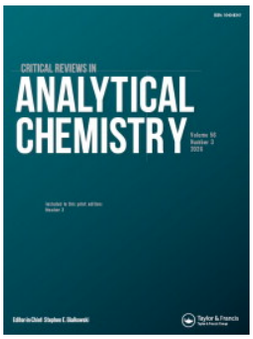

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Procedural Rigor and Reproducibility in NMR Metabolomics: Community Practices and Challenges

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ABSTRACT

Nuclear magnetic resonance (NMR) spectroscopy is a fundamental tool of metabolomics, valued for its reproducibility, quantitative accuracy and broad applicability across biological, chemical and clinical sciences. However, methodological inconsistencies, insufficient protocol reporting and limited infrastructure continue to hinder reproducibility and data sharing. To assess the current state of NMR metabolomics practice, we developed a comprehensive questionnaire and distributed it worldwide to researchers engaged in NMR-based metabolomics. We received 75 responses from a diverse cohort of investigators from academia, clinics and core facilities. The survey focused on Quality Assurance (QA) and Quality Control (QC) practices and provides an overview of the current status of NMR metabolomics and its implementation. Results reveal that while 86% of laboratories have Standard Operating Procedures (SOPs), deviations from these protocols are common and often undocumented, undermining reproducibility. QC practices, including pooled samples and system suitability checks, are widely recognized, but their implementation is inconsistent. Data accessibility remains limited, with fewer than 10% of respondents routinely depositing raw or processed spectral data in public repositories. Formal regulatory oversight and dedicated QA personnel are uncommon. Training is largely informal, with substantial gaps in areas such as data analysis and statistics, raising concerns about knowledge transfer and methodological consistency. Our findings describe a technically skilled community that is constrained by variations in NMR infrastructure and inconsistent implementation of best practices. Addressing these issues through adaptive standardization, structured training programs, and stronger institutional support is critical for advancing transparency, reproducibility and impact of NMR in metabolomics.

KEYWORDS

Best practices; metabolomics; NMR spectroscopy; quality control; quality assurance

Introduction

Nuclear magnetic resonance (NMR) spectroscopy is a fundamental analytical technique for metabolomics owing to its exceptional reproducibility, quantitative reliability, and nondestructive nature.[1] NMR enables the direct observation of metabolites in their native or near-native states, with capabilities of clinical *in vivo* evaluations through MRI scanners. This allows for capturing complex molecular

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fingerprints and metabolic profiles from a single acquisition, or through flux measurements, particularly valuable for longitudinal studies, useful for both clinical monitoring and systems biology.[2]

NMR applications span a wide range of scientific fields, including disease biomarker discovery, pharmacometabolomics, nutritional assessment, environmental toxicology, and environmental biomonitoring.[3,4] Beyond biomedical applications, NMR-based metabolomics is also widely used in plant and microbial research, food science, food authenticity and traceability studies, and the characterization of natural products and agricultural systems, underscoring its broad utility across both health-related and non-clinical domains.[5–8] Despite these technical strengths and its diverse applicability, the full potential of NMR-based metabolomics remains underexploited. A recent survey study from the Metabolomics Association of North America (MANA)[8] highlighted persistent issues, such as methodological inconsistency, insufficient metadata reporting, and the lack of standardized documentation protocols. Such challenges impede reproducibility and restrict the full realization of metabolomics in clinical applications worldwide. Community-driven initiatives, such as those led by the Metabolomics Quality Assurance and Quality Control Consortium (mQACC) and the MANA NMR Interest Group, have made significant strides in advancing standardization within the field. Developing structured best-practice recommendations encompassing all stages of NMR-based metabolomics, ranging from study design and data acquisition to processing, analysis, and reporting, can provide a clear roadmap toward improved harmonization and reproducibility in NMR metabolomics.[8–11] In parallel, the development of community resources, including public spectral and metabolite databases such as Human Metabolome Database (HMDB)[12] and Biological Magnetic Resonance Data Bank (BMRB),[13] together with associated software tools, has substantially strengthened metabolite annotation, cross-study comparison, and data reuse in NMR metabolomics. Recent harmonization and standardization efforts, including multicenter evaluations of pre-analytical variability and new reporting recommendations for NMR metabolomics studies, further highlight the growing community emphasis on inter-laboratory comparability, transparency, and reproducible implementation. Working toward these aims, the aforementioned two organizations continue to actively refine and expand these guidelines as part of an ongoing effort to strengthen methodological consistency across the metabolomics community and to define foundational training materials for students, early career scientists, and researchers new to the field.

These recommendations primarily reflect consensus guidance; however, substantial gaps remain between the advised recommendations and the routinely implemented practice, which is not solely an issue with NMR metabolomics. For instance, a 2016 *Nature* survey reported that over 60% of respondents could not replicate experiments published by other scientists,[14] and the American Society for Cell Biology also reported that more than 70% of members failed to reproduce results from published protocols due to incomplete methodological details (https://www.ascb.org/science-policy-public-outreach/advocacy-policy/ascb-examines-difficulty-reproducing-research-data/). Within NMR-based metabolomics, Powers et al.[8] analyzed 463 published studies and found that many reports lacked essential experimental details, presented incomplete, misapplied, or unvalidated statistical methods or models. These findings highlight a persistent gap between best-practice guidance and real-world implementation, echoing broader reproducibility concerns across metabolomics and other omics disciplines.[15]

From this study, we provide a comprehensive assessment of the current state and practices in NMR-based metabolomics. Specifically, we document the implementation of this methodology across a diverse range of laboratories worldwide, drawing on detailed input from the scientists directly responsible for conducting these studies. By capturing the real-world variability in instrumentation, protocols, data processing workflows, and interpretative approaches, our analysis offers an evidence-based perspective on the current state of NMR metabolomics practices and highlights areas for methodological harmonization and improvement.[4,8,15–18] To better understand the current status of NMR-based metabolomics, with focuses on quality assurance (QA) and quality control (QC) practices, we designed this targeted questionnaire and disseminated to researchers actively engaged in using NMR metabolomics from academia, government institutions, clinical research, and applied industrial sciences. The questionnaire was designed to assess NMR-based metabolomics practices from study design, execution, and management across diverse research environments. Rather than merely auditing compliance with idealized protocols, the questionnaire aimed to inform on how researchers interpret and implement methodological standards in practice. By incorporating detailed questions about both technical procedures and institutional context, this assessment highlights both alignment with best-practice expectations and areas where inconsistency or underreporting may hinder data transparency and reproducibility.

Methods

Questionnaire parameters

To assess current practices in NMR-based metabolomics for a better understanding of key methodological and reporting standards used in the field, a comprehensive questionnaire was developed and distributed to professionals actively working in the NMR metabolomics field. The questionnaire was designed to capture a broad yet detailed account of NMR metabolomics study planning, execution and management in a range of research environments. The questionnaire had over 40 questions and was structured to align with the core stages of the NMR metabolomics workflow, from study design to data dissemination.

Questionnaire analysis

To provide a comprehensive understanding of the conduct of NMR-based metabolomics across different laboratories

and institutions, the questionnaire was organized in six thematic sections (Figure 1). Each section corresponded to a key stage of the metabolomics workflow and was designed to answer specific questions about current practices, infrastructure, and training environments, as detailed below.

1. The academic/research role and relevant demographic details of the respondent who was engaged in the NMR-based metabolomics research. It contained 13 questions that capture demographic and professional information about the respondent, including their academic/research roles, institutional affiliations and years of experience in metabolomics research. This section also explored the types of biological matrices studied and provided a framework for interpreting NMR metabolomics results based on research focus and level of expertise.
2. The study designs and standard operating procedures (SOPs). This section focuses on study planning and protocols used. It includes five questions examining whether laboratories maintain formal SOPs, how familiar researchers are with these procedures and whether systems exist to document and revise protocols when deviations occur. This section evaluates the degree to which methodological planning is standardized and whether such standards are consistently integrated into practice.
3. The NMR standards used. This section investigates the use of internal referencing compounds in metabolomics workflows. It contains five questions exploring whether chemical shift standards are employed, the purpose they serve (e.g., referencing, quantification), and at which step in the workflow they are introduced. These questions help clarify the extent to which internal standards are integrated into acquisition protocols for improved spectral consistency and data comparability.
4. The data acquisition and system suitability. This section addresses questions related to ensuring analytical reliability and reproducibility. It comprises 12 questions examining whether researchers perform system checks prior to acquisition, the types of QC materials used, how the sample measurement order is determined and whether laboratory environmental conditions are monitored. Additionally, it inquires whether quality metrics are evaluated before data are handed over to researchers. This section is critical in assessing the robustness of technical procedures across labs.
5. The data accessibility and infrastructure. This section examines the institutional systems that support data stewardship and quality assurance. It consists of 12 questions examining whether laboratories upload data to public repositories, maintain traceable records through sample tracking systems and operate under formal regulatory or accreditation frameworks. It also explores whether independent QA oversight is present. The questions in this section aim to understand how well-equipped laboratories are to ensure transparency, reproducibility, and compliance.
6. Training. This section inquires how knowledge and best practices are transferred within labs and institutions. It includes five questions assessing the availability and

