The qSummary

2019/2020

October 2nd and 3rd, 2019
United States Pharmacopeial Convention, Rockville, MD

qNMR Summit | General Guidelines

- **A qNMR Summit is an international event focusing on Science and application of quantitative NMR (qNMR) and striving for the highest level of academic and professional conduct.**
- **A qNMR Summit is an event that welcomes and is respectful of all participants, regardless of race, gender, gender identity and expression, age, sexual orientation, disability, physical appearance, national origin, ethnicity, or religion.**
- **A qNMR Summit fosters the spirit of curiosity, friendliness, open-mindedness, and respect.**
- **A qNMR Summit is strictly vendor-neutral.**
- **A qNMR Summit requires the participants to obtain the permission from speakers and organizers before sharing any content including photos or recordings on social media.**
- **A qNMR Summit thrives on original content; invited speakers are expected to neither recycle the material already delivered elsewhere nor reuse the Summit presentations without making an explicit disclosure of their provenance.**

qNMR Summits | History

qNMR Summit	Date	Host	Location
1.0	Oct, 2016	USP & CENAPT	Rockville, USA
2.0	Mar, 2017	BAM	Berlin, Germany
qNMR Day	Nov, 2017	GIDRM	Bari, Italy
3.0	Jan, 2018	NIHS/JEOL/Wako	Tokyo, Japan
4.0	Oct, 2018	U of Würzburg	Würzburg, Germany
5.0	Oct, 2019	USP & CENAPT	Rockville, USA

qNMR Summit 5.0

October 2-3, 2019
USP Meetings Center, Rockville, MD USA

Speakers

1. Kristie Adams, *CEO, Steelyard Analytics*
2. Sitaram Bhavaraju, *NMR Team Lead, Reference Standard Laboratory, USP*
3. Charlotte Corbett, *Special Testing and Research Laboratory, DEA*
4. Bernd Diehl, *CEO, Spectral Service*
5. Christoph Freudenberger, *NMR Application Scientist, Bruker Biospin*
6. Klaus Fritsch, *Manager Compliance, Mettler-Toledo*
7. Gabriel Giancaspro, *VP Dietary Supplements & Herbal Medicines, USP*
8. Krish Krishnamurthy, *Applications Scientist, ChemPacker*
9. Pekka Laatikainen, *CEO, Spin Discoveries*
10. Cesar Lau-Cam, *Professor, St. John's University*
11. Michael Levy, *VP, Research & Innovation, USP*
12. Yang Liu, *Scientific Fellow, Research & Innovation, USP*
13. Gustavo Martos, *Staff Member, Organic Programme, BIPM*
14. Toru Miura, *Research Chemist, FUJIFILM Wako Pure Chemical Corporation*
15. Joo-Won Nam, *Assistant Professor, Yeungnam University*
16. Matthias Niemitz, *CEO, NMR Solutions*
17. Yuzo Nishizaki, *Researcher, Division of Food Additives, NIHS*
18. Markus Obkircher, *Head of Reference Materials R&D, Sigma-Aldrich*
19. Guido Pauli, *Professor, College of Pharmacy; Director, Center for Natural Products Research, UIC*
20. Rasika Phansalkar, *Research Chemist, Biogen*
21. Joseph Ray, *Researcher, University of Illinois at Chicago*
22. Tucker Rubino, *Lab Weighing Market Manager, Mettler-Toledo*
23. Takeshi Saito, *Chief of the Metrological Information, NMIJ*
24. Dan Sørensen, *Analytical Team Leader, Alphora Research Inc. (*currently with *Health Canada)*
25. Aaron Urbas, *Research Chemist, Organic Chemical Measurement Science Group, NIST*

Moderators

1. José Napolitano, *Senior Scientist II, Structural Chemistry Discovery Platform Technologies (DPT), AbbVie Inc.(* currently with *Genentech)*
2. Guido Pauli, *Professor, College of Pharmacy; Director, Center for Natural Products Research, UIC*

Organizers

1. Anton Bzhelyansky, *Senior Scientific Liaison, Dietary Supplements & Herbal Medicines, USP*
2. Yang Liu, *Scientific Fellow, Research & Innovation, USP*
3. Guido Pauli, *Professor, College of Pharmacy; Director, Center for Natural Products Research, UIC*

