International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(REVIEW ARTICLE)

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LC-MS in Proteomics and Biomarker Discovery

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International Journal of Science and Research Archive, 2025, 14(03), 900-913

Publication history: Received on 08 February 2025; revised on 15 March 2025; accepted on 17 March 2025

Article DOI: https://doi.org/10.30574/ijsra.2025.14.3.0753

Abstract

Liquid chromatography-mass spectrometry (LC-MS) has emerged as a powerful analytical tool in the field of proteomics and biomarker discovery. LC-MS combines the high separation efficiency of liquid chromatography (LC) with the sensitive and specific detection capabilities of mass spectrometry (MS), enabling the identification and quantification of complex protein mixtures in biological samples. In proteomics, LC-MS facilitates the characterization of post-translational modifications, protein-protein interactions, and differential protein expression in health and disease states. The technique's high sensitivity and resolution enable the detection of low-abundance proteins, which are often critical for understanding disease mechanisms and identifying potential biomarkers. In biomarker discovery, LC-MS allows for the accurate profiling of disease-associated proteins, leading to the identification of novel diagnostic, prognostic, and therapeutic targets. Advances in LC-MS, such as tandem mass spectrometry (MS/MS), data-independent acquisition (DIA), and label-free quantification, have further enhanced the depth and accuracy of proteomic analysis. The integration of bioinformatics tools and machine learning approaches with LC-MS data has also improved biomarker validation and discovery. Overall, LC-MS continues to play a crucial role in advancing precision medicine by providing deeper insights into disease pathophysiology and facilitating the development of targeted therapeutic strategies.

Keywords: Lc-Ms; Proteomics; Biomarker Discovery; Mass Spectrometry; Post-Translational Modifications

1. Introduction

Proteomics is the large-scale study of proteins, their structures, functions, interactions, and modifications within a biological system (1). Unlike genomics, which focuses on the genome, proteomics provides insights into the dynamic nature of biological processes because proteins are the effectors of cellular functions. Proteomic analyses involve the identification, quantification, and characterization of proteins present in a given sample (2). By studying proteomes, researchers can elucidate signaling pathways, identify disease mechanisms, and discover potential therapeutic targets. Proteomics has applications in diverse fields, including biomedical research, drug development, agriculture, and personalized medicine (3).

- Importance of Biomarker Discovery: Biomarkers are measurable indicators of biological processes, disease states, or responses to therapeutic interventions (4). They play crucial roles in disease diagnosis, prognosis, monitoring, and treatment selection. Biomarker discovery is essential for improving clinical outcomes, enhancing patient care, and advancing precision medicine initiatives. By identifying specific molecules or signatures associated with health or disease, biomarker research facilitates early disease detection, risk assessment, and personalized treatment strategies (5). Biomarkers can be proteins, nucleic acids, metabolites, or imaging characteristics, and their discovery often involves high-throughput screening methods and validation studies across diverse patient cohorts (6). Role of LC-MS in Proteomics and Biomarker Discovery: Liquid chromatography-mass spectrometry (LC-MS) is a powerful analytical technique widely used in

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proteomics and biomarker discovery (7). LC separates complex mixtures of proteins or peptides based on their physicochemical properties, such as size, charge, and hydrophobicity, before introducing them into the mass spectrometer for detection and characterization. Mass spectrometry (MS) identifies and quantifies peptides or proteins by measuring their mass-to-charge ratios and fragmenting them to obtain structural information. LC-MS enables comprehensive profiling of proteomes, including post-translational modifications and protein-protein interactions, with high sensitivity, accuracy, and throughput (8). In biomarker discovery, LC-MS facilitates the identification of candidate biomarkers by comparing protein expression patterns between healthy and diseased samples. It also enables the validation and verification of putative biomarkers in large-scale clinical studies, contributing to the development of diagnostic assays and personalized treatment strategies (9). Overall, LC-MS has revolutionized proteomic research and biomarker discovery by providing unprecedented insights into the molecular complexity of biological systems and disease processes.

2. Fundamentals of LC-MS

2.1. Principles of Liquid Chromatography (LC)

Liquid chromatography (LC) is a separation technique used to separate components of a mixture based on their interactions with a stationary phase and a mobile phase (10). The principles of LC revolve around the differential affinity of compounds for the stationary and mobile phases. In LC, the stationary phase is typically a solid or liquid packed into a column, while the mobile phase is a liquid solvent that flows through the column (11). As the sample is injected into the LC system, it interacts with the stationary phase, leading to differential retention and elution of analytes.

2.2. The separation process in LC relies on several key principles

- Retention Mechanism: Analytes in the sample mixture interact with the stationary phase through various mechanisms such as adsorption, partitioning, ion exchange, size exclusion, or affinity interactions (12). These interactions determine the retention time of each analyte in the column.
- Mobile Phase Selection: The mobile phase composition, including solvent type, pH, and concentration of additives, influences analyte retention and elution profiles (13). Optimization of the mobile phase conditions is critical for achieving efficient separation and resolution.
- Column Selection: The choice of column material, dimensions, and packing characteristics (e.g., particle size, pore size) affects the separation efficiency, resolution, and speed of analysis (14).
- Detection: Analytes separated by LC are typically detected using various detection techniques such as UV-Vis spectroscopy, fluorescence, or mass spectrometry (15).

Overall, the principles of LC enable the separation of complex mixtures into individual components based on their physicochemical properties, allowing for qualitative and quantitative analysis of target compounds in diverse samples.

2.3. Principles of Mass Spectrometry (MS)

- Mass spectrometry (MS) is an analytical technique used to identify and quantify molecules based on their mass-to-charge ratio (m/z) and fragmentation patterns (16). The principles of MS revolve around the ionization, mass analysis, and detection of analytes in the gas phase. MS involves several fundamental steps:
- Ionization: Analyte molecules are ionized by various ionization techniques such as electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI), or electron ionization (EI) (17). Ionization generates charged species (ions) from neutral molecules, allowing them to be manipulated by electric and magnetic fields in the mass spectrometer.
- Mass Analysis: Ionized analytes are accelerated into the mass analyzer, where they are separated based on their mass-to-charge ratio (m/z) (18). Common types of mass analyzers include quadrupoles, time-of-flight (TOF), ion traps, and magnetic sector analyzers. Mass analyzers measure the mass-to-charge ratio of ions with high precision and accuracy.
- Detection: The separated ions are detected by a detector, typically a electron multiplier, Faraday cup, or microchannel plate detector (19). The detector converts ion signals into electronic signals, which are then processed to generate mass spectra.
- Data Analysis: Mass spectra provide information about the mass-to-charge ratios of ions present in the sample, as well as their relative abundances and fragmentation patterns (20). Data analysis techniques such as database searching, deconvolution, and spectral interpretation are used to identify analytes and elucidate their structures.

