PHARMA

Combining Robustness & Flexibility - Benefits & Examples of using Benchtop NMR for Simplified Design & Risk-assessment of Analytical Quality Control Procedures

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Innovation with Integrity

Analytical Quality by Design (AQbD) principles emphasize the need for a more comprehensive understanding of all factors influencing the results of analytical procedures in quality control. By placing risk management and science at the core of procedure development, they promote "living" procedures where each variable is known, understood and controlled. The new holistic framework established by AQbD can certainly be challenging. However, besides aiming to improve the quality and robustness of procedures, it also paves the way for enhanced flexibility while introducing the concept of platform procedures. In this context, Nuclear Magnetic Resonance (NMR) stands out as a potent analytical technology to support AQbD. Based on absolute physical principles, it supports simplified management of analytical procedures, leveraging the full benefits of the platform concept. In this whitepaper, we will discuss and exemplify how such a platform can be designed for quantitative applications using the compact, cryogen-free benchtop NMR spectrometer Fourier 80 from Bruker. General considerations about the risk-assessment of quantitative NMR procedures will first be presented. Control and mitigation strategies will then be detailed, based on practical examples on how to establish Method Operable Design Region (MODR) with the Fourier 80. These can be easily leveraged by laboratories aiming at implementing flexible and streamlined quantitative NMR procedures with the Fourier 80.

Introduction

In the early 2000s, regulatory bodies emphasized the importance of Quality by Design (QbD) principles in drug manufacturing, aiming to enhance pharmaceutical product quality through systematic product and process development as well as a thorough understanding of production processes. This led to the creation of the ICH Q8 to ICH Q11 international guidelines, recently supplemented by ICH Q12 and Q13, which provide a comprehensive QbD framework. This QbD paradigm has been translated into the realm of Quality Control (QC) through the concept of Analytical Quality by Design (AQbD). AQbD integrates these principles into analytical procedures, emphasizing the importance of a risk- and science-based methodology that ensures consistent and reliable results instead of Quality by Testing approaches. Quality must be built into the analytical procedures, considered as "living" processes, and managed with a risk-assessment-based lifecycle.

The recent key guidelines USP-NF <1220>, ICH Q2(R2) and ICH Q14, support this transition. They collectively promote the need to establish a deep understanding of analytical variability and robust control strategies. This model emphasizes the importance of designing analytical procedures with a comprehensive and continuous understanding of factors affecting procedures' performance to ensure that these remain fit for purpose throughout their lifecycle. In this framework, "validation" of the procedure is merely one step of the process, where continuous control as well as pro-active knowledge gathering, and consolidation are equally important.

AQbD aims at diverging from the now so-called "traditional" approach, where analytical procedures are often developed and validated using empirical methodologies that focus on isolated validation steps. This is often seen as a check-box exercise that interprets ICH Q2 and USP-NF <1225> as mandatory steps rather than leverage them as scientific guidance. Although this conventional methodology has been used with undeniable success for decades, it can also result in a lack of systematic and comprehensive understanding of factors influencing the procedure. As such, it can lead to inconsistent performance. Besides the fact that very high confidence in analytical procedures is the cornerstone of QC, limited robustness can have a significant impact on manufacturing workflows, possibly resulting in very rigid Standard Operating Procedures (SOP), difficulties in method transfer, Out Of Specifications results due to the analytical procedure etc.

In contrast, AQbD, through the deep understanding, identification and control of potential sources of variability, aims not only to improve confidence in the reportable values but also to provide more flexibility when it comes to regulatory compliance and particularly post-approval changes. This capability can ultimately reduce the burden on QC laboratories and the pharmaceutical industry more widely.

A detailed discussion of the AQbD principles is outside the scope of this whitepaper, but it is important to mention three significant features:

1 Introduction of the concept of "Analytical Target Profile" (ATP) as a fundamental component of the analytical procedure lifecycle (see Figure 1). The ATP predefines all the desired performance characteristics of an analytical procedure. It specifies the criteria that the analytical procedure must meet to ensure the quality of the data it generates as well as that the procedure is fit for purpose. This is a significant change compared to the traditional approach, since performance criteria are defined prior to any method development and are solely based on the required quality attributes. Selection and method development are carried out afterwards, in an effort the meet the ATP. According to this new paradigm, virtually any procedure meeting the ATP criteria could be chosen, including those already in place. Notably, it also gives more flexibility, allowing changes in the design space of the procedure, as long as this has been thoughtfully investigated and demonstrated to continuously meet the ATP.
According to AQbD, the multivariate design space concept is referred to as the Method Operable Design Region (MODR) by USP-NF <1220> and ICH Q14. ICH Q14 also introduces the concept of proven acceptable ranges (PARs) for univariate parameters of analytical procedures.

2 As a direct consequence of the lifecycle approach with Quality Risk Management (QRM), enhanced understanding of the procedures and identification of the factors affecting performance is a prerequisite. It implies that a deep knowledge must be gained via detailed and comprehensive robustness studies. Thus, robustness assessment is expected to be one of the initial steps of the development and not an output of the method validation, which occurs in the traditional approach. It establishes the design space of the procedure and ultimately enhances flexibility.

3 Finally, in the logical continuation of knowledge-based management, the concept of platform procedures is introduced in both USP-NF <1220> and ICH guidelines. Providing science-based and technology-inherent justifications, a common platform procedure can be applied to several products. As a result, it is possible to significantly simplify procedure development, qualification and monitoring.

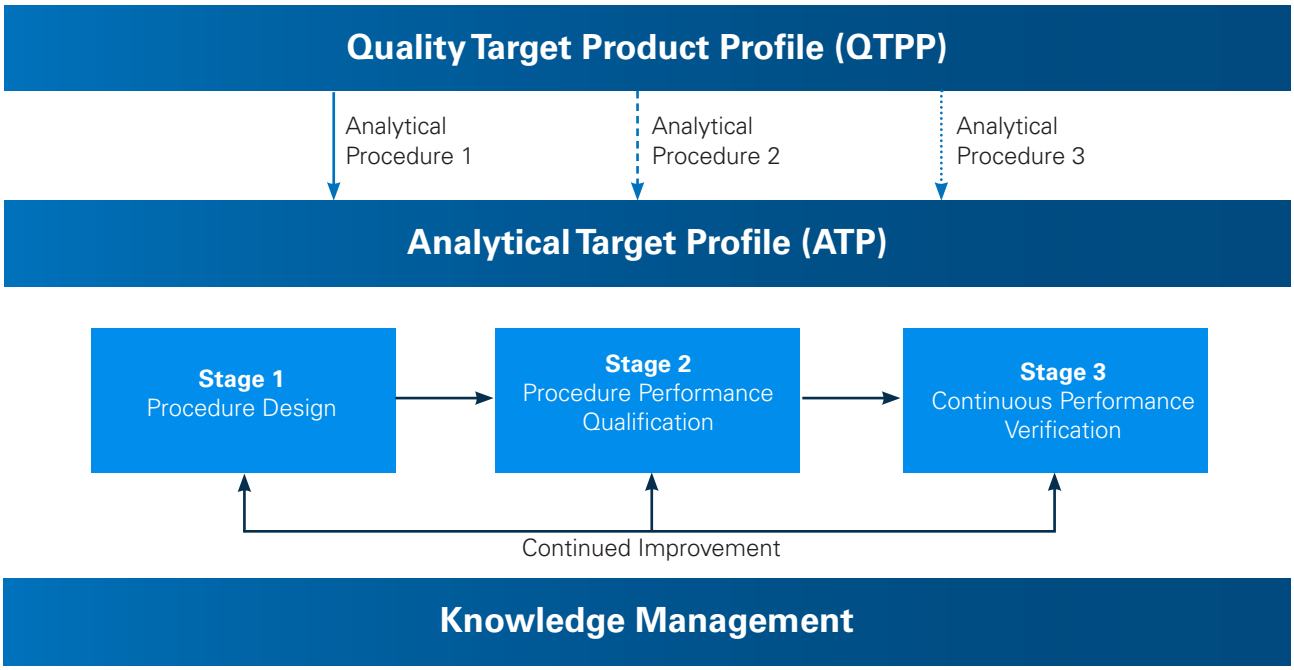

Figure 1: Management of analytical procedure lifecycle as defined in USP-NF <1220>.

