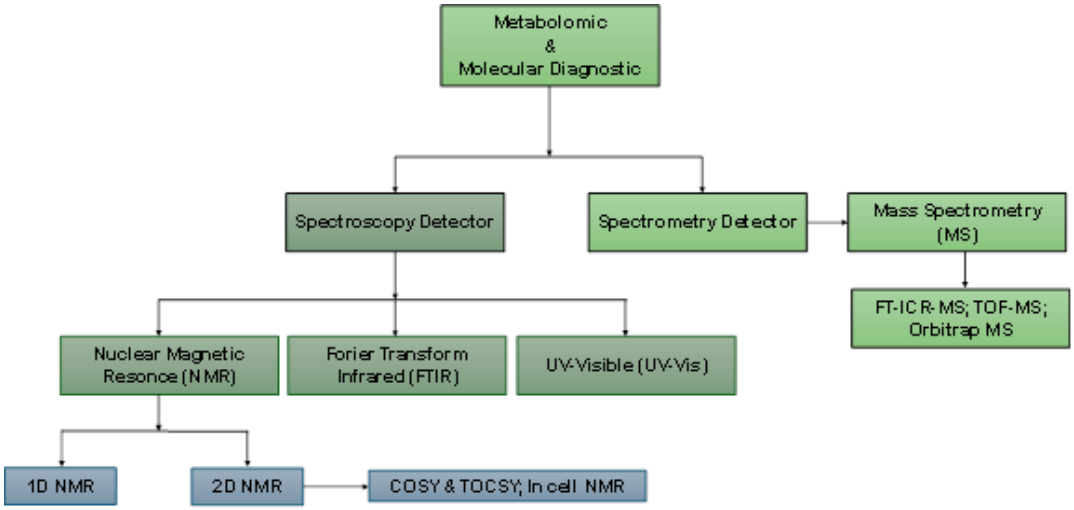
Indonesian Journal of Chemical Analysis
Homepage: https://journal.uii.ac.id/IJCA

Advancements in NMR and IR Spectroscopy: Enhancing Metabolomics and Disease Diagnostics in the Health Sector: A Comprehensive Review

Rizki Rachmad Saputra[a*], Mokhamat Ariefin[a], Meiyanti Ratna Kumalasari[a], Junita Dongoran[a], Mulani Jeni Lestari Tampubolon[a], Putri Sulistiawati[a], Sri Yulandari Simangunsong[a], Risya Ariska[a], Pandu Gizta Rapi Paksi[a], Amelia Siska[a], Jeddah Yanti[b], Luluil Maknun[c]

[a]Department of Chemistry, Universitas Palangka Raya, Palangka Raya, 73111, Indonesia
[b]Department of Geography, Universitas Negeri makassar, Makassar, 90222, Indonesia
[c]Applied Chemistry Laboratory, Nugen Bioscience Indonesia, Jakarta, 15138, Indonesia
* corresponding author: rizkirachmads@mipa.upr.ac.id
DOI: 10.20885/ijca.vol7.iss2.art6

GRAPHICAL ABSTRACT



ARTICLE INFO

Received : 19 July 2024
Revised : 20 August 2024
Published : 30 September 2024
Keywords : NMR spectroscopy; IR spectroscopy; organic compounds; disease diagnosis; biomarkers; metabolites

ABSTRACT

Metabolomics has emerged as a critical field in understanding biological processes and disease mechanisms, necessitating advancements in analytical techniques to handle complex biological samples. This review explores the global landscape of metabolomics, with a focus on the use of spectroscopy and spectrometry. Techniques such as UV-Vis and Fourier Transform InfraRed (FTIR) spectroscopy offer fast and cost-effective metabolite tracing but are limited by their sensitivity, particularly for low-abundance metabolites. Nuclear Magnetic Resonance (NMR) spectroscopy, despite being less sensitive than mass spectrometry (MS), provides unparalleled structural information, distinguishing metabolites with similar mass-to-charge ratios. NMR's capability to detect metabolites in the 1-10 µM range highlights its effectiveness in metabolomics. This review categorizes advancements in these techniques, starting with global

contributions to spectroscopy, followed by detailed discussions on FTIR strategies for metabolite tracing, and concluding with NMR's qualitative and quantitative applications in metabolomics and disease diagnostics. The review underscores the continuous development in sample preparation and data integration, enhancing the accuracy and applicability of these techniques, positioning NMR and FTIR as essential tools in modern metabolomic research.

1. INTRODUCTION

Researchers have extensively studied metabolomics in a wide range of applications. Due to the many challenges associated with complex biological samples, advancements in analytical techniques have been actively explored. A variety of techniques, such as spectroscopy and spectrometry, are reported. Both methods provide excellent information on qualitative and quantitative analysis. Although UV-Vis and Fourier Transform InfraRed (FTIR) based spectroscopy technique offers fast tracing of metabolite and cost-effectiveness, these techniques are not sensitive enough for low abundance metabolite detection. They are considered less robust [1]. Low analyte concentrations can result in weak signals that may be difficult to detect and quantify accurately. NMR effectively detect analytes in the range of 1-10 µM [2]. Although its sensitivity is lower than 10x that of mass spectrometry for metabolite detection, NMR can distinguish between metabolites with similar mass-to-charge ratios, which is a challenge for mass spectrometry (MS) [3]. Despite the challenges spectroscopic techniques like FTIR and NMR face in detecting metabolites, ongoing development in sample preparation and data integration are driving significant improvements in their accuracy and broader applicability. Countries worldwide have contributed significantly to the advancement of NMR methodologically. The global landscape of spectroscopy metabolomics is rich and varied, reflecting the challenges and opportunities inherent in this advanced analytical technique. This review is categorized into three main topics. Firstly, the global advancements in the development of spectroscopic techniques are reviewed. The second part describes the concept of FTIR and improving the metabolite tracing strategy in biofluidic samples. The third part focuses on the NMR strategy for qualitative and quantitative metabolomic and molecular diagnostic applications, with the concluding remarks in the final parts.

