PHARMA

Streamlining Identification Testing for Quality Control Using Benchtop NMR

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Innovation with Integrity

Identification is probably the most performed test by pharmaceutical Quality Control (QC) laboratories. While it appears simple, identification requires robust and efficient procedures to ensure both quality and throughput. Traditional methodologies like those found in the pharmacopeia may not be specific enough, which implicates the need to combine several tests and techniques. In this whitepaper, the benefits of implementing Nuclear Magnetic Resonance (NMR) for QC identification testing are discussed and exemplified. Using a benchtop NMR like the Fourier 80, standard-free, highly specific, and short turnaround time tests can be designed and implemented as an attractive alternative to other technologies.

1 Introduction

Identification testing is a fundamental and extensively utilized procedure in pharmaceutical quality control laboratories. Every monograph in the United States Pharmacopeia-National Formulary (USP-NF) and the European Pharmacopoeia (Ph. Eur.) includes an identification section. Additionally, all certificates of analysis (CoA) for raw materials, excipients, and active pharmaceutical ingredients (APIs) of pharmaceutical grade contain an identification section, which may be either compendial or bespoke.

In the context of pharmaceutical quality control, identification is defined as “confirming, with an acceptable degree of assurance, that the article matches the description on the label” (Ph. Eur. definition). The test thus aims to verify conformity to the label description rather than providing a comprehensive confirmation of the chemical structure or composition of the product. This difference is fundamental for the correct understanding of the identification test scope. Current proposed draft of USP <1761>[1] provides a detailed comparison between structural characterization and identification testing for chemical substance (applicable to nuclear magnetic resonance (NMR) but the scope can be extended to any other technique).

Parameter	Structural Characterization	Identification Test
Application field	Scientific process	Quality control process
Production stage	Research and development stage	Manufacturing stage
Intention of the test	Aligned with development requirements	Aligned with manufacturing process
Expertise needed	Performed by specialists	Performed by analysts and/or automated
Results decision	Involves interpretation	Meets requirements
Purpose of the work	Defines requirements and acceptance criteria	Demonstrates meeting the acceptance criteria
Final work output	Report	Certificate of analysis
Foundation of the test	Based on scientific principles	Based on comparison to reference materials and/or data

Table 1 Differences between structural characterization and identification tests as reported in current proposed draft of USP <1761>[1]

An important requirement for all materials used in drug manufacturing is that they are systematically assessed for conformity with all appropriate specifications. However, only the identification test must systematically be performed again upon material reception from the supplier and before use. Regulatory texts indeed specify that for other tests, values reported by the supplier can be used without re-testing, providing adequate compliance assurances are in place.[2] The user still needs to verify all analytical data associated with the supplier CoA but this leaves him with “only” the identification test to experimentally perform before material usage. This can still be challenging since they need to check every batch of all materials used in the production of drug substances (raw materials) and drug products (APIs, excipients).

In addition, all identification procedures, like any quality control tests, must be validated according to applicable regulatory requirements. For identification tests, this generally involves the validation of specificity and, depending on the analysis risk, robustness. With the introduction of Analytical Quality by Design (AQbD) in USP <1220> and ICH[3] Q2(R2) and ICH Q14, the initial definition of the Analytical Target Profile (ATP) and comprehensive analytical procedure life cycle management is also required, as for all quality control procedures developed in this new paradigm.

Therefore, robust, compliant, and efficient procedures are essential for identification testing to ensure smooth batch control and timely release. While compendial procedures are used for materials with existing monographs, for all other components, the most efficient strategies for in-house testing are desirable to maintain high quality standards without compromising timelines.

NMR spectroscopy has traditionally been associated with fundamental research and the drug discovery phase. It is now emerging as a powerful tool for QC, particularly for identity testing, as evidenced by the ongoing or approved revisions of pharmacopeia and international guidelines that incorporate and describe in detail how NMR can be leveraged for such procedures[4]. With the recent availability of low-cost, compact, cryogen-free benchtop NMR spectrometers, the last barriers that may have made quality control laboratories reluctant to implement NMR and incorporate it in their procedure development has been alleviated. In this whitepaper, advantages of using benchtop NMR spectrometers like the Bruker Fourier 80 for identity testing and strategies to develop and implement such procedures will be discussed.

[1] As of July 2024

[2] See for example EU GMP Part II (API) and USP <1078> (excipients)

[3] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

[4] See ICH(Q2)R2, applicable as of 14 June 2024 details the use of NMR and current draft of USP <761> and <1761> as of July 2024

2 Benefits of using NMR for identification testing

Historically, a variety of techniques have been employed to develop compendial procedures for identification testing. Monographs from the USP-NF and Ph. Eur may contain several identification tests based on different methodologies. These tests can be used either as alternatives or in mandatory combinations, depending on the specific case. The analytical techniques encompass a range of physico-chemical tests (reactivity, visual observation, melting point, optical rotation, etc.), chromatographic methods (gas, liquid, thin-layer…), mass spectrometry, and various other spectroscopic techniques, including ultraviolet, infrared (IR), Raman spectroscopy, and NMR.

