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Research Article

Advanced Chromatographic and Spectroscopic Method Development for Biomarker Identification and Validation in Clinical Biochemistry

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ABSTRACT

The application of advanced chromatographic and spectroscopic methods has revolutionized the process of biomarker identification and validation in clinical biochemistry, thus enhancing analytical sensitivity, specificity and reproducibility. Several analytical techniques like liquid chromatography–mass spectrometry (LC-MS/MS), gas chromatography–mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), Raman spectroscopy, Fourier-transform infrared spectroscopy (FTIR), and nuclear magnetic resonance (NMR), offer strong bases for the identification of complex biological molecules involved in various diseases. The present study discusses recent advances in method development, optimization and validation in the field of biomarker discovery in clinical diagnostics and personalized medicine. The combination of multi-omics, automation and advanced mass spectrometry technologies has improved clinical biomarker reliability and applicability for translation. In addition, analytical validation parameters such as sensitivity, specificity, precision and accuracy are still key for clinical acceptance and regulatory compliance. The study highlights the growing importance of advanced analytical technologies in improving early disease detection, therapeutic monitoring, and precision healthcare.

INTRODUCTION

In clinical biochemistry, huge changes have been undergone by the development of the chromatography and spectroscopy analytical techniques for identification and validation of biomarkers. Biomarkers are biological parameters that can be measured and reflect important information about physiological processes, disease course, treatment response and prognosis. With growing interest in early disease diagnosis, precision medicine, and personalized therapeutics, there has been a significant increase in the development of highly sensitive and highly selective analytical platforms for the detection of low abundance biomolecules in complex biological matrices,

(Ahmad et al., 2023; Son et al., 2024).

High performance liquid chromatography (HPLC), ultra high performance liquid chromatography (UHPLC), gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) are all essential tools in clinical biomarker analysis and are advanced chromatographic techniques. The techniques are more effective in separating metabolites, peptides, proteins, glycoproteins, and other clinically important metabolites, with increased analytical sensitivity and accurate quantitative analyses (Adaway et al., 2015; Khamis et al., 2021). Specifically, LC-MS/MS has become a gold-standard analytical platform, which has the

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Table 1: Major Biomarker Categories and Associated Analytical Techniques

Biomarker Category	Clinical Significance	Common Analytical Technique	Typical Biological Sample
Protein Biomarkers	Cancer and inflammatory diseases	LC-MS/MS	Plasma/Serum
Metabolite Biomarkers	Metabolic disorders	GC-MS, UHPLC	Urine/Serum
Glycoprotein Biomarkers	Oncology diagnostics	HPLC-MS	Blood
Salivary Biomarkers	Oral and systemic diseases	Mass Spectrometry	Saliva
Neurological Biomarkers	Neurodegenerative disorders	Raman/FTIR Spectroscopy	Cerebrospinal fluid
Proteomic Biomarkers	Kidney and cardiovascular diseases	Proteomics-MS	Plasma

capacity to offer high level of molecular characterization and absolute quantification of endogenous biomarkers in biological fluids like plasma, urine, saliva, and cerebrospinal fluid (Neubert et al., 2020; Fernández-Metzler et al., 2022).

At the same time, spectroscopic techniques such as Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy have shown to be very useful for non-invasive diagnostics of diseases and biochemical profiling. Spectroscopic technologies are able to offer rapid molecular fingerprinting for the detection of biochemical changes associated with cancer, neurological disorders, metabolic syndromes and kidney disorders (Khan, 2018; Ashraf et al., 2015). Overall, these analytical methods are being increasingly combined with the approaches of proteomics and metabolomics to facilitate the discovery of new biomarkers and the stratification of diseases (Kennedy et al., 2018; Chen et al., 2018).

