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NMR VALIDATION

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Overview of the Newsletter

On the Quantitative Nature of NMR
(by ValidNMR committee)

NMR has one feature that is not shared by any other form of spectroscopy, or chromatography – it is inherently quantitative. This means that the NMR resonance has an **intensity that is directly proportional to the number of nuclei** causing the resonance. Optical spectroscopies require a knowledge of the extinction coefficient or how strongly a functional group or a molecule absorbs light to make quantitative assessments of the composition of a given material. Chromatography requires calibration curves made from a series of solutions that vary in the concentration of the analyte to be measured to calibrate their detectors. In an NMR spectrum, **all nuclei of the same type will have resonance intensities that are proportional to their abundance.** This is true whether the nuclei are in the same molecule or in different molecules.

Some physical background

The intensity of an NMR signal depends entirely on the magnitude of the equilibrium magnetization M_0 that arises from placing the sample into a static magnetic field. In order to derive that magnitude for spin ½ nuclei one has to write down the Boltzmann factor for the two possible spin states:

$$M_0 \propto e^{\frac{E_\alpha - E_\beta}{kT}}$$

According to the "fundamental equation of NMR" the energy difference between the α and the β state is given by Planck's constant times the Larmor frequency of the nucleus:

$$\Delta E = E_\alpha - E_\beta = \hbar\omega_0$$

If we want to compare the equilibrium magnetization of one particular signal M_0 with that of second signal M'_0 we write down the ratio of the individual Boltzmann factors, which gives us an expression that depends on the frequency difference of the two NMR signals:

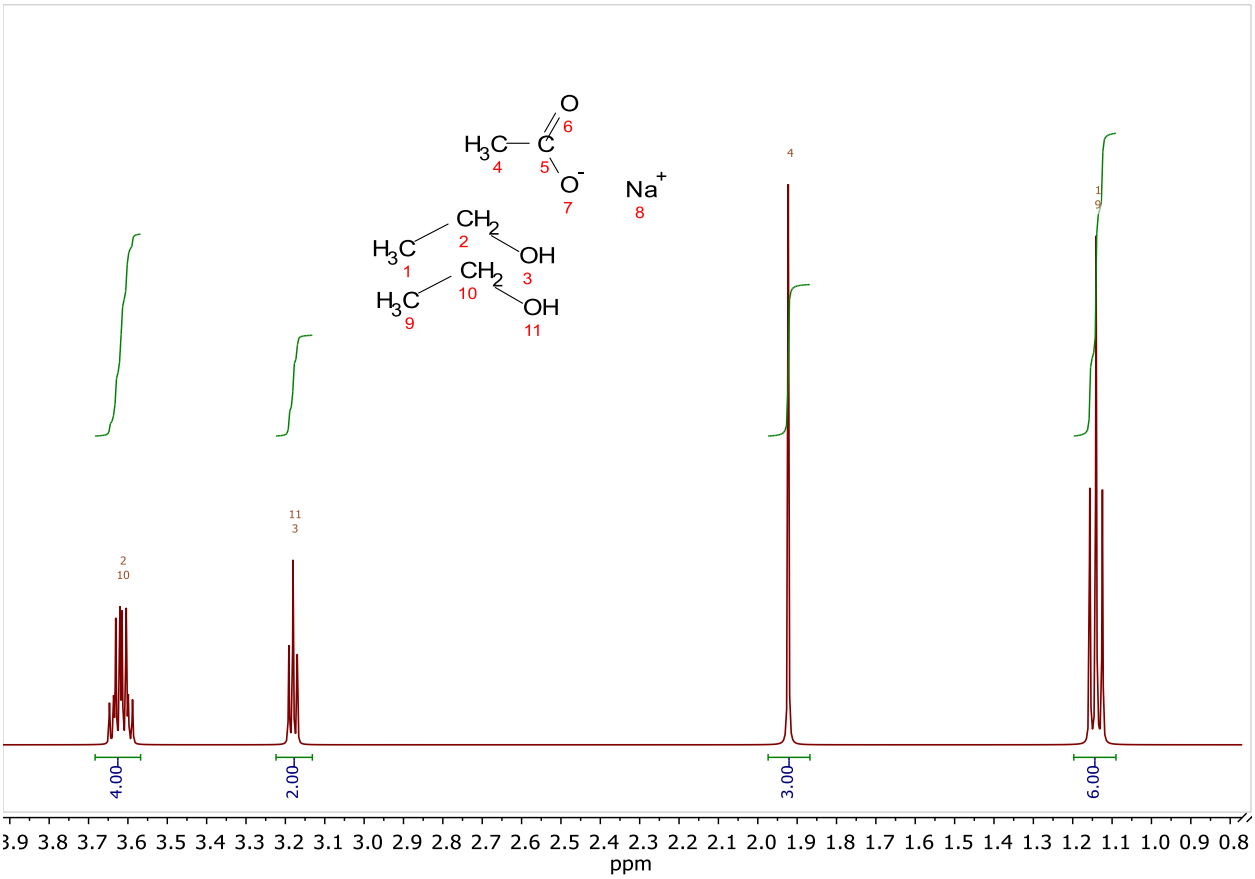
$$\frac{M_0}{M'_0} = \frac{e^{\frac{\hbar\omega_0}{kT}}}{e^{\frac{\hbar\omega'_0}{kT}}} = e^{\frac{\hbar\Delta\omega}{kT}}$$