Agenda

DAY ONE: October 2nd, 2019

8:30 – 9:00 a.m.	**Welcome & Introductions**
8:30 – 8:40 a.m.	Welcome Renee Stake, *Manager, Meetings & Marketing, USP*
8:40 – 8:50 a.m.	**Welcome – Fifth International qNMR Summit** Gabriel Giancaspro, *VP Dietary Supplements & Herbal Medicines, USP*
8:50 – 9:00 a.m.	**Introduction: qNMR Advancements and Persisters** Guido Pauli, *Director, Center for Natural Products Research (CENAPT)*
9:00 – 10:30 p.m.	**Morning Session I qNMR Methodology**
9:00 – 9:30 a.m.	**CRAFT Applications of qNMR** Krish Krishnamurthy, *Applications Scientist, ChemPacker*
9:30 – 10:00 a.m.	**Solutions for Automated Data Integrity and Integration** Tucker Rubino, *Lab Weighing Market Manager, Mettler-Toledo*
10:00 – 10:30 a.m.	**External Standardization in qNMR** Yuzo Nishizaki, *Researcher, Division of Food Additives*, *NIHS*
11:00 – 12:00 p.m.	**Morning Session II qNMR Methodology**
11:00 – 11:15 a.m.	Discussion from Morning Session I
11:150 – 12:00 p.m.	**The Most Well-Known Secrets of Quantitation by NMR** Matthias Niemitz, *CEO, NMR Solutions* Guido Pauli, *Director, CENAPT, UIC*
1:00 – 2:30 p.m.	**Afternoon Session I qNMR Applications**
1:00 – 1:20 p.m.	**Applications of qNMR at USP: Challenges and Opportunities** Sitaram Bhavaraju, *NMR Team Lead, USP*
1:20 – 1:40 p.m.	**qNMR in the Analysis of Anti-Sense Oligonucleotides (ASOs)** Rasika Phansalkar, *Research Chemist, Biogen*
1:40 – 2:00 p.m.	**Peak Purity in qNMR: Towards Solving the Challenge of Chronically Impure Proanthocyanidins Signals** Joo-Won Nam, *Assistant Professor, Yeungnam University*

2:00 – 2:20 p.m.	**Public qNMR Resources: validNMR, NMR Wiki, qNMR.org** Kristie Adams, *CEO, Steelyard Analytics*
2:20 – 2:30 p.m.	Discussion
3:00 – 4:00 p.m.	**Afternoon Session II qNMR Applications**
3:00 – 3:20 p.m.	**qNMR Challenges and Limitations** Charlotte Corbett, *Special Testing and Research Laboratory, DEA*
3:20 – 3:40 p.m.	**Improving Comparability in Organic Chemical Measurement: qNMR at NIST** Aaron Urbas, *Organic Chemical Measurement Science Group, NIST*
3:40 – 4:00 p.m.	**Sodium NMR – an Exercise in Industrial Validation** Joseph Ray, *Researcher, University of Illinois at Chicago*
4:00 – 4:45 p.m.	**qNMR Pioneers Session**
4:00 – 4:15 p.m.	**The Pioneering qNMR Work of George M. Hanna at FDA 1984-2006** Guido Pauli, *Director, CENAPT, UIC*
4:15 – 4:25 p.m.	**Working with George** Cesar Lau-Cam, *Professor, St. John's University*
4:25 – 4:35 p.m.	**Establishing a qNMR CRO Business** Bernd Diehl, *CEO, Spectral Service* "NMR is always quantitative."
4:35 – 4:45 p.m.	**USP George Hanna qNMR Award Announcement** Michael Levy, *VP, Research & Innovation, USP*
4:45 – 5:00 p.m.	**Wrap-Up Discussion: Key Topics from Day 1**