Recent developments in the field of mass spectrometry instrumentation, bioinformatic and automated analytical workflows have significantly enhanced biomarker reproducibility and clinical translation. New high-resolution mass spectrography platforms allow for comprehensive multi-omics research, which can be used to discover new diagnostic and prognostic biomarkers for oncology, cardiovascular diseases and neurodegenerative

diseases (Crutchfield et al., 2016; Son et al., 2024). In addition, the clinical application of emerging fields such as glycoproteomics, salivary biomarkers, and plasma proteomics is still expanding the application of analytical biochemistry (Wang et al., 2015; Wang et al., 2019; Zhou et al., 2020).

These technological developments leave many problems to be solved in the development of analytical procedures and in the validation of biomarkers. Despite progress in widely clinical use, problems with matrix interference, analytical variability, method accuracy and robustness, and inter-laboratory reproducibility remain (Moein et al., 2017; Ohtsu et al., 2021). Hence, strict method validation procedures such as limit of detection (LOD), limit of quantification (LOQ), precision, sensitivity, specificity, and accuracy must be performed and reported to provide analytical reliability and ensure regulatory compliance in clinical diagnostics (Suraj et al., 2018 and Fernández-Metzler et al., 2022).

Furthermore, the incorporation of artificial intelligence, machine learning algorithms, and sophisticated computational analytics further drives the biomarkers interpretation and predictive modeling capabilities in clinical chemistry. The developments are also helping to pave the way for personalized medicine, allowing for quicker detection of disease and therapeutic monitoring, as well as optimization of treatment for individual patients (Haque et al., 2025; Jannetto & Fitzgerald, 2016).

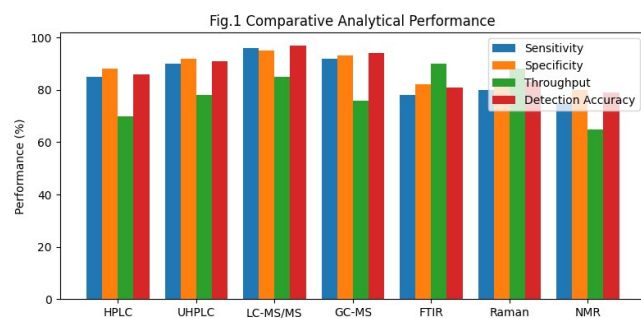


Fig 1: Comparison of sensitivity, specificity, throughput, and detection accuracy across major analytical platforms used for biomarker detection.

CHROMATOGRAPHIC METHOD DEVELOPMENT FOR BIOMARKER IDENTIFICATION

The advent of the chromatographic method has introduced an essential approach in clinical biochemistry, especially in the field of identification and validation of biomarkers, known for its high analytical sensitivity, specificity, and reproducibility. Advantageous separation and quantitative determination of complex biological compounds in plasma, serum, urine, saliva and tissue is achieved using advanced chromatographic systems coupled with mass spectrometry. The methods like high-performance liquid chromatography (HPLC), ultra-high-performance liquid



Table 2: Chromatographic Techniques and Analytical Characteristics

<i>Technique</i>	<i>Analytical Principle</i>	<i>Sensitivity</i>	<i>Major Biomarker Applications</i>	<i>Advantages</i>	<i>Limitations</i>
HPLC	Liquid-phase separation	Moderate	Drug metabolites, proteins	Good reproducibility	Longer analysis time
UHPLC	High-pressure liquid separation	High	Metabolomics, proteomics	Faster separation and high resolution	Expensive instrumentation
LC-MS/MS	Chromatography coupled with tandem mass spectrometry	Very High	Endogenous metabolites, cancer biomarkers	Excellent specificity and sensitivity	Matrix effects and ion suppression
GC-MS	Gas-phase separation with mass spectrometry	High	Volatile organic metabolites	High chromatographic efficiency	Requires derivatization for non-volatile compounds
Micro-LC-MS/MS	Miniaturized LC-MS/MS system	Very High	Low-abundance protein biomarkers	Reduced solvent use and improved sensitivity	Complex system optimization
Immunoaffinity LC-MS/MS	Antibody-assisted chromatographic enrichment	Ultra-High	Protein biomarker quantification	Highly selective enrichment	High operational cost

chromatography (UHPLC), gas chromatography–mass spectrometry (GC-MS), and liquid chromatography–tandem mass spectrometry (LC-MS/MS) are widely used in contemporary clinical diagnostics and translational biomarker studies (Adaway et al., 2015; Crutchfield et al., 2016).

