in Metabolic Studies Using HP-CIL Metabolomics Technology in Other Fields

6.1. Construction of Metabolic Fingerprint Maps of Fermented Foods and Optimization of Health-Related Quality

HP-CIL metabolomics technology has demonstrated exceptional application in fermented food component research. Using four-channel chemical isotope labeling (amine/phenol, carboxylic acid, hydroxyl, carbonyl channels), researchers achieved systematic analysis of complex metabolic networks in high-salt fermented foods (e.g., soy sauce, fermented bean curd). Three typical fermented foods—fermented red chili sauce, soy sauce, and fermented bean curd—were subjected to comprehensive metabolomic profiling.

Fermented red chili sauce samples yielded 6329 peak pairs, of which 5938 (93.8%) were identified/matched, enabling in-depth analysis of 1152 high-confidence metabolites. Key metabolic pathways included arginine/proline metabolism and amino acid–tRNA biosynthesis. Soy sauce samples revealed 9353 peak pairs (8345 identified/matched, 87.1%), covering tyrosine metabolism and alanine/aspartate/glutamate pathways. Fermented bean curd samples showed 10,076 peak pairs (8499 identified/matched, 84.3%), involving amino acid–tRNA biosynthesis and arginine/proline metabolism [59].

Comparative analysis of fermented red chili sauces with different salt contents revealed significant metabolite concentration variations. In low-salt samples, metabolite changes correlated with active microbial metabolism: microorganisms proliferated rapidly in suitable environments, driving metabolic product shifts. High-salt samples showed metabolite changes primarily influenced by physical factors (e.g., osmotic pressure). These findings provide a critical basis for food component regulation, enabling precise formula adjustment, product quality optimization, and development of consumer-tailored fermented foods.

6.2. Analysis of Plant Metabolic Diversity and Identification of Health-Related Quality in Geographical Indication Products

HP-CIL metabolomics technology exhibits unique value in trace metabolite detection within complex plant matrices, owing to its high sensitivity and wide coverage advantages. In a comparative study of mugwort from different origins, the technology identified 154 differentially expressed metabolites between Xinye and Neixiang mugwort, primarily involved in phenylpropanoid metabolism, lysine degradation, and taurine metabolism pathways. For instance, flavonoids (e.g., salvigenin, 3,6,7,3′,4′-pentamethylquercetin) were significantly more abundant in Xinye mugwort, while phenolic acids (e.g., p-coumaric acid) showed higher concentrations in Neixiang samples [60]. These differences provide a material basis for geographical indication quality authentication of mugwort and reveal correlations between metabolite accumulation and environmental adaptability.

Notably, HP-CIL metabolomics technology enabled accurate quantification of low-abundance metabolites by integrating dansylation derivatization with ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-Q-TOF/MS). In the taurine metabolism pathway, this approach detected 2.3-fold higher

taurine levels in Xinye mugwort versus Neixiang, alongside 1.8-fold lower hypotaurine concentrations. Such metabolic dynamics not only reflect plant environmental responses but also offer a novel dimension for medicinal plant quality evaluation.

6.3. Microbial Metabolic Mechanisms and Their Potential Impact on Host Health

HP-CIL metabolomics technology enables extensive applications in microbiology, providing critical technical support for dissecting microbial-host interactions and microbial community functions.

In research on plant-microorganism interactions, consider the biological control of pea root rot. HP-CIL metabolomics technology detected a 2–3-fold increase in phenylpropanoid pathway products (e.g., coumaric acid, ferulic acid) in Paenibacillus polymyxa–treated plant roots. These compounds function as plant stress-resistant signaling molecules and promote beneficial microorganism colonization [50,61]. The study also revealed that this bacterial strain disrupts pathogenic bacterial membranes by secreting cyclic lipopeptides, inducing accumulation of defense metabolites (e.g., jasmonic acid, salicylic acid) and activating plant induced systemic resistance (ISR) [50]. Figure 7 HP-CIL Technology-Driven Cross-Domain Metabolic Decoding Framework of "Plant-Food-Microbe".