2. METHOD

This review systematically investigates the advancements in Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy within the field of metabolomics and disease diagnostics. A comprehensive literature search was conducted using PubMed, ScienceDirect, and Google Scholar, focusing on peer-reviewed articles published from 2010 to 2024. The search terms included "metabolomics," "FTIR spectroscopy," "NMR spectroscopy," and "disease diagnostics," with priority given to studies reporting analytical improvements and their applications to metabolite detection. The selected studies were categorized based on their focus on either FTIR or NMR techniques. FTIR was analyzed for its rapid metabolite tracing capabilities, while NMR was examined for its structural elucidation power and ability to detect metabolites in the µM range. The review also considered advancements in sample preparation, particularly methods enhancing signal resolution and chemometric integration for improved data accuracy. A critical analysis was conducted to compare FTIR's limitations in detecting low-abundance metabolites with NMR's strengths in qualitative and quantitative metabolomics. Emerging trends in hybridization techniques and data processing innovations were also explored, emphasizing their potential to improve diagnostic sensitivity. Finally, quantitative data from the studies were collated and analyzed, allowing for a nuanced understanding of how these techniques are evolving to enhance metabolomics and disease diagnostics, positioning them as crucial tools for modern biomedical research.

3. WORlDWIDE CHARACTERIZATION OF METABOLOMIC AND MOLECULAR DIAGNOSTIC USING SPECTROSCOPIC TECHNIQUES

Numerous techniques in clinical metabolomics have been studied by Pang et al. (2019). The two commonly used techniques for clinical metabolomic analysis are spectrometry (mass spectrometry/MS) and spectroscopy (Nuclear Magnetic Resonance/NMR, Infrared/IR and UV-visible/UV-Vis). High-resolution mass spectrometry is one of the widely used techniques for metabolomic analysis around the world, including Fourier transform ion cyclotron resonance (FT-ICR), Orbitrap or Time of Flight (TOF) MS. Some journals focus on MS development for identification and quantification of metabolites in biological samples [4-7]. MS is highly sensitive, allowing for detecting a wide range of metabolites at low concentrations. Although MS is a powerful tool in clinical metabolomics, MS could only provide information primarily on molecular weight and sometimes structure through fragmentation patterns. One of the spectroscopic techniques called 'NMR' could give detailed structural information, including molecular conformations and functional groups, making it particularly valuable for identifying unknown metabolites [8]. With a reasonable effort, Wang et al. (2019) presented an approach that leverages NMR to detect molecular motifs and specific structural features within molecules which aids in the accurate and efficient identification of unknown compounds. His findings showed that the method is beneficial in uncovering novel metabolites and understanding complex biochemical pathways. A new technique combining NMR with stable isotope tracing has been developed for advanced metabolomic analysis [9]. Fan et al. (2009) explored using ^{13}C stable isotope-resolved metabolomics (SIRM) for tracking carbon atoms through metabolic pathways in lung cancer. Isotope-labelled tracers and the resulting metabolite flux maps are adequate for tracing many primary metabolites in lung cancer at higher levels. This research provides the innovative use of stable isotope-resolved metabolomics and the comprehensive analysis of metabolic pathways [10].

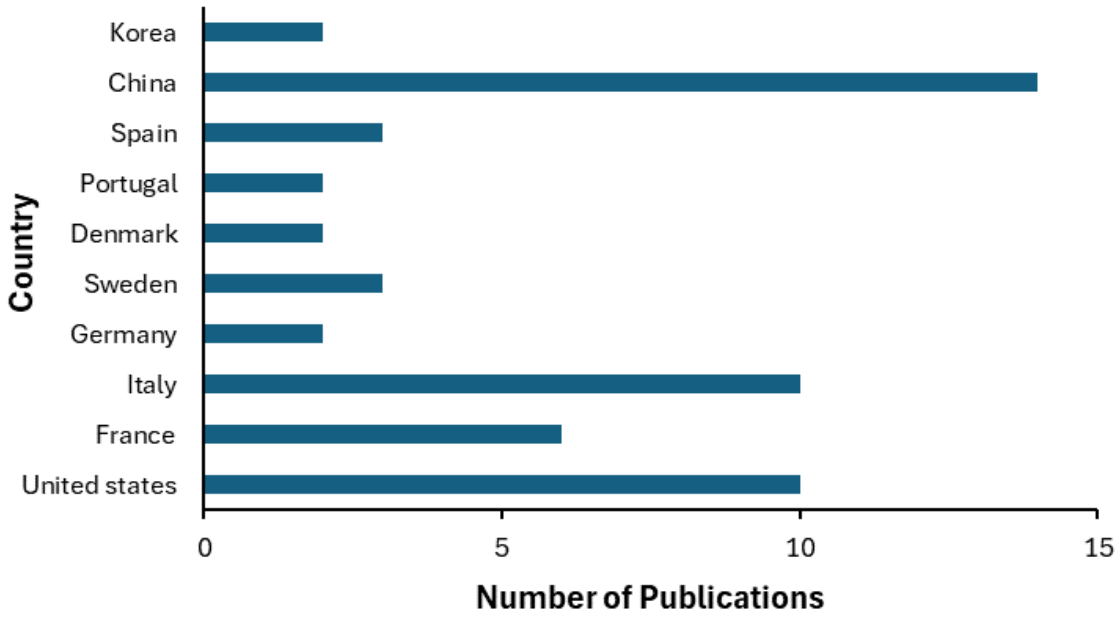


Figure 1. Number of publications by country on the use of NMR for metabolomic and molecular diagnostic (2014 – 2024)