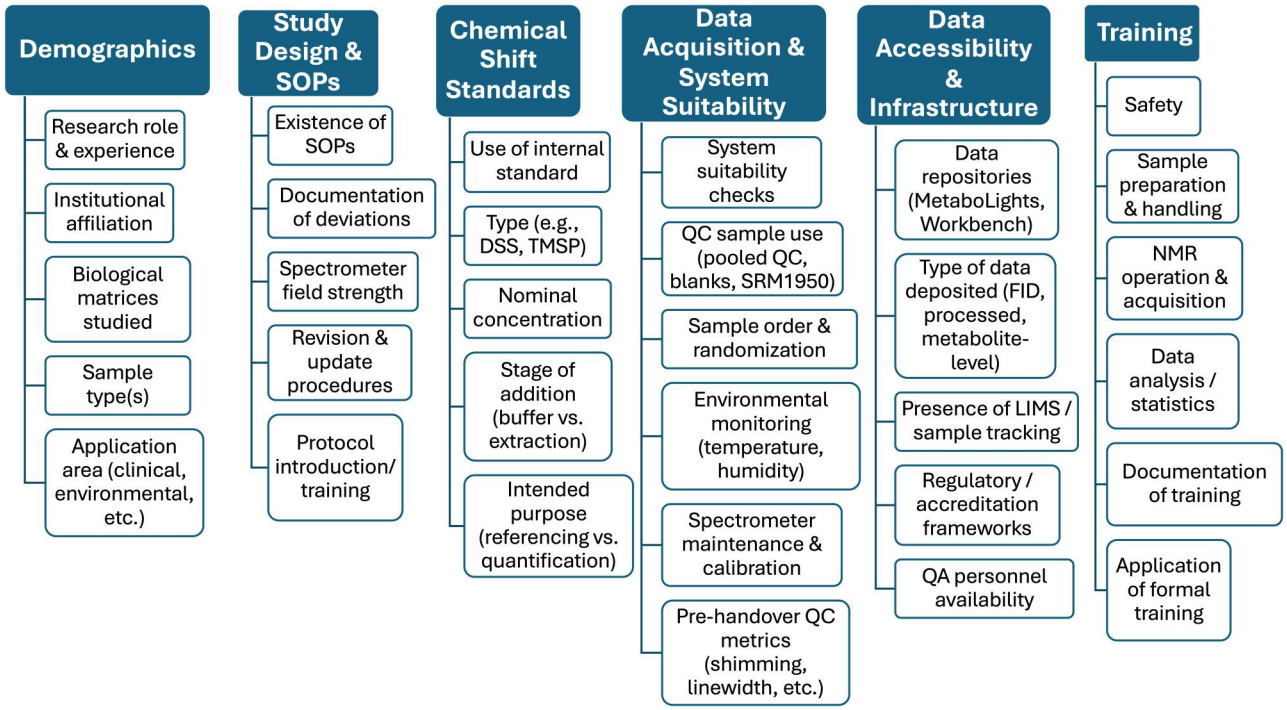

Figure 1. Overview of questionnaire domains and workflow structure. The schematic summarizes the six major sections of the mQACC NMR Metabolomics Questionnaire: (1) Background of Respondent (demographics), (2) Study Design and Standard Operating Procedures (SOPs), (3) Chemical Shift Standards, (4) Data Acquisition and System Suitability, (5) Data Accessibility and Infrastructure, and (6) Training. Each section reflects a distinct stage of the NMR-based metabolomics workflow, encompassing both technical and organizational aspects of study planning, execution, quality assurance, and data stewardship. Together, these sections provide a comprehensive framework for assessing current practices, methodological consistency, and training environments across the NMR metabolomics community.

nature of training in key areas such as sample handling, data acquisition, safety, and data analysis. It also distinguishes between informal mentorship and formally documented training programs. This section provides insight into the sustainability and scalability of methodological expertise within the community.

Together, these six sections provide an integrated view of current practices in NMR-based metabolomics, offering insight into both technical execution and institutional support systems. Rather than merely capturing whether certain practices are followed, the results illustrate how workflows are shaped by individual experience, organizational culture, and infrastructural capacity.

Questionnaire development and dissemination

The questionnaire was developed using the Qualtrics XM platform. The questions were primarily developed by the mQACC NMR Working Group, with the aim of addressing all major components necessary to achieve robust QA and QC in NMR-based metabolomics. The design of these questions was further enriched by insights from previous community surveys and published perspectives,[9,10] ensuring that the questionnaire reflected both current best practices and ongoing challenges identified by the broader metabolomics community.

Upon finalization, the questionnaire was disseminated electronically *via* the Qualtrics XM platform to a curated list of prospective respondents during the period between March 2024 and February 2025. The mailing list was compiled from multiple sources, including institutional directories, professional networks, and mailing lists associated with academic institutions, clinical laboratories, and core NMR facilities, as well as corresponding authors of NMR metabolomics publications.[8] Additionally, the questionnaire was circulated at metabolomics conferences and workshops.

The questionnaire was designed to capture insights from a broad spectrum of practitioners, including early-career and senior researchers, facility managers, and individuals in hybrid roles, thus ensuring rich representation across professional contexts.

Questionnaire responses (in anonymity) were analyzed and summarized using both Microsoft Excel and Python. Excel was employed for straightforward tabulations and simple plots, while more complex visualizations were produced with custom Python scripts. These scripts utilized the matplotlib and *seaborn* libraries, along with *numpy* for numerical handling and *textwrap* for formatting multi-line labels. The code was designed to flatten nested response structures, stack categories in bar plots, and annotate figures with role-specific labels. Formatting steps, including customized axes, gridlines, legends, and font adjustments, ensured that the resulting figures were consistent.

Results

Responses were received from 75 out of 89 participants who voluntarily started the questionnaire. Drawing from the responses of 75 participants across diverse institutional and professional backgrounds, the findings illustrate how researchers approach each stage of the metabolomics workflow, from study design to data sharing. The results are presented across the aforementioned six sections of the questionnaire, highlighting both areas of alignment with best practices and aspects where methodological transparency or infrastructure remains lacking. The analysis aims not only to quantify common practices but also to contextualize them within broader patterns of institutional support, training, and reproducibility culture. A comprehensive breakdown of the questionnaire results is provided in the Supplementary Material.

Respondent demographics

The questionnaire received responses from professionals working in NMR-based metabolomics, representing a diverse cross-section of roles, institutional contexts and levels of experience. This diversity provided a foundation for assessing the state of practice across the community. Figure 2 presents the breakdown of the respondents' background and primary role within their organization. Most respondents ($n = 42$, 56%) indicated that they were principal investigators, such as professors, directors, or heads of laboratories, while a smaller portion ($n = 8$, 10.6%) reported functioning as NMR facility managers responsible for instrument maintenance, data acquisition protocols, and user training. In addition, twenty-five respondents (33.3%) identified as metabolomics researchers directly involved in experimental design, sample preparation or data analysis. A third subset of respondents indicated having dual responsibilities, acting both as researchers and facility leads. In terms of professional experience, respondents represented a wide range of experience levels, as reported in Supplementary Figure S1. The demographic distribution shows that the vast majority (80%) of respondents have significant experience in all categories, with most respondents having worked for over a decade in the field. Only a few respondents (2%) reported having less than two years of hands-on experience in NMR-based metabolomics. The majority of our responders are from academia ($n = 38$, 76%), followed by government (14%). Overall, the distribution in terms of professional/research role, level of experience, and affiliation suggests that the questionnaire primarily reflects current practices from experienced users while also including perspectives from early-career scientists. Although the community includes both experienced and relatively new users, the questionnaire revealed a striking underrepresentation of early-career researchers (0–6 years since degree). This suggests that most NMR metabolomics activities are currently concentrated in established laboratories with long-standing expertise and infrastructure.