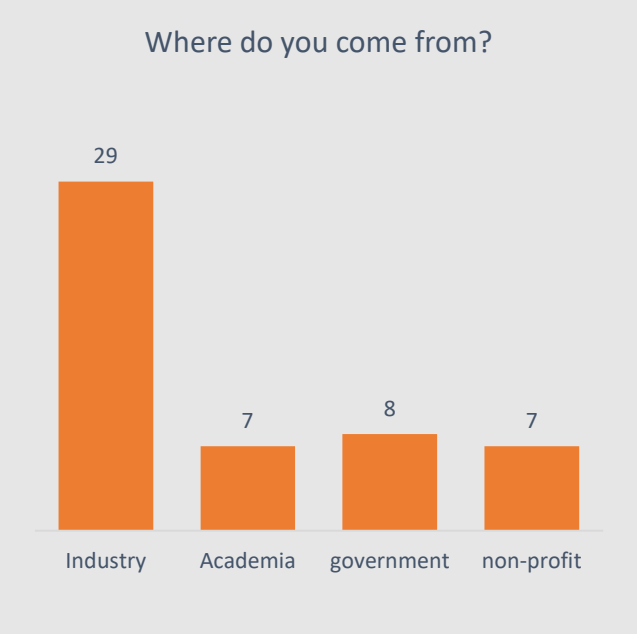
DAY TWO: October 3rd, 2019

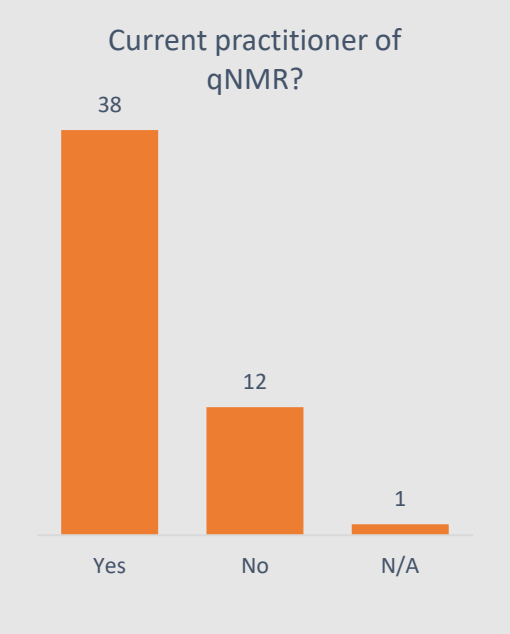
8:30 – 10:00 a.m.	**Morning Session I – Invited Talks**
8:30 – 8:50 a.m.	**The New Dimensions of Quantum Mechanical Spectral Analysis (QMSA)** Pekka Laatikainen, *Spin Discoveries*
08:50 – 09:25 a.m.	**BIPM: Advancements in qNMR Standardization** Gustavo Martos, *Organic Programme, BIPM*
09:25 – 10:00 a.m.	**Multinuclear NMR, Chirality, and Holistic Quality Analysis** Bernd Diehl, *CEO, Spectral Service*
10:30 – 12:00 p.m.	**Morning Session II – Selected Abstracts**

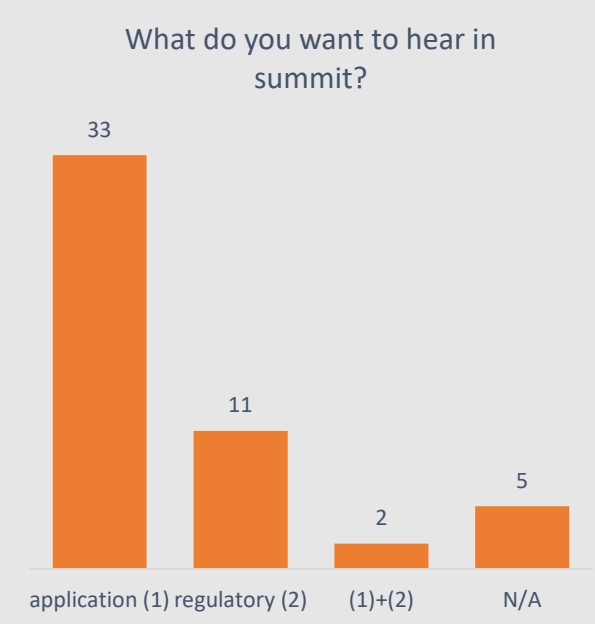
10:30 – 11:05 a.m.	**Proficiency Testing to Improve Your qNMR Laboratory Performance** Markus Obkircher, *Head of Reference Materials R&D, Sigma-Aldrich*
11:05 – 11:40 a.m.	**Good Weighing Practice for Accurate qNMR Sample Preparation** Klaus Fritsch, *Manager Compliance, Mettler-Toledo*
11:40 – 12:00 p.m.	**QbD principles to the Development of qNMR Methods** Christoph Freudenberger, *NMR Application Scientist, Bruker Biospin*
1:00 – 2:00 p.m.	**Afternoon Session I – qNMR and Regulations**
1:00 – 1:20 p.m.	**General Rules for Quantitative NMR spectroscopy (JIS K 0138:2018)** Toru Miura, *Research Chemist, FUJIFILM Wako Pure Chemical Corporation*
1:20 – 1:45 p.m.	**Progress in Proposal of an ISO Standard for Purity Assessment by qNMR** Takeshi Saito, *Chief of the Metrological Information, NMIJ*
1:45 – 2:00 p.m.	**USP General Chapters <761> and <1761> – Pertinent qNMR Topics** Yang Liu, *Scientific Fellow, Research & Innovation, USP*
2:30 – 4:45 p.m.	**Afternoon Session II – qNMR and the Future of Measurement**
2:30 – 3:00 p.m.	**qNMR in a Contract Development and Manufacturing Organization** Dan Sorensen, *Analytical Team Leader, Alphora Research Inc.*
3:00 – 5:00 p.m.	***Panel Discussion: qNMR 2019 Progress and Next Steps***