Hence, the equilibrium magnetizations of two nuclei do actually differ depending on their resonance frequencies. But let's do some math to see how "bad" it really is…
We'll assume a worst case scenario and set $\Delta\omega$ to 500 kHz and the temperature to 298 K:

$$\frac{M_0}{M'_0} = e^{\frac{\hbar\Delta\omega}{kT}} = e^{\frac{6.62\cdot10^{-34}\,Js\cdot500kHz}{1.38\cdot10^{-23}\,JK^{-1}\cdot298K}} = 1.00000008$$

Hence, the "error" is less than 10^{-7} for our worst-case scenario, for typical NMR spectra it is even smaller – definitely enough to qualify NMR as a primary ratio method.

So, in a spectrum of ethanol, CH_3CH_2OH, there will be three resonances due to the three different types of hydrogens, and the intensities will be 3:2:1. In a 2:1 mixture of ethanol and sodium acetate, CH_3COONa, the relative intensities would be 6:4:2:3 where the acetate methyl group would have an intensity of 3.



Because of this quantitative feature of NMR, it is a simple matter to determine the relative amounts of substances in a mixture; their relative concentrations can be determined by a simple ratio of the intensities of their signals corrected for the number of identical nuclei causing the signal. So, in the ethanol/sodium acetate example given above one simply needs to normalize the intensities by dividing them by the number of hydrogens they represent. The acetate methyl peak has an intensity of 3 and three protons, so division by 3 gives 1 for the normalized intensity. The ethanol methyl, methylene, and hydroxy signals have intensities of 6, 4, and 2 which, when normalized, all give 2. Hence, the ethanol to sodium acetate ratio is 2:1. This simple example can be extended to much more complicated mixtures. In fact, **quantitative NMR allows for simultaneous quantitation of multiple mixture constituents based on one sole reference standard. More importantly, the standard doesn't have to share identity with any of the analytes of interest**. This key feature makes quantitative NMR an extremely versatile technique, and enables quantitation of chemical species that could be considered rare or in short supply.

A primary method of measurement is a method having the highest metrological qualities, whose operation can be completely described and understood. For a primary method, a complete uncertainty statement can be written down in terms of SI units. As such, quantitative NMR spectroscopy is a primary ratio method, measuring the value of a ratio of an unknown to a standard of the same quantity. Its operation can be completely described by a measurement equation.