Over the past ten years, the NMR-based spectroscopic technique has gradually attracted more attention worldwide in the medical metabolomics field (Figure 1). NMR has many contributions to the metabolomic field despite its low sensitivity compared to mass spectrometry. NMR spectroscopy continues to play an important role in diverse health and disease research applications. In the United States, NMR-based metabolomics was found to be largely used for disease biomarker discovery, including cancer and cardiovascular disease [11, 12]. France has contributed significantly to

methodological advancements in NMR, particularly in developing high-field NMR and cryoprobes that enhance sensitivity and resolution. French research groups are known for integrating NMR with chemometrics and multivariate analysis to interpret complex metabolomic data [13-15]. China has rapidly adopted NMR metabolomics, with a growing number of studies focused on traditional Chinese medicine (TCM). NMR was developed to study the role of TCM in biofluids for disease treatment [16]. From Figure 1, Italy is also listed as a country to significantly contribute to NMR development in various applications in the biomedical fields, such as disease biomarker discovery, neuroscience and medicine [17-19]. Like France, Italian researchers have established strong collaborations within Europe and beyond, participating in large-scale metabolomics initiatives. With continued investment in technology and international collaboration, Italy is poised to remain a leader in the application and development of NMR metabolomics. In other countries, especially developing ones, NMR development is still rare due to the high cost of instruments and the lack of specialist experts.

In addition to NMR, FTIR is another spectroscopic technique utilized for metabolomic characterization. This technique is commonly used for qualitative analysis to identify the metabolite in biological samples. The following section will discuss a detailed review of the spectroscopic technique.

4. FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY AS FAST SCREENING METHOD FOR METABOLOMICS AND DISEASE DIAGNOSTIC ANALYSIS

4.1. The principle of FTIR: sample preparation techniques and data analysis

FTIR studies have the unique advantage of thoroughly analyzing cell metabolism without damaging the sample [20-22]. This technique can provide comprehensive data without complicated extraction or purification processes with only a few whole-cell samples. Compared to conventional methods such as MS chromatography and NMR, FTIR offers greater efficiency and convenience in metabolomics analysis [20]. The technique is known to be simple, reproducible, reagent-free, and non-destructive to samples, making it suitable for clinical study [23, 24].

FTIR spectroscopy accurately identifies bond types, functional groups, and molecular structures by analyzing their unique vibrational spectra. Each molecule possesses a distinct “spectral fingerprint,” enabling identification and characterization. The most informative spectral regions for biological analysis include the fingerprint region (1450-600 cm^{-1}), the amide I and II (1700-1500 cm^{-1}), and the higher wave number regions (3500-2550 cm^{-1}). The fingerprint region provides information about the overall molecular structure, while the amides I and II regions are related to proteins. The higher wave number regions are sensitive to hydrogen bonds and other functional groups [24].

FTIR is widely used to analyze non-aqueous samples, particularly biofluids. However, analyzing biofluids often encounters challenges due to relatively low analyte concentrations. To address this challenge, concentrating samples using a centrifugal filtration device has proven effective, enabling the measurement of analytes in aqueous conditions. Alternatively, drying the sample before analysis is another viable approach [24, 25]. However, drying can cause chemical and physical inhomogeneities, such as coffee ring effects, crack patterns, and gelation, which may reduce the sensitivity and reproducibility of the analysis. Additionally, compositional and concentration variations in dried droplets can lead to band saturation in FTIR transmission [24]. Alternatively, biofluids can be analyzed in a liquid or semi-dry state, though this may result in the loss of some spectral regions. Moreover, when analyzing biofluids such as blood samples, it is necessary to add anticoagulants like lithium heparin, EDTA, or citric acid [26]. As shown in Figure 2, The traditional sampling method involves preparing a biofluid sample with an anticoagulant and placing it on a flat surface. Modern sampling methods include transmission (where the sample is placed between IR-transparent windows), transflection (where the sample is placed on a reflective surface and IR light is reflected back), and ATR (where the sample is in contact with an ATR crystal). The corresponding spectra generated from each modern sampling method illustrate similar qualitative data across different techniques, with wavenumber on the x-axis and absorbance/transmittance on the y-axis.

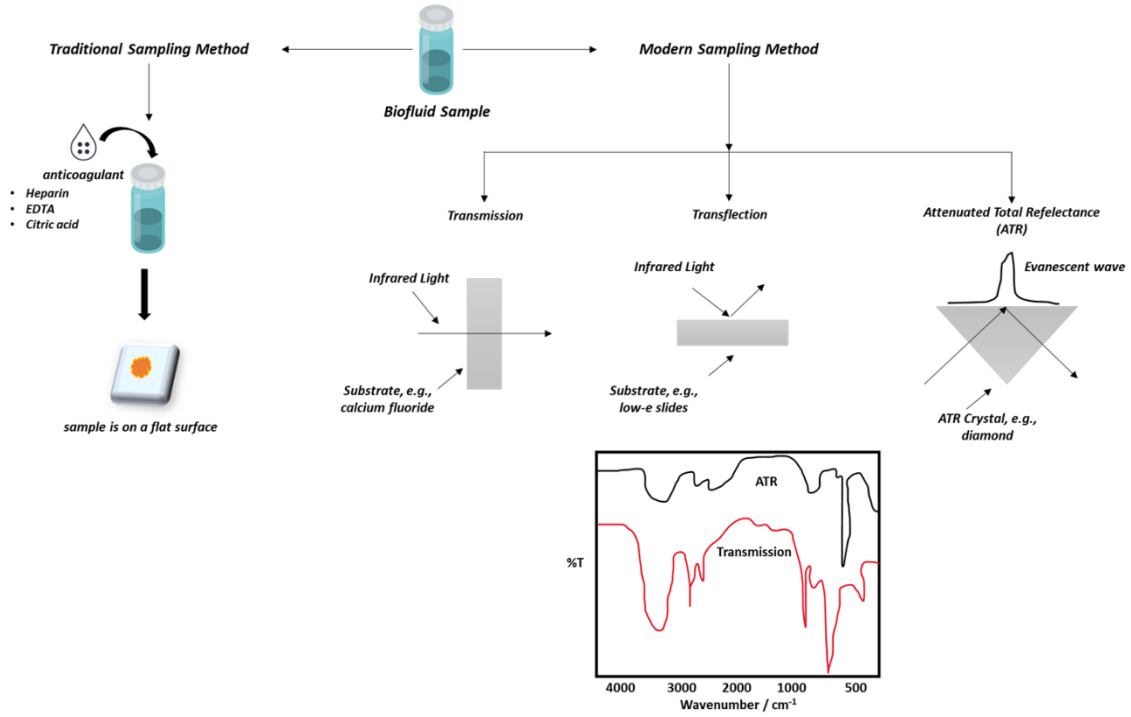


Figure 2. Overview of sampling methods for qualitative analysis using FTIR spectroscopy