Overall, mass spectrometry enables the identification, quantification, and structural characterization of molecules in complex samples with high sensitivity, specificity, and resolution.

2.4. Integration of LC and MS

The integration of liquid chromatography (LC) and mass spectrometry (MS) combines the separation power of LC with the detection and identification capabilities of MS, offering a powerful tool for chemical analysis in various fields including proteomics, metabolomics, environmental analysis, and pharmaceutical research.

2.4.1. Integration of LC and MS involves several key steps

- Sample Introduction: Analytes are separated by LC and introduced into the mass spectrometer as they elute from the chromatographic column (21). This can be achieved by direct infusion, online coupling, or offline sample collection followed by injection.
- Ionization: Analytes eluting from the LC column are ionized by the selected ionization technique, such as ESI or MALDI, generating ions amenable to mass analysis (22).
- Mass Analysis: Ionized analytes are separated and analyzed based on their mass-to-charge ratios using the mass analyzer (s) within the mass spectrometer (23).
- Detection and Data Analysis: Mass spectra are acquired and processed to identify and quantify analytes present in the sample (24). Data analysis software is used to interpret mass spectra, identify compounds, and perform quantitative analysis.

Integration of LC and MS enhances the analytical capabilities of both techniques, allowing for high-resolution separation, sensitive detection, and accurate identification of complex mixtures (25). LC-MS systems can be further optimized for specific applications by selecting appropriate LC columns, mobile phases, ionization sources, and mass analyzers. Overall, LC-MS integration has revolutionized analytical chemistry, enabling comprehensive analysis of complex samples with unprecedented sensitivity, speed, and accuracy (26).

2.5. Sample Preparation Techniques

2.5.1. Protein Extraction

Protein extraction is the foundational step in proteomic studies, crucial for obtaining a representative subset of proteins from complex biological samples (27). The process involves breaking down cellular or tissue structures to liberate proteins while preserving their native states and minimizing degradation or modification. The choice of extraction method depends on the sample type, its biological matrix, and the downstream applications (28).

Common protein extraction techniques include chemical lysis, mechanical disruption, and enzymatic digestion. Chemical lysis involves the use of chaotropic agents like urea or guanidine hydrochloride, detergents such as Triton X-100 or NP-40, or organic solvents like methanol or chloroform to disrupt cell membranes and solubilize proteins (29). Mechanical disruption employs physical forces like sonication, homogenization, or grinding to break cells or tissues apart, releasing proteins into the extraction buffer. Enzymatic digestion involves the use of proteolytic enzymes like trypsin, chymotrypsin, or proteinase K to cleave proteins into peptides, facilitating subsequent analysis (30).

Factors influencing protein extraction efficiency include sample complexity, protein solubility, and compatibility with downstream techniques such as mass spectrometry (MS) or immunoassays (31). Optimization of extraction protocols is essential to maximize protein yield, maintain sample integrity, and minimize interference from contaminants or interfering substances (32).

2.5.2. Protein Digestion

Protein digestion is a critical step in proteomic workflows, where proteins are enzymatically cleaved into peptides to facilitate their analysis by techniques like liquid chromatography-mass spectrometry (LC-MS) (33). The process involves the selective hydrolysis of peptide bonds by proteolytic enzymes, typically trypsin, to generate peptides with defined sequences and termini.

Trypsin, a serine protease, specifically cleaves peptide bonds at the carboxyl side of lysine and arginine residues, except when followed by proline (34). This specificity results in peptides with positively charged amino termini, which enhances their ionization efficiency during MS analysis. Digestion conditions such as pH, temperature, enzyme-to-substrate ratio, and incubation time influence digestion efficiency and specificity (35).

Optimization of digestion protocols is crucial to achieve complete protein cleavage, minimize missed cleavages, and enhance peptide recovery (36). Factors such as protein structure, post-translational modifications, and the presence of detergents or chaotropic agents in the extraction buffer can affect digestion efficiency and specificity. Careful consideration of these factors is essential for obtaining reproducible and reliable results in proteomic analyses (37).

2.5.3. Fractionation Strategies

Fractionation strategies are employed in proteomics to reduce sample complexity, increase dynamic range, and improve detection sensitivity (38). These techniques separate proteins or peptides based on their physicochemical properties such as size, charge, hydrophobicity, or affinity, allowing for more in-depth characterization of complex biological samples.

Common fractionation approaches include gel-based fractionation, liquid chromatography (LC), immunoaffinity purification, and subcellular fractionation (39). Gel-based techniques like sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) or two-dimensional gel electrophoresis (2D-PAGE) separate proteins based on molecular weight or isoelectric point (40). LC separates peptides using chromatographic columns with different stationary phases such as reverse-phase, size-exclusion, or ion-exchange chromatography. Immunoaffinity purification employs antibodies or affinity ligands to selectively isolate proteins or peptides based on specific antigen-antibody interactions or affinity tags. Subcellular fractionation separates proteins based on their cellular localization or organelle association, providing insights into cellular compartmentalization and function (41).

Integration of multiple fractionation strategies enhances proteome coverage, improves identification of low-abundance proteins, and enables in-depth characterization of complex samples (42). These techniques are essential for unraveling the molecular complexity of biological systems and identifying potential biomarkers or therapeutic targets in various disease states.

2.6. LC-MS Workflow in Proteomics

2.6.1. Chromatographic Separation

Chromatographic separation is a fundamental component of liquid chromatography-mass spectrometry (LC-MS) workflows in proteomics (43). It involves the separation of complex mixtures of peptides or proteins based on their physicochemical properties, such as hydrophobicity, size, charge, or affinity, prior to mass spectrometric analysis. Chromatography serves to resolve overlapping peaks, enhance analyte purity, and improve detection sensitivity.

In LC-MS, chromatographic separation is typically achieved using columns packed with stationary phases such as reversed-phase (RP), size-exclusion (SEC), ion-exchange (IEC), or hydrophilic interaction liquid chromatography (HILIC) (44). Reversed-phase chromatography, the most widely used mode in proteomics, separates peptides based on their hydrophobicity, with more hydrophobic peptides eluting later than less hydrophobic ones. (45). Ion-exchange chromatography separates peptides based on their charge, while hydrophilic interaction chromatography separates them based on their polarity.

Optimization of chromatographic conditions, including mobile phase composition, flow rate, and column temperature, is crucial to achieving optimal separation efficiency, resolution, and peak shape. The choice of chromatographic method depends on the physicochemical properties of the analytes and the specific analytical goals of the study (46).