In addition to **relative quantitation, absolute determinations**, such as the purity of substances, can be determined by weighing a standard of known purity into a solution containing the known weight of the analyte. NMR provides the most universally applicable form of **direct purity determination without need for reference materials of impurities or the calculation of response factors** but only exhibiting suitable NMR properties. Since both materials are in the same solvent, the relative molar concentrations can easily be determined. With the knowledge of the MW of both components, determination of the weight percent of the analyte can be made. That NMR-determined weight, and the known weight used to make the solution, are used to give the purity of the analyte.

The same determination can be made with separate solutions of the reference standard and the analyte, but in that case the concentrations of the two materials must be known, not just the weights. This so-called external reference method is usually slightly less accurate and precise because in addition to the errors from weighing, one encounters volume measurement errors.

To assess how quantitative NMR is, the largest error in the purity measurements can be shown to be sample weighing, even when 5 or 6-place balances are used.

Are calibration curves ever used in NMR? Yes, there are some rare, special applications when a calibration curve is needed. One example would be when you have an analyte of unknown MW, such as a polymer, that is appearing as a contaminant in a solution of another polymer. If a sample of the contaminating polymer is available, a calibration curve made from different weights of this material can be constructed that will give the weight-% of the contaminant.

The occasional use of calibration curves defines the difference between validation of linearity in the compound-specific method versus the validation of linearity of the instrument as part of the performance qualification. In chromatography, every method is compound-specific and requires calibration curves for validation of linearity. Because the NMR instrument is more akin to an analytical balance (for qNMR applications), the validation of linearity can in general be done as part of the instrument performance qualification (i.e., based on the acceptable target measurement uncertainty and its dependence on the Signal/Noise ratio). For the method itself, it should then be sufficient to verify that the S/N of the measured signals meet the PQ specifications (i.e., performing a standard ^{1}H sensitivity test as a system suitability test and determining the S/N of the relevant signals in the test sample).

Another question that is sometimes asked by people very familiar with chromatography, but less familiar with NMR, is whether the current measurement is contaminated by the prior measurement. This is easily possible with chromatography where samples are successively injected onto the same column. It is not possible in NMR since every sample is placed in its own NMR tube. Cross contamination could only occur during sample preparation and, if it did, this would be an example of poor laboratory practices.

In summary, NMR is an inherently quantitative technique that does not require calibration curves, except for special cases.

Who is the ValidNMR committee?

To broaden our horizons, we expanded our core team to include members from both
academic and industrial backgrounds. Your feedback is important to us!

Listed below is the NMR Validation core group, with the newest members highlighted in green.

Torsten Schönberger
Bundeskriminalamt

Michael Maiwald
Bundesanstalt für Materialforschung und -prüfung (BAM)

Kristie M. Adams
Steelyard Analytics, Inc.

Dan Sorensen
Eurofins Alphora

Claudia M. Boot
Colorado State University

José G. Napolitano
AbbVie

Joseph Ray
Baxter Healthcare

Christoph Freudenberger
Bruker BioSpin

Elina Zailer
Spectral Service AG & University Würzburg

Announcement of the next **Bruker Fellowship for Excellence in NMR Validation presented by PANIC**

Details: Will be presented soon on our homepage www.validnmr.com

Term of appointment: Autumn 2018

Is the NMReDATA proposal suitable for qNMR?

(by Mike Bernstein, Mestrelab)

It is fair to say that the qNMR community has quite a good understanding of the requirements for a successful purity analysis, but is constantly grappling with issues surrounding matters such as accreditation, compliance, standardisation, data archival, and metadata standards. At the recent qNMR summit in Japan, a draft document to achieve an ISO standard procedure was discussed at great length. The PANIC qNMR Workshop in 2017 identified the areas requiring clear guidance or standards for qNMR to be more widely adopted. Guidance documents like the recently-published MHRA "Guidance on GxP data integrity" [1] is, for example, very useful in providing definitions and guidance on all key issues relating to analytical data. But there is still some distance to wide-spread adoption of qNMR because of the missing standard procedures.