When non-selective or poorly selective techniques are employed, combinations of several tests are often required, as frequently observed in compendial monographs. However, NMR stands out as one of the best techniques for identification testing, offering a unique combination of features:

- Each analyte will exhibit numerous NMR spectroscopic characteristics. Given that NMR is highly sensitive to the chemical and magnetic environment, each non-equivalent nucleus yields a specific resonance in an NMR spectrum,[5] characterized not only by its frequency (chemical shift), but also by its shape and intensity. This can be compared, to a certain extent, to infrared spectroscopy, but NMR offers a much higher level of detail. As a result, NMR is arguably the most specific spectroscopic technique.
- NMR is universal: a given analyte under a specific set of analytical conditions will consistently yield the same spectroscopic characteristics (e.g., the same fingerprint, see Figure 1). Therefore, spectra of qualified reference standards need to be recorded only once and then used as a reference for routine testing (regardless of the spectrometer used, as long as the magnetic field strength is identical). This not only saves time but also eliminates the need for the recurrent supply of costly qualified reference standards, contrasting starkly with other spectroscopic and chromatographic techniques used for identification. This is underpinned but to on-going effort of USP to develop and promote digitalized reference standards as introduced in the current draft of USP <11>. Lastly, as NMR is the most used analytical tool during the R&D stage for structure elucidation and confirmation, knowledge and data acquired at these stages can be directly leveraged for the development of the identification procedure.
- For proton-based identification methods (see next section), data acquisition often takes only a few minutes, even on a low-field benchtop NMR spectrometer. In addition, since it is a non-destructive, contactless technique, no conditioning of the system is required when changing between different methods.[6] This provides very short turnaround times (TAT) and a very high capacity per instrument, even when dealing with dozens of methods on the same system.
- In the context of Analytical Quality by Design, NMR procedures fit all the criteria of the platform analytical procedure concept.[7] Thus, provided sound justification, several steps of the analytical procedure life cycle can be made common to all NMR-based procedures (like system suitability testing), which simplifies and streamlines management.
- Finally, as NMR is the most powerful tool for structural characterization and elucidation, investigations when Out Of Specification (OOS) or Out Of Trend (OOT) results are obtained can be directly initiated and possibly solved using the initial experimental results. Additional data can also be directly acquired on the same sample if required for the investigation, as NMR is not destructive.

[5] If the nuclei are NMR active. This is the case for most of the elements found in organic substances (H, C, N, F, P) where at least one isotope is NMR active. Only oxygen cannot be directly recorded.

[6] Except if different temperatures are used, but for routine identification testing this is unlikely.

[7] Defined as "An analytical procedure that is suitable to test quality attributes of different products without significant change to its operational conditions, system suitability and reporting structure" in ICHQ14 and "multiproduct method suitable to test quality attributes of different products without significant change to its operational conditions, system suitability, and reporting structure" in USP <761> draft as of July 2024.

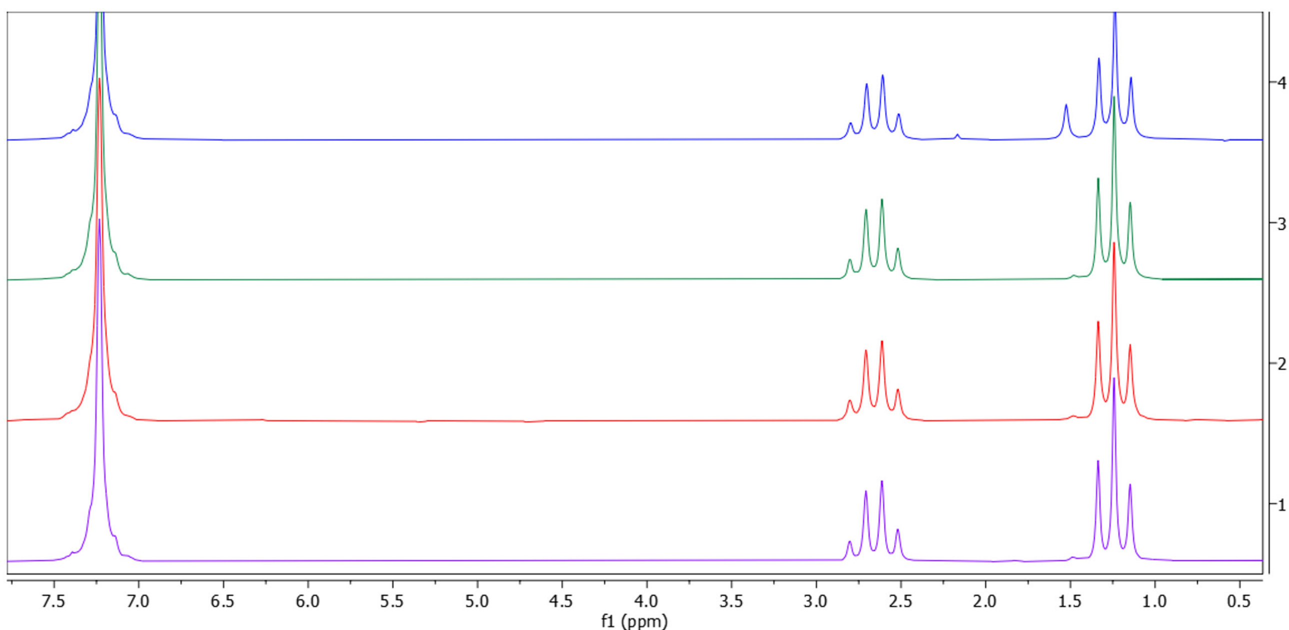

Figure 1: Overlays of four ^{1}H NMR spectra of the same compound (ethylbenzene) recorded on four different Fourier 80 spectrometers after dissolution in $CDCl_3$. The spectroscopic features associated of the substance are strictly identical (observable differences between the spectra are due to variable content of impurities such as trace water at about 1.5 ppm).

Despite these significant advantages, only a few dozen compendial identification tests use NMR. Pharmacopeia methodologies indeed tend to rely on the most "accessible" procedures in terms of equipment and expertise. For a long time, NMR was perceived as a technique to avoid for compendial and more generally QC procedures due to the cost of the systems and the level of expertise required. Although NMR rapidly established itself as one of the fundamental analytical techniques in academia, its availability in the private sector was much more limited, and GMP-compliant systems were even rarer. This paradigm is now changing quickly. Spectrometers are simpler to use and can be operated on a routine basis by non-experts, due to significant improvements in user interfaces, fully automated procedures, and simplified maintenance operations. The recent progress in GMP compliant NMR spectrometers and the introduction of benchtop systems have further accelerated the process.