The high selectivity and low limits of detection of LC-MS/MS make it one of the most reliable analytical platforms for quantification of endogenous biomarker metabolites. The technique is useful for targeted and untargeted metabolomic profiling, which can be used to elucidate disease-associated molecular signatures for cancer, cardiovascular disorders, kidney disease and neurological disease (Kennedy et al., 2018; Khamis et al., 2021). A new trend in chromatographic separation has also been the improvement of separation speed, reduction of matrix effects, and robustness of the separation method for clinical use (Son et al., 2024).

The optimization of chromatograph method development involves optimization of many analytical parameters such as stationary phase selection, mobile phase composition, pH control, flow rate, injection volume, and the conditions of gradient elution. In addition to the above, sample preparation procedures like protein precipitation, liquid-liquid extraction and solid-phase extraction are also crucial to minimize biological interferences and analyte recovery (Moein et al., 2017). Immunoaffinity enrichment coupled with LC-MS/MS has been pivotal in the discovery of low abundant protein biomarkers, particularly in oncology and inflammatory disease research (Neubert et al., 2020).

A lot of interest has been directed to micro-LC-MS/MS

systems which can lower solvent usage and enhance analytical sensitivity and resolution. These are especially beneficial for the simultaneous quantification of several biomarkers from small biological specimens. Suraj et al., 2018, successfully applied micro-LC-MS/MS for high precision and reproducibility quantitative analysis of endothelial dysfunction protein biomarkers in murines plasma.

In metabolomics-based biomarker discovery, chromatographic techniques facilitate the separation of structurally related metabolites prior to mass spectrometric detection. Untargeted metabolomic workflows have become increasingly valuable for identifying novel biomarkers associated with metabolic syndromes, cancer progression, and rare diseases (Kennedy et al., 2018). Similarly, plasma proteomics studies have utilized advanced chromatographic platforms to discover early-stage gastric cancer biomarkers and other clinically relevant molecular indicators (Zhou et al., 2020).

The integration of chromatographic systems with high-resolution mass spectrometry has further strengthened personalized medicine and clinical diagnostics. Advanced analytical workflows now support multiplex biomarker profiling, automated data acquisition, and improved quantification accuracy. However, challenges such as matrix variability, ion suppression, biomarker instability, and inter-laboratory reproducibility continue to affect analytical standardization and clinical implementation (Fernández-Metzler et al., 2022; Ohtsu et al., 2021).

Moreover, chromatographic techniques play a significant role in glycoproteomics and glycosylation analysis for

cancer biomarker discovery. Aberrant glycosylation patterns identified through chromatographic-mass spectrometric methods have shown strong diagnostic and prognostic relevance in several malignancies (Wang et al., 2019). Salivary biomarker analysis using mass spectrometry-based chromatographic platforms has also emerged as a promising non-invasive diagnostic approach for systemic and oral diseases (Wang et al., 2015).

The continued advancement of chromatographic technologies, including automation, miniaturization, and AI-assisted analytical interpretation, is expected to enhance biomarker discovery efficiency and clinical applicability. Future developments in multidimensional chromatography and hybrid mass spectrometric systems may further improve analytical sensitivity, throughput, and reproducibility in clinical biochemistry and precision medicine (Haque et al., 2025; Son et al., 2024).