Another crucial aspect of biofluid analysis is the selection of the appropriate analytical technique. Three primary sampling methods exist: transmission, transflection, and attenuated total reflection (ATR). Each method has its own set of advantages and disadvantages. Transmission and transflection, for instance, require more complex sample preparation and exhibit a low signal-to-noise ratio [27]. On the other hand, ATR is user-friendly, generates robust signals, and causes minimal sample damage [26]. However, it also presents limitations, such as potential sample damage upon contact with the ATR crystal and interference from air trapped between the sample and the crystal [26, 28]. Therefore, the sample must be in direct contact with the internal reflection element (IRE), which is the ATR crystal. The incident IR beam angle must exceed the critical angle to induce total internal reflection; otherwise, the resulting spectrum will combine ATR and external refraction [26].

The interpretation of FTIR spectra can be significantly enhanced by integrating multivariate analysis approaches, particularly principal component analysis (PCA) and cluster analysis. These methods play a crucial role in identifying patterns and groupings within the dataset, thereby enabling more meaningful interpretation and more precise sample classification. After data preprocessing, the selection of an appropriate chemometric method becomes crucial for qualitative or quantitative data analysis. Seven statistical methods are commonly used in chemometric analysis to extract useful information from chemical data. Infrared spectra can also be classified by applying qualitative analysis techniques based on pattern recognition methodology. These classification techniques fall into two categories: supervised and unsupervised. Unsupervised methodologies, such as PCA and hierarchical cluster analysis (HCA), are highly effective for organizing spectra without requiring prior sample information. They provide an overview of dataset complexity, heterogeneity, and similarity, aiding outlier detection. Additionally, these techniques ensure measurement reproducibility and offer an overview of group separation [29].

4.2. Application of FTIR for metabolomic and molecular diagnostic

FTIR has been discussed in several publications for its applications, as shown in Table 1. This technique is suitable for fast screening techniques of metabolomic analysis. Banerjee et al. (2022) employed Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopy, Raman spectroscopy, and mass spectrometry to profile metabolites in serum samples from patients

with pituitary adenomas. From the result, they identified specific metabolic patterns and biomarkers associated with pituitary adenomas through spectral analysis. The quantification of particular metabolites was done using mass spectrometry, allowing the levels of various biomarkers to be measured in the sample [30].

TABLE 1. Qualitative strategies for metabolomic and molecular diagnostic analysis using Fourier transform infrared (FTIR)

Application	Qualitative strategy	Emerging technologies	Limitations of previous method	Key findings	References
Pituitary Adenomas (PAs)	Identification of differential regions (nucleic acids, lipids)	Advanced chemometrics for detailed analysis	Limited sensitivity and specificity in traditional methods	High accuracy in classification, sensitive detection of molecular changes	[30]
Digestive Tract Cancers (DTC)	PLS-DA with 100% sensitivity, over 95% specificity	Infrared Molecular Fingerprints (IMFs)	Conventional tests are time-consuming and less detailed	Highly sensitive and specific detection across cancer types	[31]
Metabolic Syndrome	Sensitivity and specificity not specifically detailed	Chemometrics with ATR-FTIR	Previous methods lack comprehensive metabolic profiling	Effective differentiation of metabolic syndrome in blood plasma	[32]

Guo et al. (2022) used ATR-FTIR spectroscopy to identify distinct spectral patterns and biomarkers associated with various digestive tract cancers. These patterns revealed differences in the biochemical composition of blood samples from patients with different types of cancers, allowing for the qualitative differentiation of cancerous and non-cancerous samples. For digestive tract cancers (DTC), ATR-FTIR, combined with advanced technologies like 2D-SD-IR and BP Neural Networks, delivered a perfect 100% sensitivity and over 95% specificity [31]. Souza et al. (2022) also employed ATR-FTIR to identify distinct spectral patterns associated with metabolic syndrome, showcasing the technique's potential in differentiating complex metabolic profiles. Although specific quantitative sensitivity and specificity data were not provided, the integration with chemometrics demonstrated the technique's qualitative capability [32].

While valuable for qualitative analysis, several limitations have been found for quantitative analysis using FTIR, such as peak overlap and matrix interference in complex samples, which could lead to inaccurate measurement. Despite these limitations, FTIR can still be effective for quantification when used with appropriate calibration methods and sample preparation techniques.

5. QUALITATIVE AND QUANTITATIVE STRATEGY FOR METABOLOMICS AND DISEASE DIAGNOSTIC ANALYSIS USING NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

5.1. Type of Nuclear Magnetic Resonance (NMR): Concept and Limitation

Nuclear Magnetic Resonance (NMR) spectroscopy has become a pivotal tool in metabolomics, offering qualitative and quantitative insights crucial for comprehensive metabolic analysis and disease diagnostics. NMR's dual functionality—accurate quantification of metabolite concentrations and detailed structural analysis—makes it indispensable for understanding metabolic processes and identifying potential disease biomarkers. This review explores the capabilities of NMR spectroscopy, its applications, emerging technologies, limitations of previous methods, and key findings that underscore its importance in metabolomics.