Even though the questionnaire captures experienced users well, this imbalance may suggest that emerging practices or challenges faced by newer labs may be underrepresented. As a result, the conclusions may be unintentionally biased toward workflows, sample-handling approaches, and analytical strategies typical of well-established groups, potentially

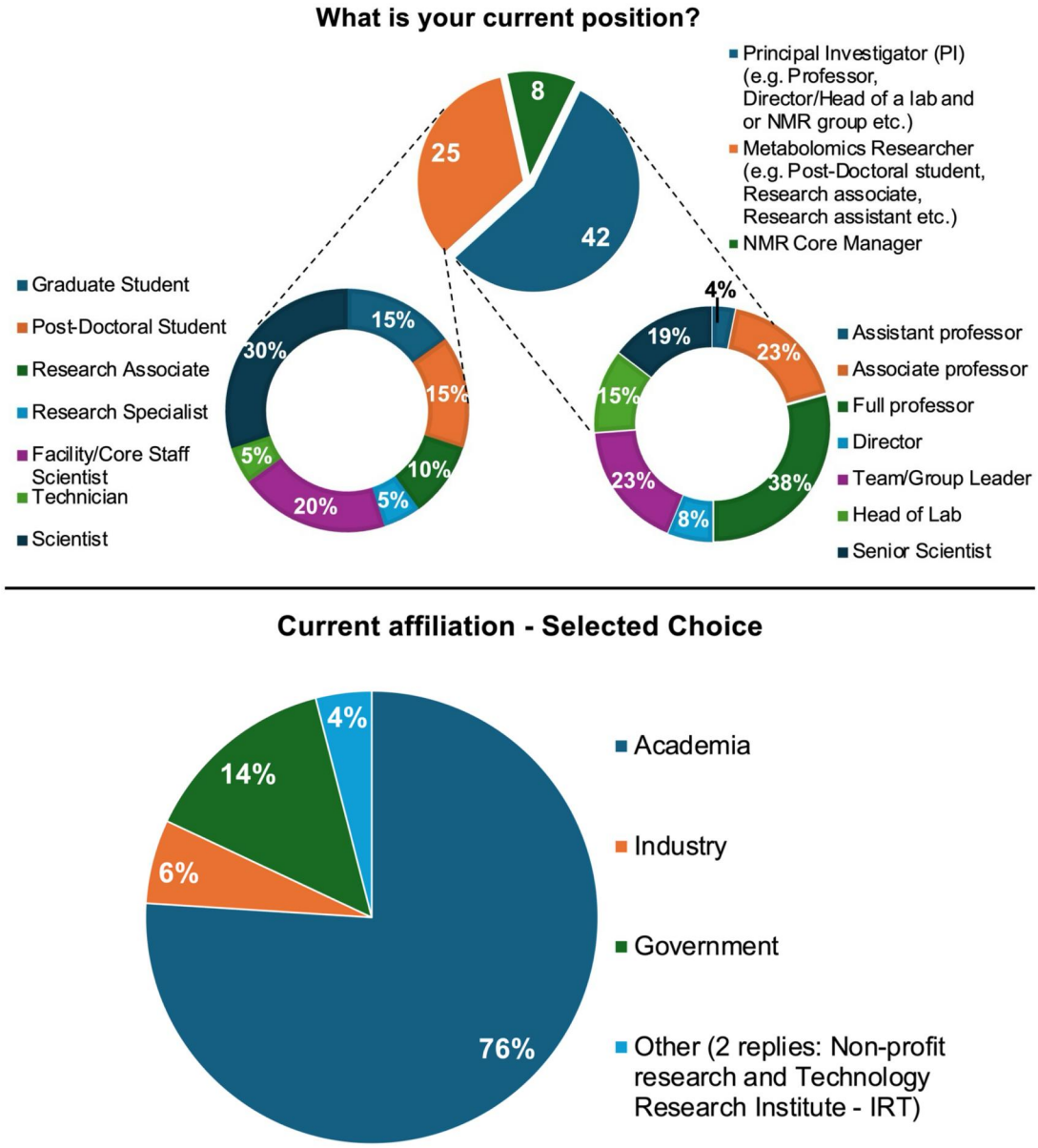

Figure 2. Respondents' current professional roles and organizational affiliations (n = 75). (Upper panel) Distribution of respondents according to current professional role, including researchers, facility managers, and individuals with dual responsibilities. The two lower pie charts within the upper panel provide more detailed role information; respondents were allowed to select multiple options for these questions. (Lower panel) Distribution of respondents according to organizational affiliation (n = 75).

overlooking innovative methods, resource limitations, or adoption barriers encountered by early-stage labs.

The questionnaire also explored the range of biological samples routinely analyzed by respondents. Using a select-all-that-apply format, respondents identified a wide array of sample types, from canonical biofluids such as plasma, urine, and saliva to more specialized or challenging biomaterials, including milk, synovial, cerebrospinal and seminal fluids, feces, digesta, rumen fluid and exhaled breath condensate. Environmental and non-human biological materials were also reported, including fermentation liquids, coral tissue, insects, pollen, and soil. The breadth of sample types underscores the versatility of NMR as a platform for metabolomics and highlights the inherent challenges of standardization across such a heterogeneous research landscape. Details of the different types of samples the respondents reported can be found in Supplementary Figure S2.

The respondents were asked about the types of NMR-based metabolomics applications performed as reported in Supplementary Figure S3. The vast majority (98%) reported using solution-state NMR for their metabolomics work. High-resolution magic angle spinning (HRMAS) was used by 20% of respondents, while solid-state cross-polarization/magic angle spinning (CP-MAS, NMR technique that boosts signal and sharpens spectra in solids by transferring magnetization between nuclei and spinning the sample at the magic angle to reduce line broadening) was uncommon, with only 2% indicating its use. These numbers reflect the dominance of solution-state NMR in the metabolomics field, but also indicate that solid and semi-solid matrices are being explored using specialized techniques.

Study design and standard operating procedures

A proper study design is the foundational element of any successful metabolomics investigation.[16] It shapes the scope, reproducibility, reliability, and interpretability of the results and outcomes. Most respondents reported 600 MHz NMR spectrometers for their metabolomics experiments. Supplementary Figure S4 provides a distribution of NMR instrumentation used by metabolomics researchers. The questionnaire also explored whether researchers and facilities implemented formal planning procedures and maintained structured protocols such as SOPs to assess the consistency of NMR-based metabolomics study designs across laboratories.

As shown in Figure 3, most respondents indicated the existence of their laboratory-maintained SOPs for NMR metabolomics workflows. Approximately 86% reported the availability of SOPs, and 69% confirmed a formal introduction of these protocols to new personnel as part of their training or onboarding process. This suggests that structured procedures are available in most institutions and are being actively communicated to researchers conducting metabolomics. However, when asked about procedural adherence, the responses revealed a more fragmented picture. Over half of the respondents (54%) reported that deviations from SOPs occur during routine work, indicating a level of methodological flexibility or inconsistency. Of those acknowledging deviations, only 67% stated that such departures are documented and used to update existing protocols. This implies that while many laboratories operate with procedural guidelines in place, the mechanisms for maintaining and evolving those standards are not uniformly applied. Without systematic documentation of deviations, it becomes difficult to track methodological drift or ensure that future users are working from the most current and validated version of a procedure.

These results indicate a disconnect between the presence of formal procedures and their consistent implementation during the execution of NMR metabolomics studies. While SOPs are widely adopted, they often function more as static reference documents than as dynamic tools that evolve with laboratory practices. Collectively, these results underscore the need for greater alignment and enforcement between written protocols and real-time decision-making in study execution. Strengthening the integration between SOPs and post-study documentation would help bridge this gap and support more transparent, reproducible, and methodologically robust NMR metabolomics research.

Recommendations

Project objectives and scientific hypotheses should be explicitly defined during the planning phase and reported in a manner consistent with the chosen study design. Targeted studies should clearly state all predefined hypotheses and analytical endpoints, whereas untargeted or exploratory studies must explicitly describe their hypothesis-generating intent to avoid ambiguity. Untargeted studies should ideally be followed by targeted validation as biological understanding improves. Experimental design should include a justified sample size and number of biological replicates that reflect the expected statistical power, biological variability, sample type, and high dimensionality of metabolomics data, as underpowered studies—particularly in clinical settings—can limit interpretability and contribute to reproducibility and reliability concerns. While analytical replicates may be constrained by cost and throughput, biological replication and appropriate controls remain essential for robust data interpretations. In addition, randomized sample-run order should be implemented to minimize systematic bias during sample preparation and data acquisition. Finally, comprehensive reporting of experimental design decisions enables readers

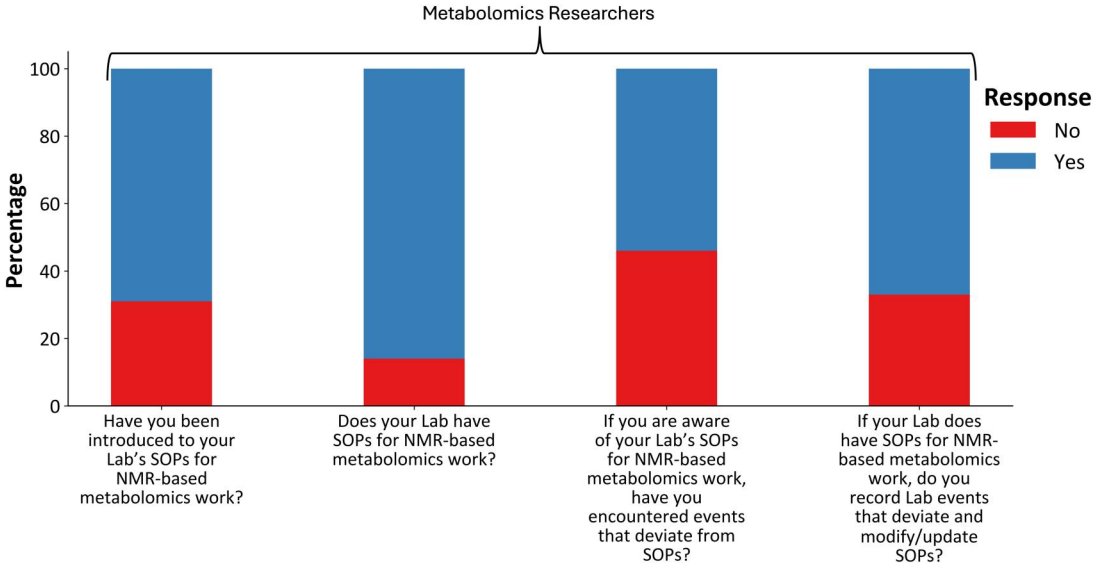

Figure 3. Responses from Metabolomics Researchers subgroup to questions about the use of Standard Operating Procedures (SOPs) in NMR metabolomics workflows (number of respondents for each question:7, 6, 13, 13). The chart shows that while SOPs are widely present (86%), adherence and documentation of deviations are inconsistent, suggesting a gap between formal protocol existence and dynamic protocol usage.

to evaluate whether the study is adequately powered and methodologically aligned with its stated objectives.