SPECTROSCOPIC APPROACHES IN BIOMARKER DETECTION

Spectroscopic techniques have emerged as highly valuable analytical tools for biomarker detection and characterization in clinical biochemistry due to their high sensitivity, rapid analytical performance, minimal sample preparation requirements, and non-destructive analytical capabilities. These approaches provide detailed molecular and structural information that supports disease diagnosis, therapeutic monitoring, and personalized medicine. Modern spectroscopic platforms are increasingly integrated with chromatographic and mass spectrometric systems to enhance biomarker specificity and quantitative reliability in complex biological matrices (Son et al., 2024). Among the most widely applied spectroscopic methods are Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, nuclear magnetic resonance (NMR), ultraviolet-visible (UV-Vis) spectroscopy, and mass spectrometry-based spectroscopic systems. These analytical techniques enable the identification of proteins, metabolites, nucleic acids, glycoproteins, and

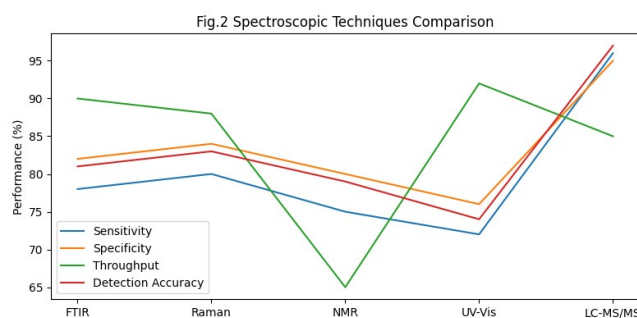


Fig 2: Performance trends illustrating diagnostic efficiency among commonly used spectroscopic methods in biomarker analysis.

lipid-associated biomarkers linked to cancer, neurological disorders, cardiovascular diseases, kidney diseases, and metabolic syndromes (Ahmad et al., 2023).

FTIR spectroscopy has gained considerable importance in clinical diagnostics because of its ability to generate molecular fingerprint profiles from biological samples such as plasma, saliva, urine, and tissues. The technique detects vibrational changes in molecular bonds, enabling differentiation between healthy and diseased states. FTIR has shown promising applications in cancer diagnostics and metabolic disease monitoring due to its rapid spectral acquisition and reproducibility (Ashraf et al., 2015). Similarly, Raman spectroscopy provides highly specific molecular information through inelastic light scattering mechanisms and has demonstrated strong potential in neurological biomarker analysis, tumor classification, and non-invasive disease screening (Khan, 2018).

Nuclear magnetic resonance spectroscopy is another important spectroscopic platform used extensively in metabolomics-based biomarker discovery. NMR allows simultaneous qualitative and quantitative analysis of multiple metabolites within biological systems while maintaining excellent reproducibility and minimal sample destruction. The technique has become increasingly valuable for metabolic profiling, disease stratification,

Table 3: Spectroscopic Techniques and Their Clinical Biomarker Applications

<i>Spectroscopic Technique</i>	<i>Analytical Principle</i>	<i>Biomarker Application</i>	<i>Major Advantage</i>
FTIR Spectroscopy	Molecular vibration analysis	Cancer and metabolic biomarkers	Rapid fingerprint profiling
Raman Spectroscopy	Inelastic light scattering	Neurological disorders	Non-invasive analysis
NMR Spectroscopy	Magnetic resonance of nuclei	Metabolomics profiling	High reproducibility
UV-Visible Spectroscopy	Light absorption measurement	Routine biochemical assays	Cost-effective
LC-MS/MS	Mass-to-charge ratio detection	Proteomics and metabolomics	Ultra-high sensitivity



and therapeutic response assessment in clinical research (Kennedy et al., 2018). In addition, UV-Visible spectroscopy continues to support routine biomarker quantification because of its simplicity, low operational cost, and compatibility with automated clinical analyzers.

Mass spectrometry-integrated spectroscopic techniques represent one of the most advanced developments in clinical biomarker detection. Technologies such as LC-MS/MS and immunoaffinity LC-MS/MS provide ultra-sensitive detection and absolute quantification of endogenous biomarkers in complex biological matrices. These systems are widely used for proteomics, glycomics, and metabolomics investigations due to their superior selectivity, sensitivity, and multiplexing capabilities (Khamis et al., 2021). Furthermore, tandem mass spectrometry platforms have significantly improved the identification of low-abundance biomarkers associated with early-stage diseases and personalized therapeutic interventions (Crutchfield et al., 2016).