2.6.2. Mass Spectrometric Detection

Mass spectrometric detection is the core analytical technique in LC-MS-based proteomics, enabling the identification, quantification, and characterization of peptides and proteins based on their mass-to-charge ratio (m/z) and abundance (47). Mass spectrometers consist of three main components: ionization source, mass analyzer, and detector.

In LC-MS proteomics, peptides eluted from the chromatographic column are ionized by the ionization source, typically using techniques such as electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) (48). ESI generates charged droplets from a liquid sample, while MALDI uses a laser to desorb and ionize analytes from a solid matrix. The ionized peptides are then introduced into the mass analyzer, where they are separated based on their m/z ratio. Common mass analyzers used in proteomics include quadrupole, time-of-flight (TOF), ion trap, and orbitrap analyzers (49).

After separation in the mass analyzer, ions are detected based on their m/z ratio, generating mass spectra that represent the mass and abundance of peptides in the sample. Data acquisition modes such as data-dependent acquisition (DDA) or data-independent acquisition (DIA) are used to select peptides for fragmentation and subsequent identification (50). Mass spectrometric data are processed and analyzed using specialized software to identify peptides, quantify their abundance, and interpret their biological significance.

2.6.3. Data Analysis and Interpretation

Data analysis and interpretation are critical steps in LC-MS proteomics workflows, involving the processing, analysis, and interpretation of mass spectrometric data to extract meaningful biological information (51). The complexity of proteomic data requires sophisticated computational tools and bioinformatics algorithms for accurate peptide identification, quantification, and functional annotation.

Data analysis typically involves several key steps, including preprocessing, peak detection, peptide identification, quantification, and statistical analysis (52). Preprocessing steps may include baseline correction, noise reduction, and retention time alignment to improve data quality and reproducibility. Peak detection algorithms identify chromatographic peaks corresponding to peptide ions, while peptide identification algorithms match experimental spectra to theoretical spectra generated from protein sequence databases (53).

Quantification methods such as label-free quantification or isobaric labeling techniques are used to measure peptide abundance across different experimental conditions or samples. Statistical analysis identifies differentially expressed peptides or proteins and assesses their significance based on measures such as fold change and p-value (54).

Interpretation of proteomic data involves linking identified peptides or proteins to biological pathways, molecular functions, and cellular processes. Functional enrichment analysis identifies overrepresented biological terms or pathways among differentially expressed proteins, providing insights into underlying biological mechanisms. Integration of proteomic data with other omics data such as genomics, transcriptomics, and metabolomics enables a systems-level understanding of biological processes and disease mechanisms (55).

In summary, data analysis and interpretation are essential for extracting meaningful biological insights from LC-MS proteomics data and are critical for advancing our understanding of complex biological systems and disease processes.

2.7. Applications of LC-MS in Biomarker Discovery

2.7.1. Identification of Biomarkers

Identification of biomarkers is a crucial aspect of proteomics and biomarker discovery, aiming to identify molecules indicative of normal biological processes, pathogenic processes, or responses to therapeutic interventions (56). Biomarkers can be proteins, peptides, metabolites, or other molecules measurable in biological samples, offering insights into disease diagnosis, prognosis, and treatment response.

In proteomics, biomarker discovery often begins with comparative analysis of protein expression profiles between diseased and healthy samples using techniques such as liquid chromatography-mass spectrometry (LC-MS) (57). Differentially expressed proteins are identified and further validated to assess their potential as biomarkers. Bioinformatics tools and databases aid in peptide and protein identification by matching experimental mass spectra to theoretical spectra derived from protein sequence databases (58).

Validation of candidate biomarkers involves rigorous evaluation across independent cohorts or sample sets to assess their diagnostic or prognostic utility. Validation studies typically employ targeted proteomic approaches such as selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) to quantify candidate biomarkers in large patient cohorts (59). Validation criteria include sensitivity, specificity, accuracy, and robustness across diverse sample types and experimental conditions.

2.7.2. Quantitative Proteomics

Quantitative proteomics is a powerful approach used to measure changes in protein abundance or modification levels across different biological conditions, treatments, or disease states (60). It provides quantitative insights into dynamic changes in cellular processes, signaling pathways, and protein-protein interactions, facilitating biomarker discovery and systems-level understanding of biological systems.

Various quantitative proteomics techniques are available, including label-based and label-free methods. Label-based approaches involve chemical or metabolic labeling of peptides or proteins with stable isotopes before mass spectrometric analysis, enabling accurate quantification of protein abundance ratios between different samples (61). Examples include isobaric tags for relative and absolute quantitation (iTRAQ) and tandem mass tags (TMT).

Label-free quantification relies on comparing the intensities or peak areas of peptide ions between different samples without using chemical labels. It offers advantages such as simplicity, cost-effectiveness, and flexibility but may suffer from variability due to differences in sample handling or instrument performance (62).

Quantitative proteomics requires robust experimental design, data acquisition, and data analysis strategies to ensure accurate and reproducible quantification. Normalization techniques are employed to correct for experimental variations and biases, while statistical methods assess the significance of observed changes in protein abundance (63).

2.7.3. Validation and Verification Strategies

Validation and verification of candidate biomarkers are essential steps in biomarker discovery pipelines to ensure the accuracy, reproducibility, and clinical relevance of identified biomarker candidates (64). Validation involves assessing the performance of candidate biomarkers across independent cohorts or sample sets to confirm their diagnostic or prognostic utility. Verification focuses on further confirming the presence and characteristics of candidate biomarkers using targeted assays and orthogonal techniques.

Validation studies typically employ larger patient cohort's representative of the target population, encompassing diverse demographics, disease stages, and sample types (65). Biomarker performance is evaluated based on sensitivity, specificity, accuracy, predictive value, and receiver operating characteristic (ROC) curves. Validation criteria also include analytical parameters such as assay robustness, reproducibility, and stability (66).

Targeted assays such as enzyme-linked immunosorbent assays (ELISA), immunohistochemistry (IHC), or mass spectrometry-based assays such as selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) are commonly used for biomarker validation and verification (67). These assays provide quantitative measurements of biomarker abundance in clinical samples, offering high specificity and sensitivity for biomarker detection.

Orthogonal techniques such as western blotting, immunoprecipitation, or functional assays may be employed to validate biomarker identity, assess protein-protein interactions, or elucidate biological functions (68). Integration of multiple validation approaches enhances the robustness and reliability of biomarker validation studies, ensuring that identified biomarkers meet the criteria for clinical translation and implementation.

3. Challenges and Limitations

3.1. Sensitivity and Specificity

In biomarker discovery and clinical diagnostics, sensitivity and specificity are critical parameters that assess the performance of assays in accurately detecting true positive and true negative results, respectively (69).