With GMP-compliant benchtop NMR systems like the Fourier 80 NMR spectrometer with the Bruker GxP kit, the benefits of NMR can be achieved in a low-cost, compact, cryogen-free system. The reduced footprint of the benchtop system allows for more versatility in QC labs and manufacturing facilities where available space for a high-field system can be impractical. With virtually no maintenance, these systems allow uninterrupted operation by non-experts, making them very attractive for both quantitative and qualitative quality control applications. In the case of identification testing, procedures can be easily designed and implemented, leveraging all the benefits from an NMR-based procedure.

3 Design and implementation of NMR identification tests

A. General considerations

As a common principle, spectroscopic and chromatographic identification tests are typically conducted by comparing the batch under investigation with a qualified reference standard. The analytical features examined during the procedure must be specific enough to conclude, with reasonable assurance (e.g. as determined by a risk-assessment approach), the unequivocal identity of the substance. If a method lacks sufficient selectivity, an additional procedure must be implemented, either as a supplement or a replacement.

It is important to note that the specificity assessment for identification is not purely technical (e.g., risk of interferences) but must also be based on a risk-assessment approach. The procedure must be capable of clearly differentiating closely related substances that may be present from the manufacturing process or the supply chain. A prime example from NMR is enantiomers. NMR spectra of enantiomers will be strictly identical and thus cannot be differentiated. If a substance must be identified as a specific enantiomer and there is a risk of the presence of the other, then NMR procedures alone is not sufficient. The implementation of an additional testing procedure will be required to address this gap.

It may be puzzling to refer to the currently applicable compendial NMR methods for identification when trying to understand how to design a bespoke method since the description of the analytical procedure and acceptance criteria found in the pharmacopeia range from extremely detailed prescriptions to only a few lines of indications. To illustrate these discrepancies, two extreme examples can be cited:

- The NMR identification test described in the heparin sodium monograph of USP-NF is highly detailed, including a system suitability section. Several criteria need to be met with clear and strict numerical limits. To some extent, even if reported in the identification section, this test goes beyond stated requirements and incorporates quantitative impurity testing ("No unidentified signals greater than 4% of the mean of signal height of 1 and 2 are present"). Thus, verification of this procedure by a laboratory before its first use (as required by USP <1226>) will probably require additional criteria validation compared to a purely qualitative procedure. Such drastic testing arose from the so-called "heparin crisis". Around 2008, batches of heparin induced severe adverse events for patients under heparin therapy. After extensive investigations, it appeared that the heparin batches were contaminated with oversulfated chondroitin sulfate even if they conformed with the monograph in force at the time.[8] Thus, deep revisions of the compendial procedures were undertaken to ensure detection of this contaminant to avoid any new occurrences.
- On the other end of the spectrum, the first identification test for the antibiotic tobramycin described in Ph. Eur. 0645 only comprises a few lines. It merely states the concentration to be used for sample preparation and "comparison" to the chemical reference standard is the only indicated criterium. Such oversimplified prescriptions leave the responsibility of defining the acceptance criteria to the laboratory, possibly introducing subjectivity, operator dependence, and poor control over the procedure.

Even if the purpose of the identification testing is intrinsically qualitative, defining quantitative criteria for the examination of the spectroscopic attributes or features in comparison to the reference is highly preferable. Considering two sets of spectroscopic features to be identical should not be based on subjective assessments like visual inspection, but instead on defined and justified criteria. Figure 2 illustrates this problem. Using the same sample preparation and NMR spectrometer, but slightly modified magnetic field homogeneity, two comparable, yet not exactly superposable proton(^{1}H) NMR spectra, are obtained. The slight visual differences may lead a non-expert operator to a subjective conclusion if the sole criteria are "comparable fingerprints" or "identical fingerprints." Spectroscopic features (resonance frequency, shape, intensity, etc.), however, are in-fact strictly identical with regards to the technique and tolerated variations,[9] leading to direct conclusions if used as criteria.

[8] See for example: Liu *et al.* Nat Prod Rep. 2009; 26(3); 313–321

[9] Which must be documented and justified.