The NMReDATA detailed proposal has been published [2] and is supported by a Wiki [3]. The purpose of the proposed file standard is to "generate, store and share the data extracted from set of NMR spectra". The focus is on NMR data and spectrum-to-atom assignments.
Fundamentally, we would like to save all the information that relates to a qNMR analysis so that a reviewer can easily see how the result was obtained, and potentially repeat the analysis to verify the stated result.

Can this new record proposal meet the requirements of GxP for qNMR, or maybe come close to doing so?

The "NMR record"

The interested reader should carefully read the available literature that supports the NMReDATA standard (see, above).

The NMReDATA "NMR record" is a compressed SDF file – a well-established chemical data standard. The SDF file is popular because its makeup is well understood, it describes 1 or more molecules in a standard way ("MOL"), and it allows any additional tags to be part of the associated data it can hold.

There are good, free tools for manipulating chemical structure files, and the popular OpenBabel is recommended. Other supporting capabilities are described: ALATIS [4] is used for consistent molecule atom numbering via the InChI string.

The structure(s), and metadata tags have been specified that address some acquisition, processing, and analysis properties. The DOI is required, as the proposal attempts to meet "Open Data" standards. Importantly the raw, experimental data are also included.

<u>The compound record</u>

Each compound has its own record, comprising:

- Molecular structure
- NMR extracted data
    - Property tags that describe the sample (solvent, temperature, concentration)
    - Compilations of chemical shift and scalar coupling information (assignments, J-data)
    - Descriptions of 1D and 2D spectra, including peak and multiplet positions, and integrals

This results in a lot of supported tags!

<u>Of special interest to a qNMR analysis</u>

First, it is useful that data for >1 compound can be specified. This approach could, therefore, accommodate the popular "internal concentration standard" type qNMR experiment, where one would describe the concentration reference compound, and another the analyte. We can focus on these available tags as being useful for qNMR.

Property tags relating to the sample are:

- Concentration (mM)
- Solvent
- Temperature
- pH

The NMR experiment

It is expected that other data are extracted from the primary NMR data, but these can be explicitly stated:

- pointer to the original spectrum, with FID, acquisition, and processing parameters. The crude files produced by the manufacturer are specified.
- spectrum type (1D 1H, etc.)
- pulse programme
- decoupling status
- HTML link to where the NMReDATA file can be downloaded

Extracted analysis

The most useful tag in this category is "NMREDATA_1D_1H", as it specifies the critical information for quantitation: integral range(s), crude integral, and the normalisation of the integral to one proton.

Elaborating NMReDATA to make it suitable for qNMR

This section is my interpretation, and I welcome your thoughts.
I think that the NMReDATA record is an excellent starting point for a qNMR record which allows it to meet regulatory requirements. I can envisage it meeting all the needs for the range of qNMR experiments, and replicates and repeats.

I suggest that these additional tags would make the record complete for qNMR:

- qNMR experiment type
- Physical sample identifier
- Compound mass (mg)
- Purity (%)
- (Molecular mass is determined from the structure)
- Spectrometer constant (for when there is no standard)
- Electronic reference signal position and "concentration"
- NMREDATA_1D_1H • add the SNR (signal-to-noise ratio) for a range

In the same way that the current standard provides a framework for assignment (NMR_ASSIGNMENT), I suggest that a class of tags is made available to show how the purity was determined (multiplet ranges, crude integral, normalised area, SNR) – NMR_QNMR. The important acquisition and processing values have been specified often, but most recently by Monakhova and Diehl [5]. It should also be possible to specify the statistical analysis results – averages, SD, and %RSD. This should be possible within a single-, and across multiple measurements. The latter would accommodate repeats and replicates.

Because not all vendors' raw data formats are easily read to extract parameters, it could be argued that the most critical acquisition and processing parameters should also be explicitly tagged (e.g., repetition time). Indeed, this is also a limitation for most data processing software files, even when the file format is open.

Conclusions

The NMReDATA proposal is an excellent framework for any NMR data description and storage. It starts to address the needs for "Open Data" and broad regulatory requirements, although further security measures and data security are out of scope. For the purposes of this discussion, I suggest that a small number of additional and optional tags should be used especially for qNMR experiments, and that this would be relatively easy to implement.
As with any standard, it would then be up to software vendors and individual labs to put this into practice and make it a de facto standard across the industry.