Even if the AQbD concept has matured over several years and can be seen as a logical, straightforward extension of QbD principles, it is also undeniably a ground-breaking change for QC laboratories. Several years will probably be needed to fully apprehend and harmonize practical implementations of AQbD in laboratories, especially for the traditional QC technologies that have been used for decades in line with the traditional framework. In this regard, a fully comprehensive understanding of analytical procedures can appear daunting when dealing with somewhat empirical techniques.

On the other hand, this new AQbD paradigm is well-aligned with more recent analytical technologies. Revision R2 of the ICH Q2 introduces and exemplifies several new tools besides those based on chromatography, and Nuclear Magnetic Resonance (NMR) stands out as a highly valuable technology. It combines several benefits and technology-inherent justifications that allow procedure management to be simplified. In effect, NMR relies on universal and absolute physical principles. This means that knowledge gathering and robustness assessment can be drastically simplified, as many of the factors influencing the results are already known and are universally applicable. In that sense, NMR is probably one of the very few solutions where the platform procedure concept can be fully leveraged to establish a robust and versatile analytical foundation that can be easily applied and transferred to several tests within a laboratory. Additionally, NMR solves the issue of authentic standards availability. Any surrogate can be used for quantitative NMR applications (qNMR), with the additional benefit of supporting direct metrological traceability[1]. For qualitative applications such as identification,[2] reference data only need to be recorded once. Therefore, continuous availability of reference materials is not required, opening the doors to the use of digital references, as introduced in the current draft of USP-NF <11>.

The unique advantages of NMR for QC applications have been recently recognized. For example, ICH Q2(R2) specifically details NMR method validation for quantitative applications, and the current drafts of USP-NF <761> and <1761> directly incorporate the AQbD principles into NMR systems as well as analytical procedure management. This broad acceptance coincides with the availability of low-cost, compact, cryogen-free benchtop NMR spectrometers, such as the Fourier 80. Benchtop systems can be placed in traditional laboratories or manufacturing environments because of their limited footprint and cryogen-free, permanent magnet configuration. Dedicated, specialized rooms that are typically required by conventional NMR instruments are not needed, removing the last barriers to adopting NMR in QC laboratories without compromising on the intrinsic benefits of NMR technology.

[1] Several certified reference materials are commercially available with a direct metrological traceability to primary standard for mass fraction purity.

[2] See for example our whitepaper V. Poirier *et al*, Streamlining Identification Testing for Quality Control Using Benchtop NMR, **July 2024**

Some practical examples of NMR procedures for QC using the Fourier 80 have been recently described in other white-papers to illustrate the benefits and overall simplicity provided by benchtop NMR.[3] In the present publication we will focus on the initial stage of the analytical procedure lifecycle, i.e. the risk-assessment and robustness study, as part of the QRM of the analytical procedure. This will highlight how the intrinsic features of NMR can be leveraged to significantly accelerate key steps while supporting the implementation of a robust, quantitative analytical platform. This insight supplements the extensive discussion provided in the current draft of USP-NF <1761> (and the associated stimuli article) to demonstrate that all prior knowledge on NMR is perfectly applicable to benchtop solutions and can therefore be leveraged.

General Considerations for Quantitative NMR

qNMR is a well-established method for quantitative analysis. Under suitable conditions, NMR signals are directly proportional to the content of chemical species in the sample under investigation, making this powerful solution ideal for absolute quantitation. In addition, qNMR alleviates the need for identical reference material, as mentioned earlier. General benefits and considerations for qNMR methods have been extensively documented in the growing body of scientific literature[4]. The ongoing revisions of USP-NF <761> and <1761> also provide a comprehensive description of NMR systems and applications, including those for quantitative purposes.

As NMR is a very versatile technology, numerous analytical strategies based on mono or multidimensional experiments are possible. Furthermore, various nuclei (or spins) can be studied by NMR, supporting flexibility in method design. Thus, a detailed description of all possible strategies and considerations of qNMR falls outside the scope of the present white-paper. This document will focus on the most used and reported approach for quantitative NMR: proton (^{1}H) qNMR using the simplest monodimensional (1D) NMR sequence, e.g. single pulse sequence. Nonetheless, many of the general considerations discussed hereafter can be extended to other NMR-active nuclei and potentially to other sequences.

In a very simplified overview, there are three main categories of quantitative qNMR procedures:

1. Relative quantitation to simply determine the ratio of two or more components in a mixture. This can be typically used for the determination of the molar or weight ratio of components from polymeric species.[5] This is the most straightforward application of qNMR, but it does not deliver absolute values.

2. Absolute quantitation with external calibration: this method requires simple sample preparation because the sample and internal calibrant (IC) are recorded separately (it can be seen as a single-point calibration methodology). However, it provides lower accuracy compared to internal calibration.

3. Absolute quantitation with internal calibration: this method offers the highest accuracy. It is most commonly used for assays and involves a more demanding sample preparation, as it is necessary to mix a known amount of IC with a sample prior to any analysis.[6]

[3] See our page dedicated to QC applications using the Fourier 80.

[4] See for example Diehl *et al.* J Pharm Biomed Anal. **2020**, *177*, 112847 ; Pauli et al. Magn Reson Chem. **2021**, *59*, 7

[5] See for example our other whitepapers: V. Poirier *et al*, Benchtop NMR as a Versatile Tools for Quality Control: Example of Poloxamer Compendial Testing, **June 2024**; V. Poirier, Adapting and Streamlining Compendial Procedures to the Fourier 80 Benchtop NMR spectrometer – Example of the Betadex testing, **July 2024**.

[6] See for example our other whitepaper V. Poirier *et al*, Quantitative Assays with Fourier 80 Benchtop NMR: Benefits and Examples, **June 2024**

Figure 2 presents a representative ^{1}H NMR spectrum used in a qNMR procedure with the Fourier 80. Figure 3 provides a schematic representation of the NMR sequence utilized for single pulse 1D qNMR, illustrating the key terms referenced throughout this whitepaper.

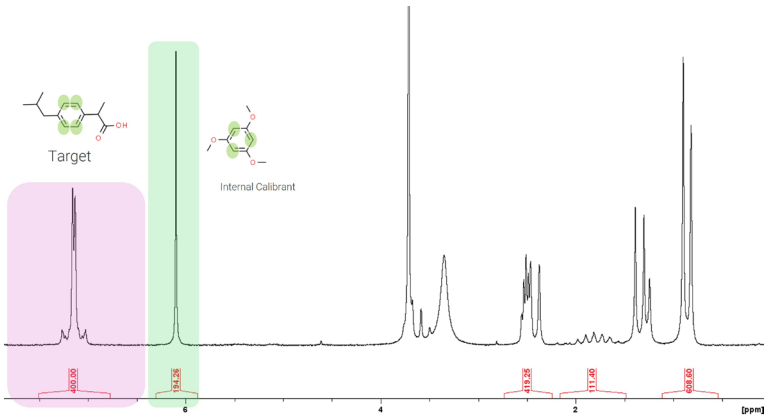

Figure 2: Example of a ^{1}H spectrum for quantitative measurements performed on a model drug product with IC using Bruker's Fourier 80 NMR spectrometer. Resonances used for quantitation are highlighted in purple (target) and green (IC).