Proteomic and glycoproteomic biomarker studies have particularly benefited from spectroscopic advancements. Aberrant glycosylation patterns associated with cancer progression are now routinely investigated using high-resolution mass spectrometric techniques coupled with advanced spectral analysis algorithms (Wang et al., 2019). Similarly, plasma proteomics and salivary biomarker profiling have expanded diagnostic opportunities for gastric cancer, oral diseases, and systemic disorders through highly sensitive spectroscopic detection systems (Wang et al., 2015; Zhou et al., 2020).

Despite these advancements, several analytical challenges remain in spectroscopic biomarker detection. Variability in sample preparation, instrument calibration, matrix interference, spectral overlap, and standardization protocols can significantly affect analytical reproducibility and biomarker validation outcomes. Therefore, robust analytical validation procedures involving accuracy, specificity, precision, sensitivity, and limit-of-detection assessment are critical for ensuring clinical reliability and regulatory acceptance (Fernández-Metzler et al., 2022). Method optimization and validation strategies remain essential components in translating spectroscopic biomarker discoveries into clinical diagnostic applications (Moein et al., 2017; Ohtsu et al., 2021).

Recent developments in artificial intelligence, machine learning, and automated spectral processing have further enhanced the diagnostic potential of spectroscopic techniques. AI-assisted spectral interpretation enables rapid classification of complex biochemical patterns and improves disease prediction accuracy in clinical settings. The integration of advanced spectroscopy with multi-omics platforms is expected to further accelerate biomarker discovery, precision medicine implementation,

and next-generation clinical diagnostics (Haque et al., 2025).

BIOMARKER VALIDATION AND ANALYTICAL STANDARDIZATION

Biomarker validation and analytical standardization are critical stages in the translation of biomarker discoveries into reliable clinical applications. Advanced chromatographic and spectroscopic techniques such as liquid chromatography–tandem mass spectrometry (LC-MS/MS), gas chromatography–mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), and Raman spectroscopy have significantly enhanced the sensitivity and specificity of biomarker detection in clinical biochemistry. However, the increasing complexity of biological matrices and analytical workflows requires rigorous validation procedures to ensure reproducibility, accuracy, and clinical reliability (Khamis et al., 2021; Fernández-Metzler et al., 2022).

Analytical validation involves systematic evaluation of assay performance characteristics including precision, accuracy, selectivity, sensitivity, robustness, linearity, limit of detection (LOD), and limit of quantification (LOQ). These parameters are essential for ensuring that biomarker assays produce reproducible and clinically meaningful results across different laboratories and patient populations (Moein et al., 2017). In mass spectrometry-based biomarker studies, matrix effects, ion suppression, sample instability, and instrument variability remain major challenges affecting assay consistency and quantitative reliability (Adaway et al., 2015).

The validation of endogenous biomarkers is particularly complex because biological samples naturally contain varying concentrations of target analytes. Absolute quantification methods using isotope-labeled internal standards and calibration strategies have therefore become important for improving analytical precision in LC-MS/MS workflows (Khamis et al., 2021). Similarly, immunoaffinity LC-MS/MS platforms have demonstrated improved selectivity and sensitivity for protein biomarker quantification in clinical laboratories (Neubert et al., 2020). These approaches have facilitated the reliable measurement of low-abundance biomarkers associated with cancer, metabolic disorders, kidney disease, and cardiovascular dysfunction (Chen et al., 2018; Zhou et al., 2020).