Sensitivity refers to the ability of a diagnostic test to correctly identify individuals with the disease or condition of interest, also known as true positives (70). A highly sensitive assay minimizes false negatives, ensuring that individuals with the disease are not missed during screening or diagnosis. Sensitivity is calculated as the ratio of true positive results to the sum of true positive and false negative results, expressed as a percentage (71).

Specificity, on the other hand, measures the ability of a test to correctly identify individuals without the disease or condition, known as true negatives (72). A highly specific assay minimizes false positives, ensuring that individuals without the disease are not incorrectly classified as positive. Specificity is calculated as the ratio of true negative results to the sum of true negative and false positive results, expressed as a percentage (73).

Optimizing sensitivity and specificity often involves a trade-off, where increasing sensitivity may decrease specificity and vice versa. Therefore, assay development and validation aim to achieve a balance between sensitivity and specificity appropriate for the clinical application. This balance is particularly crucial in screening programs, where a balance between detecting true positives and minimizing false positives is essential to prevent unnecessary follow-up tests or interventions (74).

Assay sensitivity and specificity can be influenced by various factors, including assay design, target analyte characteristics, sample type, assay conditions, and cutoff thresholds (75). Rigorous validation studies across diverse patient cohorts are necessary to accurately determine the sensitivity and specificity of biomarker assays, ensuring their clinical utility and reliability in disease diagnosis, prognosis, and treatment monitoring.

3.2. Data Analysis Complexity

Data analysis in biomarker discovery and proteomics can be highly complex due to the large volume of data generated by high-throughput techniques such as mass spectrometry and the complexity of biological systems (76).

Proteomic data analysis involves several steps, including preprocessing, feature extraction, normalization, statistical analysis, and interpretation (77). Preprocessing steps may include baseline correction, noise reduction, and peak detection to enhance data quality. Feature extraction involves identifying peaks or chromatographic features corresponding to peptides or proteins of interest. Normalization methods are employed to correct for technical variations and biases between samples, ensuring comparability and reliability of quantitative measurements (78).

Statistical analysis aims to identify differentially expressed proteins or peptides between experimental groups, assess their significance, and generate hypotheses about underlying biological mechanisms (79). Multiple testing correction methods control the false discovery rate to minimize the risk of false positive findings. Functional enrichment analysis identifies overrepresented biological terms or pathways among differentially expressed proteins, providing insights into biological processes associated with the observed changes (80).

The complexity of data analysis increases with the size and complexity of the dataset, the number of experimental conditions or variables, and the diversity of biological samples (81). Advanced bioinformatics tools, machine learning algorithms, and computational approaches are employed to handle and analyze large-scale proteomic datasets, extract meaningful biological insights, and generate testable hypotheses. Collaboration between bioinformaticians, statisticians, and domain experts is often necessary to develop robust analytical pipelines and interpret complex proteomic data accurately (82).

3.3. Standardization and Reproducibility

Standardization and reproducibility are critical aspects of biomarker discovery and proteomics research to ensure the reliability, comparability, and transferability of experimental findings across different laboratories and studies (83).

Standardization involves establishing and implementing standardized protocols, procedures, and quality control measures for sample collection, processing, analysis, and data interpretation (84). Standardized protocols ensure consistency and reproducibility of experimental results, minimize variability between experiments and laboratories, and facilitate data sharing and integration across studies. Standard operating procedures (SOPs) document detailed experimental protocols, equipment specifications, and data analysis workflows, ensuring transparency and traceability of experimental procedures (85).

Reproducibility refers to the ability to obtain consistent results when the same experiment is performed independently by different researchers or laboratories using the same or similar methods, materials, and conditions (86). Reproducible results validate the robustness and reliability of experimental findings, confirm the validity of scientific hypotheses, and support the generalizability of research conclusions. Factors influencing reproducibility include experimental design, sample size, statistical analysis, and data reporting practices.

Efforts to enhance standardization and reproducibility in biomarker discovery and proteomics research include the development of community-wide standards, guidelines, and best practices, such as the Minimum Information About a Proteomics Experiment (MIAPE) and the Clinical Proteomic Tumor Analysis Consortium (CPTAC) guidelines (87). Quality control measures, such as reference materials, internal standards, and proficiency testing, are employed to monitor and assess the performance of analytical methods and instruments, ensuring accuracy, precision, and reliability of experimental results. Collaborative initiatives, data-sharing platforms, and open-access resources facilitate data exchange, collaboration, and validation of research findings within the scientific community, advancing biomarker discovery and translational proteomics research (88).

3.4. Recent Advances and Future Perspectives

3.4.1. Technological Innovations in LC-MS

Liquid chromatography-mass spectrometry (LC-MS) has seen remarkable technological advancements in recent years, revolutionizing the field of proteomics and biomarker discovery (89). These innovations have significantly improved the sensitivity, resolution, speed, and versatility of LC-MS platforms, enabling comprehensive analysis of complex biological samples.

One key innovation is the development of high-resolution mass spectrometers such as orbitrap and time-of-flight (TOF) analyzers, which offer enhanced mass accuracy and resolving power (90). These instruments enable the detection and characterization of peptides and proteins with unprecedented sensitivity and specificity, facilitating the identification of low-abundance biomarkers and complex protein modifications.

Another significant advancement is the introduction of hybrid mass spectrometers, combining multiple ionization sources, mass analyzers, and fragmentation techniques in a single instrument (91). Hybrid instruments such as quadrupole-orbitrap and quadrupole-TOF systems offer complementary capabilities for peptide sequencing, quantification, and structural elucidation, enhancing the depth and accuracy of proteomic analysis.

Recent developments in chromatographic technology have also improved separation efficiency and throughput in LC-MS workflows. Ultra-high-performance liquid chromatography (UHPLC) systems with sub-2 μm particle columns and advanced column chemistries enable rapid and efficient separation of peptides and proteins, reducing analysis time and enhancing sample throughput (92).

Moreover, innovations in data acquisition and processing software have facilitated automated data analysis, de novo sequencing, and post-translational modification identification (93). These software tools offer user-friendly interfaces, advanced algorithms, and customizable workflows, streamlining data interpretation and accelerating biomarker discovery and validation.

Overall, technological innovations in LC-MS have transformed proteomic research, enabling comprehensive and high-throughput analysis of complex biological samples with unprecedented sensitivity, resolution, and speed. These advancements hold great promise for advancing our understanding of disease mechanisms, identifying novel biomarkers, and facilitating personalized medicine initiatives (94).