On the qualitative front, NMR spectroscopy plays a crucial role in providing detailed information on the molecular structure and dynamics of metabolites and proteins. Techniques such as in-cell NMR, which allows for real-time tracking of metabolites and investigation of protein structures in environments close to their natural state, are essential for understanding metabolic pathways and their alterations in disease states [33, 34]. In vivo magnetic resonance spectroscopy (MRS) further extends these qualitative capabilities by enabling whole-organism metabolic monitoring and visualizing the spatial distribution of metabolites and targeted proteins. This holistic

approach provides a deeper understanding of metabolic changes at both cellular and systemic levels [33, 34].

Moreover, NMR-based metabolomics has widespread applications across diverse fields, including agriculture, veterinary science, medicine, and pharmacology. Its ability to deliver quantitative and qualitative data makes it a powerful tool for comprehensive metabolomic studies and disease diagnostics, offering a complete picture of metabolic alterations associated with various conditions [35].

NMR spectroscopy is renowned for its quantitative precision, enabling the accurate measurement of metabolite concentrations with minimal sample preparation [36]. his precision is essential for tracking metabolic changes in biological samples and identifying biomarkers linked to various diseases [35]. One-dimensional (1D) NMR techniques, particularly valuable for generating spectra that allow for the determination of both relative and absolute metabolite concentrations, play a crucial role in identifying disease biomarkers and providing significant insights into disease mechanisms and progression [37].

However, ^{1}D NMR techniques face notable challenges, such as peak or spectral overlap, particularly in ^{1}H NMR spectra with a limited chemical shift range [38]. This overlap can hinder accurate quantification, especially in complex biological mixtures where metabolites with similar structures may be closely positioned [39]. Advanced strategies such as two-dimensional (2D) NMR methods (e.g.,^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC) have been developed to address these challenges. These methods distribute the signal across a second, more dispersed chemical shift range, reducing overlap and improving the identification of metabolites [40]. Nowadays, more advanced 2D-NMR, ^{13}C-^{13}C CT-TOCSY NMR, has been increasingly used for faster tracing an analyte in complex samples. As shown in Figure 3, the ^{13}C-^{13}C CT-TOCSY NMR provides better resolution than 1D NMR, which often suffers from peak overlap. ^{13}C-^{13}C CT-TOCSY NMR spectrum offers superior sensitivity and resolution by precisely mapping carbon-carbon correlations, like the isotope labelling strategy used in mass spectrometry (MS). This technique accurately identifies and differentiates complex structures within biological samples.

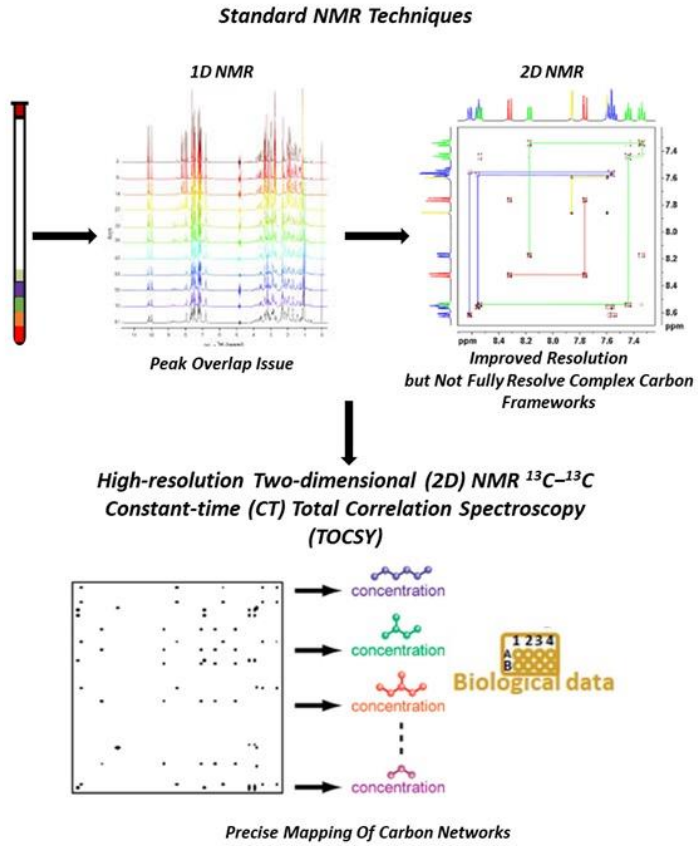


Figure 3. Comparative Overview of Standard NMR Techniques and ^{13}C-^{13}C CT-TOCSY NMR.

Despite its strengths, NMR spectroscopy faces limitations, particularly in sensitivity. To overcome this challenge, advanced techniques such as dynamic nuclear polarization (DNP) and parahydrogen-induced polarization (PHIP) have been developed [33, 34]. These methods significantly enhance signal strength, enabling the detection of low-abundance metabolites and the real-time monitoring of metabolic activities. DNP enhances signal detection by transferring high polarization from electronic spins of radicals to nearby nuclear spins. At the same time, PHIP involves the transfer of nuclear spin polarization from parahydrogen to other nuclei, enhancing signal detection, particularly in proton NMR studies. These advancements improve the quantitative and qualitative aspects of NMR-based metabolomics, expanding the technique's scope in studying complex biological systems and identifying subtle metabolic changes linked to disease [33, 34].

Traditional NMR methods, while powerful, have encountered limitations, particularly in analyzing complex mixtures and detecting low-abundance metabolites. For instance, 1D NMR techniques often suffer from peak overlap, making distinguishing between metabolites with similar chemical structures difficult. Additionally, abundant proteins in biological samples like human serum or plasma can interfere with metabolite detection in NMR spectra [38]. Conventional sample preparation methods, such as ultrafiltration and organic solvent precipitation, have been used to remove proteins, but these techniques can also remove metabolites, complicating the analysis [41].