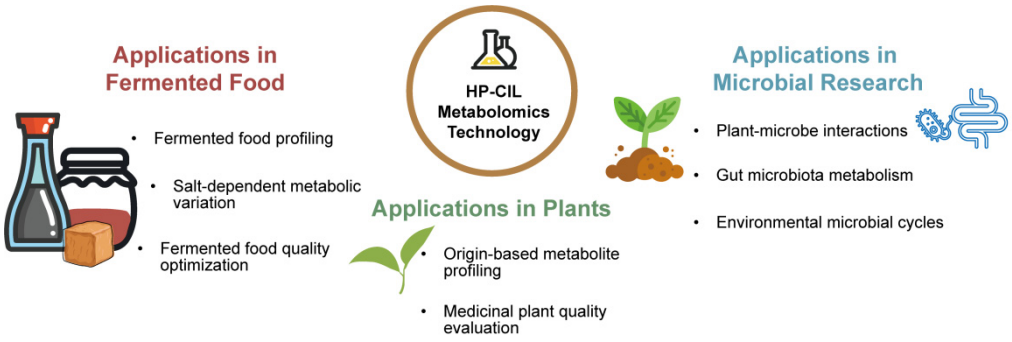

Figure 7. HP-CIL Technology-Driven Cross-Domain Metabolic Decoding Framework of "Plant-Food-Microbe".

For investigations into microbial plant growth promotion, HP-CIL metabolomics technology confirmed that Bacillus subtilis regulates plant nitrogen metabolism, leading to significant increases in root nitrogen transport metabolites (e.g., glutamine, asparagine). This enhances ammonium nitrogen transformation efficiency in rhizosphere soil, with plant hormones and extracellular enzymes secreted by *B. subtilis* boosting root nitrogen absorption efficiency by up to 35% [62].

In the research on gut microbiota, HP-CIL metabolomics technology enables nanomolar-level detection of key metabolites (e.g., short-chain fatty acids SCFAs, bile acids) in gut microbiota studies, addressing detection limitations of traditional methods for low-concentration analytes [23,63]. For instance, a dietary fiber intervention study using HP-CIL metabolomics technology revealed significant increases in SCFA levels (e.g., acetic acid, propionic acid), which positively correlated with improvements in host blood glucose and cholesterol parameters [63]. In marine carbon cycle research, HP-CIL metabolomics technology quantified carbon transfer efficiency between phytoplankton and heterotrophic bacteria (e.g., SAR11, Roseobacter) via isotope labeling/tracking, confirming that their metabolic activities are modulated by light cycles and nutrient concentrations [64]. In soil microbiology, HP-CIL metabolomics technology analysis of pea root rot biocontrol identified over 20 distinct metabolites (e.g., cyclic lipopeptides, polyketides) from Bacillus subtilis–based antagonistic strains—detecting threefold more metabolites than traditional methods and enabling tracking of antibacterial substance secretion dynamics [50]. Pea rhizosphere soil studies further showed that high-fertility soil harbored fiber-degrading microbiota producing elevated SCFA levels, with metabolic products directly linked to soil carbon/nitrogen cycling functions. This approach effectively overcomes single-gene sequencing limitations in functional verification.

7. Challenges and Prospects of HP-CIL Metabolomics Technology Application

7.1. Challenges

7.1.1. Fluctuations in Labeling Efficiency Mediated by Derivatizing Reagents and Limitations in Functional Group Adaptation