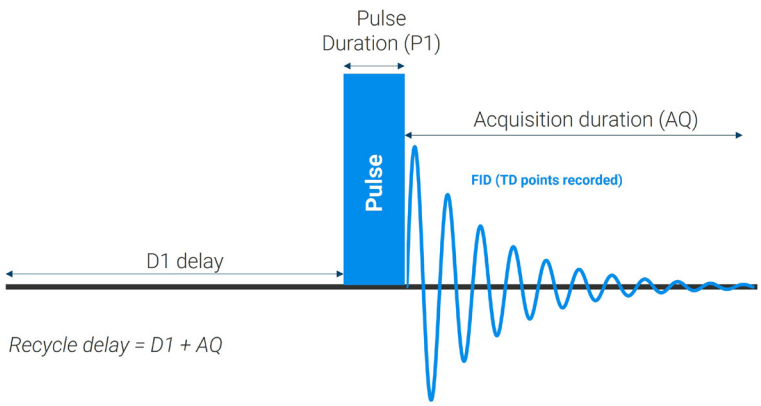

Figure 3: Schematic representation of the single pulse NMR sequence (zg) commonly used for ^{1}H qNMR.

Risk-Assessment for qNMR Procedures

The three categories of qNMR procedure described in the previous section share many elements. As an initial step in the risk assessment, a general process map for qNMR procedures can be established, as discussed in the current draft of USP-NF <761> and illustrated in Figure 4. This generic process map represents the majority of qNMR processes, though specific procedures may include additional or different sub-steps. It highlights why NMR enables platform procedures: except for sample preparation, all steps are common to any application. This means that process understanding and knowledge can be shared and leveraged across multiple procedures, significantly simplifying management and development efforts.

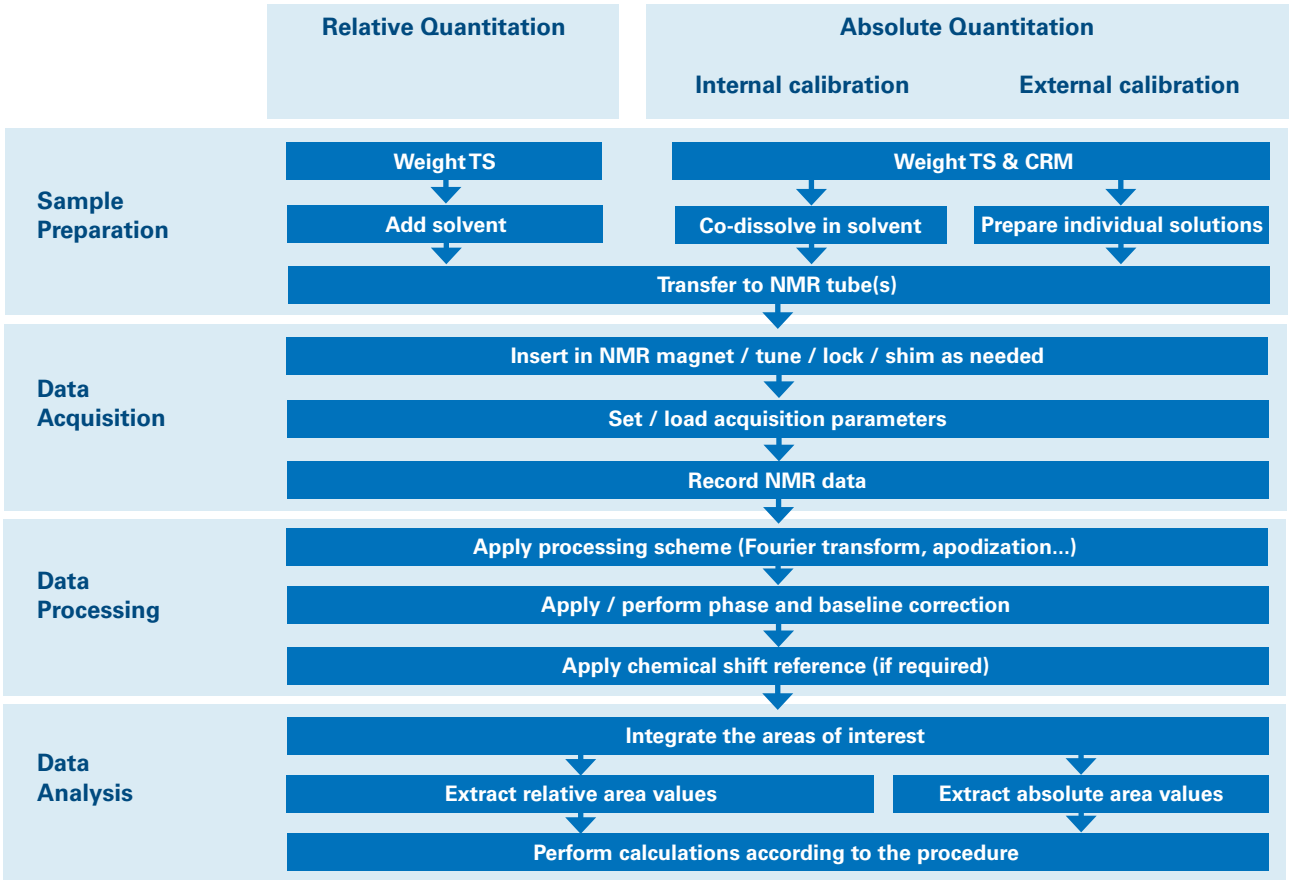

Figure 4: Example of typical process map for qNMR adapted from current draft of USP-NF <761> as of July 2024 (TS: Test Sample, CRM: Certified Reference Material).

The process map is a valuable entry point for the risk assessment, as it helps to understand and define all steps and sub-steps of the process. With additional tools, such as Ishikawa diagrams, the exhaustive identification of possible variables of qNMR procedures is then possible. Again, many of these will be common to all NMR-based analytical quantitative procedures, and the example of an Ishikawa diagram in Figure 5 will probably apply to the vast majority of qNMR procedures.

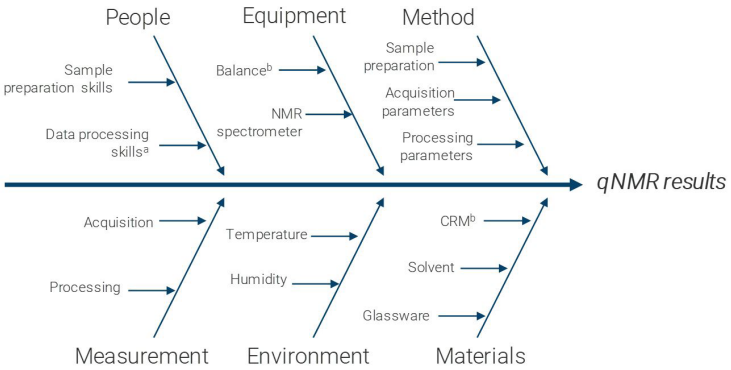

Figure 5: Example of Ishikawa diagram to identify potential variables in qNMR procedures (a: only for non-automated process, b: only for absolute quantification process).

Each item in Figure 5 can be further divided into sub-items. For example, the acquisition and processing parameter categories encompass many underlying variables, necessitating a more detailed diagram to display all the individual variables. Nonetheless, this generic diagram highlights general variable categories and helps identify risk associated with each.

These risks can then be scored and ranked according to the required performance attributes of the procedure and, ultimately, the ATP. This assessment is driven by prior knowledge and scientific expertise. As discussed in USP-NF <1220>, a heat map is a valuable tool to visualize risks and their scoring. This, in turn, helps designing and implementing appropriate mitigation strategies. Based on inherent technology features, such a heat map can be established on a general basis for qNMR procedures, to help the quick identification of "critical risks" associated with the analytical method. Annex 1 contains a detailed example of a heat map for qNMR procedures, demonstrating that most risks are associated with qNMR as a platform and can thus be addressed and managed in a similar way across many applications by adopting standard practices. Only a few risks are purely sample-dependent, such as hygroscopicity and stability, which need to be addressed on a case-by-case basis. Additionally, some components of the platform procedure present risks that can exhibit sample dependency, more specifically, where the NMR characteristics of the specific sample must be known to mitigate the risk. However, these can be simply assessed during the first extension of the platform to a new target, and this strategy can be incorporated into the design of the platform procedure. In this sense, adding new products into a platform procedure can be seen as an additional variable in the MODR, and the procedure can be managed accordingly.