Spectroscopic biomarker validation has also gained importance in neurological and metabolic disease diagnostics. Techniques such as FTIR, Raman spectroscopy, and NMR provide rapid and non-destructive molecular profiling; however, spectral overlap, signal variability, and data interpretation remain key standardization challenges (Khan, 2018). Advanced chemometric algorithms

Table 4: Major Validation Parameters and Their Clinical Significance in Biomarker Assays

<i>Validation Parameter</i>	<i>Description</i>	<i>Importance in Clinical Biochemistry</i>	<i>Common Analytical Techniques</i>
Accuracy	Closeness of measured value to true value	Ensures reliable diagnosis and monitoring	LC-MS/MS, HPLC
Precision	Reproducibility of repeated measurements	Maintains assay consistency	GC-MS, LC-MS/MS
Sensitivity	Ability to detect low biomarker concentrations	Supports early disease detection	Mass Spectrometry
Specificity	Ability to distinguish target analyte from interference	Reduces false-positive results	Immunoaffinity LC-MS/MS
Linearity	Proportional analytical response across concentration range	Enables quantitative reliability	HPLC, Spectroscopy
Limit of Detection (LOD)	Lowest detectable analyte concentration	Enhances trace biomarker identification	LC-MS/MS
Limit of Quantification (LOQ)	Lowest quantifiable analyte concentration	Improves quantitative confidence	GC-MS, UHPLC
Robustness	Resistance to analytical variability	Supports routine clinical application	Automated analytical systems
Selectivity	Ability to isolate specific biomarkers in complex matrices	Improves biomarker discrimination	Proteomics platforms
Stability	Preservation of analyte integrity during analysis	Prevents biomarker degradation	Bioanalytical workflows

and artificial intelligence-based analytical models are increasingly being integrated into spectroscopic workflows to improve biomarker classification accuracy and diagnostic reproducibility (Ahmad et al., 2023).

Regulatory agencies and bioanalytical guidelines emphasize the need for standardized validation frameworks before biomarkers can be adopted in clinical practice or drug development. Important considerations include sample handling procedures, instrument calibration, inter-laboratory reproducibility, and quality control strategies (Ohtsu et al., 2021). Biomarker assays intended for precision medicine applications require stringent validation due to their direct influence on patient diagnosis, therapeutic monitoring, and personalized treatment decisions (Son et al., 2024).

Recent advances in proteomics, metabolomics, and glycomics have further accelerated biomarker discovery and validation. Untargeted metabolomics approaches have enabled comprehensive profiling of disease-associated metabolites, while glycoproteomic analyses have improved cancer biomarker identification through aberrant glycosylation profiling (Kennedy et al., 2018; Wang et al., 2019). Moreover, salivary and plasma biomarker investigations using advanced mass spectrometric platforms continue to expand opportunities for minimally invasive clinical diagnostics (Wang et al., 2015).

The integration of automated analytical systems, high-resolution mass spectrometry, and computational

bioinformatics is improving assay throughput and clinical applicability. Nevertheless, harmonization of analytical protocols across laboratories remains essential for ensuring global reproducibility and translational reliability. Future biomarker validation strategies are expected to increasingly incorporate multi-omics integration, machine learning-assisted interpretation, and ultra-sensitive analytical instrumentation to support next-generation personalized medicine and precision diagnostics (Haque et al., 2025; Son et al., 2024).

CLINICAL APPLICATIONS AND EMERGING TECHNOLOGIES

With their ability to discover, quantify and validate a large variety of disease related biomarkers in a high resolution environment, advanced chromatographic and spectroscopic platforms have emerged as key tools in modern clinical biochemistry. Proteomics- and metabolomics-based LC-MS/MS workflows have been extensively applied in clinical oncology for the identification of protein signatures of the tumor and metabolic changes in the circulation, thereby enhancing early detection and prognosis (Zhou et al., 2020; Crutchfield et al., 2016). Likewise, glycoprotein profiling, as well as aberrant-glycosylation analysis, has improved the specificity of cancer markers, especially in complex cancers where standard assays have low sensitivity



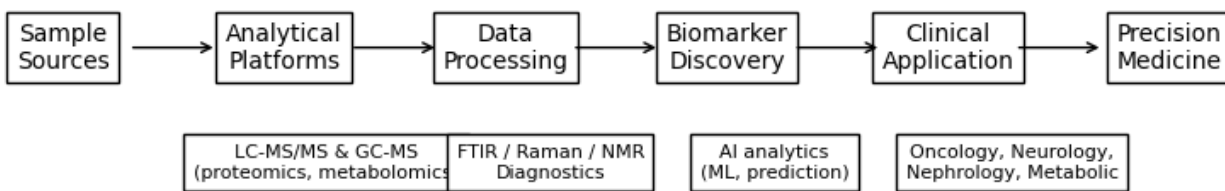


Fig 3: Conceptual framework showing the progression from sample analysis to biomarker discovery and precision medicine applications through advanced analytical and AI-driven technologies.