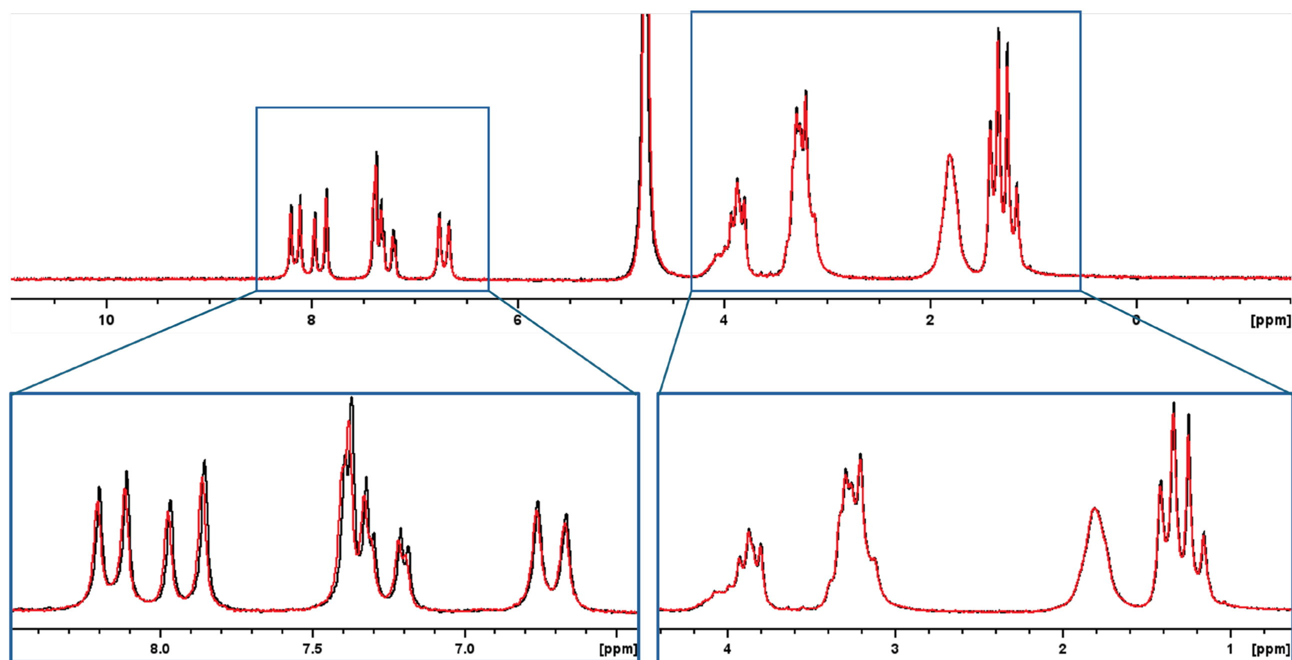

Figure 2: Overlays of two ^{1}H NMR spectra of hydroxychloroquine in D_2O recorded on Fourier 80 with a very slight change of the magnetic field homogeneity (+/- 10 points on the z shim). The zoomed-in views demonstrate that while extremely close, the two spectra are not superposable.

The current proposed draft of USP <1761>[1] directly addresses this issue, stating that "critical quality attributes of the NMR spectrum should be identified, justified, and specified with numerical limits." This implies that during the development of the NMR identification procedure, all necessary spectral features allowing unambiguous identification must be enumerated, and criteria for each must be defined based on the expected non-critical and critical variations.

While this may seem like an extensive and time-consuming approach, in practice, it is significantly streamlined since NMR is a universal technique. The NMR spectroscopic features of each substance are indeed predictable and can be easily collected experimentally on the qualified reference standard. Furthermore, the sources of variations in NMR are known and predictable. Knowledge from robustness studies can be leveraged for several procedures, and substance-specific sources of variation can be readily identified, ensuring accurate and reliable identification processes while streamlining procedure development.

B. Typical design and implementation steps

Leveraging prior experimental data and/or knowledge acquired during R&D stages, the development of an NMR procedure for identity testing for a specific substance can be summarized as follows:[10]

1. Selection of the most appropriate nucleus to be recorded. This step is straightforward as the proton nucleus is the most suitable choice with only few exceptions. Protons are highly prevalent in organic compounds, and they offer the highest sensitivity in NMR. On the Fourier 80, such a spectrum can be recorded in just a few minutes, providing very short TATs. Even though its spectral width is more limited than other, less sensitive nuclei, ^{1}H NMR resonances exhibit sophisticated patterns from spin-spin coupling. This is beneficial for identification testing as it provides specific characteristic features, further ensuring the specificity of the method. Depending on the chemical structure, other nuclei such as phosphorous (^{31}P) or fluorine (^{19}F) may be exploited; however, a procedure based solely on ^{31}P or ^{19}F NMR may not provide enough specificity to differentiate between closely related structures. Finally, carbon (^{13}C)-based approaches may prove useful due to the very large spectral width, allowing very similar compounds to be differentiated, but due to the low sensitivity of ^{13}C, acquisition times can be significant. Identification tests based on ^{13}C will thus be rare and implemented only in specifically challenging cases.

[10] This section focuses on the technical considerations of the procedure development , after definition of the ATP and initial risk assessment.

2. Selection of the sample preparation methodology: In NMR, this will usually be as simple as selecting the solvent to fully dissolve the chemical under investigation, without any additional steps. The chemical must be stable in the selected solvent, which can be easily assessed since any evolution will have noticeable effect on the NMR spectrum. When possible, protonated solvents such as water or methanol should be avoided as they can introduce more sources of variability (e.g., pH sensitivity) necessitating a more demanding robustness assessment.

3. Selection of data acquisition and processing parameters: For identification purposes, this is straightforward since typical, standard parameters are usually suitable and could be easily assessed during method development.

4. Recording reference data using a qualified reference standard: This can be either sourced from commercial sources (with suitable traceability) or in-house qualified, for example using NMR methodologies to unambiguously demonstrate the structure of the product (see example two in the next section).

5. Defining the critical attributes of the resulting spectrum to be exploited and associated acceptance criteria. Usually, these will be a combination of:

> a. For small molecules, determining chemical shift, multiplicity, and intensity of each NMR resonance associated with the structure. For larger molecules and polymers, this may not always be practical, especially using benchtop NMR spectrometers. A sound description of the features should then be performed like "chemical shift of an adequate selection of the patterns" (USP <1761> draft[1]). More sophisticated approaches based on multivariate procedures may also be considered but are more complex to implement and validate and thus lies out of the scope of this whitepaper.
>
> b. Assessing the expected variation of each of these values considering the general and specific sources of variability (e.g. perform a risk assessment).
>
> c. Defining the acceptance criteria required to ensure a specific identification procedure. They will be based on the spectroscopic features specific to the substance, associated source of variability, and a risk assessment of the manufacturing process and/or the supply chain. All expected interferences should also be described to avoid erroneous OOS results. In ^{1}H NMR this will typically be the resonances from water and traces of non-fully deuterated solvent.

6. Formally validate[11] the procedure according to the applicable regulatory requirements.

From this list, steps 5b and 5c appear the most challenging, particularly for those without NMR experience; however, in practice these steps can straightforwardly addressed, leveraging the general features of NMR and evaluating prior knowledge. Having a platform procedure approach is desirable, as most of the justification will be common to all analytes, facilitating procedure management.

The table below provides some examples of sources of variation for ^{1}H NMR testing depending on the exploited features. Even if features should be re-assessed on a case-by-case basis, the general considerations in Table 2 will hold true for most small molecules, both on low- and high-field NMR spectrometers.