As a final note in this example of a generic risk assessment for qNMR procedures, it should be highlighted that all considerations discussed and the variables identified apply to both high-field and benchtop NMR systems. Therefore, a global, platform approach for both could also be conceivable.

Design Space and Mitigation Strategies for qNMR Procedures with the Fourier 80

To enable flexible procedures, implement appropriate mitigation strategies, and ensure the robustness of the analytical method, a full understanding of its design space or MODR is essential. Beside prior knowledge and scientific expertise, this understanding can be achieved through well-designed experiments, similar to the Design of Experiment (DoE) approach of QbD. While this may initially seem tedious, it can prove highly beneficial for two main reasons: 1) it continuously ensures a fit-for-purpose procedure and 2) it provides flexibility and the ability to perform minor changes in the procedure without necessitating an extensive risk re-evaluation each time.

As a platform-compatible technology, NMR procedures for quantitative applications benefit from large design spaces, where most variables are identified and either known, predictable or easily manageable through the investigation and control of the platform MODR as well as technology-inherent justifications. The current draft of USP-NF <1761> provides detailed explanations and examples of MODR evaluation for qNMR procedures in this regard.

To demonstrate that the benefits of high-field NMR extend to benchtop NMR and provide additional examples of design space evaluation in NMR, a series of experimental tests were performed on the Fourier 80. Using model samples, several variables were experimentally studied to evaluate MODRs and PARs of qNMR procedures, commonly referred to as "design space" hereafter, using the Fourier 80. These tests focused on the data acquisition and data processing tasks of the qNMR workflow, as these are common to all qNMR procedure categories (Figure 4) and can be easily transposed to any laboratory. In contrast, variables in the sample preparation step are mostly laboratory-dependent and will not be discussed here.

Importantly, all the tests described in the following section were performed with fully automated acquisition and processing, eliminating all possible operator contributions, which is highly desirable for QC procedures. In fact, all results shown incorporate contributions from these automated processes.

Unless otherwise stated, most of the tests discussed were performed on a model sample: a 1M sample preparation of ethyl crotonate in $CDCl_3$. The typical ^1H spectrum of this sample on the Fourier 80 is illustrated below. Relative integrations of the resonances from the same molecule were investigated, as the expected ratios are accurately known, since they directly correspond to the ratio between each non-equivalent proton. This approach eliminates sample preparation uncertainties while enabling direct translation of the results to absolute quantification, as the acquisition and processing tasks are identical for both relative and absolute quantification.

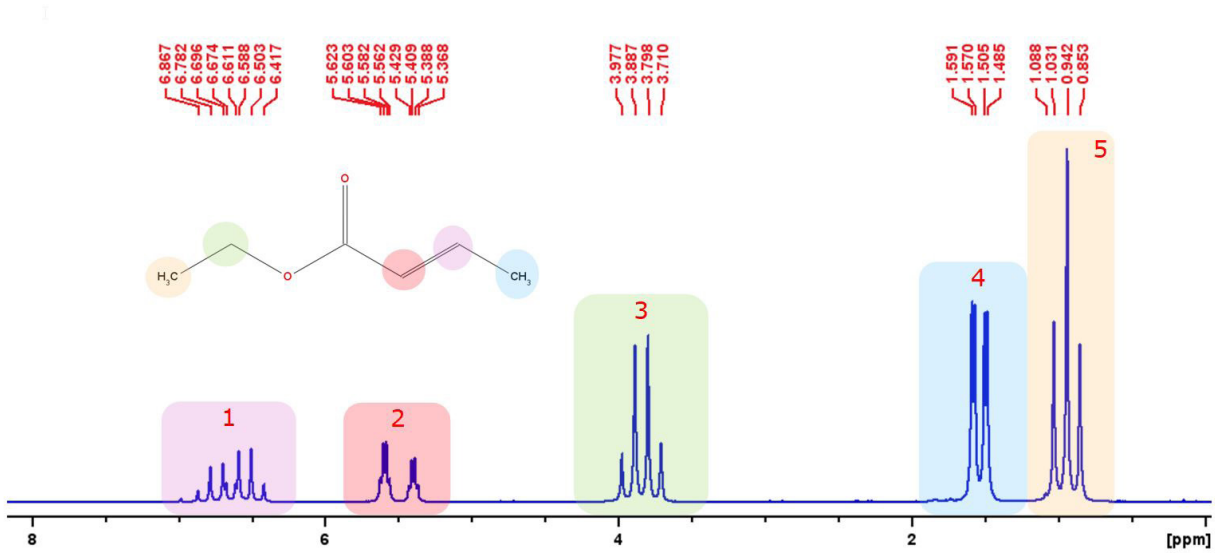

Figure 6: Example of the ^1H spectrum obtained with Fourier 80 of 1M ethyl crotonate in $CDCl_3$. Structural attribution is represented as colored areas. Resonances are arbitrarily numbered to facilitate further discussion. Most of the tests were done using resonances 3 and 4, since resonance 5 suffers from interference and spins yielding resonances 1 and 2 are subject to long relaxation time.

A) Uniformity of Instrument Response

To perform effective quantitative measurements with NMR, it is critical that the response is fully uniform across the entire spectral region used for quantitative applications. This uniformity is achieved through the combination of two main elements: the uniformity of the pulse excitation and the uniformity of the receiver. Referring to the example in Figure 2, verification across the 5 to 8 ppm range would suffice for this specific case. However, for a platform approach, it is highly advisable to verify uniformity across the entire range of possible chemical shifts for ^{1}H. Figure 7 illustrates the typical ^{1}H uniformity of the Fourier 80 with a remarkable relative standard deviation (RSD) of 0.1% across the useful range of -2 to 13 ppm. It worth mentioning that this residual uncertainty is most probably due to the test itself (including the data processing) rather than the instrument uniformity.

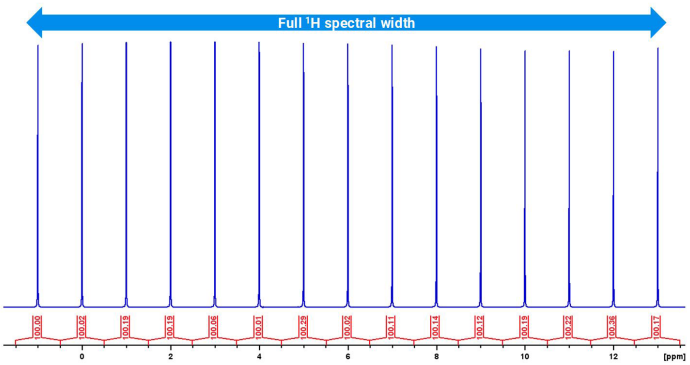

Figure 7: Example of ^{1}H uniformity of response verification on a Fourier 80 spectrometer using incremental change of the transmitter offset on a 5% H_2O/D_2O, 0.6mM $CuSO_4$ sample at 25°C (the chemical shift scale is reversed compared to the conventional one).

Achieving such uniformity is standard for modern NMR systems, including the benchtop Fourier 80. Drift of this variable is not expected, except in cases of system failure, which would also present other symptoms. Consequently, this test is typically part of the system's operational qualification and does not need continuous monitoring, supporting a straightforward platform approach to the full range of ^{1}H chemical shifts for this variable, as reported in Annex 1.