The HP-CIL technology encounters multidimensional technical bottlenecks during derivatization, with core challenges focusing on labeling efficiency stability and metabolite functional group compatibility. On one hand, labeling efficiency is significantly affected by environmental factors. For instance, when the temperature increases

from 25 ℃ to 37 ℃, the labeling efficiency of dansyl chloride for amine/phenolic metabolites (e.g., xanthine) drops from 92% to 78%, directly causing detection failure in 12% of Alzheimer's disease (AD) saliva samples containing low-abundance amine metabolites (e.g., 5–10 nM phenylproline) [9,25]. Regarding bromide labeling of DmPA for hydroxyl metabolites, a mere 0.5-unit pH fluctuation (from 8.0 to 7.5) results in a 12% decrease in labeling efficiency. This reduces signal response and quantitative repeatability (CV rising to 18%) for hydroxyl biomarkers like 3-hydroxycanine in Parkinson's disease serum. These issues compound the inherent detection limitations of hydroxyl metabolites in four-channel CIL technology, further restricting metabolic coverage [18,25]. Additionally, existing derivatization reagents demonstrate limited adaptability to specific functional group metabolites. For instance, the dyesulfonamide hydrazine labeling of carbonyl metabolites shows only 65% reaction efficiency for low-molecular-weight aldehydes, significantly lower than the 90% efficiency observed with ketone metabolites. This results in a 30% reduction in detection rates for energy metabolism biomarkers like acetaldehyde in sports medicine research. Similarly [55], the dansyl chloride derivatization commonly used in anti-doping tests exhibits batch-to-batch variations ±10% in labeling efficiency for amine-containing stimulants such as 1,3-dimethylpentylamine [56]. Furthermore, subtle variations in instrument stability (e.g., temperature fluctuations ±2 ℃ in mass spectrometry ion sources) may cause signal loss of certain derivatives. This issue becomes particularly evident in trace metabolite analyses like serum bile acid detection, further compromising data integrity and failing to meet the demands for simultaneous and precise analysis of diverse metabolites in complex biological samples [15,25,51].

7.1.2. Methodological Limitations of Precise Quantification of All Metabolites

The HP-CIL technology employs a four-channel chemical isotope labeling strategy for metabolite group analysis, specifically detecting four major categories: amine/phenolic (A), carboxylic (C), carbonyl/keto/alkaloid (K), and hydroxyl (H) metabolites. Theoretically, this approach can cover 94.7% of the metabolome (primarily comprising five chemical subgroups: amine, phenol, hydroxyl, carboxylic, and carbonyl) [18]. However, in practical applications, the technique still faces significant challenges including incomplete coverage of chemical subgroups, insufficient isotope internal standards, and matrix effects. Particularly notable is the substantial detection gap for hydroxyl metabolites, resulting in actual coverage rates that fall significantly below theoretical projections.

The Parkinson's Disease (PD) serum metabolomics study (baseline n = 85) revealed [25] that among the eight metabolites used to differentiate PD from early-stage dementia, some belonging to the "four-channel detection deficiency subgroup" (e.g., amines such as putrescine ^{2}H), faced challenges due to the lack of commercialized ^{13}C-labeled internal standards. When using external reference materials for quantification, the batch-to-batch variation coefficient reached 18.2%, significantly higher than the 4.5% observed with metabolites using proprietary internal standards.

Human Metabolome Database data [40] show, the HP-CIL commonly used $^{12}C/^{13}C$ labeled endogenous markers showed high coverage of the four-channel dataset for major chemical subgroups, but significantly reduced coverage of under-detected hydroxyl metabolites and minor subgroups (such as sulfur-containing compounds and heterocyclic compounds). Notably, endogenous markers for gut microbiota-specific metabolites (e.g., thiopterine, predominantly belonging to hydroxyl or other minor subgroups) achieved only 38% coverage. In dietary fiber intervention studies, the key biomarker propionate (10 nM, carboxylic acid class) faced challenges due to the absence of specific endogenous markers. When using alternative markers (propionate- $^{13}C_3$) for quantification, recovery rates fluctuated between 8% to 12% [23], severely compromising the reliability of metabolic pathway correlation analyses. Furthermore, matrix effects caused quantitative deviations that showed significant variations across different chemical subgroups: The abstract indicates that the range of matrix effects in QC samples ranges from 77.9% to 120.2%. For instance, when quantifying keto deoxycholic acid (20 nM, potentially belonging to hydroxyl or carbonyl categories) in rheumatoid arthritis patients' serum using external reference standards, inter-batch detection fluctuations reached ±15% [42], far exceeding the stability requirements for clinical research data (±5%).