[1]https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/687246/MHRA_GxP_data_integrity_guide_March_edited_Final.pdf
[2] https://doi.org/10.1002/mrc.4737
[3] http://nmredata.org/wiki/Main_Page
[4] http://alatis.nmrfam.wisc.edu/
[5] Monakhova, Y. B.; Diehl, B. W. K. Magn. Reson. Chem. 2017, 55 (11), 996–1005

Follow-up action after qNMR Summit 2018 in Tokyo, Jan. 29 & 30

(by Takako Suematsu, JEOL RESONANCE Inc.)

The international standardization of qNMR methodologies has been selected as one of the projects, "Initiative to Promote Strategic International Standardization", supported by the Ministry of Economy, Trade and Industry in Japan since 2017. This project will continue to achieve the goal for three years.

The qNMR has already been officially adopted to Japan's Specifications and Standards for Food Additives (JSSFA) and Japanese Pharmacopoeia (JP). In addition, the general rules for qNMR were issued on January 22, 2018 in Japanese Industrial Standards (JIS). At the same time, our efforts to standardize qNMR methodologies internationally are already underway to attain the international recognition of the International Standards Organization (ISO).
A draft proposal to ISO was reviewed at the qNMR Summit 2018 in Tokyo by the professionals from all over the world. After the summit, one of the working groups for ISO in the qNMR Japanese Committee refined it. The document is now in final stage the preparation to be submitted to the ISO technical committee. Meanwhile, an international collaborative study started in June and it will be completed at the end of September. The activities will be updated at the qNMR Summit in Würzburg in October.

qNMR SUMMIT 2018

Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

We invite you!
Be part of the next qNMR summit in Würzburg (10-11 Oct 2018) and register here:
https://www.uni-wuerzburg.de/index.php?id=203750

First Day (Wednesday), October 10th

9.00 – 11.00 qNMR-Workshop (MNova)
(Dr. Michael Bernstein, Mestrelab Research)

10.15 **Registration, Coffee break**

10.45 Welcome address (Prof. Dr. Ulrike Holzgrabe, University of Würzburg)

11.00 Dr. James Hook, University of New South Wales Sydney, Australia
"What is ClON NMR and How to Q it"

12.15 **Lunch break / Poster session**

13.30 **Workshop: Automatic qNMR**

13.30 – 14.00 Dr. Rainer Kerssebaum (Bruker BioSpin)
14.00 – 14.30 Dr. Takako Suematsu (JEOL)
Dr. Michael Bernstein (Mestrelab Research)
14. 30 –15.00 Common discussion
15.00 – 15.30 Dr. Rainer Kerssebaum (Bruker BioSpin)
"GMP in qNMR"
15.30 – 16.00 Dr. Takako Suematsu (JEOL)
"ISO Standardization of qNMR"
16.00 – 16.30 Dr. Matthias Weber (EDQM)
"The use of qNMR for the establishment of Ph. Eur. reference standards"
16.30 – 17.00 Dr. Matthias Abele (Evonik Resource Efficiency GmbH)
"Practical aspects of introducing automated qNMR for product release"
17.00 – 17.30 Dr. Christian Geletneky (Roche Diagnostics GmbH)
"qNMR in Diagnostics and Pharma, from Reference Methods to Market registration"
17.30 – 18.00 Dr. K. Fritsch, Mettler-Toledo
"Good weighing practice for accurate qNMR sample preparation"
18.00 – 18.30 Panel Discussion (all speakers)
19.30 Wine tasting at Juliusspital (Juliuspromenade 19, Würzburg)