B) Linearity of Instrument Response and Number of Accumulated Scans

The management and validation of linearity for NMR methods has been an issue for a long time. Previous guidelines, such as ICH Q2(R1), have typically been more focused on chromatographic techniques. As such, they explicitly required validation of linearity, which might seem redundant to NMR experts, since linearity is inherent to quantitative this analytical method. Additionally, it was not always clear whether the requirement was for instrumental linearity or procedural linearity, and whether correlation models that do not indicate linear relationships could be accepted.

The R2 revision of ICH Q2 addresses these ambiguities. The linearity criterion is now included under the general "response" criterion, and the revised guidelines explicitly state for ^{1}H qNMR that "validation tests are typically not necessary" for the reportable range. Nonetheless, the current draft of USP-NF <761> indicates that general linearity verification should be performed as part of the qualification test procedure. This test can be performed using samples of various concentrations, but it presents a dual challenge: 1) it includes uncertainties arising from sample preparation and 2) the dynamic range of NMR is very high, requiring numerous preparations to cover it.

As the NMR response is directly proportional to concentration and the number of accumulated scans (as its square root), another strategy to verify the linearity of the NMR response is to study a sample across multiple accumulated scans to demonstrate the expected perfectly linear correlation (thus mimicking changes in concentration). Figure 8 illustrates such a verification on a Fourier 80. This was performed using a single resonance sample with an increasing number of scans. The corresponding RSD after normalization is very low (0.4% in this instance) and likely includes additional sources of uncertainty due to the test itself.

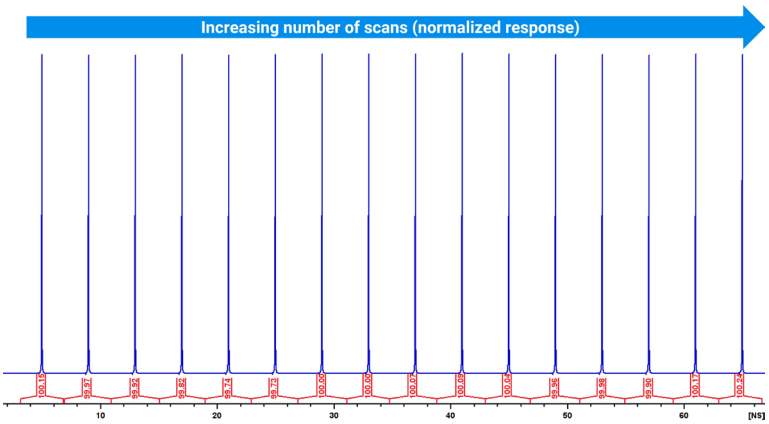

Figure 8: Example of ^{1}H linearity response verification on a Fourier 80 spectrometer using incremental increase of accumulated scans on a 5% H_2O/D_2O, 0.6mM $CuSO_4$ sample at 25°C. Response is normalized by the square root of the number of scans.

Taken from another study examining the design space, Figure 9 illustrates the impact of the number of scans on results for both internal and external calibration models. This test uses the same series of acquired data, varying only the number of scans, and applies two different methods for exploiting the integration values. This strategy delivers a combined, general verification:

- The number of acquired scans does not impact the results. This stands true provided there is a sufficient signal-to-noise ratio. In the study presented here, the slight increase in error for a low number of scans can probably be attributed to this factor, yet the error remains marginal.
- There is a fully linear response, demonstrated by the external calibration model. This test involves normalization by the square root of the number of scans. Nonetheless, results are consistent across the range of tested values (excluding a negligible contribution for the lowest number of scans due to a lower signal-to-noise ratio), evidencing the perfect linearity of the response.

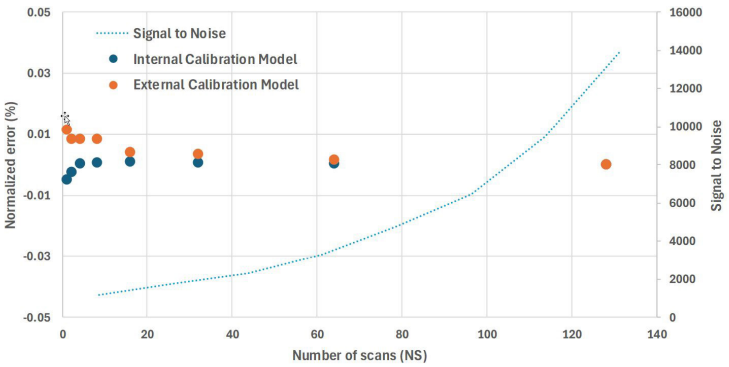

Figure 9: Example of ^{1}H linear response verification on a Fourier 80 using incremental increase of accumulated scans on a 1M ethylcrotonate in $CDCl_3$ sample at 25°C . It uses the integration of resonance 3 (Figure 6) relative to that of resonance 4 for the internal calibration model. Integration of the spectrum acquired with NS = 1 is used for the external calibration model after normalization by the square root of the number of scans. In both cases, error is normalized against the result of the spectrum acquired with NS = 128 to remove potential bias. Signal-to-noise of resonance 3 is measured using an automatic 2 ppm noise range.

Overall, such straightforward tests explicitly demonstrate both the linearity of the system and the procedure, as well as the complete decorrelation of results concerning the number of scans, provided that a minimal signal-to-noise ratio is reached. This results in a very large design space, as only the minimal number of scans needs to be controlled, and this can be directly applied to any ^{1}H qNMR procedure in a platform approach, as considered in Annex 1.

C) Magnetic Field Homogeneity

Homogeneity of the magnetic field is a critical factor in NMR, as it strongly influences the lineshape of the resonances, and thus resolution and sensitivity. Achieving the best possible homogeneity is done by setting the so-called shim currents, which correct for any detected deviation through a complex, multivariate measurement and adjustment process. Due to the complexity of this process, it is usually fully automated on high-field systems. On the Fourier 80, this is also done automatically. However, in contrast to floor-standing spectrometers, it does not need to be performed with every new sample tube, as the impact of this in the benchtop NMR is very limited. Instead, adjustments on a predefined schedule (e.g. once a day) are often sufficient, saving significant time.

Nonetheless, shim current variations can be seen as critical variables in the qNMR measurement process, requiring their management with suitable controls. Figure 10 illustrates the impact of deviations in shim currents from the optimal settings (as automatically determined) on the lineshape of a singlet (expressed as its width at half height). This example only considers the two most influential shims (X, Z), yet it demonstrates that resonances quickly broaden with any change in these shims. If only referring to this factor, one might conclude that the design space for these variables is very limited and that only the exact optimal setting is acceptable.

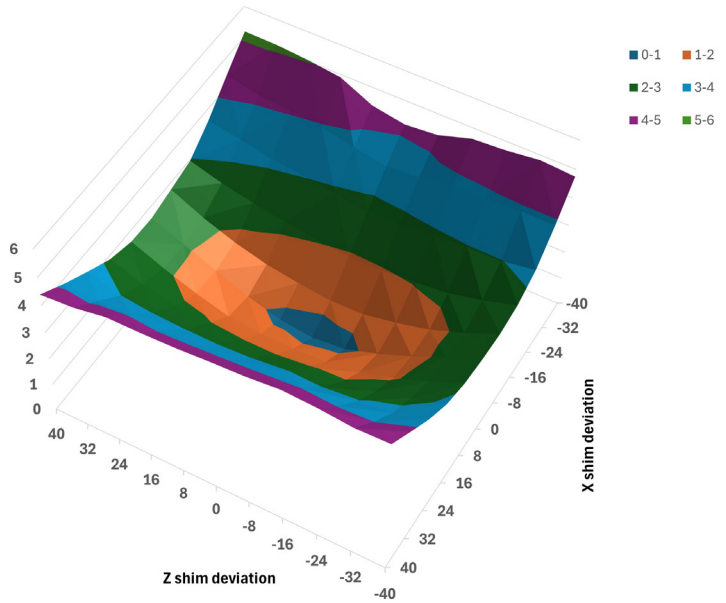

Figure 10: Example of an experimental plot of the full width at half maximum (FWMH) of the water resonance on a 5% H_2O/D_2O, 0.6mM $CuSO_4$ sample at 25°C, as recorded on a Fourier 80 when varying the z and x shim current from the optimal settings.