While HP-CIL technology corrects for partial matrix effects and instrument drift through isotope internal standard calibration via $^{12}C/^{13}C$-Diallyl sulfonyl chloride derivatization, existing alternatives still face limitations when addressing "underdetected hydrocarbon metabolites" and "minor chemical subgroups". For instance, when using "structurally similar substitute internal standards" for microbial metabolites in these categories, quantitative results may still show deviations of 10% to 15% due to differences in matrix response between substitutes and target metabolites. This remains insufficient to meet the high precision requirements of clinical diagnostics.

7.1.3. Reliability Bottleneck of Large-Scale Data Interpretation

The "signal-noise" separation challenge in metabolomics data is particularly pronounced in long-term cohorts. Among the 10^4peak pairs identified by HP-CIL single-sample detection, only 35–40% can be clearly

annotated, while the structural uncertainty of remaining peaks elevates the false positive rate in machine learning models (e.g., random forest) to 25% [11,25]. Although a combination of five metabolites including oxalate showed an AUC = 0.955 in a single cohort for Parkinson's disease serum, multi-center studies face challenges due to instrument drift (e.g., mass spectrometer resolution decreasing from 120,000 to 80,000 FWHM), with characteristic peak intensities fluctuating ±20%. Dynamic calibration with over 1000 samples is required to maintain diagnostic efficacy [25]. In chronic hepatitis B patients, the cross-cohort AUC values of biomarkers like 2-methyl-3-oxopentanoic acid decreased from 0.98 to 0.81 [25], influenced by disease progression (Hrp/Lerp subtypes) and treatment history, highlighting the technical difficulty in separating data standardization from biological variations.

The low annotation rate of metabolites and data variability constrain the reliability of large-scale data interpretation using HP-CIL technology. Although the Human Metabolome Database (HMD) has nearly tripled the number of fully annotated metabolites [40], the annotation rate of HP-CIL detected metabolites in clinical samples remains only 35–40%, with the existence of numerous unknown metabolites directly impacting data interpretation depth. In biomarker studies for Alzheimer's disease (AD) and Parkinson's disease (PD), unannotated characteristic peaks account for 58% and 63% respectively, forcing machine learning models to exclude nearly half of detected signals during construction, which indirectly increases false positive rates above 25%. Instrumental drift-induced fluctuations in peak intensity further exacerbate this issue. Continuous analysis of quality control samples over 10 days revealed uncorrected metabolite peak area coefficients of variation (Covariates) reaching 15–20%. Such volatility is more pronounced in long-term cohort studies, as seen in chronic hepatitis B patients' metagenomic research where biomarkers like [43], 2-methyl-3-oxy-pentanoic acid experienced AUC values dropping from 0.98 to 0.81 during cross-cohort validation. This was primarily attributed to fluctuations ±20% in peak intensity caused by changes in instrument resolution (from 120,000 to 80,000 FWHM) across different time points. Additionally, among the 10^4peak pairs generated per HP-CIL detection, only 35–40% can be definitively annotated [11], while structural uncertainty of remaining peaks significantly increases data interpretation difficulty, leading to missed potential metabolic pathway associations. Building HP-CIL-specific databases and implementing dynamic calibration algorithms (such as LOESS) for quality control have become essential solutions. The former enhances metabolite annotation accuracy through accumulated HP-CIL spectral data, while the latter effectively mitigates instrument stability impacts on quantitative results. For instance, in a multicenter cardiovascular disease study, inserting one QC sample every 20 samples and applying LOESS correction reduced batch-to-batch variation coefficients from 22% to 8% [10], significantly improving data reliability.