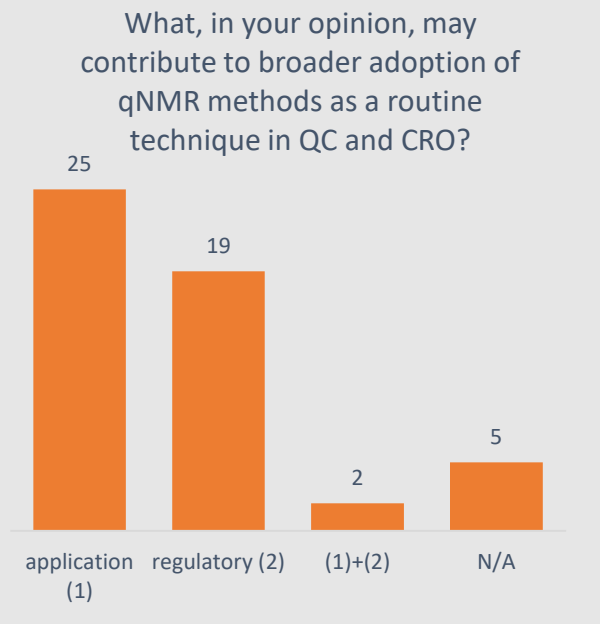
Survey

Survey Conducted at Registration





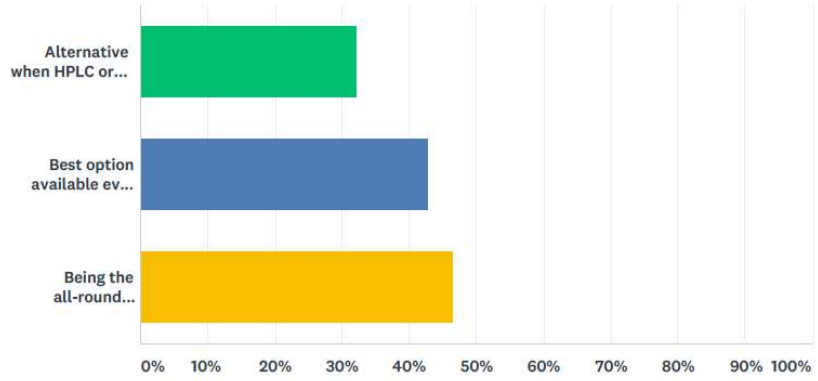




Survey on the 2nd Day

Q1 What is the nature of your current or anticipated qNMR use? (Select multiple options, if necessary)

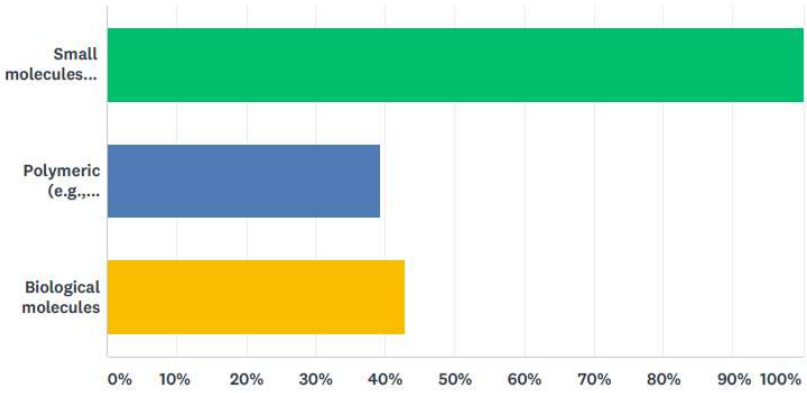
Answered: 28 Skipped: 0



ANSWER CHOICES	RESPONSES	
Alternative when HPLC or other technique is not feasible	32.14%	9
Best option available even though other viable technique exists	42.86%	12
Being the all-round preferred option for a wide range of targets	46.43%	13
Total Respondents: 28		

Q2 Types of molecular targets (Select multiple options, if necessary)

Answered: 28 Skipped: 0



ANSWER CHOICES	RESPONSES	
Small molecules (e.g., simple medicinal chemicals, natural products and food ingredients)	100.00%	28
Polymeric (e.g., excipients)	39.29%	11
Biological molecules	42.86%	12
Total Respondents: 28		

Synopses of the Presentations

Welcome – Fifth International qNMR Summit

Gabriel Giancaspro, *VP Dietary Supplements & Herbal Medicines, USP*

USP is committed to the qNMR community and is working towards the standardization of qNMR methodology through the development of documentary standards, reference standards and serving as a convener by originating and supporting the tradition of the qNMR Summit.

Introduction: qNMR Advancements and Persisters

Guido Pauli, *Director, Center for Natural Products Research (CENAPT), UIC*

The field of Quantitative NMR (qNMR) has grown significantly over the last 20 years; however, it is difficult to quantify this growth as a substantial volume of unpublished and/or proprietary qNMR data has been and is constantly being generated.

qNMR is used actively in many fields such as metrology, pharmacopoeias, industry, and academia. qNMR applications expand and challenge NMR instrumentation, thereby leading to the production of new acquisition and processing tools specifically for qNMR. A widespread belief renders qNMR less financially viable than other analytical techniques; however, when factoring in the cost of necessary progress and science, head-to-head cost comparison between instruments, and true costs per analytical problem solved, this belief may not be totally justified.

Depending on how data is acquired, NMR is simultaneously a qualitative and a quantitative tool. Generally, quantitative NMR (qNMR) comes" free" with the structural NMR data. Data sharing is critical for the development and growth of qNMR as a field and NMR in general, and there is substantial movement within the scientific community towards active sharing of raw NMR data.