However, while lineshape tests are useful for understanding the interrelation of various variables, they do not directly reflect the impact of shim deviations on qNMR measurements, as lineshape may not be as critical for this application of NMR. In fact, during the processing of NMR data, a mathematical broadening is usually deliberately introduced (via the apodization function, see section E). Therefore, it is preferable to assess the impact of shim deviations directly on qNMR results, as illustrated in Figure 11. In the example below, deviations of ±10 points for both shims, which are significant, result in an experimental error that includes other uncertainty factors while remaining well below 0.5%. The design space is thus much larger than the single optimal settings point for the shims. Thus, a control strategy based both on the required resolution between signals and sensitivity can be implemented, rather than solely relying on lineshape measurements.

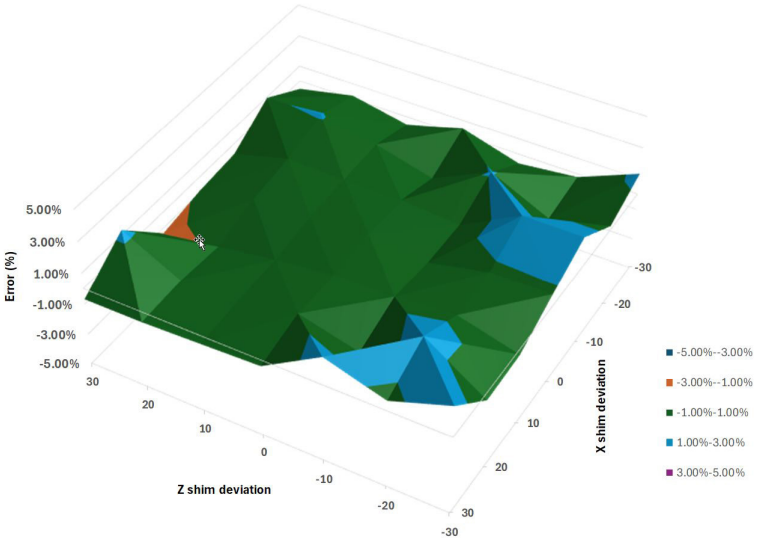

Figure 11: Example of shim deviation impact on quantitative results on a 1M ethylcrotonate in CDCl3 sample at 25°C using the integration of resonance 3 (Figure 6) relative to that of resonance 4 as recorded on a Fourier 80 when varying the z and x shim current from the optimal settings.

D) Flip Angle and Recycle Delay

The critical impact of flip angle and recycling delay is well known to NMR experts. For any quantitative application of NMR, full relaxation must be achieved; that is, the system of spins must return to its equilibrium state before the subsequent pulse. The flip angle directly influences the required recycle delay for a given set of spins, since a higher flip angle necessitates a longer relaxation time. However, there is no universal setting for the recycle delay, as it is also dependent on the longitudinal relaxation time of the spins (T1), which varies based on the spins and experimental conditions (solvent, temperature, etc.). The surface plot in Figure 12 illustrates the relationship between the flip angle and recycle delay, demonstrating a large design space available for these two variables, provided that:

1) The recycle delay is sufficiently long relative to the flip angle used.

2) The flip angle is high enough to achieve a sufficient signal-to-noise ratio.

This second conclusion can only be inferred from Figure 12, and plotting the signal-to-noise ratio against the same variables, as shown in Figure 13, further highlight the direct relationship between flip angle and achieved sensitivity. While this relationship is apparent to NMR experts, such a plot helps define the acceptable design space. For instance, one could set a minimum signal-to-noise ratio of 1000:1, leaving the green part of the surface as the acceptable combination of flip angle and recycle delay.

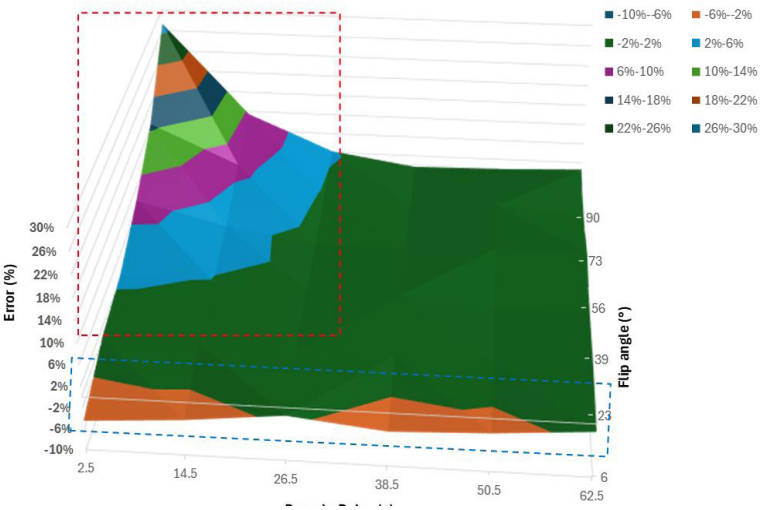

Figure 12: Example of error on quantitative results on a 1M ethylcrotonate in $CDCl_3$ sample at 25°C using the integration of resonance 3 (Figure 6, T1 of associated spin is ca. 8.3 s) relative to that of resonance 4 (T1 of associated spin is ca. 5.4 s) as recorded on a Fourier 80 when varying the flip angle and the recycle delay. The dashed red square indicates the area where the combination of recovery delay and flip angle is not adequate. The dashed blue square indicates the area where, due to a small flip angle, the signal-to-noise ratio is low, resulting in higher error.

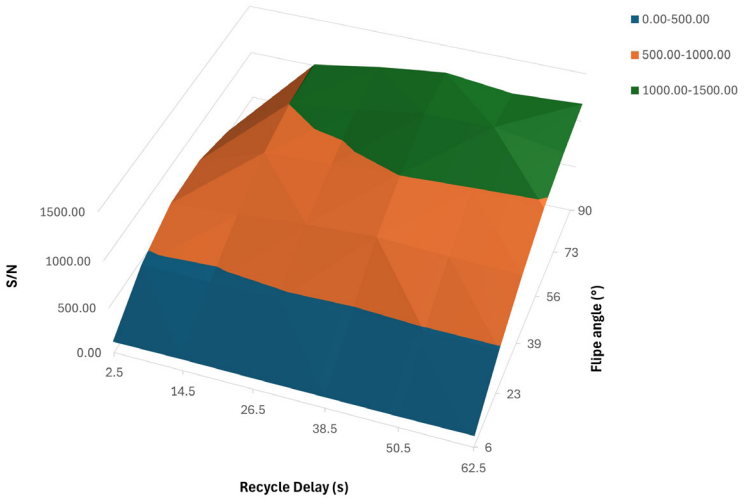

Figure 13: Example of signal-to-noise of resonance 3 dependency on flip angle and recovery delay for resonance 3 of a 1M ethylcrotonate in $CDCl_3$ sample at 25°C (Figure 6, T1 of associated spin is ca. 8.3 s) as recorded on a Fourier 80 when varying the flip angle and the recycle delay.

The experimental results shown in Figure 12 are predictable, as they result from known, mathematical relationships between the flip angle, T1 and the recycle delay. Based on Equation 1, the theoretical surface for the error arising from inadequately setting the recovery delay with respect to the flip angle can be plotted as illustrated in Figure 14.

$$M_{z,t} = M_0 \frac{1 - e^{-\frac{t}{T_1}}}{1 - e^{-\frac{t}{T_1}cos\theta}}$$

Equation 1: Relationship between the magnetization along the z axis after a recycle delay t (Mz,t), initial magnetization (M_0) and flip angle (θ) for a given T_1.