Advanced sample preparation methods have been developed to address these limitations. Nanoparticle-assisted protein removal and ultrafiltration have shown excellent results in recovering metabolites and generating cleaner NMR spectra. These methods enhance the accuracy of metabolomics analysis, particularly in complex biological systems [41].

5.2. Application of NMR for metabolomic and molecular diagnostic

Nuclear Magnetic Resonance (NMR) spectroscopy is a crucial tool in metabolomics and molecular diagnostics due to its non-destructive nature, quantitative precision, and ability to provide detailed molecular insights, as shown in Table 2. NMR allows for the accurate quantification of metabolite concentrations and the structural elucidation of complex biomolecules, making it indispensable for comprehensive metabolic analysis and disease diagnostics [35, 36]. One-dimensional (1D) NMR techniques are commonly used for metabolomics because they provide quantitative data on metabolite concentrations with minimal sample preparation. This capability is essential for identifying disease biomarkers and understanding metabolic processes [37]. However, 1D NMR faces limitations such as peak overlap, particularly in complex biological samples where metabolites with similar structures may appear close together [38]. This issue has led to more advanced two-dimensional (2D) NMR techniques, such as ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC, which spread the signal across a second chemical shift range, reducing overlap and enhancing metabolite identification [40].

Beyond quantitative capabilities, NMR also provides valuable qualitative insights into molecular structures and dynamics. In-cell NMR and in vivo magnetic resonance spectroscopy (MRS) are not just tools; they are windows into the real-time tracking of metabolic processes and protein structures in environments close to their natural state, providing critical information on metabolic pathways and their alterations in disease states. These techniques are particularly useful for visualizing the spatial distribution of metabolites and understanding systemic metabolic changes in disease conditions [33, 34].

Emerging technologies, such as dynamic nuclear polarization (DNP) and parahydrogen-induced polarization (PHIP), have not just enhanced but significantly enhanced the sensitivity of NMR, enabling the detection of low-abundance metabolites and improving both quantitative and qualitative analyses [33, 34]. These advancements have expanded the scope of NMR in metabolomics and diagnostics, allowing for a more comprehensive understanding of complex biological systems and subtle metabolic changes linked to disease. Despite its strengths, traditional NMR methods have faced challenges in analyzing complex mixtures and detecting low-abundance metabolites. Advanced sample preparation techniques, such as nanoparticle-assisted protein removal combined with ultrafiltration, have been developed to address these issues, improving the accuracy of metabolomics analysis [41]. NMR spectroscopy remains a cornerstone in metabolomics and molecular diagnostics, with advanced techniques such as ^{13}C-^{13}C CT-TOCSY NMR, in-cell NMR, and emerging polarization technologies driving discoveries. These methods enhance our understanding of metabolic processes and improve the accuracy of disease biomarker identification, making NMR an essential tool in the field [35, 36].

TABLE II. Qualitative and quantitative strategies for metabolomic and molecular diagnostic analysis using nuclear magnetic resonance (NMR) from the past 10 years (2014 – 2024)

Type of NMR technique	Application	Quantitative strategy	Qualitative strategy	Emerging technologies	Limitations of previous method	Key findings	References
1D NMR	Metabolite concentration measurement	Direct quantification using signal intensities. Quantification with detection limit around micromolar range	Identification of metabolites based on chemical shift		Limited sensitivity, spectral overlap. Limited sensitivity (~micromolar), spectral overlap	Provides precise quantitative data on metabolite concentrations, but spectral overlap can limit accuracy	[36, 38]
^{1}H NMR	Metabolomics analysis of biological samples	Quantification using peak intensities and internal standards	Comprehensive view of all detectable compounds		Overlap in spectra, interference from abundant proteins	Widely used in metabolomics, but faces challenges with peak overlap and sensitivity	[38, 39]
2D NMR (COSY, HSQC)	Structural elucidation of complex mixtures	Improved quantification by spreading signals over two dimensions with detection limit around nanomolar range	Identification of molecular structures and interactions		Requires advanced data interpretation, still limited sensitivity than 1D	Resolves spectral overlap, improves accuracy in complex mixtures	[38, 40]
^{13}C-^{13}C CT-TOCSY NMR	Metabolite quantification with labeling	Full ^{13}C labeling for accurate quantification	Structural insights through ^{13}C-^{13}C correlation		Requires extensive labeling, expensive	Offers precise quantification and structural information through labeled metabolites	[38]
^{31}P NMR	Energy state studies in cells (in vivo/ex vivo)	Quantification of phosphorylated compounds	Analysis of energy-related metabolites		Signal overlap from phosphorylated compounds	Provides insights into cellular energy states, but faces challenges with signal overlap	[38]
In-cell NMR	Real-time tracking of metabolites and protein structures	Not primarily used for quantification	Investigates protein structures and dynamics in natural environments		Limited to cell-based systems; less applicable for whole-organism studies	Offers real-time insights into metabolic pathways in native environments	[33, 34]
In vivo MRS	Whole-organism metabolic monitoring	Not primarily used for quantification	Visualizes spatial distribution of metabolites and proteins		Low resolution, limited by tissue heterogeneity	Enables holistic understanding of systemic metabolic changes	[33, 34]

DNP - enhanced NMR	Enhancement of NMR signal sensitivity. Detection of low-abundance metabolites	Enhanced signal detection through hyperpolarization	Improved structural analysis due to increased sensitivity	Dynamic nuclear polarization (DNP)	Requires specialized equipment; may not be applicable for all metabolite types	Significantly boosts signal detection, allowing for study of low-abundance metabolites	[33, 34]
PHIP - enhanced NMR	Enhancement of NMR signal sensitivity. Proton NMR studies	Enhanced signal through parahydrogen -induced polarization	Better detection of subtle structural features	Parahydrogen-induced polarization (PHIP)	Requires specific conditions for parahydrogen production; less suited for some applications. Complex sample preparation, limited to specific reactions	Enhances signal particularly in proton NMR; useful in studying enzymatic reactions and cell signaling	[33, 34]
Integration with MS	Comprehensive metabolomic analysis	Combines quantitative NMR with sensitive MS data			NMR sensitivity issues; MS may require sample preparation and complex data interpretation	Leverages strengths of both techniques for more robust metabolite profiling and biomarker identification	[36, 38]