Chemical shift standard

The questionnaire evaluated the use and intended purpose of internal chemical shift standards across participating laboratories. The results, summarized in Figure 4(a), illustrate the distribution of reported applications. Most respondents who indicated they use chemical shift standards do so to establish the chemical shift reference, whereas a smaller proportion reported employing them for sample normalization or for other purposes. Figure 4(b) illustrates the type of chemical shift standards used by respondents, with deuterated 4,4-dimethyl-4-silapentane-1-sulfonic acid (d6-DSS) and 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid (d4-TMSP) being the most frequently used, while some laboratories indicated the use of alternative standards. Figure 4(c) highlights the nominal concentrations at which these standards were employed, revealing substantial variability: some laboratories consistently used fixed concentrations such as 1 mM, while others varied the concentration depending on the project or sample. Figure 4(d) focuses on the point at which the standard was introduced into the workflow. Strikingly, eleven out of twelve responses from laboratories reported using a standard added during buffer preparation immediately prior to analysis, whereas only one reported that they introduced the standard earlier in the workflow, such as before extraction or drying. This distinction is significant, since adding the standard at an earlier stage allows for correction of potential compound losses during preparation, while later introduction supports chemical shift referencing, but does not enable absolute quantification.

Recommendations

To improve consistency, quantitative rigor, and cross-study comparability in NMR metabolomics projects, laboratories should adopt clearly defined practices for the use of internal chemical shifts and other types of standards, and report the type, concentration, and intended purpose of these standards, as well as the point at which it is introduced into the workflow. When absolute or relative quantification is a study objective, standards should be added as early as practicable in the sample preparation process to account for potential analyte losses, rather than only during final buffer preparation. Conversely, when standards are used solely for chemical shift referencing or annotations, this limitation should be clearly stated.

Data acquisition and system suitability

Data acquisition is the essential component of a robust NMR-based metabolomics study. Careful optimization of acquisition parameters is critical to reduce technical variability and

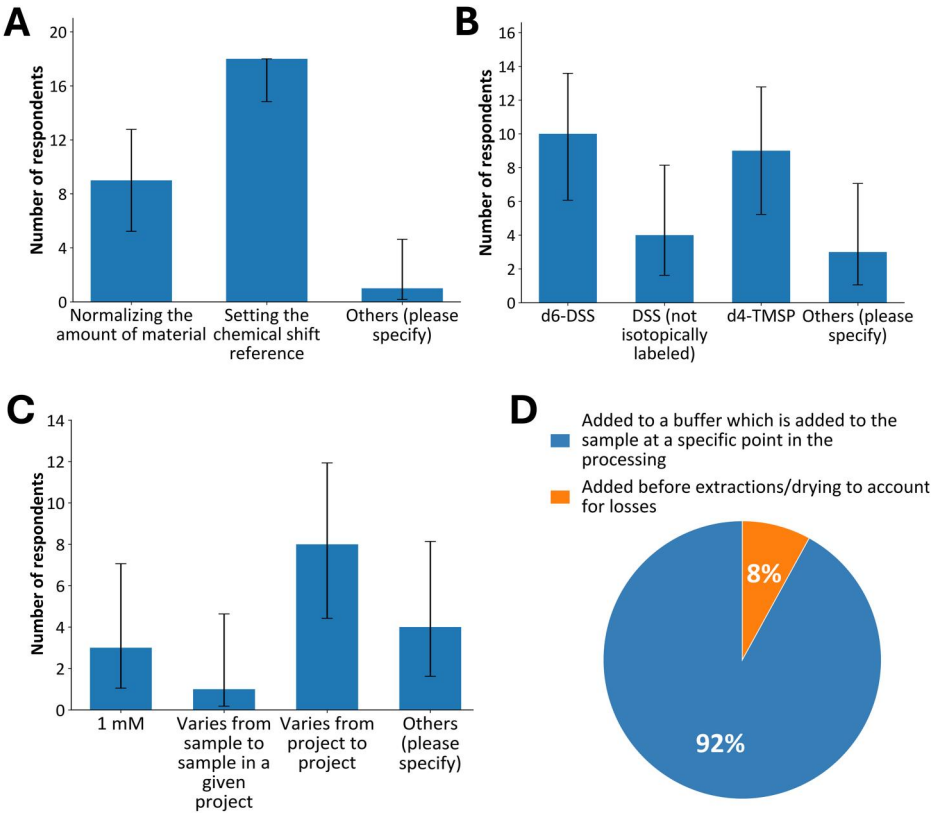

Figure 4. Reported use of chemical shift standards among NMR metabolomics laboratories. Panel A ($n = 28$) summarizes the reported uses of chemical shift standards, and panel B ($n = 26$) reports the types of standards. Panel C ($n = 16$) shows the nominal concentrations and Panel D ($n = 12$) reports the methods of introduction of the chemical shift standard into the sample. Only 24% of respondents routinely employed standards, most commonly adding them during buffer preparation. This reflects limited implementation of internal standards, which has implications for quantification, accuracy and spectral referencing. Error bars indicate 95% confidence intervals.

capture biologically relevant differences among samples.[17] To assess the current practices, the questionnaire included a series of questions concerning approaches ranging from system suitability assessments to acquisition consistency.

Thirty (73%) respondents confirmed that they or their labs actively performed system suitability checks prior to collecting NMR spectra for metabolomics projects. As outlined in Figure 5, these system checks included common practices such as performing pulse calibrations and running a sample from the previous session. It should be noted that respondents who opted for the "Other" option mentioned running a pooled QC sample before starting the NMR acquisition and optimizing the water suppression signal for each experiment. Twenty-four respondents indicated that QC materials were used to assess technical reproducibility (e.g., sample extraction and acquisition); conversely, thirteen of them reported not using QC materials, indicating a continued gap in reproducibility assessment practices. Among those using QC materials, pooled QC samples were the most commonly reported ($n = 45$ out of 67), followed by solvent or buffer blanks ($n = 39$) and processed extraction blanks ($n = 36$). Reference materials, whether in-house or commercial, were used by 34 respondents, and certified reference materials such as NIST SRM1950[18] were used by 17 respondents. Intra-study technical replicates were reported by 17 respondents, and "other" materials were selected by only three respondents. These results indicate a strong preference for accessible QC strategies like pooled samples and blanks, while more formal reference materials and technical replicates remain underutilized across the field (see Supplementary Figure S5).

Sample ordering was another acquisition-related consideration examined by the questionnaire. As seen in Figure 6(a), a total of 69% of respondents indicated that the order in which samples are processed is considered during experimental design or analysis. To mitigate run-order effects, 78% of respondents indicated randomization when determining sample order within a batch and 33% reported interleaving study group samples to distribute potential drift evenly across groups. Seventy-seven percent of respondents also reported interleaving QCs at regular intervals. These

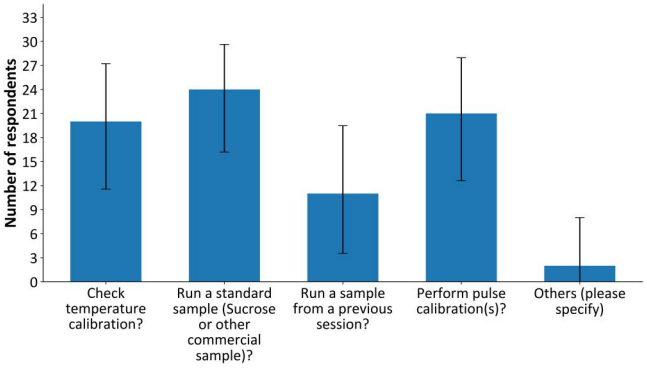

Figure 5. System suitability practices performed prior to analytical NMR runs. Most respondents report engaging in at least one quality control procedure (e.g., perform pulse calibrations, run a sample from the previous session), demonstrating general awareness of pre-acquisition checks, though adoption of more rigorous standards remains variable ($n = 75$). Error bars indicate 95% confidence intervals.

considerations reflect awareness of possible spectrometer drift effects across long acquisition batches that may influence data interpretation. Lastly, it should be noted that when participants were asked to confirm if their NMR Core Facility routinely evaluates quality metrics before transferring data to researchers, only four respondents answered positively. As summarized in Supplementary Figure S6, shimming and solvent suppression were the most frequently checked parameters. followed by linewidth, and a few respondents reported checking either phasing or baseline. Thus, while some quality checks are performed, the scope appears limited and not consistently employed across NMR core facilities. In fact, when asked which NMR data quality metrics were routinely evaluated, shimming and baseline quality were the most reported ($n = 25$), followed by phasing and solvent suppression ($n = 23$ each) and linewidth ($n = 24$). Several respondents also specified other metrics such as pulse stability, quantitation of internal standards, and detection of peak splitting, indicating additional lab-specific checks beyond standard benchmarks. These observations highlight both strengths and existing gaps in the standardization of acquisition best practices. The widespread adoption of quality control protocols and chemical shift standards by NMR facilities remains a need.