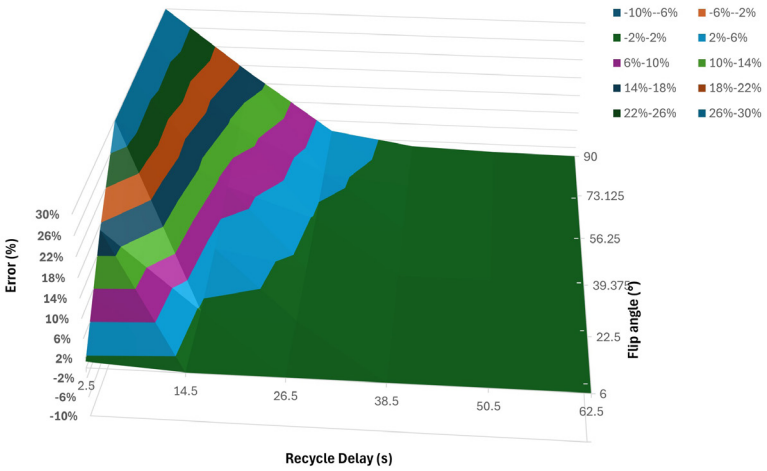

Figure 14: Example of error modeling on ^{1}H quantitative results for ethylcrotonate for the integration of resonance 3 (Figure 6, T1 of associated spin is ca. 8.3 s) when varying the flip angle and the recycle delay based on Equation 1. Error scale is not shown beyond 30%.

A comparison between Figure 14 and Figure 12 can only be qualitative, as Figure 14 models the relaxation of spins yielding resonance 3, while Figure 12 includes the relaxation of spins yielding resonances 3 and 4, along with other variables (signal-to-noise ratio, processing, etc.). Nonetheless, they qualitatively match, highlighting that many NMR variables can be mathematically computed or predicted, and serve as justification for the design space of the NMR procedure.

These considerations are obvious to NMR experts. It is well established that for quantitative applications, a recycle delay of at least 5 to 7 times the longest T1 of the spins under investigation must be used, and that flip angles of 90° are preferable for ^{1}H. However, this type of multivariate investigation and comparison to models supports the definitive justification of the design space and associated controls for an NMR procedure. For an ^{1}H qNMR procedure, this could be for example:

1) A compulsory experimental measurement of T1 for the relevant spins for each new product and/or preparation.

2) A default instruction of a 90° flip angle and a recovery delay of 7 times the longest T1 but allowing variations within the possible design space, provided that any other implemented controls are met.

These types of prescriptions could apply to any analyte and thus support a truly platform approach concept as leveraged in Annex 1.

Lastly, it should be mentioned that the relationship between the flip angle and pulse duration (see Figure 3) is partially empirical and must be determined experimentally for a given spectrometer. It is generally part of the initial operational qualification but can drift over time. Therefore, it must be controlled appropriately with a formal annual verification, for example. Indirect controls via System Suitability Tests (SST) at a higher frequency, such as regular system sensitivity testing, are also desirable to proactively detect any drift. Nonetheless, having validated a design space means that it is possible to handle deviations within the permitted ranges. Thus, a drift may result in acceptable results, provided all variables fall within this design space. This capability highlights the benefits of such approaches.

E) Acquisition and Processing

In this final example of studying ^{1}H qNMR MODR, a series of tests were performed on variables affecting the quality of the resulting spectra. Initially, the number of experimental acquired points (TD) and the number of points used in the Fourier transform (SI) were systematically varied, as illustrated in Figure 15. In this example, a large space with errors below 0.5% is available. Some values of SI and TD are however very low and do not actually yield spectra of acceptable quality (see Figure 16). Similarly, it is not advisable to use an SI value lower than half of TD, as this results in data truncation. This means that other variables besides TD and SI contribute to spectral quality and must be accounted for when implementing and delimiting the design space. Nonetheless, this example demonstrates the flexibility offered by NMR procedures, indicating that strict definitions of parameters such as TD and SI are not required. Instead, defining a range of acceptable values is preferable.

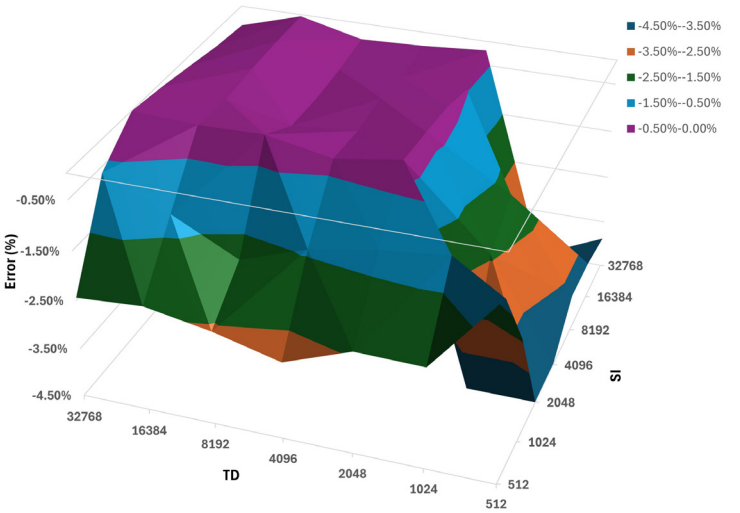

Figure 15: Example of error (absolute value) on quantitative results on a 1M ethylcrotonate in $CDCl_3$ sample at 25°C using the integration of resonance 3 (Figure 6) relative to that of resonance 4 as recorded on a Fourier 80 when varying TD and SI parameters. Errors below -4.5% are not shown.

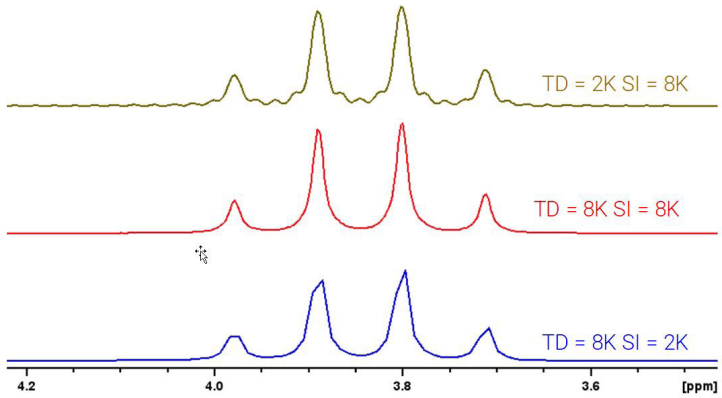

Figure 16: Zoom on the ^1H resonance 3 of 1M ethylcrotonate in $CDCl_3$ sample at 25°C as recorded on a Fourier 80 when varying TD and SI parameters. All sets of parameters result in acceptable quantitative results, as plotted in Figure 15. Only the red spectrum is suitable based on the spectral quality, as the brown spectrum suffers from FID truncation (TD too low) as indicated by wiggle artifacts, while the digital resolution of the blue one is not optimal (SI too low) resulting in insufficient points per peak.

Using the same series of experimental data, it is also possible to study and understand the impact of other processing factors. In ^1H NMR processing, a mathematical correction, known as the apodization function, is often applied to the NMR raw data to improve signal-to-noise ratio. This is typically an exponential decay function, set using a line broadening factor (LB). This is usually 0.3 Hz. After Fourier Transform, two additional processing steps - phase correction and baseline correction - are typically required before integration (see Figure 4). For a QC procedure, these need to be performed automatically to avoid operator contribution, and several algorithms are available. Figure 17 examines the variation of LB and some of the processing algorithms available in Topspin. This study highlights that 1) there is no clear correlation between the LB value and the performance of the algorithms, as the same trends are observed in all three studied combinations, and 2) there is again a large design space of acceptable values. In this example, values of LB even above 1 still result in errors below 0.5% when using the "APK0/ABS" and the "APK/APBK -bo" combination for processing. Conversely, the "APBK" algorithm appears less suitable, as deviations occur more frequently and with an apparently random character.