6. FUTURE PERSPECTIVE OF SPECTROSCOPY'S TECHNIQUE FOR METABOLOMIC AND MOLECULAR DIAGNOSTIC ANALYSIS

NMR spectroscopy leverages the magnetic properties of specific nuclei, such as ^{1}H, ^{13}C, ^{31}P, and ^{19}F, to absorb and emit energy, enabling detailed analysis of biological samples [37]. The technique's ability to perform both one-dimensional (1D) and more complex two-dimensional (2D) and three-dimensional (3D) analyses enhances its utility in metabolomics. While 1D NMR techniques are primarily used for quantitative analysis, providing essential data on metabolite concentrations, 2D and 3D techniques offer detailed insights into molecular structures and interactions. Databases like HMDB, BMRB, TOCCATA, and COLMAR are crucial in ensuring accurate metabolomics analysis by providing standard spectra and molecular information [13]. However, these databases need more compound coverage. Efforts like the NIH Common Fund Centers' establishment of a metabolomics data repository and the COSMOS initiative, which is developing improved data standards and infrastructure, are addressing these limitations. Open-source platforms like MVAPACK have also emerged to support NMR metabolomics data analysis, facilitating adherence to best practices in metabolic fingerprint analysis [38].

7. CONCLUSION

Spectroscopy techniques remain an indispensable tool in metabolomics, offering a unique combination of quantitative precision and qualitative insights. Its wide application in identifying and characterizing biomarkers underscores its critical role in disease diagnostic analysis. Advances in hyperpolarization techniques, along with the complementary use of other analytical methods such as mass spectrometry (MS), further highlight the evolving landscape of metabolomics research. Together, these techniques promise continued advancements in our understanding of metabolic processes and the discovery of disease biomarkers, solidifying NMR's role as a fundamental tool in the field.

References

[1] D. A. Dias *et al.*, "Current and future perspectives on the structural identification of small molecules in biological systems," vol. 6, no. 4, p. 46, 2016.

[2] L. C. Heather, X. Wang, J. A. West, J. L. J. J. o. m. Griffin, and c. cardiology, "A practical guide to metabolomic profiling as a discovery tool for human heart disease," vol. 55, pp. 2-11, 2013.

[3] T. M. Domenick, E. L. Gill, V. Vedam-Mai, and R. A. J. A. C. Yost, "Mass spectrometry-based cellular metabolomics: current approaches, applications, and future directions," vol. 93, no. 1, pp. 546-566, 2020.

[4] M. Manzi, G. Riquelme, N. Zabalegui, M. E. J. J. o. P. Monge, and B. Analysis, "Improving diagnosis of genitourinary cancers: Biomarker discovery strategies through mass spectrometry-based metabolomics," vol. 178, p. 112905, 2020.

[5] S.-a. Qi *et al.*, "High-resolution metabolomic biomarkers for lung cancer diagnosis and prognosis," vol. 11, no. 1, p. 11805, 2021.

[6] I. Menkovic, M. Boutin, A. Alayoubi, F. E. Mercier, G.-É. Rivard, and C. J. I. J. o. M. S. Auray-Blais, "Identification of a reliable biomarker profile for the diagnosis of Gaucher disease type 1 patients using a mass spectrometry-based metabolomic approach," vol. 21, no. 21, p. 7869, 2020.

[7] Y. Hövelmann, A. Jagels, R. Schmid, F. Hübner, and H.-U. J. E. j. o. n. Humpf, "Identification of potential human urinary biomarkers for tomato juice intake by mass spectrometry-based metabolomics," vol. 59, pp. 685-697, 2020.

[8] I. Garcia-Perez *et al.*, "Identifying unknown metabolites using NMR-based metabolic profiling techniques," pp. 1-30, 2020.

[9] C. Wang *et al.*, "Accurate and efficient determination of unknown metabolites in metabolomics by NMR-based molecular motif identification," vol. 91, no. 24, pp. 15686-15693, 2019.

[10] T. W. Fan *et al.*, "Altered regulation of metabolic pathways in human lung cancer discerned by 13 C stable isotope-resolved metabolomics (SIRM)," vol. 8, pp. 1-19, 2009.

[11] D. Lingli *et al.*, "Combining NMR and LC/MS Using Backward Variable Elimination: Metabolomics Analysis of Colorectal Cancer, Polyps, and Healthy Controls," 2016.

[12] A.-H. Emwas *et al.*, "Recommendations and standardization of biomarker quantification using NMR-based metabolomics with particular focus on urinary analysis," vol. 15, no. 2, pp. 360-373, 2016.

[13] P. Giraudeau and G. Remaud, "Advanced NMR Methods for Position-Specific Isotope Analysis," in *Handbook of Isotopologue Biogeochemistry*: Springer, 2024, pp. 1-31.

[14] C. Palaric *et al.*, "Processing of NMR and MS metabolomics data using chemometrics methods: a global tool for fungi biotransformation reactions monitoring," vol. 15, pp. 1-13, 2019.

[15] L. Lecuyer *et al.*, "NMR metabolomic profiles associated with long-term risk of prostate cancer," vol. 17, pp. 1-10, 2021.

[16] Q. Song, A.-h. Zhang, G.-l. Yan, L. Liu, and X.-j. J. R. a. Wang, "Technological advances in current metabolomics and its application in tradition Chinese medicine," vol. 7, no. 84, pp. 53516-53524, 2017.