Environmental monitoring was also addressed. Fourteen respondents stated that their NMR facilities monitor environmental factors such as temperature, pressure, or humidity, parameters known to influence magnetic field stability and spectral quality. These responses suggest that many laboratories recognize the importance of maintaining consistent acquisition conditions, particularly in high-throughput or long-duration studies. These results have been summarized in Supplementary Figure S7. As presented in Figure 6(b), 19 respondents reported maintaining a formal schedule for preventative maintenance, calibration, and tuning of their NMR instruments, which amounts to 86% of those who answered the question. This indicates a strong awareness of the need for long-term system stability and consistent performance. Also, the majority ($n = 10$ out of 13) of researchers reported collecting additional spectra for compound identification, underscoring the continued role of NMR in structural elucidation beyond primary metabolic profiling.

In summary, the majority of respondents demonstrated awareness of and engagement with acquisition reliability practices. Routine system suitability assessments, attention to sample order, and environmental monitoring were reported by a substantial portion of the community.

Recommendations

To ensure interpretability, comparability, and reproducibility of NMR metabolomics data, all studies should comprehensively report spectrometer specifications, sample preparation conditions, data acquisition and pre- and post-processing parameters. At a minimum, this includes sample temperature, buffer composition (type, concentration, pH, osmolality), solvent system, chemical shift reference, and the specific 1D and/or 2D pulse sequences used, as these variables directly influence peak positions and intensities,

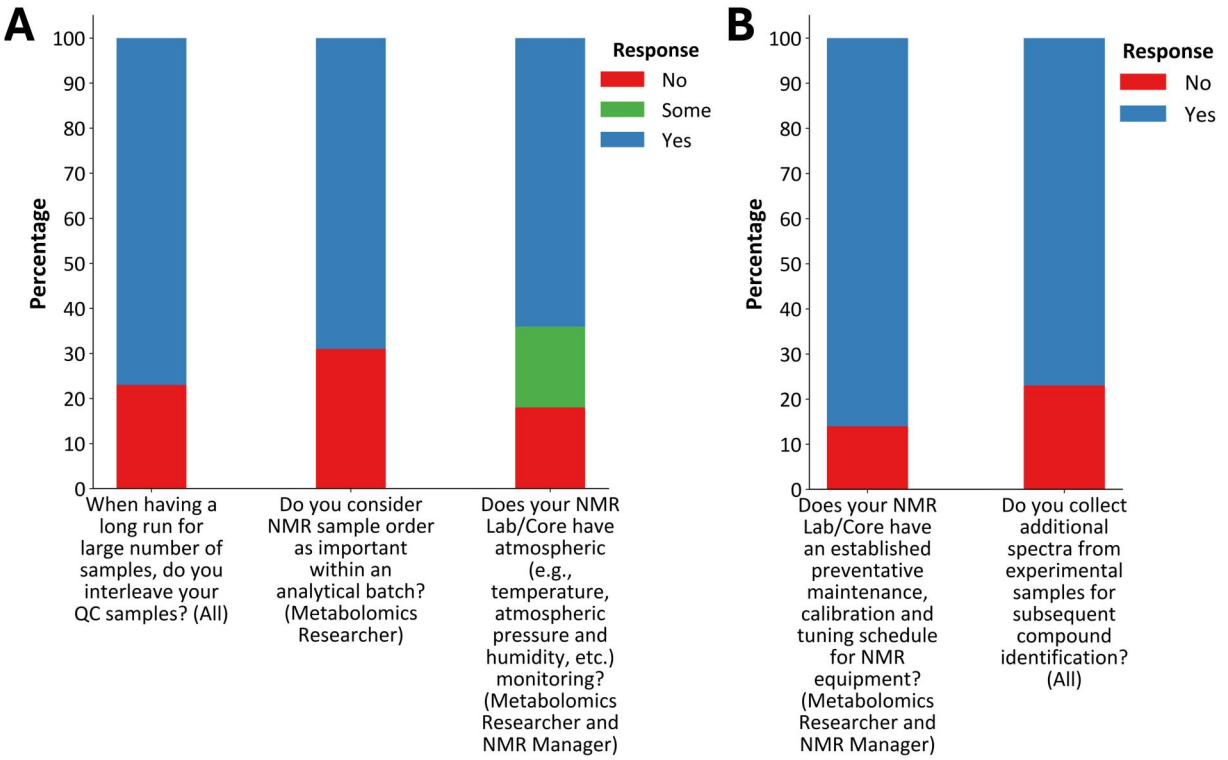

Figure 6. Approaches to NMR data acquisition and quality assurance practices. (A) Sample ordering and quality control strategies, highlighting widespread use of randomization (78%) and interleaved QC samples (77%) to reduce run-order effects and drift (number of respondents for each question: 13, 13, 18). (B) Advanced acquisition practices, including preventative maintenance scheduling, use of additional spectra for compound identification, and pre-handover data quality evaluation (number of respondents for each question: 13, 13, 18). Together, responses indicate strong foundational maintenance and QC practices, with increasing, though variable, adoption of spectral evaluation metrics to ensure reproducibility and unbiased acquisition.

linewidths and spectral patterns. Processing parameters like apodization functions, baseline correction, linear prediction, zero filling and solvent removal similarly impact the overall quality and interpretation of the resulting NMR spectra. Reporting only the use of 1D or 2D experiments is insufficient; complete experimental details are required, particularly for profiling studies where methodological variability is high. Studies should also provide a clear summary of the full sample set, including the number of biological replicates, QC samples and cohort composition, ideally in a dedicated table or figure. Similarly, alignment, denoising normalization, scaling, and model validation protocols should be reported for univariate and multivariate statistical models and machine-learning/AI approaches. Finally, metrics demonstrating spectral quality and instrument performance should be reported where appropriate and any recommended parameter that is not applicable should be explicitly stated rather than omitted, to support transparency, harmonization, and reproducible reuse of NMR metabolomics data.

Data accessibility and infrastructure

In addition to experimental and analytical workflows, reliable NMR metabolomics research depends on the presence of institutional infrastructure that supports data stewardship, traceability, and regulatory compliance. To evaluate the degree to which such systems are in place, the questionnaire included questions related to data accessibility, formal regulation, quality assurance procedures, and sample tracking infrastructure. Fifty-seven percent of metabolomics researchers reported that their laboratories upload study data to public repositories, such as MetaboLights,[19] Metabolomics Workbench,[20] or institutional repositories. Among those who do share data, only a small number report uploading raw FID data ($n = 4$), processed spectral data ($n = 3$), or interpreted metabolite-level data ($n = 6$) (Figure 7). These low numbers suggest that, despite over half of respondents indicating data sharing, in reality, data deposition remains limited. This points to ongoing barriers in repository use, including metadata preparation, formatting challenges, or institutional support.[8,10,11,15] In terms of institutional oversight, only 4% of facilities operated under any formal regulatory or accreditation framework, and they specified compliance with Good Laboratory Practice (GLP) framework. Thus, the vast majority of NMR metabolomics facilities operate without any regulatory oversight. The presence of QA personnel was similarly limited. Only five respondents reported that their institution employs a designated individual or team responsible for overseeing quality assurance in NMR metabolomics workflows. In most cases, QA was embedded within research or facility roles, rather than treated as an independent function. Finally, only a small number ($n = 14$) of laboratories indicated that sample traceability and digital infrastructure were addressed through a Laboratory Information Management Systems (LIMS) or equivalent sample tracking tools.

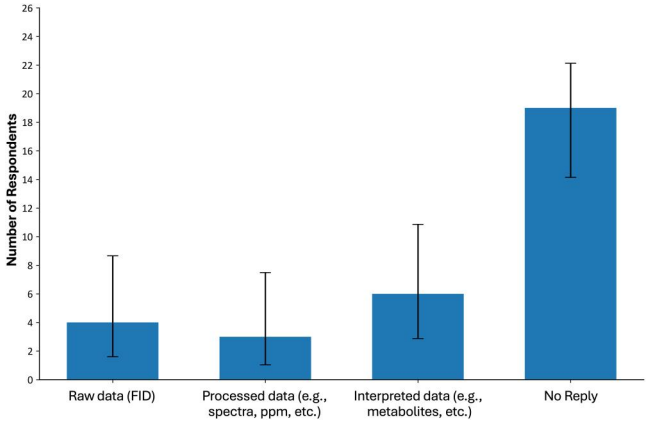

Figure 7. Types of metabolomics data that are reported to be uploaded to public repositories by the respondents ($n = 13$). While 57% of respondents indicated some form of data sharing, the figure reveals that raw FID data and processed spectral files are infrequently deposited. This highlights practical and institutional barriers to open data practices within the community. Error bars indicate 95% confidence intervals.