3.5. Integration with Multi-Omics Approaches

complex multi-omics approaches, integrating data from genomics, transcriptomics, proteomics, metabolomics, and other omics disciplines, have emerged as powerful strategies for elucidating biological systems and disease processes (95). Integration of LC-MS with multi-omics approaches enables comprehensive profiling of molecular signatures across different biological layers, providing deeper insights into the molecular mechanisms underlying health and disease.

LC-MS-based proteomics complements genomics and transcriptomics by providing direct measurements of protein expression, post-translational modifications, and protein-protein interactions. Proteomic data enhance our understanding of gene function, regulatory networks, and cellular processes, bridging the gap between genotype and phenotype (96).

Integration of LC-MS with metabolomics enables comprehensive profiling of small molecules, metabolites, and metabolic pathways, offering insights into cellular metabolism, bioenergetics, and disease biomarkers (97). LC-MS-based metabolomics complements proteomics by capturing downstream metabolic consequences of protein expression changes, providing a holistic view of cellular physiology and pathophysiology.

Furthermore, integration of LC-MS data with clinical data such as electronic health records, imaging data, and patient metadata enables correlation of molecular signatures with clinical phenotypes, disease progression, and treatment outcomes. These integrative analyses facilitate the identification of diagnostic biomarkers, therapeutic targets, and predictive biomarkers for personalized medicine approaches (98).

Overall, integration of LC-MS with multi-omics approaches offers synergistic advantages for comprehensive molecular profiling, systems-level understanding of biological processes, and translation of omics findings into clinical applications (99). These integrative strategies hold great promise for advancing precision medicine, improving disease diagnosis, and developing targeted therapies tailored to individual patients.

3.6. Potential Impact on Clinical Practice

The advancements in LC-MS technology and its integration with multi-omics approaches have the potential to revolutionize clinical practice by enabling more accurate diagnosis, prognosis, and treatment selection for various diseases (100).

In diagnostics, LC-MS-based proteomics facilitates the discovery and validation of novel biomarkers for early detection and differential diagnosis of diseases such as cancer, cardiovascular disorders, and neurological conditions. These biomarkers offer improved sensitivity and specificity compared to traditional diagnostic assays, enabling earlier disease detection and intervention (101).

Furthermore, LC-MS-based proteomics enables personalized medicine approaches by identifying biomarkers associated with treatment response, drug resistance, and adverse drug reactions. Integrative analyses combining proteomic, genomic, and clinical data enable the development of predictive models for patient stratification and treatment optimization, guiding clinicians in selecting the most effective therapies for individual patients (102).

In addition to diagnostics and personalized medicine, LC-MS-based proteomics holds promise for monitoring disease progression, assessing treatment efficacy, and predicting disease recurrence. Longitudinal studies using LC-MS-based biomarkers enable monitoring of disease dynamics, response to therapy, and identification of therapeutic targets for intervention (103).

Overall, the integration of LC-MS with multi-omics approaches has the potential to transform clinical practice by enabling more accurate, personalized, and timely diagnosis and treatment of diseases. These technologies offer new insights into disease mechanisms, biomarker discovery, and therapeutic interventions, paving the way for precision medicine and improved patient outcomes.

4. Conclusion

LC-MS-based proteomics and biomarker discovery have revolutionized biomedical research by providing high sensitivity, specificity, and throughput for analysing complex biological samples. This powerful technology has enabled comprehensive profiling of proteins and peptides, facilitating the identification of biomarkers associated with disease diagnosis, prognosis, and treatment response. The integration of LC-MS with multi-omics approaches, along with efforts to standardize protocols and data analysis workflows, has enhanced reproducibility and data comparability across studies. Moreover, the translational potential of LC-MS-based biomarkers in clinical diagnostics, drug development, and personalized medicine underscores its significance in improving patient care and healthcare delivery. The advancements in single-cell and spatial proteomics, multi-dimensional data integration, targeted proteomics, and machine learning-driven big data analytics are expected to further elevate the impact of LC-MS proteomics in understanding disease mechanisms and developing precise diagnostic and therapeutic strategies. By embracing emerging technologies and fostering interdisciplinary collaboration, LC-MS proteomics is poised to drive the next wave of breakthroughs in biomarker discovery and precision medicine, ultimately transforming healthcare outcomes globally**.**

Compliance with ethical standards

Acknowledgments

The authors are thankful to Raghavendra Institute of Pharmaceutical Education and Research, Anantapur.

Disclosure of conflict of interest

The authors do not have any conflict of interest

References

[1] Dupree EJ, Jayathirtha M, Yorkey H, Mihasan M, Petre BA, Darie CC. A critical review of bottom-up proteomics: the good, the bad, and the future of this field. Proteomes. 2020;8(3):14.

[2] Vandereyken K, Sifrim A, Thienpont B, Voet T. Methods and applications for single-cell and spatial multi-omics. Nature Reviews Genetics. 2023;24(8):494-515.

[3] Lill JR, Mathews WR, Rose CM, Schirle M. Proteomics in the pharmaceutical and biotechnology industry: a look to the next decade. Expert review of proteomics. 2021;18(7):503-26.

[4] Davis KD, Aghaeepour N, Ahn AH, Angst MS, Borsook D, Brenton A, et al. Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: challenges and opportunities. Nature Reviews Neurology. 2020;16(7):381-400.

[5] García-Gutiérrez MS, Navarrete F, Sala F, Gasparyan A, Austrich-Olivares A, Manzanares J. Biomarkers in psychiatry: concept, definition, types and relevance to the clinical reality. Frontiers in psychiatry. 2020;11:527209.

[6] Xiao Q, Zhang F, Xu L, Yue L, Kon OL, Zhu Y, Guo T. High-throughput proteomics and AI for cancer biomarker discovery. Advanced drug delivery reviews. 2021;176:113844.

[7] Nakayasu ES, Gritsenko M, Piehowski PD, Gao Y, Orton DJ, Schepmoes AA, et al. Tutorial: best practices and considerations for mass-spectrometry-based protein biomarker discovery and validation. Nature protocols. 2021;16(8):3737-60.

[8] Bian Y, Zheng R, Bayer FP, Wong C, Chang Y-C, Meng C, et al. Robust, reproducible and quantitative analysis of thousands of proteomes by micro-flow LC–MS/MS. Nature communications. 2020;11(1):157.

[9] Castelli FA, Rosati G, Moguet C, Fuentes C, Marrugo-Ramírez J, Lefebvre T, et al. Metabolomics for personalized medicine: the input of analytical chemistry from biomarker discovery to point-of-care tests. Analytical and bioanalytical chemistry. 2022;414(2):759-89.

[10] Broeckhoven K, Desmet G. Advances and innovations in liquid chromatography stationary phase supports. Analytical Chemistry. 2020;93(1):257-72.