[11] In USP <1220> the term "procedure validation" is replaced by "procedure qualification" to highlight that this step is only a part of the whole life cycle process

Feature	Possible source of variation	Possible mitigation strategy
Chemical shift of non-labile proton	Solvent	Solvent must be specified. Slight modification of the solvent quality usually has little to no effect, except for the water content. Assess the worst-case scenario and define it as a limit in the procedure.
	Concentration	Only significant concentration changes will impact chemical shifts. Define a concentration to be used with a typical 10% tolerance or assess extreme cases of the design space of the procedure.
	Temperature	Temperature during acquisition may have a significant effect. Define a temperature to be used with a narrow tolerance. If a larger design space is needed, test extreme cases to assess the tolerance to be applied to each exploited resonance.
	Chemical shift referencing	For identification tests, using an internal chemical shift reference like tetramethylsilane (TMS) is desirable. Referencing strategy must be clearly specified in the procedure.
Chemical shift of labile proton	Several	Chemical shifts of labile protons are influenced by many factors and are usually not reliable. It is advisable to exclude them from the features that will be assessed.
Multiplicity / Coupling constants	Magnetic field homogeneity	Resolution and the ability to measure coupling constants and determine multiplicity is intrinsically linked to the magnetic field homogeneity of the spectrometer during acquisition. Define features based on the typical homogeneity achievable, as externally controlled by performance qualification tests. Extensive coupling constants or multiplicity description may not be required and can be simplified as long as the procedure remains specific.
	Concentration	See chemical shift of non-labile proton.
	Data acquisition & processing	Suitable acquisition and processing parameters are specified. Some may require only minimal values (e.g. number of acquisition points, spectral width etc.) while others may need specific settings (e.g. apodization function and associated parameters).
Integration	Data acquisition & processing	See multiplicity / coupling constants. Identification tests do not aim at quantification – large tolerances can be applied since the goal is only to discriminate between integer values (e.g. number of nuclei).

Table 2 Example of risk-assessment for an NMR-based identification procedure. Typical sources of variation for each NMR feature exploited for identification are listed and possible mitigation strategies illustrated.

Considering the potentially vast and overwhelming array of NMR features for a particular substance, it is crucial to remember that only a „minimal" set may be required to accomplish a specific procedure, within the context of its intended use. Therefore, the procedure should not be construed as an exhaustive characterization of the NMR spectrum. Instead, it should clearly delineate which features need to be controlled and the associated acceptance criteria to ensure a specific, yet overall streamlined, identification testing process.

C. Examples

Example 1: ibuprofen

To illustrate the general approach discussed in the previous section, ibuprofen will be first considered as a simple model example, using Fourier 80 to develop an NMR identification procedure. The model reference spectrum obtained, after selection of DMSO-d_6 as the solvent, is presented in Figure 3. Semi-automated processing was performed to identify the associated features using Mnova software. Results are reported in Table 3.

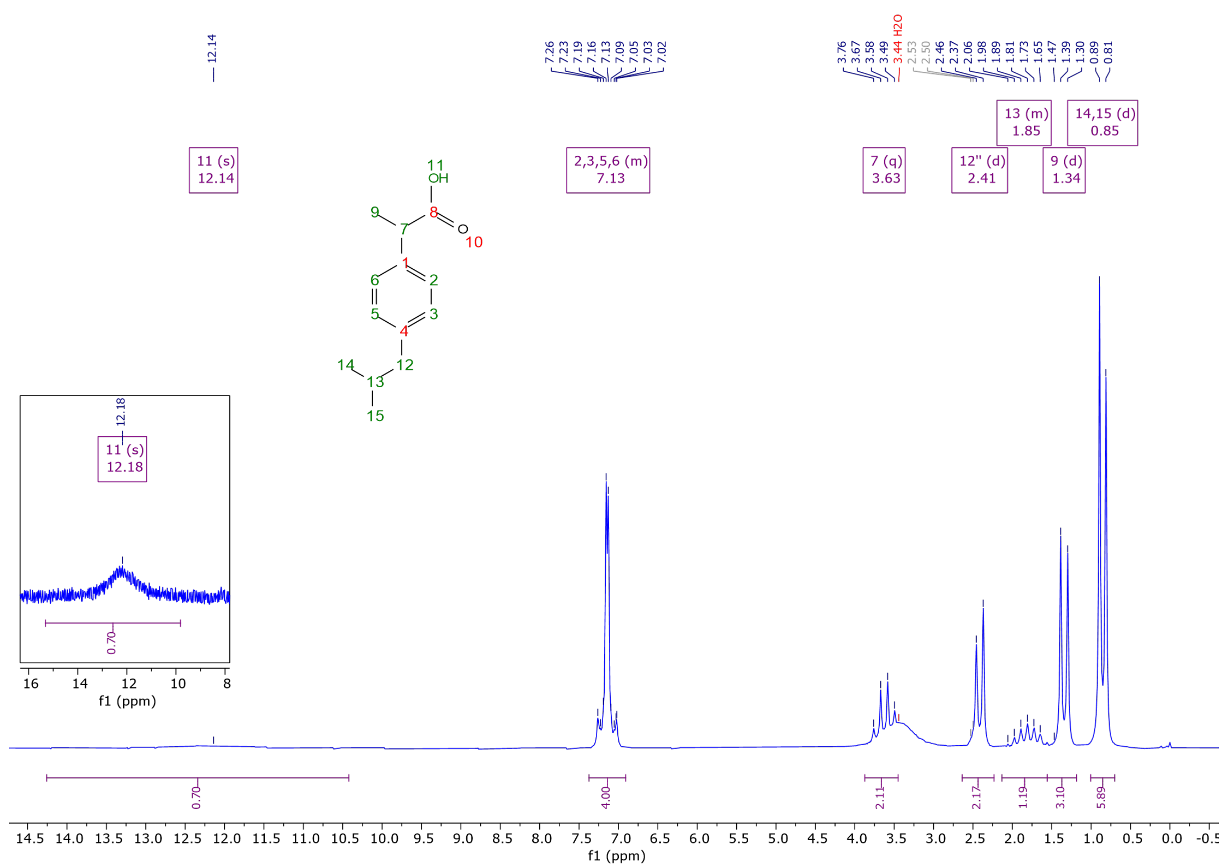

Figure 3: ^{1}H NMR spectrum of ibuprofen in DMSO-d_6 recorded on a Fourier 80. Purple boxes report the spectral features of the resonances of ibuprofen and their attributions are reported in Table 3.