Collectively, these findings suggest that the supporting infrastructure for data stewardship and institutional quality remains fragmented. Greater investment in centralized data management systems, independent QA mechanisms, and routine repository submission could strengthen the foundation of the field and improve long-term traceability and reproducibility.

Recommendations

To strengthen reproducibility, transparency, and long-term reuse of NMR metabolomics data, studies should deposit acquired spectra together with complete, structured metadata in publicly accessible repositories appropriate for NMR data. Where feasible, submissions should include raw and processed spectra, as well as QC and reference material data, to enable independent assessment of data quality and cross-laboratory comparability in alignment with FAIR principles.[21] Given the persistently low rates of meaningful data deposition, authors should clearly state the type of data deposited, provide persistent identifiers, and explicitly justify any restrictions due to privacy (i.e., HIPA), proprietary constraints, or regulatory requirements. Institutional infrastructure must also be strengthened to support these practices, including adoption of sample tracking systems (e.g., LIMS: Laboratory Information Management System), dedicated or clearly defined QA oversight and standardized data stewardship workflows. Finally, reporting and deposition expectations should be adapted to reflect differences between quantitative and profiling NMR metabolomics, while continued development of accessible, open-source tools and harmonized metadata standards will be critical to making spectral data deposition both practical and scientifically valuable.

Training

Training plays a central role in ensuring the methodological rigor and reproducibility of NMR-based metabolomics. Accordingly, the questionnaire assessed whether respondents had received instruction across several core sections, including safety protocols, sample preparation and handling, use of NMR instrumentation, data acquisition, and data analysis. In this regard, the questionnaire was structured for better understanding the conducted formal and informal training across the metabolomics research community. Figure 8 presents the distribution of responses while distinguishing between general training provided by a PI or a Core Manager. It also summarizes the presence of formalized documentation of training and indicates whether such training was subsequently applied. Overall, most respondents reported receiving general training from a PI or Core Manager. Specifically, 23 respondents indicated receiving general instruction in sample preparation, sample handling, and the use of NMR equipment. Twenty-four individuals reported training in data acquisition, and eighteen respondents stated that they had received safety training. In contrast, only sixteen respondents reported receiving training in workflows, which may be less emphasized during routine training.

The formal documentation of training is an important element for the standardization and accountability of metabolomics data.[22] Unfortunately, it was not commonly reported. Only three to six individuals noted that their training had been formally documented. For instance, just four respondents indicated formal documentation for training in sample preparation and use of NMR instruments, while overall experimental procedure safety training was formally documented in six cases. Application for formalized training was reported even less frequently, ranging from one to five individuals across all sections. Over forty respondents did not report on any formalized training and over twenty respondents did not report any application of said training, indicating either a lack of formal processes in many labs or uncertainty about training procedures among respondents. Taken together, these findings suggest that training within the NMR metabolomics community remains largely informal, decentralized, and highly variable in scope.

While general competency in laboratory tasks appears to be transmitted through mentorship or internal lab instruction, there is a marked lack of systematic training practices and consistent documentation. The low uptake of structured instruction and the sparse application of formalized training raise concerns about consistency and knowledge transfer, particularly in areas such as data interpretation and quality assurance. This gap in formalized training infrastructure points to a broader need for institutional investment in standardized educational resources. The development of comprehensive training protocols, ideally co-led by core facilities and research groups, could promote greater methodological uniformity, facilitate reproducibility, and ensure that newer members of the field are equipped with the necessary skills to conduct robust and interpretable metabolomics research.

Recommendations

Laboratories and institutions should implement structured, documented training programs that span the full metabolomics workflow, including safety, sample preparation, NMR

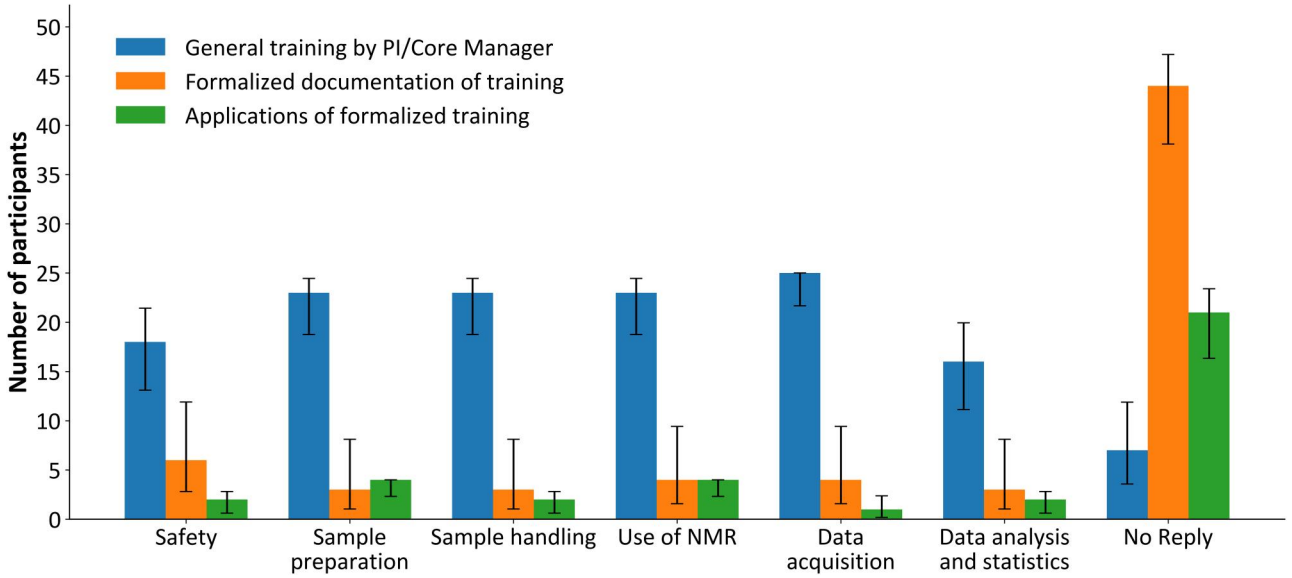

Figure 8. Training types and delivery modes reported by respondents across key stages of the NMR workflow. Most individuals received informal training from supervisors, formal documentation and structured application were rare. Data analysis training was notably underreported, indicating a critical gap in computational skills development (blue: $n = 25$, yellow: $n = 6$, green: $n = 4$). Error bars indicate 95% confidence intervals.

instrumentation, data acquisition, and data analysis. While mentorship-based instruction remains valuable, reliance on informal training alone is insufficient, particularly for data processing, statistics, and quality assurance, which requires consistent and verifiable competency. Training activities should be formally documented, periodically reviewed, and linked to clear expectations for application in routine practice to support accountability and continuity. Core facilities and research groups should collaboratively develop standardized training materials and onboarding protocols, and institutions should invest in educational infrastructure that supports reproducible workflows and equips early-career researchers with the skills necessary to generate robust, interpretable, and reusable NMR metabolomics data. As a proposed strategy, structured training programs covering the full NMR metabolomics workflow could be developed through coordinated efforts across the scientific community. These may include online and in-person workshops, courses, and webinars organized in collaboration with initiatives such as the Metabolomics Society, MANA, and mQACC, which promote best practices and harmonization. At the practical level, such programs could provide hands-on or interactive training in safety, sample preparation, NMR instrumentation, and data acquisition, integrated within graduate and doctoral training schemes. The use of real-world datasets from repositories such as MetaboLights and Metabolomics Workbench could further support applied learning.

Discussion

Our distributed questionnaire provides an empirical overview of prevailing practices, methodological heterogeneity, and infrastructure limitations in the NMR metabolomics community. While the results demonstrate substantial methodological awareness, they also reveal persistent gaps in SOP implementation, reproducibility, training, and institutional support.

Although 86% of respondents reported having SOPs, more than half acknowledged deviations during routine work, and only 67% of those deviations were documented. This indicates that the presence of SOPs alone does not ensure methodological rigor, and that undocumented procedural drift may undermine reproducibility and data accuracy. These findings are consistent with broader concerns regarding "protocol inertia" in metabolomics, where SOPs are insufficiently updated or integrated into daily practice.[8,10,15]

Responses related to system suitability, QC materials, and acquisition parameters indicate strong technical awareness but limited consensus on best practices. Pooled QCs and process blanks were widely used, whereas certified reference materials and technical replicates were adopted less frequently, likely reflecting barriers related to cost, access, or perceived necessity. Although 70% of respondents assessed data quality prior to analysis, the heterogeneity of metrics used (e.g., shimming, solvent suppression, linewidth) highlights the lack of standardized benchmarks. Only 24% of respondents routinely used chemical shift standards, underscoring an emphasis on relative rather than absolute quantification. While appropriate for exploratory studies, this practice may limit cross-study comparability and quantitative rigor and raises concerns related to chemical shift variability and QC assessment.

Training was identified as a major limitation. Most respondents received informal instruction from supervisors or facility managers, but such training was rarely documented or institutionally recognized. Particularly concerning was the low level of training reported in data analysis and statistics,[23] which are essential for metabolomics studies relying on complex univariate and multivariate approaches.[24] These gaps are likely to become more problematic with the increasing incorporation of AI-based

methods in metabolomics,[25] as insufficient training can compromise data interpretation, reproducibility, and biological conclusions.