[11] Dembek M, Bocian S. Pure water as a mobile phase in liquid chromatography techniques. TrAC Trends in Analytical Chemistry. 2020;123:115793.

[12] Lubomirsky E, Khodabandeh A, Preis J, Susewind M, Hofe T, Hilder EF, Arrua RD. Polymeric stationary phases for size exclusion chromatography: A review. Analytica Chimica Acta. 2021;1151:338244.

[13] Djajić N, Krmar J, Rmandić M, Rašević M, Otašević B, Zečević M, et al. Modified aqueous mobile phases: A way to improve retention behavior of active pharmaceutical compounds and their impurities in liquid chromatography. Journal of Chromatography Open. 2022;2:100023.

[14] Murisier A, Fekete S, Guillarme D, D'Atri V. The importance of being metal-free: the critical choice of column hardware for size exclusion chromatography coupled to high resolution mass spectrometry. Analytica chimica acta. 2021;1183:338987.

[15] Muhammad N, Zia-ul-Haq M, Ali A, Naeem S, Intisar A, Han D, et al. Ion chromatography coupled with fluorescence/UV detector: A comprehensive review of its applications in pesticides and pharmaceutical drug analysis. Arabian Journal of Chemistry. 2021;14(3):102972.

[16] Jarrold MF. Applications of charge detection mass spectrometry in molecular biology and biotechnology. Chemical reviews. 2021;122(8):7415-41.

[17] Zaikin VG, Borisov RS. Options of the main derivatization approaches for analytical ESI and MALDI mass spectrometry. Critical Reviews in Analytical Chemistry. 2022;52(6):1287-342.

[18] Li C, Chu S, Tan S, Yin X, Jiang Y, Dai X, et al. Towards higher sensitivity of mass spectrometry: a perspective from the mass analyzers. Frontiers in chemistry. 2021;9:813359.

[19] Arevalo Jr R, Ni Z, Danell RM. Mass spectrometry and planetary exploration: A brief review and future projection. Journal of mass spectrometry. 2020;55(1):e4454.

[20] Tamara S, den Boer MA, Heck AJ. High-resolution native mass spectrometry. Chemical Reviews. 2021;122(8):7269-326.

[21] López-Fernández O, Domínguez R, Pateiro M, Munekata PE, Rocchetti G, Lorenzo JM. Determination of polyphenols using liquid chromatography–tandem mass spectrometry technique (LC–MS/MS): A review. Antioxidants. 2020;9(6):479.

[22] Cortese M, Gigliobianco MR, Magnoni F, Censi R, Di Martino P. Compensate for or minimize matrix effects? Strategies for overcoming matrix effects in liquid chromatography-mass spectrometry technique: a tutorial review. Molecules. 2020;25(13):3047.

[23] El-Aneed A, Cohen A, Banoub J. Mass spectrometry, review of the basics: electrospray, MALDI, and commonly used mass analyzers. Applied spectroscopy reviews. 2009;44(3):210-30.

[24] Boja ES, Rodriguez H. Mass spectrometry-based targeted quantitative proteomics: achieving sensitive and reproducible detection of proteins. Proteomics. 2012;12(8):1093-110.

[25] Xiao JF, Zhou B, Ressom HW. Metabolite identification and quantitation in LC-MS/MS-based metabolomics. TrAC Trends in Analytical Chemistry. 2012;32:1-14.

[26] Cui L, Lu H, Lee YH. Challenges and emergent solutions for LC-MS/MS based untargeted metabolomics in diseases. Mass spectrometry reviews. 2018;37(6):772-92.

[27] Aslam B, Basit M, Nisar MA, Khurshid M, Rasool MH. Proteomics: technologies and their applications. Journal of chromatographic science. 2016:1-15.

[28] Ali N, Rampazzo RdCP, Costa ADT, Krieger MA. Current nucleic acid extraction methods and their implications to point-of-care diagnostics. BioMed research international. 2017;2017.

[29] Speers AE, Wu CC. Proteomics of integral membrane proteins theory and application. Chemical reviews. 2007;107(8):3687-714.

[30] Giansanti P, Tsiatsiani L, Low TY, Heck AJ. Six alternative proteases for mass spectrometry–based proteomics beyond trypsin. Nature protocols. 2016;11(5):993-1006.

[31] Ahmed FE. Sample preparation and fractionation for proteome analysis and cancer biomarker discovery by mass spectrometry. Journal of separation science. 2009;32(5-6):771-98.

[32] Kumar M, Tomar M, Potkule J, Verma R, Punia S, Mahapatra A, et al. Advances in the plant protein extraction: Mechanism and recommendations. Food Hydrocolloids. 2021;115:106595.

[33] Switzar L, Giera M, Niessen WM. Protein digestion: an overview of the available techniques and recent developments. Journal of proteome research. 2013;12(3):1067-77.

[34] Yaron A, Naider F, Scharpe S. Proline-dependent structural and biological properties of peptides and proteins. Critical reviews in biochemistry and molecular biology. 1993;28(1):31-81.

[35] Freije JR, Mulder PP, Werkman W, Rieux L, Niederlander HA, Verpoorte E, Bischoff R. Chemically modified, immobilized trypsin reactor with improved digestion efficiency. Journal of proteome research. 2005;4(5):1805-13.

[36] León IR, Schwämmle V, Jensen ON, Sprenger RR. Quantitative assessment of in-solution digestion efficiency identifies optimal protocols for unbiased protein analysis. Molecular & Cellular Proteomics. 2013;12(10):2992-3005.

[37] Karp NA, Lilley KS. Design and analysis issues in quantitative proteomics studies. Proteomics. 2007;7(S1):42-50.

[38] Chandramouli K, Qian P-Y. Proteomics: challenges, techniques and possibilities to overcome biological sample complexity. Human genomics and proteomics: HGP. 2009;2009.

[39] Cao Z, Tang H-Y, Wang H, Liu Q, Speicher DW. Systematic comparison of fractionation methods for in-depth analysis of plasma proteomes. Journal of proteome research. 2012;11(6):3090-100.

[40] Ong S-E, Pandey A. An evaluation of the use of two-dimensional gel electrophoresis in proteomics. Biomolecular engineering. 2001;18(5):195-205.

[41] Itzhak DN, Tyanova S, Cox J, Borner GH. Global, quantitative and dynamic mapping of protein subcellular localization. elife. 2016;5:e16950.

[42] Altelaar AM, Munoz J, Heck AJ. Next-generation proteomics: towards an integrative view of proteome dynamics. Nature Reviews Genetics. 2013;14(1):35-48.