7.1.4. Matrix Interference and Preprocessing Bottleneck in Microscale Clinical Samples

The analysis of HP-CIL in microscale clinical samples (e.g., cerebrospinal fluid ≤ 10 μL, neonatal heel blood ≤ 50 μL, and premature infant cerebrospinal fluid ≤ 5 μL) remains a core technical challenge. This is due to multiple overlapping interference factors: First, non-specific adsorption by high-abundance substances. For instance, albumin at 1 g/L in cerebrospinal fluid strongly adsorbs low-abundance metabolites (e.g., 10 nM 1,4-diaminobutane) through hydrophobic interactions, resulting in a 15% loss rate of target metabolites—a phenomenon confirmed in metabolic analyses of brain tissue and cerebrospinal fluid from AD mouse models [9,25,39]. Second, high-abundance hemoglobin (approximately 120 g/L concentration) in neonatal heel blood binds to amine and phenolic metabolites, reducing the detection rate of breast cancer-related lipid biomarkers by 25% [28]. Additionally, high-molecular-weight impurities in samples (e.g., mM-level urea in urine and 1–5 mg/mL mucin in saliva) compete with target metabolites for derivatization reagents. For example, urea in urine reduces the labeling efficiency of diacyl chloride in amine metabolites by 18%, leading to quantitative deviations exceeding 15% for neurodegenerative disease markers like 1,4-diaminobutane. This mechanism aligns with the matrix interference observed during O-phosphoryl ethanolamine detection in bladder cancer urine samples [24,25]. Secondly, the adaptability and efficiency of pretreatment processes remain inadequate. Traditional methods such as solid-phase extraction and centrifugation often suffer irreversible losses—due to insufficient sample volumes in analyses of premature infant cerebrospinal fluid (≤5 μL). For instance, conventional 15000 g centrifugation (10 min) results in over 30% loss of target metabolites due to sample adhesion, a phenomenon also documented in toxicological metabolomics studies of microscale hepatocyte samples [25,54]. Residual inorganic salts (e.g., NaCl from sweat) and small molecular impurities in sample matrices exacerbate mass spectrometry ion suppression effects, reducing signal response for trace metabolites like O-phosphatidylethanolamine (ELA, detection limit 5 nM) in bladder cancer urine samples by 40%. Similar matrix interference challenges exist in hydrophilic interaction chromatography (HIC) quantitative analysis [16,24]. Furthermore, detection rates for gut microbiota metabolites (e.g., nanomolar-level short-chain fatty acids) in microfecal samples are below 60% due to

pretreatment losses and matrix interference, severely limiting HP-CIL technology's application in clinical microsample scenarios such as newborn disease screening and minimally invasive diagnostics [23,25,28].

7.1.5. The Fault Line between Basic Research and Clinical Transformation

The insufficient validation of single cohort studies and poor cross-scenario applicability have created a significant gap between the foundational research achievements of HP-CIL technology and their clinical translation. In bladder cancer biomarker studies [24], although the ethanolamine O-phosphate identified by HP-CIL technology demonstrated diagnostic value in a single cohort (AUC = 0.709), its sensitivity dropped from 82% to 65% when validated on 500 samples across five clinical centers due to urinary matrix variations (with urea concentration fluctuations of 2–3 times). This failure to meet the FDA's clinical effectiveness threshold (requiring AUC $\geq$ 0.8) currently limits HP-CIL to an adjuvant diagnostic tool rather than a direct component of clinical treatment protocols.

Similar challenges exist in studies of polyamine metabolic biomarkers for oral cancer detection [33]. The polyamine pathway key metabolite identified by HP-CIL technology achieved an 85% early detection rate in a single cohort. However, during multicenter validation, the rate dropped to 72% due to variations in sample preprocessing procedures across institutions (e.g., storage temperature and processing time). Combining this with DNA methylation testing was required to elevate the detection rate above 95%. Moreover, the metabolic profiles of chronic hepatitis B patients are significantly influenced by disease progression (Hrp/Lerp subtypes) and treatment history [43], further highlighting the disconnect between basic research and clinical translation.