CRAFT Applications of qNMR

Krish Krishnamurthy, *Applications Scientist, ChemPacker*

CRAFT is an unconventional data processing tool. Unlike the conventional tools that work in *frequency domain,* CRAFT processes the data in *time domain*. In the frequency domain, proper integration of the NMR signal is the key to valid qNMR results. The integral limits definition in turn depends on the linewidth of the resonance. Hence the measured integral of a signal depends significantly on its isolation from other signals (resolution) and its linewidth. Peak overlap is a common problem when quantifying by spectral matching, as different linewidths with different signals occur in the same spectrum. Modeling approaches in frequency domain are also constrained by a range of linewidths and (more often than not) sub-Lorentzian line shapes of different resonances. Moreover, phase and baseline corrections are unchallenged prerequisites for

accurate quantification in the frequency domain. Dr. Krishnamurthy showed a spectrum of serum sample wherein signals of formate, alanine and lactate overlap with very broad background arising from the protein resonances and pointed to the challenge of quantifying such signals by conventional frequency domain processes. It was explained by basic principles that, in the time domain (FID), the four parameters (frequency, amplitude, decay rate and phase) that describe an NMR resonance are orthogonal and variations in one does not impede the estimation of the other. Considering that phase is orthogonal to amplitude, phase correction (if any) is moot. Similarly, it was explained that the baseline issues in the spectra are no more than error in the first few points of the FID. CRAFT exploits this orthogonality of the NMR properties and uses a Bayesian probability approach to convert the FID to a table of frequency/amplitude/decay-rate/phase components. The applicability of this approach to complex spectra (as in the serum) was demonstrated. In summary, CRAFT redefines overlap, phase-correction and baseline-correction – the three banes in methodologies based on frequency-domain analysis.

Solutions for Automated Data Integrity and Integration

Tucker Rubino, *Lab Weighing Market Manager, Mettler-Toledo*

Why does balance practice influence on qNMR – To answer the question what is the reference point for quantitation? One cannot escape the relevance of proper balance weighing and data integrity. Improper data integrity can be very expensive, to both your bank account and your reputation. Both quality and integrity must be considered, that are contingent on the creation of correct data and the proper management of complete data.

What is ALCOA - ALCOA describes the pre-conditions for data integrity. This system of upholding integrity is broken down into the terms ***a***ttributable, ***l***egible, ***c***ontemporaneous, ***o***riginal and ***a***ccurate. Competition of data keeping hinges on the availability of the original metadata, for example, the sample ID, instrument, operator, and date and time.

Data management - Using technology, such as automated record-keeping balances, that facilitates proper data management, removing human error when possible, is a practical way of reducing the occurrence of error and improving the likelihood of being able to trace back the source of any problems. The ultimate goal of data management is to improve the quality and integrity of *all* data.

External Standardization in qNMR

Yuzo Nishizaki, *Researcher, Division of Food Additives*, *NIHS*

Among analytical methodologies for purity determination, quantitative ^{1}H NMR (qHNMR) is one of the promising candidate tools. When sample quantities are severely limited, and internal calibration is unsuitable for the sample preparation, qHNMR employing external calibration (EC-qHNMR), a non-destructive and non-contaminative approach, can be a powerful tool for purity assessment. We have applied the principle of reciprocity to the EC-qHNMR and aimed to evaluate EC-qHNMR feasibility using the validation parameters, such as accuracy and precision. The principle of reciprocity relates to two variables, i.e., signal intensity (or

probe quality factor Q) and value of the 90° pulse width (90PW). The signal intensity is inversely proportional to the values of the 90PW. In practice, efficient tuning-and-matching (T&M) and reliable 90PW measurement can enhance signal intensity and probe behavior, and, thus improve accuracy and precision of qHNMR determination. To verify the EC-qHNMR methodology, certified reference materials, caffeine and dimethyl sulfone (aka. DMSO2), were selected as analyte and external calibrant, respectively. Initially, an automatic determination of the 90PW calibration was developed. The result of the optimal nutation experiments was identical with the outcome that is derived from the precise measurement of 360° pulse width manually. Using the automatic 90PW calibration, EC-qHNMR was performed. Both measured values via automatic and manual T&M equaled to caffeine certified value within 1% bias. Although the T&M can be performed prior to and/or after shimming, the operation sequence appears to be absolutely critical: to achieve full equilibrium at the set temperature, sample solution requires extra time to reduce the temperature gradients of the airflow inside the probe. When an analyte and the external calibrant were dissolved in different deuterated solvents, bias errors increased to 2% or greater. From our perspective, different solvent thermal conductivities may cause a discrete increase between two solutions, and, thus introduce a bias in intensities of measured signals. So qHNMR result cannot be attenuated to a negligible level.

The Most Well-Known Secrets of Quantitation by NMR

Matthias Niemitz, *CEO, NMR Solutions*
Guido Pauli, *Professor; Director, CENAPT, UIC*

The first well-known secret of qNMR is that the purity of NMR signals/peaks is unknown. This is because signals may represent multiple resonances. Achieving confidence in the quantitative evidence of qNMR goes hand-in-hand with achieving an understanding of the complete NMR spectrum of the analytes, considering all observed signals/peaks. Being able to also understand the correlations between the resonances within a given spin system(s) further increases the confidence. This serves to improve the resulting statistics, especially in the presence of signal overlap and higher-order effects.