Signal	Attibution	Shift (ppm)	Range (ppm)	Integral	Multiplicity[a]	Coupling constant (Hz)
1	14,15	0.85	1.01-0.70	5.89	d	6.44
2	9	1.34	1.59-1.19	3.10	d	7.10
3	13	1.85	2.13-1.56	1.19	m	/
4	12	2.41	2.64-2.23	2.17	d	6.98
5	7	3.63	3.87-3.45	2.11	q	7.09
6	2,3,5,6	7.13	7.37-6.91	4.00	m	/
7	11	12.14	14.26-10.42	0.70	s	/

a) d: doublet, q: quartet, s: singlet, m: multiplet.

Table 3: Experimental NMR features of Ibuprofen determined from the spectrum in Figure 3 as extracted after processing using Mnova.

From these experimental data, if one considers that the batch under investigation is a qualified reference standard, the definition of acceptance criteria for a positive identification could be, as an example:

Signal	Attibution	Shift (ppm)	Integral	Multiplicity[a]	Coupling constant (Hz)	Remark
1	14,15	0.9	6[b]	**d**	6.4	/
2	9	1.3	3	**d**	7.1	/
3	13	2.2-1.5	(1)	m	/	Integral for information only (possible interference from 1)
4	12	2.4	(2)	**d**	7.0	Integral for information only (possible interference from DMSO-d_5)
5	7	3.6	(1)	**q**	7.1	Integral for information only (possible interference from residual water)
6	2,3,5,6	7.4-6.9	4	m	/	/
(7)	11	(~12)	/	(ls)	/	Labil proton, to report for info if observed

a) d: doublet, q: quartet, s: singlet, m: multiplet. b) relative integration reference set to 6

Acceptance criteria: Resonances in this table must be detected at the reported chemical shift (for well-defined multiplicity in bold) or within the chemical shift range for non-resolved multiplet (m). Relative integration values, multiplicity and coupling constants must match the reported values, using resonance 1 as a relative integration reference set to 6. All values in parenthesis are not defined as criteria and are reported for information only.

Other expected resonances are DMSO-d_5 at 2.5 ppm (multiplet) and residual water at 3.3 ppm (large singlet).

Any other resonance detected with a relative integration above 0.5 should be reported as a potential contaminant and investigated.

Table 4: Example of defining acceptance criteria for ibuprofen ^{1}H NMR identification in DMSO-d_6 at 80 MHz

The acceptance criteria defined here are an example to illustrate how an identification method could be defined. In the present case, the following strategy was applied:

- Integration of resonances with potential interferences are not exploited.
- Labile protons are excluded from any criteria.
- Values used as criteria were judiciously rounded to provide the required tolerances, e.g. 0.05 ppm for chemical shift, 0.5 for integration and 0.05 Hz for coupling constant. These are typical tolerances that could be used but as previously mentioned, they must clearly be justified based on the risk analysis, robustness study and/or positive and negative tests.
- Expected interferences are listed and excluded.
- A limit is set to define an alert threshold in case unexpected resonances are detected. Again, this criterion should be justified. In the present case, the rationale was to apply the same tolerance as for the integration of the expected resonances (0.5) so an OOS would be raised in both cases (one must keep in mind that the goal of an identification tests is not the quantification of impurities).

Comparing the spectra in Figure 3 to Figures 1 and 2 clearly evidence that the spectroscopic features defined as criteria are more than enough to easily discriminate ibuprofen form other chemicals and these do not constitute challenging negative controls. Two more interesting features could be either the use of a wrong solvent to dissolve the substance (Figure 4, negative example A) or the presence of an unusual amount of water (Figure 5, negative example B).

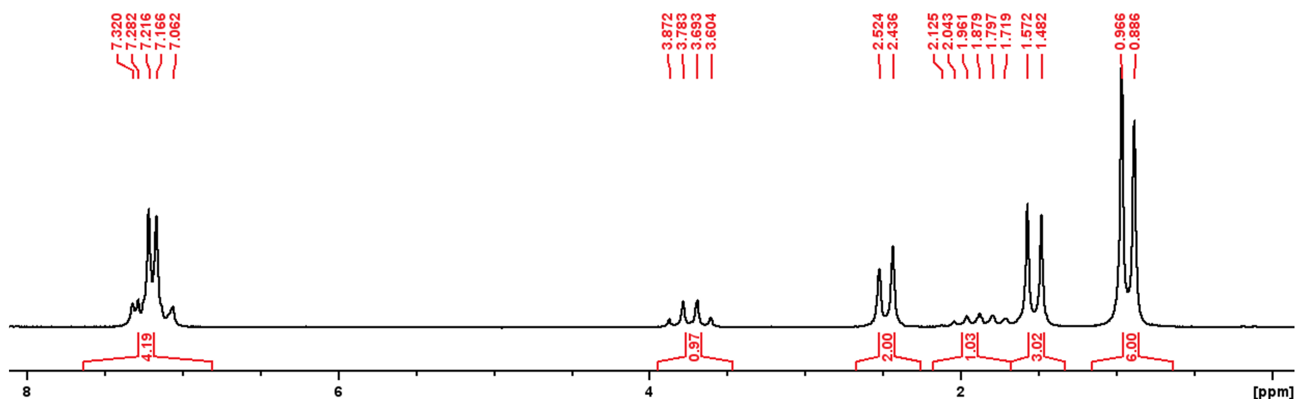

Figure 4: Negative example A - ^{1}H NMR spectrum of ibuprofen in $CDCl_3$ recorded on Fourier 80.