The core reasons for this gap lie in three aspects: First, the high homogeneity of single cohort samples in basic research fails to reflect clinical population heterogeneity (such as age, gender, and comorbidity differences). Second, there is a lack of standardized clinical translation pathways for metabolic biomarkers, particularly missing subgroup validation processes across ethnicities and disease stages. Third, insufficient analysis has been conducted on the correlation between HP-CIL detection results and clinical indicators, with most studies focusing solely on metabolite-disease associations without thoroughly verifying the causal relationship between metabolic changes and clinical outcomes (such as treatment response or survival). Future efforts should establish a multicenter validation network, develop clinical translation guidelines for HP-CIL biomarkers, and specifically address how different population subgroups influence detection results. Expanding sample sizes and subgroup analyses will enhance translation reliability, while strengthening research on metabolite-clinical prognosis correlations will provide stronger evidence-based support for clinical applications. A comprehensive evaluation of HP-CIL technology development requires addressing both challenges and potential opportunities, which can be more intuitively grasped through the panoramic framework shown in Figure 8.

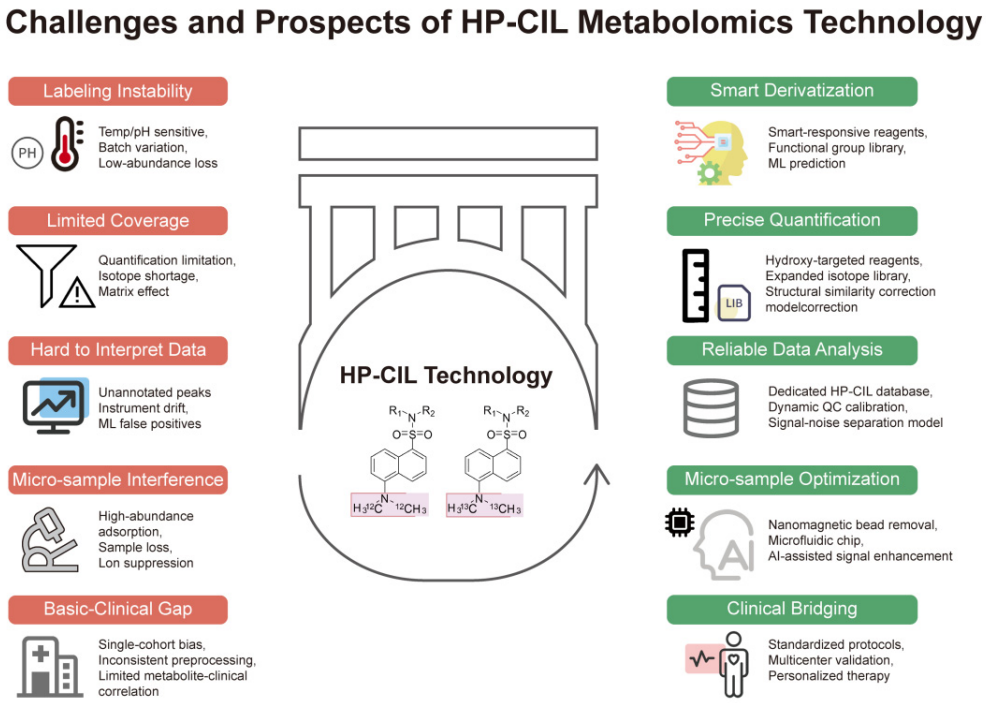

Figure 8. Challenge-Opportunity Landscape of HP-CIL Technology.

7.2. Future Trends

7.2.1. Technical Bottleneck and Optimization Direction of Derivatization

In the derivatization process of HP-CIL technology, labeling efficiency is susceptible to environmental factors (temperature, pH) and functional group adaptability: Elevated temperatures reduce the labeling efficiency of pamoic acid chloride on hypoxanthine [9], pH fluctuations significantly interfere with brominated DmPA in labeling hydroxyl metabolites [25], and some aldehyde metabolites exhibit lower labeling efficiency [55]. To address these challenges, researchers can develop smart-responsive derivatization reagents (e.g., temperature/pH-sensitive polymer probes) [56], expand the "functional group-reagent" matching library (e.g., designing sulfonic acid chloride derivatives with long hydrophobic chains for hydroxyl targeting) [59], and integrate machine learning models to predict labeling efficiency [51]. These optimizations provide crucial support for advancing subsequent technological development.