[17] A. Vignoli *et al.*, "NMR-based metabolomics for the assessment of inhaled pharmacotherapy in chronic obstructive pulmonary disease patients," vol. 19, no. 1, pp. 64-74, 2019.

[18] R. Gitto *et al.*, "Discovery of neuroprotective agents based on a 5-(4-Pyridinyl)-1, 2, 4-triazole scaffold," vol. 13, no. 5, pp. 581-586, 2022.

[19] A. Cerulli *et al.*, "Metabolite profiling of “green” extracts of Corylus avellana leaves by 1H NMR spectroscopy and multivariate statistical analysis," vol. 160, pp. 168-178, 2018.

[20] S. Correa-García, M. Bermúdez-Moretti, A. Travo, G. Déléris, and I. J. A. M. Forfar, "FTIR spectroscopic metabolome analysis of lyophilized and fresh Saccharomyces cerevisiae yeast cells," vol. 6, no. 6, pp. 1855-1861, 2014.

[21] M. C. Crocco *et al.*, "ATR-FTIR spectroscopy of plasma supported by multivariate analysis discriminates multiple sclerosis disease," vol. 13, no. 1, p. 2565, 2023.

[22] A. Poonprasartporn and K. A. J. B. e. B. A.-M. C. R. Chan, "Live-cell ATR-FTIR spectroscopy as a novel bioanalytical tool for cell glucose metabolism research," vol. 1868, no. 7, p. 119024, 2021.

[23] M. M. Neves *et al.*, "Perspectives of FTIR as Promising Tool for Pathogen Diagnosis, Sanitary and Welfare Monitoring in Animal Experimentation Models: A Review Based on Pertinent Literature," vol. 12, no. 4, p. 833, 2024.

[24] K. Senevirathna, N. U. Jayawardana, R. D. Jayasinghe, B. Seneviratne, A. U. J. M. Perera, and C. R. 435, "Di-agnostic Value of FTIR Spectroscopy, Metabolomic Screening and Molecular Genetics in Saliva for Early Detection of Oral Squamous Cell Carcinoma (OSCC)," vol. 454, 2021.

[25] M. J. Baker *et al.*, "Clinical applications of infrared and Raman spectroscopy: state of play and future challenges," vol. 143, no. 8, pp. 1735-1757, 2018.

[26] M. Paraskevaidi, P. L. Martin-Hirsch, F. L. J. N. C. T. f. B. Martin, and M. Diagnosis, "ATR-FTIR spectroscopy tools for medical diagnosis and disease investigation," pp. 163-211, 2018.

[27] S. Magalhães, B. J. Goodfellow, and A. J. A. S. R. Nunes, "FTIR spectroscopy in biomedical research: How to get the most out of its potential," vol. 56, no. 8-10, pp. 869-907, 2021.

[28] A. Mazumder, S. Banerjee, F. Zevictovich, S. Ghosh, A. Mukherjee, and J. J. S. L. Chatterjee, "Fourier-transform-infrared-spectroscopy based metabolomic spectral biomarker selection towards optimal diagnostic differentiation of diabetes with and without retinopathy," vol. 51, no. 7, pp. 340-349, 2018.

[29] M. Al-Kelani, N. J. S. R. Buthelezi, and Technology, "Advancements in medical research: Exploring Fourier Transform Infrared (FTIR) spectroscopy for tissue, cell, and hair sample analysis," vol. 30, no. 6, p. e13733, 2024.

[30] A. Banerjee *et al.*, "Metabolomics profiling of pituitary adenomas by raman spectroscopy, attenuated total reflection-fourier transform infrared spectroscopy, and mass spectrometry of serum samples," vol. 94, no. 34, pp. 11898-11907, 2022.

[31] S. Guo *et al.*, "Fast and deep diagnosis using blood-based ATR-FTIR spectroscopy for digestive tract cancers," vol. 12, no. 12, p. 1815, 2022.

[32] N. M. P. de Souza *et al.*, "Detection of metabolic syndrome with ATR-FTIR spectroscopy and chemometrics in blood plasma," vol. 288, p. 122135, 2023.

[33] B. Peng, H. Li, X.-X. J. P. Peng, and cell, "Functional metabolomics: from biomarker discovery to metabolome reprogramming," vol. 6, no. 9, pp. 628-637, 2015.

[34] Y. Peng, Z. Zhang, L. He, C. Li, M. J. A. Liu, and B. Chemistry, "NMR spectroscopy for metabolomics in the living system: recent progress and future challenges," vol. 416, no. 9, pp. 2319-2334, 2024.

[35] J. G. M. Pontes, A. J. M. Brasil, G. C. Cruz, R. N. de Souza, and L. J. A. m. Tasic, "NMR-based metabolomics strategies: plants, animals and humans," vol. 9, no. 7, pp. 1078-1096, 2017.

[36] A.-H. M. J. M. M. Emwas and protocols, "The strengths and weaknesses of NMR spectroscopy and mass spectrometry with particular focus on metabolomics research," pp. 161-193, 2015.

[37] D. Kruk *et al.*, "Dynamics of Solid Proteins by Means of Nuclear Magnetic Resonance Relaxometry," vol. 9, no. 11, p. 652, 2019.

[38] J. L. Markley *et al.*, "The future of NMR-based metabolomics," vol. 43, pp. 34-40, 2017.

[39] A. A. Crook and R. J. M. Powers, "Quantitative NMR-based biomedical metabolomics: Current status and applications," vol. 25, no. 21, p. 5128, 2020.

[40] J. McCullagh and F. J. C. O. i. C. B. Probert, "New analytical methods focusing on polar metabolite analysis in mass spectrometry and NMR-based metabolomics," vol. 80, p. 102466, 2024.

[41] G. Nagana Gowda and D. J. A. C. Raftery, "Recent advances in NMR-based metabolomics," vol. 89, no. 1, pp. 490-510, 2017.