(Wang et al., 2019). Proteomic-based LC-MS strategies facilitate discovery of kidney injury markers to facilitate early intervention and better patient stratification in nephrology (Chen et al., 2018).

Mass spectrometry has been expanded to therapeutic drug monitoring and endocrine and metabolic disorder diagnostics, with its high level of analytical precision and multiplexing capabilities (Jannetto & Fitzgerald, 2016; Adaway et al., 2015). Salivary and minimally invasive diagnostic biomarker platforms are also being explored that may be further developed for point-of-care applications and for population scale screening (Wang et al., 2015). Moreover, spectroscopic methods like FTIR and Raman spectroscopy are being increasingly used in diagnostics of neurological diseases that provide rapid biochemical fingerprinting of diseased tissues without labels (Khan, 2018).

Emerging technologies, increasingly, are geared towards deepening the analysis and clinical translatability. The untargeted metabolomics and high throughput proteomics are allowing for profiling of the entire biomarker profile, and immunoaffinity LC-MS/MS are improving quantification of low abundance proteins in complex biological matrices (Kennedy et al., 2018; Neubert et al., 2020). Another recent advancement is the discussion of better assay validation strategies to guarantee the accuracy, reliability, and clinical relevance of diagnostics that rely on biomarkers (Moein et al., 2017; Fernández-Metzler et al., 2022; Ohtsu et al., 2021).

The insertion of artificial intelligence and machine learning (AI-ML) in the pipelines of chromatographic and spectroscopic data analysis is growing and facilitates the automatic detection of peaks, pattern recognition and prediction of biomarker models. This integration supports precision medicine by linking molecular signatures to individualized therapeutic responses (Ahmad et al., 2023; Son et al., 2024). Additionally, advancements in ultra-high-resolution mass spectrometry and micro-LC-MS platforms are improving sensitivity for low-abundance biomarkers in small-volume clinical samples (Suraj et al., 2018; Haque et al., 2025).

CONCLUSION

The development of advanced chromatographic and spectroscopic methods has become integral to the clinical biochemistry of today, with the ability to identify disease-related biomarkers with high sensitivity and selectivity. The introduction of liquid chromatography–tandem mass spectrometry (LC-MS/MS), gas chromatography–mass spectrometry (GC-MS) and other complementary spectroscopic platforms has greatly increased the accuracy and scope of biomolecular profiling of complex biological samples. Nevertheless, it is difficult to obtain absolute quantification of endogenously produced biomarkers, as matrix effects, analytical variability, and strict standardization of workflows are all significant challenges (Khamis et al., 2021).

The reliability and reproducibility of measurements of biomarkers in clinical research and diagnostics has been enhanced by recent improvements in the design and validation of assays and regulatory guidance. Platforms based on mass spectrometry have proven good performances for multiplexed biomarker quantification, where stringent validation protocols are being put in place for clinical translatability and consistency between labs (Fernández-Metzler et al., 2022; Ohtsu et al., 2021). In addition, the continuously evolving method development strategies and bioanalytical validation principles have improved the robustness of the clinical assays, facilitating their wider application in precision medicine and in translation research (Moein et al., 2017; Crutchfield et al., 2016).

Virtually, the combination of sophisticated chromatographic separation methods, high-resolution spectroscopic analysis, and uniform validation systems is bringing about tremendous advances in the discovery and implementation of biomarkers in the clinical setting. Analytical throughput, inter-laboratory variability and integration of multi-omics datasets are likely to be the focus of future developments to enable comprehensive and personalized diagnostics of diseases.

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