The second qNMR secret is that NMR itself is based on quantum mechanical theory. The NMR spectra can be fully calculated based on the chemical shifts (delta), and coupling constants (J), and line width values, which also determines the relative intensities of all observed signals and, hence, makes NMR intrinsically quantitative. A complete understanding of the quantum mechanical (QM) calculation is not required for calculating (q)NMR spectra - appropriate spin simulation software is available. The NMR spectrum can be interpreted and analyzed completely with the QM-based calculated spectra, both qualitatively and quantitatively. Hence the term ‘multiplet’ is not a necessary term anymore to describe very complex signal patterns.

The third secret is that NMR parameters such as chemical shifts (expressed in ppm) and coupling constants (expressed in Hz) are field-independent. Spectra of a particular sample measured under similar conditions give the same NMR parameters at any field strength. Thus, qNMR spectra of a given sample, measured at different field strength are based on the identical parameters (chemical shifts and coupling constants). This

means that even low-field benchtop NMR can be used to perform qNMR analysis where normal integration is not feasible. Collectively, this makes qNMR methodology portable between instruments. Moreover, its QM basis makes NMR spectra highly reproducible and (q)NMR libraries universal.

The fourth secret is that, if the separation of signals is insufficient, the integrity of integrals in qNMR becomes doubtful and requires careful re-consideration.

The fifth secret is that generic deconvolution techniques are usually NMR-unaware and, therefore, cannot take advantage of the correlations between peaks belonging to the same spin system. Accordingly, generic deconvolution in qNMR faces a high degree of freedom that can readily lead to ambiguous or problematic results.

Applications of qNMR at USP: Challenges and Opportunities

Sitaram Bhavaraju, *NMR Team Lead, USP*

The use of qNMR is primarily for purity assessment to support reference standards such as qualification of raw materials, research and method development, aimed at producing high-quality chemical and documentary compendial standards. Specific applications of qNMR include estimation of polymer chain lengths, quantitation of material purity, and assessment of counter ions in drug standards. A counter ion is typically used in synthetic drug preparations to better the physiochemical property of the active pharmaceutical ingredient (API) and is usually prepared in solid state. Both cations and anions can serve as counter ions to facilitate and maintain electrical neutrality of the accompanying API. Hence, they invariably have impact on the drug substance and drug formulation thereof. qNMR serves as a better tool to handle questions related to quantification of counter ions, the accompanying API, interestingly both measurements done in one shot. The conventional methods of analysis, such as chromatography and titrimetry, require customized method development and test procedures highly specific to drug product, and may not be easily available. qNMR readily fills the gap and the presentation showed examples of the assessment of (a) counter ion, (b) main compound, (c) formula weight, and (d) verification of stoichiometry [ratio of (a):(b)]. Knowing the exact formula weight and stoichiometry of a drug standard is highly useful, as it allows to set and monitor limits of product and process related impurities in drug manufacturing.

qNMR in the Analysis of Anti-Sense Oligonucleotides (ASOs)

Rasika Phansalkar, *Research Chemist, Biogen*

Speaker did not provide permission to distribute extended talk details

- Anti-sense oligonucleotides (ASOs) are investigated as new therapies for neurodegenerative disorders.
- Similar to peptides, ~ 20-mer ASOs are synthesized on a solid phase support in 3’ to 5’ direction with the base sequence complementary to the target mRNA

- Synthetic modifications, such as alkylation of the sugar moiety /base and replacement of phosphodiester (P=O) linkage with phosphorothionate linkage (P=S), improve potency, nuclease stability and reduce immunogenicity.
- The presence of a phosphorothionate linkage introduces a phosphorus chiral center. Several chiral phosphorus atoms can yield thousands of diastereomers leading to a high chemical complexity and therefore complex NMR spectra.
- A distinct group of NMR resonances belonging to the anomeric ribose protons are well resolved from other signals which can be leveraged for qHNMR giving the first opportunity in the complexity!
- In the event of spectral overlap from the impurities, the second opportunity in addressing complexity is ^{31}P qNMR.
- Purity in terms of total oligonucleotide content (includes shorter and longer impurities) can be determined using ^{31}P qNMR with either phosphonoacetic acid or triphenyl phosphate as an internal calibrant. Additionally, P=O to P=S linkages can be accurately determined using ^{31}P NMR

Peak Purity in qNMR: Towards Solving the Challenge of Chronically Impure Proanthocyanidins Signals