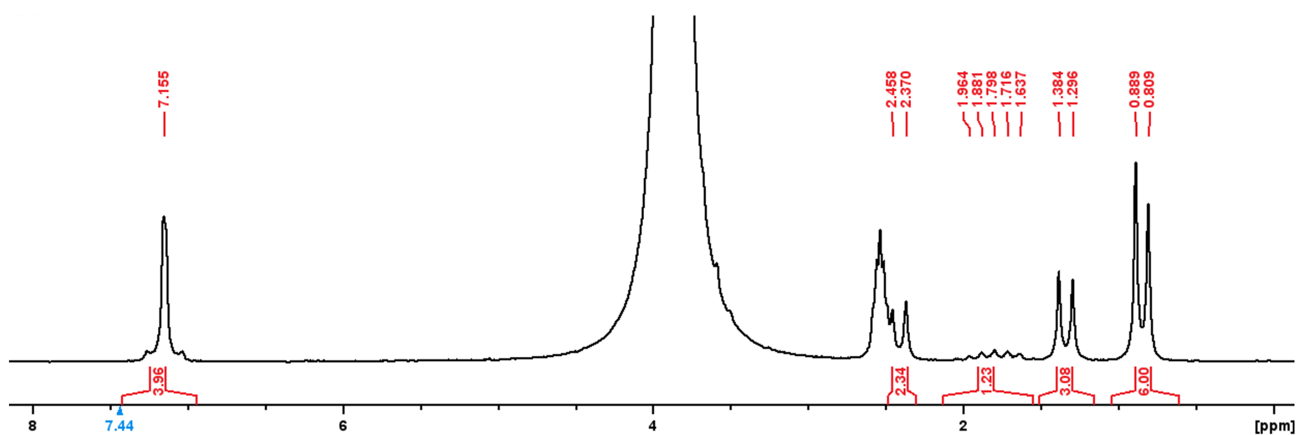

Figure 5: Negative example B - ^{1}H NMR spectrum of ibuprofen in DMSO-d_6 doped with water recorded on a Fourier 80.

In both cases, the identification according to the example acceptance criteria would fail. In the negative example A, all features related to the chemical structure itself (multiplicity, integration) are matching the criteria; however, using a different solvent (which should constitute a clear violation of the SOP) results in slightly different chemical shifts. For example, resonances 2 and 4 are now respectively at 1.5 and 2.5 ppm, falling outside of the defined acceptance criteria. This would correctly result in an OOS.

In negative example B, all detected features exactly match their associated criteria (chemical shifts are the same as the reference spectrum); however, resonance 5 is not detectable anymore due to the massive interference of water, resulting again in an OOS. This is by design: water content like this should probably be investigated as a possible contaminated solvent used during sample preparation or water uptake from the batch or aliquot. This negative example B also illustrates that resonance of the residual DMSO-d_5 could also interfere with resonance 4. If this was partially anticipated in Table 4, this interference could ultimately make multiplicity or coupling constant measurements impossible, resulting in an OOS. This may not be desirable so the corresponding SOP should specify both a minimum concentration and a minimal solvent quality to be used to avoid any false negative.

Example 2: brucine

A strategy comparable to the first example can be used on much more complex molecules like brucine. In a previous application note,[12] the use of a Fourier 80 for the structure verification of this structurally complex natural alkaloid was demonstrated. This work typically exemplified the required steps for reference standard qualification using NMR, allowing ab initio demonstration of the chemical nature of the substance. By leveraging the data acquired during this study, it is possible to define the acceptance criteria in Table 5 for the identification brucine based on its reference ^{1}H NMR spectrum (in $CDCl_3$, Figure 6) in a similar way to the previous example.