Despite methodological advances, the questionnaire highlights substantial deficiencies in institutional infrastructure. Data accessibility, long-term storage, and deposition in public repositories remain largely decentralized. Only 4% of respondents reported operating under a formal regulatory framework, and access to dedicated QA personnel was rare. Limited adoption of Laboratory Information Management Systems (LIMS) further restricts metadata tracking and traceability across complex studies. Without coordinated investment in digital infrastructure and QA oversight, reproducibility efforts will remain fragmented.

The wide range of sample types and applications reported reflects the versatility of NMR metabolomics but also poses challenges for standardization.[26] Protocols optimized for specific matrices may not translate across applications, highlighting the need for modular standardization based on shared core principles such as QC, documentation, and sample traceability rather than rigid technical uniformity. In this context, the mQACC Living Guidance document aims to provide flexible, workflow-spanning best practices for untargeted metabolomics across analytical platforms, supporting data quality and reproducibility.[27] Related community initiatives, including mQACC and MANA, could further contribute through shared SOP libraries, reference datasets, and open repositories.

Our findings closely align with the recent survey and perspective by Andersson et al.,[11] which identified major deficiencies in standardized reporting of experimental parameters, SOP adherence, QC practices, and data accessibility in NMR metabolomics. Importantly, our results suggest that these shortcomings originate at the level of routine laboratory practice, not solely during publication, reinforcing the need for both top-down policy adoption and bottom-up cultural change.

The patterns observed in this study underscore a continuing need for improved standardization and transparency in NMR-based metabolomics. Despite methodological advances, critical experimental details—including sample preparation, acquisition parameters, normalization procedures, and statistical modeling—are still inconsistently implemented or reported,[6] limiting reproducibility, cross-study comparability, data reuse, and the development of widely accepted best practices.

This variability is particularly impactful given the expanding scope of metabolomics, which spans diverse biological systems and study designs, from plant and microbial studies to large human cohorts and both targeted and untargeted analyses.[28] Because experimental choices in sample handling, spectral acquisition, and data interpretation are inherently context dependent,[29,30] the absence of clear reporting standards makes these decisions difficult to assess or reproduce.

As recently highlighted by a MANA NMR Special Interest Group study,[11] several reporting recommendations have been proposed to address these challenges by providing guidance on critical factors affecting reproducibility and reliability in NMR metabolomics. These include appropriate study design, detailed reporting of sample preparation, spectral acquisition, and data processing and analysis methods, as well as the deposition of spectral data in public repositories. The variability in these practices, and the broader heterogeneity within the NMR metabolomics community, is further highlighted by the findings of the present study. Therefore, addressing these challenges will require coordinated efforts to consolidate and adopt existing guidelines, such as the mQACC framework, which provides structured, consensus-based recommendations for NMR metabolomics workflows.[27] In parallel, the development of shared benchmarking resources—including standardized metadata templates, open-access spectral libraries,[31] and multi-laboratory intercomparison studies[32]—would support harmonized practices across instrumentation, data processing, and interpretation. Together, these measures would improve reproducibility, facilitate integration with complementary omics platforms, and accelerate progress toward FAIR-compliant (Findable, Accessible, Interoperable, and Reusable) metabolomics datasets.

Limitations

A practical limitation of our study is the challenge of defining the exact "denominator" of the global NMR metabolomics community, which complicates a formal assessment of representativeness. Although our survey was distributed to a broad network of individuals associated with various applications of NMR in metabolomics, this list likely included a significant number of researchers who use NMR only as a secondary, occasional tool.

To contextualize our 75 responses, we performed a bibliometric review (based upon PubMed and Google Scholar databases) of the literature from the past decade to identify the "active core" of the field—defined as independent laboratories (last/corresponding authors) consistently publishing at least one NMR-based metabolomics study per year. This analysis suggested that the specialized global community consists of approximately 120–150 dedicated research groups. Within this context, our 75 responses likely capture the perspectives of a majority of the groups in the field.

Nevertheless, we acknowledge that our distribution strategy may have favored certain geographical regions or a narrow network. Consequently, the findings presented here should be interpreted as reflecting the views of this reached community rather than an exhaustive, fully representative census of the entire community that performs NMR-based metabolomics community. However, these insights provide a robust snapshot of current practices-challenges and future efforts could benefit from even wider multi-society collaborative outreach to capture the most emerging or peripheral nodes of the community.

Conclusion

Results from our questionnaire provide an important summary of current practices in NMR-based metabolomics. Most researchers engage with core best practices, such as SOP usage, system suitability checks, and quality control protocols, critical gaps remain in areas such as procedural documentation, formal statistical training, and long-term data stewardship. The inconsistent use of public repositories limits regulatory oversight, and sparse application of structured training frameworks point to a need for stronger institutional support and clearer community-wide expectations. Given the methodological diversity of the field, efforts toward standardization must remain adaptable rather than prescriptive. Moving forward, meaningful progress will depend on both organizational investment and cultural shifts toward increased transparency, traceability, and shared responsibility. Community-led efforts, such as the mQACC Living Guidance document, are crucial for establishing adaptive, widely-adopted best practices that will help ensure that NMR metabolomics reaches its full potential as a reproducible, scalable, reliable, and integrative tool in biomedical and environmental research.

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Authors contributions

CRediT: **Leo L. Cheng**: Conceptualization, Supervision, Writing – review & editing; **Robert Powers**: Conceptualization, Supervision, Writing – review & editing; **Robert B. Brua**: Writing – review & editing; **Fabio Casu**: Writing – review & editing; **Valérie Copié**: Writing – review & editing; **Teklab Gebregiworgis**: Writing – review & editing; **Goncalo Jorge Gouveia**: Writing – review & editing; **Gagandeep Kaur**: Writing – review & editing; **Wimal Pathmasiri**: Writing – review & editing; **Marie M. Phelan**: Writing – review & editing; **David Rovnyak**: Writing – review & editing; **Tracey B. Schock**: Writing – review & editing; **Anastasios Theodorou**: Data curation, Visualization, Writing – original draft; **Mario Uchimiya**: Writing – review & editing; **Panteleimon G. Takis**: Conceptualization, Data curation, Supervision, Visualization, Writing – original draft, Writing – review & editing.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, PGT, upon reasonable request.

References

[1] Takis, P. G.; Ghini, V.; Tenori, L.; Turano, P.; Luchinat, C. Uniqueness of the NMR Approach to Metabolomics. *TrAC – Trend Anal. Chem.* **2019**, *120*, 115300. DOI: 10.1016/j.trac.2018.10.036.

[2] Vignoli, A.; Ghini, V.; Meoni, G.; Licari, C.; Takis, P. G.; Tenori, L.; Turano, P.; Luchinat, C. High-Throughput Metabolomics by 1D NMR. *Angew Chem. Int. Ed. Engl.* **2019**, *58*, 968–994. DOI: 10.1002/anie.201804736.

[3] Bedia, C. Metabolomics in Environmental Toxicology: Applications and Challenges. *Trends Environ. Anal. Chem.* **2022**, *34*, e00161. DOI: 10.1016/j.teac.2022.e00161.

[4] Marchev, A. S.; Vasileva, L.; V; Amirova, K. M.; Savova, M. S.; Balcheva-Sivenova, Z. P.; Georgiev, M. I. Metabolomics and Health: From Nutritional Crops and Plant-Based Pharmaceuticals to Profiling of Human Biofluids. *Cell Mol. Life Sci.* **2021**, *78*, 6487–6503. DOI: 10.1007/s00018-021-03918-3.

[5] Ocampos, F. M. M.; de Souza, A. J. B.; Ribeiro, G. H.; Almeida, L. S.; Cônsolo, N. R. B.; Colnago, L. A. NMR-Based Plant Metabolomics Protocols: A Step-by-Step Guide. *Front. Nat. Prod.* **2024**, *3*, 1414506. DOI: 10.3389/fntpr.2024.1414506.

[6] Moco, S. Studying Metabolism by NMR-Based Metabolomics. *Front Mol. Biosci.* **2022**, *9*, 882487. DOI: 10.3389/fmolb.2022.882487.

[7] Wang, D.-G.; Hu, J.-Q.; Wang, C.-Y.; Liu, T.; Li, Y.-Z.; Wu, C. Exploring Microbial Natural Products through NMR-Based Metabolomics. *Nat. Prod. Rep.* **2025**, *42*, 1459–1488. DOI: 10.1039/D4NP00065J.

[8] Powers, R.; Andersson, E. R.; Bayless, A. L.; Brua, R. B.; Chang, M. C.; Cheng, L. L.; Clendinen, C. S.; Cochran, D.; Copié, V.; Cort, J. R.; et al. Best Practices in NMR Metabolomics: Current State. *Trend Anal. Chem.* **2024**, *171*, 117478. DOI: 10.1016/j.trac.2023.117478.