Joo-Won Nam, *Assistant Professor, Yeungnam University*

Work on Oligomeric Proanthocyanidins (OPACs) was showcased by explaining the nature of these substances in HPLC and NMR analyses. OPACs are flavanol derivatives found in numerous plants. In the USP monographs on grape seed extract and pycnogenol (pine bark extract) containing OPACs as major constituents, non-separated 'humps' of OPACs are observed in the HPLC chromatograms. Therefore, an alternative method is required to enable both quantitative and qualitative analysis of OPACs. To overcome the limitations of chemical analysis of OPACs using chromatography, NMR has been proposed as an alternative. However, there are many inherent obstacles (e.g. signal overlap, atropisomerism, near- identical spectra) in interpreting NMR OPAC spectra. Due to subtle differences in the structures of monomeric units and the interflavan linkage also matters in formation of different OPACs, near-identical spectra emerge. The signals in the ^{1}H NMR spectra of the OPACs were also very broad due to atropisomerism. Therefore, low temperature (-40^{o}C) has to be maintained to restrict rotation, such that sharp signals are obtained. The low-temperature ^{1}H NMR spectra indicated the presence of conformers. The in-depth analysis of the 1H NMR spectra of PACs revealed that hydrogens on the 6, 8 positions on their structures are prone to exchange with deuterium (on standing in methanol for few days; exchange rate of H-8 > H-6 in procyanidins). Hence, the resulting changes in spectra may lead to erroneous quantitation results. Also illustrated were the quantum mechanical calculations of some OPACs to explain virtual coupling effects and/or higher order spin systems resulting from close resonance frequencies of two strongly coupled protons and arising from mixtures of deuterated vs. non-deuterated species. Finally, it was suggested that the use of qCNMR might represent a better strategy for the quantification of these metabolites.

Public qNMR Resources: validNMR, NMR Wiki, qNMR.org

Kristie Adams, *CEO, Steelyard Analytics*

Initially, what qNMR represents was briefly reviewed. Then, a summary of available qNMR methodology and validation resources was presented. Eurolab, for example, has made some method and validation resources that are freely available to the public. Additional qNMR sources are nmrwiki.org and Open NMR Project, however despite some relevant information, they have not been updated recently with the exception of the QA platform. Some other qNMR resources and information were curated by Dr. Guido Pauli, which includes freely available spreadsheets that are ideal for new qNMR users, are found at qNMR.org, which is regularly updated.

qNMR Challenges and Limitations

Charlotte Corbett, *Special Testing and Research Laboratory, DEA*

Speaker did not provide permission to distribute extended talk details

- Why is any other method used for quantitation?
- Improvements are needed in acquisition and processing of mixtures.
- At least one clean signal region cannot always be achieved.
- Improved signal-to-noise is required for low-purity components.

Improving Comparability in Organic Chemical Measurement: qNMR at NIST

Aaron Urbas, *Organic Chemical Measurement Science Group, NIST*

Speaker did not provide permission to distribute extended talk details

- qNMR/NMR is powerful for both quantification and structure determination/verification and underpins many of NISTs SRM products in the organic chemistry space
- NIST PS1 Primary Standard for qNMR is a realization of SI Organic Chemical Measurement Units
- New Bayesian Approaches to Chemical Measurement Uncertainty are Being Developed (https://nist2.shinyapps.io/purity_app/)

Sodium NMR – an Exercise in Industrial Validation

Joseph Ray, *Researcher, University of Illinois at Chicago*

Sodium (q)NMR introduction: The rationale for developing the technique is the high volume, over a billion bags, of saline produced by Baxter each year. Dr. Ray had been spearheading an effort to bring in low-field NMR that could be used for quality control in plants.

Baxter has two sodium products – Saline and Renal solutions, the former is traditionally qualified by titration and the latter by flame photometry and both had issues with their quantification.

Details on sodium (q)NMR adoption - Key must-haves for the adoption of the technique include a total error less than 2.5% at a 95% confidence interval, compliance with 21 CFR Part 11, a run time less than 10 minutes

and matrix independence. Additional aspects that benefit from adoption include simple operation, low cost, easy sample preparation, inexpensive standards and run times less than 5 minutes. Three instruments (Bruker, ORS and NanoNord) available for this type of work were tested.

Compliance with pharmacopeial standards (USP <1210> and <1225> was required. eNOVAL software was used to determine the Analytical Target Profile and served as a framework for method adoption. One must consider the method, performance requirements and scope of a method that is to be validated, must demonstrate that the method is suitable, provide evidence that future results will remain within the confidence limits and provide substantiation of those claims.

The logic behind an easy, inexpensive and reliable analytical technique becomes even more appealing when considering some of the logistical constraints of the saline solution supply chain. It is very expensive to ship large volumes of liquid around the world. Thus, shipping concentrated solutions, requiring subsequent reconstitution, to remote areas becomes a favorable option, but requires a method to confirm the concentration after dilution, and qNMR can provide that.