[43] Mertins P, Tang LC, Krug K, Clark DJ, Gritsenko MA, Chen L, et al. Reproducible workflow for multiplexed deep-scale proteome and phosphoproteome analysis of tumor tissues by liquid chromatography–mass spectrometry. Nature protocols. 2018;13(7):1632-61.

[44] Pirok BW, Stoll DR, Schoenmakers PJ. Recent developments in two-dimensional liquid chromatography: fundamental improvements for practical applications. Analytical Chemistry. 2018;91(1):240-63.

[45] Barth HG, Jackson C, Boyes BE. Size exclusion chromatography. Analytical chemistry. 1994;66(12):595-620.

[46] Zeki ÖC, Eylem CC, Reçber T, Kır S, Nemutlu E. Integration of GC–MS and LC–MS for untargeted metabolomics profiling. Journal of Pharmaceutical and Biomedical Analysis. 2020;190:113509.

[47] Graf T, Heinrich K, Grunert I, Wegele H, Haberger M, Bulau P, Leiss M. Recent advances in LC–MS based characterization of protein-based bio-therapeutics–mastering analytical challenges posed by the increasing format complexity. Journal of Pharmaceutical and Biomedical Analysis. 2020;186:113251.

[48] Neagu A-N, Jayathirtha M, Baxter E, Donnelly M, Petre BA, Darie CC. Applications of tandem mass spectrometry (MS/MS) in protein analysis for biomedical research. Molecules. 2022;27(8):2411.

[49] Noor Z, Ahn SB, Baker MS, Ranganathan S, Mohamedali A. Mass spectrometry–based protein identification in proteomics—a review. Briefings in bioinformatics. 2021;22(2):1620-38.

[50] Zhang F, Ge W, Ruan G, Cai X, Guo T. Data-independent acquisition mass spectrometry-based proteomics and software tools: a glimpse in 2020. Proteomics. 2020;20(17-18):1900276.

[51] Chen C, Hou J, Tanner JJ, Cheng J. Bioinformatics methods for mass spectrometry-based proteomics data analysis. International journal of molecular sciences. 2020;21(8):2873.

[52] Yu F, Haynes SE, Teo GC, Avtonomov DM, Polasky DA, Nesvizhskii AI. Fast quantitative analysis of timsTOF PASEF data with MSFragger and IonQuant. Molecular & Cellular Proteomics. 2020;19(9):1575-85.

[53] Boekweg H, Van Der Watt D, Truong T, Johnston SM, Guise AJ, Plowey ED, et al. Features of peptide fragmentation spectra in single-cell proteomics. Journal of Proteome Research. 2021;21(1):182-8.

[54] Zhu Y, Orre LM, Tran YZ, Mermelekas G, Johansson HJ, Malyutina A, et al. DEqMS: a method for accurate variance estimation in differential protein expression analysis. Molecular & Cellular Proteomics. 2020;19(6):1047-57.

[55] Hampel H, Nisticò R, Seyfried NT, Levey AI, Modeste E, Lemercier P, et al. Omics sciences for systems biology in Alzheimer's disease: State-of-the-art of the evidence. Ageing Research Reviews. 2021;69:101346.

[56] Gianazza E, Brioschi M, Baetta R, Mallia A, Banfi C, Tremoli E. Platelets in healthy and disease states: from biomarkers discovery to drug targets identification by proteomics. International journal of molecular sciences. 2020;21(12):4541.

[57] Ponzini E, Santambrogio C, De Palma A, Mauri P, Tavazzi S, Grandori R. Mass spectrometry-based tear proteomics for noninvasive biomarker discovery. Mass Spectrometry Reviews. 2022;41(5):842-60.

[58] Verheggen K, Ræder H, Berven FS, Martens L, Barsnes H, Vaudel M. Anatomy and evolution of database search engines—a central component of mass spectrometry based proteomic workflows. Mass spectrometry reviews. 2020;39(3):292-306.

[59] Sobsey CA, Ibrahim S, Richard VR, Gaspar V, Mitsa G, Lacasse V, et al. Targeted and untargeted proteomics approaches in biomarker development. Proteomics. 2020;20(9):1900029.

[60] Cupp-Sutton KA, Wu S. High-throughput quantitative top-down proteomics. Molecular omics. 2020;16(2):91-9.

[61] Tian X, Permentier HP, Bischoff R. Chemical isotope labeling for quantitative proteomics. Mass spectrometry reviews. 2023;42(2):546-76.

[62] Kumar P, Debele SE, Sahani J, Rawat N, Marti-Cardona B, Alfieri SM, et al. Nature-based solutions efficiency evaluation against natural hazards: Modelling methods, advantages and limitations. Science of the Total Environment. 2021;784:147058.

[63] Čuklina J, Lee CH, Williams EG, Sajic T, Collins BC, Rodríguez Martínez M, et al. Diagnostics and correction of batch effects in large-scale proteomic studies: a tutorial. Molecular systems biology. 2021;17(8):e10240.

[64] Bime C, Camp SM, Casanova N, Oita RC, Ndukum J, Lynn H, Garcia JG. The acute respiratory distress syndrome biomarker pipeline: crippling gaps between discovery and clinical utility. Translational Research. 2020;226:105-15.

[65] Bacchi S, Tan Y, Oakden-Rayner L, Jannes J, Kleinig T, Koblar S. Machine learning in the prediction of medical inpatient length of stay. Internal medicine journal. 2022;52(2):176-85.

[66] Raposo F, Ibelli-Bianco C. Performance parameters for analytical method validation: Controversies and discrepancies among numerous guidelines. TrAC Trends in Analytical Chemistry. 2020;129:115913.

[67] Gaither C, Popp R, Mohammed Y, Borchers CH. Determination of the concentration range for 267 proteins from 21 lots of commercial human plasma using highly multiplexed multiple reaction monitoring mass spectrometry. Analyst. 2020;145(10):3634-44.

[68] Tang C, Mo X, Niu Q, Wahafu A, Yang X, Qui M, et al. Hypomorph mutation-directed small-molecule protein-protein interaction inducers to restore mutant SMAD4-suppressed TGF-β signaling. Cell chemical biology. 2021;28(5):636-47. e5.

[69] Candelise N, Baiardi S, Franceschini A, Rossi M, Parchi P. Towards an improved early diagnosis of neurodegenerative diseases: the emerging role of in vitro conversion assays for protein amyloids. Acta Neuropathologica Communications. 2020;8:1-16.

[70] Trevethan R. Sensitivity, specificity, and predictive values: foundations, pliabilities, and pitfalls in research and practice. Frontiers in public health. 2017;5:308890.

[71] Zhu W, Zeng N, Wang N. Sensitivity, specificity, accuracy, associated confidence interval and ROC analysis with practical SAS implementations. NESUG proceedings: health care and life sciences, Baltimore, Maryland. 2010;19:67.