7.2.2. Methodological Improvement of Accurate Quantification of All Metabolites

HP-CIL technology has limitations in comprehensive metabolite quantification, including insufficient coverage of hydroxy metabolites, low coverage of isotope internal standard libraries for microbial-specific metabolites, and significant deviations in alternative internal standard quantification [18,25]. Currently, the development of exclusive labeling reagents for hydroxy groups has significantly increased the detection of hydroxy metabolites in yeast samples [59]. The expansion of isotope internal standard libraries [18] and the supplementation of sub-group internal standards from gut microbiota and plant sources have improved coverage. Additionally, the establishment of a "structural similarity correction model" [51] has effectively reduced quantification deviations in alternative internal standards, providing a reliable data foundation for multi-omics integration and AI analysis.

7.2.3. Improved Reliability of Interpretation of Large-Scale Metabolome Data

Current HP-CIL technology achieves annotation for only 35–40% of detected peaks in single analyses, with inconsistent instrument stability causing data fluctuations that increase false positive rates in machine learning models [25,51]. To address this, we have established a dedicated HP-CIL metabolite database integrating measured retention times and MS/MS spectral annotation rates [40]. The implementation of dynamic QC sample calibration algorithms (e.g., LOESS and SVR) has reduced batch-to-batch variation coefficients [24]. Furthermore, our "signal-noise separation model" classifies unannotated peaks through correlation analysis between peak profiles and metabolic pathways [28], providing robust support for real-time monitoring and AI-driven data mining.

7.2.4. Matrix Interference and Pretreatment Optimization of a Small Clinical Sample

The high-performance chromatographic immunoassay (HP-CIL) analysis of trace clinical samples such as cerebrospinal fluid and neonatal heel blood faces challenges including adsorption of high-abundance substances (e.g., cerebrospinal fluid albumin, heel blood hemoglobin) [9,24,39], loss during traditional sample pretreatment, and matrix ion suppression [16,53]. The developed miniaturized matrix removal devices (such as surface-modified specific ligand nanomagnetic beads) [28] can eliminate high-abundance interfering compounds. The microfluidic chip integrates a "pre-treatment-derivatization-separation" workflow [56] to achieve stable detection of trace samples. By combining computational mass spectrometry for matrix interference prediction [59], the signal response of target metabolites is significantly enhanced, enabling real-time analysis of minimally invasive samples and ensuring data reliability through AI-driven approaches.

7.2.5. The Fault Line between Basic Research and Clinical Transformation

The HP-CIL technology faces a disconnect between basic research and clinical translation, with insufficient validation across single cohorts and inconsistent preprocessing procedures hindering achievement of clinical standards [25,51]. By establishing a multicenter validation network with standardized sample protocols [39], bladder cancer biomarkers demonstrated improved AUC (area under the curve) across trials. The development of the "HP-CIL Metabolic Markers Clinical Translation Guidelines" [25] standardized subgroup validation protocols, reducing variability in oral cancer biomarker detection rates. Furthermore, the creation of a "metabolic markers-clinical prognosis" correlation model [56] confirmed positive correlations between metabolites and treatment response rates in type 2 diabetes, providing evidence for multi-omics integration and personalized therapy development.

8. Conclusions

HP-CIL metabolomics technology has achieved systematic breakthroughs in metabolite detection sensitivity, coverage, and quantitative precision via its $^{12}C/^{13}C$ dual-labeling strategy, offering innovative solutions across multiple domains.