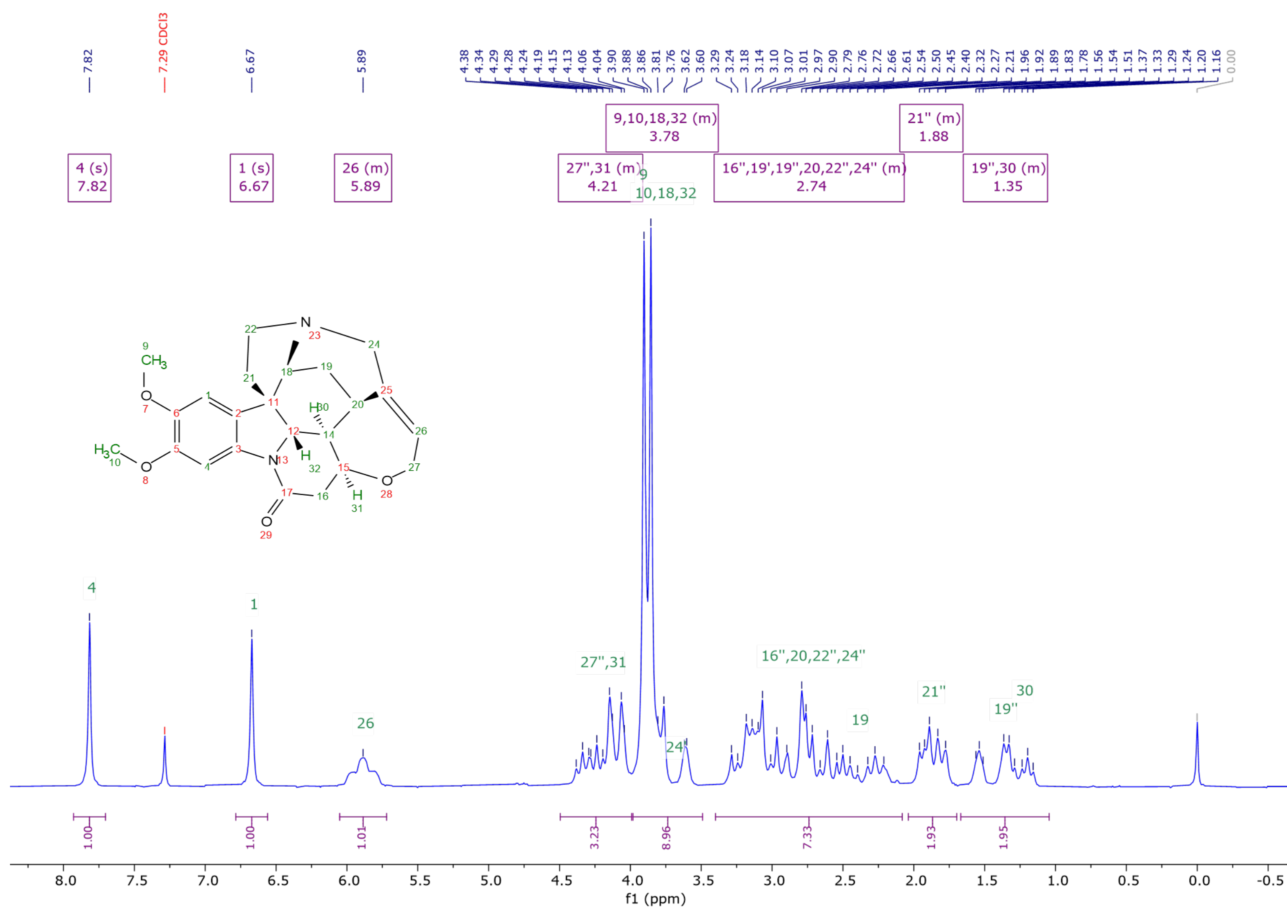

Figure 6: ^{1}H NMR spectrum of brucine in $CDCl_3$ recorded on a Fourier 80. Purple boxes report the spectral features of the resonances of brucine and their structural attribution (according to ref. 12).

[12] A. Beaton, Analysis of Brucine on Fourier 80 Benchtop Spectrometer Using ^{1}H and ^{13}C NMRs spectroscopy, **Dec 2023**

Signal	Attibution	Shift (ppm)	Integral	Multiplicity[a]	Additional Criteria
1	19', 30	1.65-1.05	/	m	Resolved pseudo large singlet at 1.54 ppm Possible interference from residual water, report integration value for information only
2	21	1.69-2.06	2	m	/
3	16,19',20,22,24	3.40-2.08	7	m	/
4	9,10,18,24',32	4.00-3.50	9	m	Must be dominated by two singlets at 3.90 and 3.85 ppm. Resolved pseudo large singlet at 3.61 ppm
5	27',31	4.50-4.00	3	m	/
6	26	6.05-5.70	1	m	/
7	1	6.67	1	s	/
8	4	7.82	1[b]	s	/

a) s: singlet, m: multiplet or mix of resonances. b) relative integration reference set to 1

Acceptance criteria: Resonances in this table must be detected at the reported chemical shift (for well-defined multiplicity in bold) or within the chemical shift range for non-resolved multiplet or mix of resonances with a 0.03 ppm tolerance. Relative integration values, multiplicity and coupling constants must match the reported values, using resonance 8 as relative integration reference set to 6.

Other expected resonances are $CHCl_3$ at 7.3 ppm (singlet) and TMS (chemical shift reference at 0.00 ppm, singlet) residual water will be overlapped with signal 1.

Any other resonance detected with a relative integration above 0.5 should be reported as a potential contaminant and investigated.

Table 5: Example of defining acceptance criteria for brucine ^{1}H NMR identification in DMSO-d_6 at 80 MHz

In this example, given the spectral complexity, it is not possible to clearly define multiplicities or list any coupling constants as criteria. A significant portion of the spectrum can only be divided in areas to determine the corresponding relative integration value. If this appears permissive, one must still consider that overall, 23 unique criteria are defined in Table 5 (8 areas, with 7 corresponding integration values, 2 specific multiplicities (singlet), and 4 additional criteria with specific descriptions for 2 of the areas). In addition, the tolerance for chemical shift is more rigorous than in the previous ibuprofen example (0.03 ppm instead of 0.05 ppm). This combination is thus actually very specific for an identification method[13] and finding another chemical fitting the criteria of Table 5 would be challenging. Furthermore, considering the steric constraints of brucine, even a slight structure modification would probably have drastic effect of the ^{1}H spectrum and yield an OOS (as it should). Nonetheless, for a validated procedure, this should be formally justified. For example, if the risk analysis indicates that a closely related structure may be present during manufacturing and/or supply chain, it should be tested as a negative control to ultimately demonstrate the specificity of the analytical procedure.

In this unlikely scenario, thanks to the versatility of NMR, several additional strategies could be used to meet the required specificity without having to change the analytical technique. For example, additional data could be acquired using two-dimensional NMR. Detailed explanation is out of the scope of this white paper, but using a ^{1}H-^{13}C HSQC experiment, Table 5 could be completed, in a very reasonable amount of acquisition time, with ^{13}C chemical shifts, adding at least another 16 unique features to the acceptance criteria list (and even more if multiplicity-edited HSQC is used instead). Other examples of alternative strategies include using a different solvent for dissolution or changing the temperature during acquisition. Each of these alternative or complementary solutions can easily be tested and implemented on the Fourier 80.

[13] For example, identification based on LC-UV may combine as little as 2 criteria: retention time and maximal UV absorption.

4 Conclusion

Seemingly trivial, identification testing can remain demanding due to the large number of tests that need to be performed by a quality control laboratory. Efficient and robust analytical procedures are thus highly desirable. NMR brings many benefits for such testing, with the unique feature that qualified reference material is needed only once during procedure development and validation. It can deliver very specific methods, with minimal development, which can be streamlined leveraging the modern concept of platform analytical procedure. With very short TAT, no conditioning, and full automation possible, NMR is a very potent candidate to replace the more traditional approaches for new identification testing procedures. With the benchtop Fourier 80 NMR spectrometer, all these benefits are now available in a virtually no maintenance system with a small footprint, as it was exemplified in this white paper. Supported by the extensive Bruker GxP readiness kit allowing streamlined compliance, the Fourier 80 is thus perfectly tailored for quality control laboratories where the highest level of quality must be combined with efficiency and robustness.

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