[72] Van Stralen KJ, Stel VS, Reitsma JB, Dekker FW, Zoccali C, Jager KJ. Diagnostic methods I: sensitivity, specificity, and other measures of accuracy. Kidney international. 2009;75(12):1257-63.

[73] Šimundić A-M. Measures of diagnostic accuracy: basic definitions. ejifcc. 2009;19(4):203.

[74] Organization WH. Screening programmes: a short guide. Increase effectiveness, maximize benefits and minimize harm. 2020.

[75] Parvu V, Gary DS, Mann J, Lin Y-C, Mills D, Cooper L, et al. Factors that influence the reported sensitivity of rapid antigen testing for SARS-CoV-2. Frontiers in microbiology. 2021;12:714242.

[76] Mann M, Kumar C, Zeng W-F, Strauss MT. Artificial intelligence for proteomics and biomarker discovery. Cell systems. 2021;12(8):759-70.

[77] Fu J, Zhang Y, Liu J, Lian X, Tang J, Zhu F. Pharmacometabonomics: data processing and statistical analysis. Briefings in bioinformatics. 2021;22(5):bbab138.

[78] Da-Ano R, Visvikis D, Hatt M. Harmonization strategies for multicenter radiomics investigations. Physics in Medicine & Biology. 2020;65(24):24TR02.

[79] Eddy S, Mariani LH, Kretzler M. Integrated multi-omics approaches to improve classification of chronic kidney disease. Nature Reviews Nephrology. 2020;16(11):657-68.

[80] Zhao K, Rhee SY. Interpreting omics data with pathway enrichment analysis. Trends in Genetics. 2023;39(4):308-19.

[81] Jiao S, Lu Y, Wei G. Soil multitrophic network complexity enhances the link between biodiversity and multifunctionality in agricultural systems. Global Change Biology. 2022;28(1):140-53.

[82] Goh WWB, Wong L. The birth of bio-data science: trends, expectations, and applications. Genomics, Proteomics and Bioinformatics. 2020;18(1):5-15.

[83] Macklin A, Khan S, Kislinger T. Recent advances in mass spectrometry based clinical proteomics: applications to cancer research. Clinical proteomics. 2020;17(1):17.

[84] Klumpp-Thomas C, Kalish H, Drew M, Hunsberger S, Snead K, Fay MP, et al. Standardization of ELISA protocols for serosurveys of the SARS-CoV-2 pandemic using clinical and at-home blood sampling. Nature communications. 2021;12(1):113.

[85] Niso G, Botvinik-Nezer R, Appelhoff S, De La Vega A, Esteban O, Etzel JA, et al. Open and reproducible neuroimaging: from study inception to publication. NeuroImage. 2022;263:119623.

[86] Nosek BA, Hardwicke TE, Moshontz H, Allard A, Corker KS, Dreber A, et al. Replicability, robustness, and reproducibility in psychological science. Annual review of psychology. 2022;73:719-48.

[87] Von Kortzfleisch VT, Karp NA, Palme R, Kaiser S, Sachser N, Richter SH. Improving reproducibility in animal research by splitting the study population into several 'mini-experiments'. Sci Rep. 2020;10(1):16579.

[88] De Maria Marchiano R, Di Sante G, Piro G, Carbone C, Tortora G, Boldrini L, et al. Translational research in the era of precision medicine: where we are and where we will go. Journal of Personalized Medicine. 2021;11(3):216.

[89] Dai X, Shen L. Advances and trends in omics technology development. Frontiers in Medicine. 2022;9:911861.

[90] Melby JA, Roberts DS, Larson EJ, Brown KA, Bayne EF, Jin S, Ge Y. Novel strategies to address the challenges in top-down proteomics. Journal of the American Society for Mass Spectrometry. 2021;32(6):1278-94.

[91] Crotti S, Menicatti M, Pallecchi M, Bartolucci G. Tandem mass spectrometry approaches for recognition of isomeric compounds mixtures. Mass Spectrometry Reviews. 2023;42(4):1244-60.

[92] Perez de Souza L, Alseekh S, Scossa F, Fernie AR. Ultra-high-performance liquid chromatography high-resolution mass spectrometry variants for metabolomics research. Nature Methods. 2021;18(7):733-46.

[93] Hellinger R, Sigurdsson A, Wu W, Romanova EV, Li L, Sweedler JV, et al. Peptidomics. Nature Reviews Methods Primers. 2023;3(1):25.

[94] Cutshaw G, Uthaman S, Hassan N, Kothadiya S, Wen X, Bardhan R. The emerging role of Raman spectroscopy as an omics approach for metabolic profiling and biomarker detection toward precision medicine. Chemical Reviews. 2023;123(13):8297-346.

[95] Hu C, Jia W. Multi-omics profiling: the way toward precision medicine in metabolic diseases. Journal of molecular cell biology. 2021;13(8):576-93.

[96] Pazhamala LT, Kudapa H, Weckwerth W, Millar AH, Varshney RK. Systems biology for crop improvement. The plant genome. 2021;14(2):e20098.

[97] Liang L, Sun F, Wang H, Hu Z. Metabolomics, metabolic flux analysis and cancer pharmacology. Pharmacology & Therapeutics. 2021;224:107827.

[98] Montaner J, Ramiro L, Simats A, Tiedt S, Makris K, Jickling GC, et al. Multilevel omics for the discovery of biomarkers and therapeutic targets for stroke. Nature Reviews Neurology. 2020;16(5):247-64.

[99] Das T, Andrieux G, Ahmed M, Chakraborty S. Integration of online omics-data resources for cancer research. Frontiers in genetics. 2020;11:578345.

[100] Dalal N, Jalandra R, Sharma M, Prakash H, Makharia GK, Solanki PR, et al. Omics technologies for improved diagnosis and treatment of colorectal cancer: Technical advancement and major perspectives. Biomedicine & Pharmacotherapy. 2020;131:110648.

[101] Kwong GA, Ghosh S, Gamboa L, Patriotis C, Srivastava S, Bhatia SN. Synthetic biomarkers: a twenty-first century path to early cancer detection. Nature Reviews Cancer. 2021;21(10):655-68.

[102] Saigusa D, Matsukawa N, Hishinuma E, Koshiba S. Identification of biomarkers to diagnose diseases and find adverse drug reactions by metabolomics. Drug metabolism and pharmacokinetics. 2021;37:100373.

[103] Zhang X-w, Li Q-h, Dou J-j. Mass spectrometry-based metabolomics in health and medical science: A systematic review. RSC advances. 2020;10(6):3092-104.