<u>Second Day (Thursday), October 11th</u>
9.00 – 10.00 Dr. Yang Liu
United States Pharmacopeial Convention (USP)
10.00 Short talks
11.00 Coffee break
11.30 "Low-field NMR in qNMR"
Magritek, Nanalysis, Oxford, Bruker BioSpin
12.30 Keynote
Prof. Dr. Bernd Diehl, Spectral Service AG, Cologne
13.00 Lunch break and poster session
14.00 Open space discussion
15.00 Summaries of discussion
16.00 Concluding remarks and farewell

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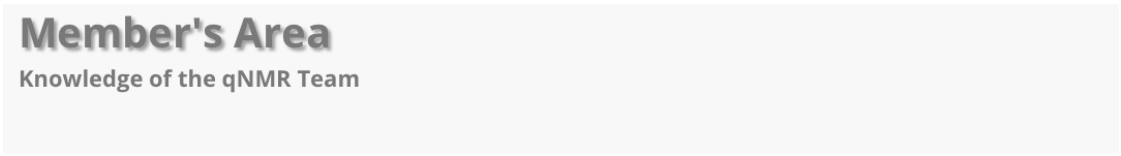
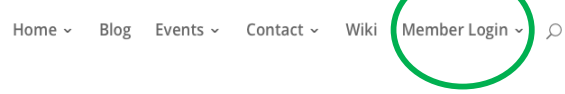
What is new?

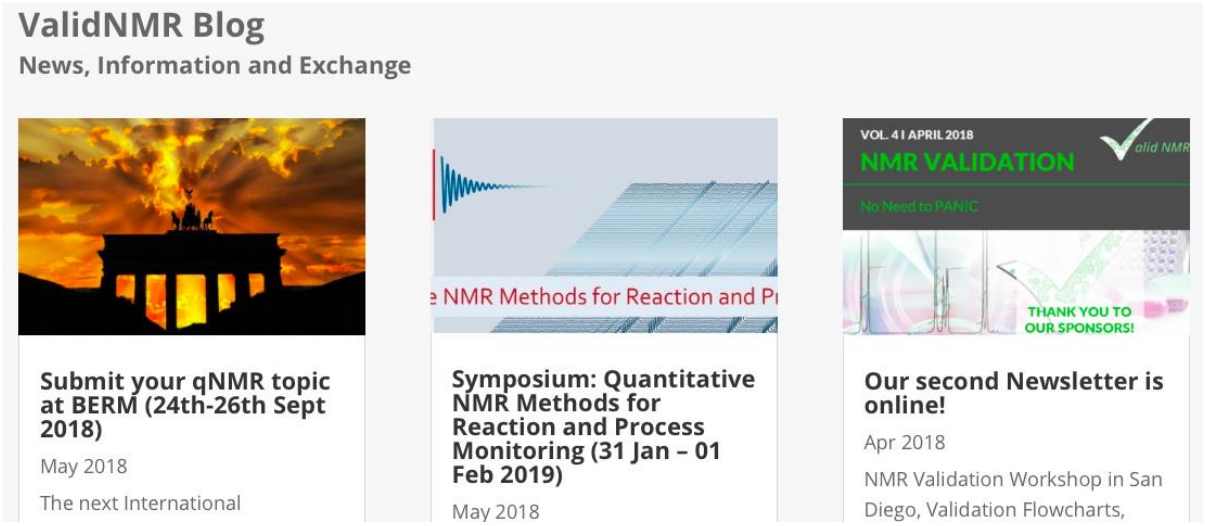


Welcome to your Member's Area! Here, you will be able to gain access to Member's only content such as presentations from meetings and conferences, documents concerning validating qNMR spectroscopy and tutorials for the wiki. On this page, the knowledge of the ValidNMR group is collected. In this context, we would like to thank all authors for providing their documents to give us the opportunity to share it with you. Hence, we ask you not to forward any documents with third parties!

How to get access to the Member's Area?

Contact **elina@validnmr.com** and receive your individual login data.





Want to be featured in the next ValidNMR newsletter?

The deadline for submissions and constributions to the next newsletter is September 15, 2018.

Please contact us at committee(at)validnmr.com!

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Practical Applications of NMR in Industry Conference PANIC