HP-CIL metabolomics technology demonstrates significant application value across diverse medical domains. In oncology, it enables labeling of amine/phenol metabolites in urine samples, offering a novel non-invasive approach to early diagnosis for diseases like bladder cancer. Specifically, lipid metabolism analysis of minimal breast cancer cell populations uncovers key metabolic nodes in tumor progression. In neurodegenerative disease research, saliva/serum metabolomic profiling via HP-CIL metabolomics technology allows precise differentiation of Alzheimer's disease stages and Parkinson's disease subtypes. For cardiovascular diseases, the technology has elucidated the regulatory mechanisms of targeted therapies on postoperative cardiac metabolism and the metabolomic reprogramming effects of drugs in diabetic patients, providing metabolic targets for personalized treatment. In immunology and inflammation fields, HP-CIL metabolomics technology-identified metabolite panels exhibit exceptional diagnostic performance in rheumatoid arthritis and chronic hepatitis B, significantly enhancing disease-specific diagnostic efficacy.

In nutrition and health research, HP-CIL metabolomics technology offers precise metabolomic solutions for food component analysis, safety assessment, and nutritional intervention. For food component studies, metabolic pathway analysis of fermented foods enables optimization of low-salt fermentation processes, driving the development of healthy food products. In food safety assessment, it uncovers potential risk associations between food components and human health, providing theoretical support for establishing a novel "food-metabolism-health" evaluation system. For nutritional intervention efficacy evaluation, the constructed multi-lipid scoring system provides a quantifiable metabolic basis for personalized dietary guidance. Additionally, it screens nutritional metabolism-related biomarkers to support disease prevention strategies.

In sports health research, HP-CIL metabolomics technology serves as a core tool for decoding exercise metabolism mechanisms and supporting athlete wellness. It accurately captures dynamic tricarboxylic acid cycle metabolite changes during endurance sports and purine metabolism profiles post–high-intensity interval training, providing critical insights to deepen understanding of exercise metabolic adaptation mechanisms. By analyzing metabolite fluctuations in athlete sweat and blood samples, HP-CIL metabolomics technology enables precise monitoring of fatigue and metabolic status. Integrating metabolomics data with other physiological parameters allows prediction of sports performance, forming a scientific basis for tailoring training plans and competition strategies. In sports-related disease prevention, analysis of dynamic patterns in exercise fatigue–associated metabolites facilitates formulation of personalized nutritional and training strategies. These interventions effectively mitigate exercise-induced fatigue while providing a precise basis for sports-mediated prevention and management of metabolic syndrome.

In drug development, HP-CIL metabolomics technology has established a comprehensive technical chain spanning from trace component detection to metabolic marker verification. For anti-doping analysis, it effectively overcomes urine matrix interference to enable high-sensitivity detection of low-concentration stimulants. In biomarker-driven drug discovery, the technology provides critical support for drug target screening and efficacy evaluation. Additionally, by investigating dietary impacts on human metabolism, it offers novel perspectives for drug development strategies.

Despite challenges in data standardization, database construction, and clinical applicability, HP-CIL metabolomics technologydemonstrates broad prospects through in-depth integration with single-cell RNA sequencing, spatial proteomics, and artificial intelligence. Future technological innovation and interdisciplinary integration are expected to establish a "detection-analysis-intervention" closed-loop system. Prevention stage: Wearable devices enable real-time monitoring of metabolic fingerprints (e.g., sweat hypoxanthine for exercise fatigue warning); Diagnosis stage: Combined with single-cell RNA sequencing and spatial proteomics, it analyzes tumor microenvironment metabolic heterogeneity (e.g., lung cancer glutamine metabolism subtypes); Treatment stage: Generative adversarial networks (GANs) simulate individual drug metabolic responses to enable customized targeted therapies (e.g., predicting metformin efficacy in diabetic patients).

Notably, its deep collaboration with Artificial Intelligence, such as using Transformer models to mine dynamic metabolic networks—will drive precision medicine's shift from "disease diagnosis" to "health prediction". This provides critical technical support for complex disease prevention, personalized medicine, and the big health industry, serving as a core bridge between basic research and clinical practice.

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