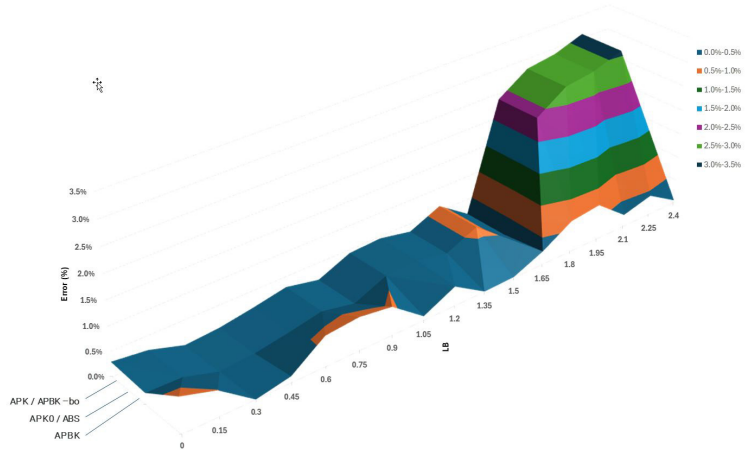

Figure 17: Example of error on quantitative results on a 1M ethylcrotonate in $CDCl_3$ sample at 25°C using the integration of resonance 3 (Figure 6) relative to that of resonance 4 as recorded on a Fourier 80 when varying the LB parameters and the combination of algorithms for phase and baseline correction. For each combination, errors are normalized against the result with LB = 0.3 (reference point). The raw data was recorded with TD = 32K and processing with SI=32K.

There is an obvious analyte and preparation dependency to these results, as the shape and proximity of the resonances to be integrated will vary. Nonetheless, this type of study can help drawing general trends, designing control strategies, and implementing case-by-case recommendations. For example, since the results shown in Figure 17 are generated from a single raw data set, they can be easily generated for any new reference spectrum. This can be implemented as a required step when extending a procedure to a new analyte/preparation, as indicated in the example of Annex 1.

Conclusions

The AQbD framework can be seen as an additional challenge for QC laboratories. Nonetheless, besides elevating the control and confidence over the quality of the product, it can actually prove beneficial for analytical procedure management, supporting greater flexibility and science-based operations instead of offering a rigid framework. Leveraging new technologies, it also supports the introduction of platform approaches, for which NMR is well-suited.

Based on intrinsic, well-known physical principles, NMR can significantly simplify the lifecycle management of analytical procedures, especially in the context of AQbD, as it benefits from large, easily controlled design spaces. A benchtop NMR spectrometer, such as Bruker's Fourier 80, brings these benefits to QC laboratories through a compact, cryogen-free system. As the analytical framework of this solution does not differ from conventional, high field systems, the same know-how and well-established considerations for quantitative NMR procedures apply. Risk assessment and QRM of NMR procedures using the Fourier 80 are thus simple, as technology-inherent justifications and collective knowledge can be leveraged. Global approaches can be deployed to study and control the design space of general platform procedures. This offers unparalleled flexibility and adaptability to QC laboratories while increasing the confidence in the reportable values.

Acknowledgements

Benjamin Goerling, Mark Howard and Robin Stein

Annex 1: Example of a general risk-assessment for quantitative NMR procedures, including a color-coded scoring of the risks (heat map) and possible mitigation strategies. Adapted from current draft of USP-NF <1761> (as of July 2024).

Analytical Procedure Component	Risk Factor	Potential Hazard	Accuracy Level	Precision Level	Platform management[a]	Control Strategy
Sample preparation	Analyst skill	Inaccurate sample preparation[d]	Strong	Strong	X	Appropriate analyst training and periodic verification (QMS)
	Weighing amount of the sample and reference material	Inaccurate weighing[d]	Strong	Strong	X	Define minimum weight based on balance uncertainty. Ensure appropriate environment and tools. Balance managed via the laboratory QMS to ensure compliance
	Hygroscopic sample or reference material				X	Use of appropriate CRM
					X	Control laboratory humidity
						Assess sample hygroscopicity
	Additional preparation steps, e.g. mixing, sonication	Inappropriate steps leading to inaccurate preparation	Strong	Strong	(X)	Assessed on given preparation protocol MODR
	Quality of solvent	Low deuteration, high water content, and impurities overlapping with the response of interest	Medium	Medium	X	Minimal quality defined Continuous quality assessment (QMS)
	Quality of reference material	High expanded uncertainty of reference material purity	Medium	Strong	X	Minimal expanded uncertainty defined
		High water content and impurities overlapping with the response of interest	Strong	Medium	X	Minimal quality defined Minimal expanded uncertainty defined
	Quality of NMR tubes	Poor tube quality (e.g., non-uniform internal diameter) resulting in quantitation error (external method) and/or poor line shape	Medium	Medium[b]	X	Minimal quality defined Continuous quality assessment (QMS)
	Stability	Mixture of sample and/or reference material in the selected solvent may be unstable	Strong	Strong		Assessed by a stability study – Timeframe defined for subsequent analysis
Data acquisition[b]	Linearity of the instrument	Nonlinearity	Strong	Minor	X	OQ MODR
	Uniformity of response factor across the relevant spectral region	Nonuniformity	Strong	Minor	X	OQ MODR
	Sample temperature	Changes in peak chemical shifts	Minor	Minor	(X)	OQ MODR
	Shimming	Low sensitivity, poor line shape	Medium	Medium	X	OQ/ PQ MODR SST
	Probe tuning	Low sensitivity, inaccurate flip angle	Minor[c]	Strong	X	OQ/PQ SST Not relevant for Fourier 80
	Flip angle	Low sensitivity	Minor	Strong	X	Delfine minimal values MODR OQ / PQ SST

Analytical Procedure Component	Risk Factor	Potential Hazard	Accuracy Level	Precision Level	Platform Management[a]	Control Strategy
Data acquisition[b]	Relaxation delay	Incomplete relaxation leading to inaccuracy	Strong	Minor	(X)	Define minimal values based on experimental T1 measurement MODR
	Spectral Width / Carrier Frequency	Truncated resonances	Strong	Strong	(X)	Define minimal margin based on experimental spectra MODR
	Time domain	Truncated FID	Medium	Medium	(X)	Define minimal margin based on experimental result MODR
	Receiver gain	Overloaded receiver	Strong	Medium	X	Define control strategy MODR Not relevant for Fourier 80
	Number of scans	Low sensitivity	Minor	Strong	X	Define minimal values. MODR
Data processing[b]	Zero filling	Low digital resolution	Medium	Medium	X	Define minimal values MODR SST
	Apodization	Line broadening	Medium	Minor	(X)	Define acceptable range MODR SST
	Baseline correction	Distortion, inappropriate correction	Medium	Strong	(X)	Define a procedure MODR SST
	Integral range	Incorrect integration range setting	Medium	Minor	(X)	Define a procedure MODR SST

a: In platform procedure management, items noted as X can be fully managed via the platform. Items noted (X) can have some sample dependency but are manageable via a validated MODR. Other items are fully dependent on the sample and must be managed on a case-by-case basis.
b: Considering a fully automated process.
c: For internal standard. For external standard, the impact could be significantly higher
d: Only for absolute quantitation procedure. For relative quantitative, these items are not relevant.
QMS: Quality Management System, OQ: Operational Qualification, PQ: Performance Qualification

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GxP
Solution Page