[9] Beger, R. D.; Dunn, W. B.; Bandukwala, A.; Bethan, B.; Broadhurst, D.; Clish, C. B.; Dasari, S.; Derr, L.; Evans, A.; Fischer, S.; et al. Towards Quality Assurance and Quality Control in Untargeted Metabolomics Studies. *Metabolomics* **2019**, *15*, 4. DOI: 10.1007/s11306-018-1460-7.

[10] Gouveia, G. J.; Head, T.; Cheng, L. L.; Clendinen, C. S.; Cort, J. R.; Du, X.; Edison, A. S.; Fleischer, C. C.; Hoch, J.; Mercaldo, N.; et al. Perspective: Use and Reuse of NMR-Based Metabolomics Data: What Works and What Remains Challenging. *Metabolomics* **2024**, *20*, 41. DOI: 10.1007/s11306-024-02090-6.

[11] Andersson, E. R.; Bayless, A. L.; Brua, R. B.; Casu, F.; Cheng, L. L.; Choo, M.; Edison, A. S.; Eghbalnia, H. R.; Fleischer, C. C.; Gouveia, G. J.; et al. Securing the Future of NMR Metabolomics Reproducibility: A Call for Standardized Reporting. *Anal. Chem.* **2025**, *97*, 20655–20666. DOI: 10.1021/acs.analchem.5c03274.

[12] Wishart, D. S.; Guo, A.; Oler, E.; Wang, F.; Anjum, A.; Peters, H.; Dizon, R.; Sayeeda, Z.; Tian, S.; Lee, B. L.; et al. HMDB 5.0: The Human Metabolome Database for 2022. *Nucl. Acid Res.* **2022**, *50*, D622–D631. DOI: 10.1093/nar/gkab1062.

[13] Hoch, J. C.; Baskaran, K.; Burr, H.; Chin, J.; Eghbalnia, H. R.; Fujiwara, T.; Gryk, M. R.; Iwata, T.; Kojima, C.; Kurisu, G.; et

al. Biological Magnetic Resonance Data Bank. *Nucl. Acid Res.* **2023**, *51*, D368–D376. DOI: 10.1093/nar/gkac1050.

[14] Aarts, A. A.; Anderson, J. E.; Anderson, C. J.; Attridge, P. R.; Attwood, A.; Axt, J.; Babel, M.; Bahník, Š.; Baranski, E.; Barnett-Cowan, M.; et al. Estimating the Reproducibility of Psychological Science. *Science. (1979)* **2015**, *1979*, 349. () DOI: 10.1126/science.aac4716.

[15] Cochran, D.; NourEldein, M.; Bezdekova, D.; Schram, A.; Howard, R.; Powers, R. A Reproducibility Crisis for Clinical Metabolomics Studies. *Trends Anal. Chem.* **2024**, *180*, 117918. DOI: 10.1016/j.trac.2024.117918.

[16] Tzoulaki, I.; Ebbels, T. M. D.; Valdes, A.; Elliott, P.; Ioannidis, J. P. A. Design and Analysis of Metabolomics Studies in Epidemiologic Research: A Primer on-Omic Technologies. *Am. J. Epidemiol.* **2014**, *180*, 129–139. DOI: 10.1093/aje/kwu143.

[17] Nagana Gowda, G. A.; Raftery, D. Can NMR Solve Some Significant Challenges in Metabolomics? *J. Magn. Reson.* **2015**, *260*, 144–160. DOI: 10.1016/j.jmr.2015.07.014.

[18] Mandal, R.; Zheng, J.; Zhang, L.; Oler, E.; LeVatte, M. A.; Berjanskii, M.; Lipfert, M.; Han, J.; Borchers, C. H.; Wishart, D. S. Comprehensive, Quantitative Analysis of SRM 1950: The NIST Human Plasma Reference Material. *Anal. Chem.* **2025**, *97*, 667–675. DOI: 10.1021/acs.analchem.4c05018.

[19] Yurekten, O.; Payne, T.; Tejera, N.; Amaladoss, F. X.; Martin, C.; Williams, M.; O'Donovan, C. MetaboLights: Open Data Repository for Metabolomics. *Nucl. Acid Res.* **2024**, *52*, D640–D646. DOI: 10.1093/nar/gkad1045.

[20] Sud, M.; Fahy, E.; Cotter, D.; Azam, K.; Vadivelu, I.; Burant, C.; Edison, A.; Fiehn, O.; Higashi, R.; Nair, K. S.; et al. Metabolomics Workbench: An International Repository for Metabolomics Data and Metadata, Metabolite Standards, Protocols, Tutorials and Training, and Analysis Tools. *Nucl. Acid Res.* **2016**, *44*, D463–D470. DOI: 10.1093/nar/gkv1042.

[21] Zulfiqar, M.; Crusoe, M. R.; König-Ries, B.; Steinbeck, C.; Peters, K.; Gadelha, L. Implementation of FAIR Practices in Computational Metabolomics Workflows—A Case Study. *Metabolites* **2024**, *14*, 118. DOI: 10.3390/metabo14020118.

[22] Winder, C. L.; Witting, M.; Tugizimana, F.; Dunn, W. B.; Reinke, S. N., Committee, the M. S. E. and T. Providing Metabolomics Education and Training: Pedagogy and Considerations. *Metabolomics* **2022**, *18*, 106. DOI: 10.1007/s11306-022-01957-w.

[23] Idkowiak, J.; Dehairs, J.; Schwarzerová, J.; Olešová, D.; Truong, J. X. M.; Kvasnička, A.; Eftychiou, M.; Cools, R.; Spotbeen, X.; Jirásko, R.; et al. Best Practices and Tools in R and Python for Statistical Processing and Visualization of Lipidomics and Metabolomics Data. *Nat. Commun.* **2025**, *16*, 8714. DOI: 10.1038/s41467-025-63751-1.

[24] Blaise, B. J.; Correia, G. D. S.; Haggart, G. A.; Surowiec, I.; Sands, C.; Lewis, M. R.; Pearce, J. T. M.; Trygg, J.; Nicholson, J. K.; Holmes, E.; et al. Statistical Analysis in Metabolic Phenotyping. *Nat. Protoc.* **2021**, *16*, 4299–4326. DOI: 10.1038/s41596-021-00579-1.

[25] Chi, J.; Shu, J.; Li, M.; Mudappathi, R.; Jin, Y.; Lewis, F.; Boon, A.; Qin, X.; Liu, L.; Gu, H. Artificial Intelligence in Metabolomics: A Current Review. *Trends Anal. Chem.* **2024**, *178*, 117852. DOI: 10.1016/j.trac.2024.117852.

[26] Emwas, A.-H.; Zacharias, H. U.; Alborghetti, M. R.; Gowda, G. A. N.; Raftery, D.; McKay, R. T.; Chang, C.; Saccenti, E.; Gronwald, W.; Schuchardt, S.; et al. Recommendations for Sample Selection, Collection and Preparation for NMR-Based Metabolomics Studies of Blood. *Metabolomics* **2025**, *21*, 66. DOI: 10.1007/s11306-025-02259-7.

[27] Mosley, J. D.; Schock, T. B.; Beecher, C. W.; Dunn, W. B.; Kuligowski, J.; Lewis, M. R.; Theodoridis, G.; Ulmer Holland, C. Z.; Vuckovic, D.; Wilson, I. D.; et al. Establishing a Framework for Best Practices for Quality Assurance and Quality Control in Untargeted Metabolomics. *Metabolomics* **2024**, *20*, 20. DOI: 10.1007/s11306-023-02080-0.

[28] Bifarin, O. O.; Yelluru, V. S.; Simhadri, A.; Fernández, F. M. A Large Language Model–Powered Map of Metabolomics Research. *Anal. Chem.* **2025**, *97*, 14088–14096. DOI: 10.1021/acs.analchem.5c01672.

[29] Emwas, A.-H.; Roy, R.; McKay, R. T.; Tenori, L.; Saccenti, E.; Gowda, G. A. N.; Raftery, D.; Alahmari, F.; Jaremko, L.; Jaremko, M.; et al. NMR Spectroscopy for Metabolomics Research. *Metabolites* **2019**, *9*, 123. DOI: 10.3390/metabo9070123.

[30] Emwas, A.-H.; Saccenti, E.; Gao, X.; McKay, R. T.; dos Santos, V. A. P. M.; Roy, R.; Wishart, D. S. Recommended Strategies for Spectral Processing and Post-Processing of 1D 1H-NMR Data of Biofluids with a Particular Focus on Urine. *Metabolomics* **2018**, *14*, 31. DOI: 10.1007/s11306-018-1321-4.

[31] Steinbeck, C.; Kuhn, S. NMRShiftDB – Compound Identification and Structure Elucidation Support through a Free Community-Built Web Database. *Phytochemistry* **2004**, *65*, 2711–2717. DOI: 10.1016/j.phytochem.2004.08.027.

[32] Viant, M. R.; Bearden, D. W.; Bundy, J. G.; Burton, I. W.; Collette, T. W.; Ekman, D. R.; Ezernieks, V.; Karakach, T. K.; Lin, C. Y.; Rochfort, S.; et al. International NMR-Based Environmental Metabolomics Intercomparison Exercise. *Environ. Sci. Technol.* **2009**, *43*, 219–225. DOI: 10.1021/es802198z.