The Pioneering qNMR Work of George M. Hanna at FDA 1984-2006

Guido Pauli, *Professor; Director, CENAPT, UIC*

Dr. George M. Hanna worked between 1984-2006 at the New York regional laboratory of the FDA. From his work, a total of 34 publications resulted on analysis of drugs by NMR during his tenure. In the early years (1984-1998), Hanna performed his NMR work using a Varian EM-390 90-MHz instrument. His first publication in *JAOAC* was on the analysis of formulations of dicyclomine by NMR using maleic acid as an internal standard. The overlapping triplet & quartet signals (associated with methylenes attached to nitrogen) were used for quantification. The values for commercial samples varied by <1% relative to the USP-recommended titrimetric method. Dr. Hanna's next publication was on the identification and quantification of diphenhydramine with isopropanol as internal standard, where he used the *N,N*-dimethyl group signal for quantification by NMR. Further, the degradation products were also identified and quantitated during this process, thus showcasing the advantage of qNMR as a tool for analysis. Around 1.5% of degradation products were identified in the drug. In 1988, carbachol in ophthalmic solution was studied, and Hanna's work provided its first reference to the explanation of complex methylene H multiplets (AA'XX'), while the trimethylamino group was chosen for quantification. In the same year, simultaneous determination of quinidine and dihydroquinidine in quinidine sulfate tablets was achieved by qNMR. The H-9 signal was selected for finding the ratio of unsaturated and saturated species. From 1989, he studied several chiral drugs (tranylcypromine, indacrinone, tramadol, carprofen, methylphenidate, timolol maleate, ibuprofen) by employing lanthanide shift reagents [[Eu(hfc)$_3$]] to determine the enantiomeric purity of racemic drugs. Dr. Hanna clearly established the orthogonality of NMR as an alternative to colorimetric, titrimetric, and IR spectroscopic methods. Various aspects during the early years of study was to quantify single APIs and mixtures, determine purity and stability, and enantiomeric purity. Later, from 1999 until his last publication in 2006, all the publications report that the analysis was performed at 400 MHz in response to ICH guidelines on impurities in new drug

substances. The high-field NMR work started with the analysis of 22 lots of trimethoprim from manufacturers in China, Israel, and the US. Two byproducts were isolated and identified. The total impurities were found to be between 0.1-2.1% by HPLC. However, quantitation of these impurities was not performed by qNMR. In 2000, NMR assessment of the enantiomers of prilocaine was performed. The *S* enantiomer was found to undergo slow hydrolysis, whereas the *R* counterpart rapidly hydrolyzed to toluidine, which is known to cause methemoglobinemia. Hanna publications continued to report on applications (enantiomeric purity, mixtures, stability/purity and impurity identification) of NMR, just as for his 90 MHz work. Dr. Hanna was a pioneer in qNMR. He recognized the quantitative ability of NMR - even at 90 MHz. He utilized multiple internal calibrants to solve the challenge of signal overlap. Further, in several publications, N-alkyl hydrogen signals were employed for quantitation (as a "hook" for qNMR analysis). The progress in utilizing the quantitative aspects of NMR has not kept pace considering the advancements in instrumentation. The early years of data produced by Dr. Hanna clearly demonstrates that qNMR is fully feasible on present day benchtop NMR spectrometers (mainly 40 to 80 MHz).

Working with George

Cesar Lau-Cam, *Professor St. John's University*

Prof. Cesar Lau-Cam shared his experience on qNMR work and some anecdotes about Dr. George Hanna when they worked at FDA.

Establishing a qNMR CRO Business

Bernd Diehl, *CEO, Spectral Service*

"NMR is always quantitative." Looking back on the founding of his company has afforded Bernd the opportunity to offer some advice. "Trust and believe in a method that never lies." The first contract was using qPNMR to measure lung surfactants. In 2009 when he acquired his first 600-mHz cryoprobe equipped NMR with assistance from Bruker, his turnaround time dramatically decreased and he was able to grow the business more rapidly. Early GLP certification was important but their GMP certification after 5 years was significant due to relevance for working with pharmaceutical companies. Bernd Diehl's venture is defined by his curiosity that led him out on a limb, trusting in the integrity of qNMR —"a method that never lies" — to initiate an NMR service that can be expanded into the future. All the while dreaming of the black boxes of science fiction, Bernd continues to lay the foundation for qNMR services with present-day applications.

USP George Hanna qNMR Award Announcement

Michael Levy, *VP Research & Innovation, USP*

Mr. Michael Levy explained USP's commitment to the investigation of the qNMR use for drug analysis since 2016. He related the contributions of Dr. George M. Hanna's work on NMR methodologies of various types of drug substances including enantiomer and diastereomeric impurities, botanicals, process impurities, and relevance of those methods in pharmacopoeial analysis. During our efforts to locate Dr. Hanna, we found that

he is no longer with us. However, we made a contact with Prof. Cesar Lau-Cam who was a co-author with Dr. Hanna in 21 out of 34 publications on drug analysis by qNMR. He acknowledged Dr. Lau-Cam for his contributions. Later, an announcement about USP's new commitment of honoring a person who practices qNMR with an award named after Dr. George Hanna, a qNMR pioneer in drug analysis, who was 30 years ahead of his time, once every two years. The first award will be presented at the next qNMR Summit in 2021 at USP headquarters, Rockville, USA. An award committee would be created, and candidate selection and other criteria notified. Nominations should be sent to Anton Bzhelyansky (anb@usp.org). Self-nominations will also be accepted. A prototype of the award was shown.

The New Dimensions of Quantum Mechanical Spectral Analysis (QMSA)

Pekka Laatikainen, *CEO, Spin Discoveries*

Spin systems of coupled protons spins are floating in a "bubble bath" of electrons and thus, the transition energies and relative line intensities obey the laws of quantum mechanics perfectly: even complex spectra can be modelled using coupling constants and chemical shifts, and into very details adding line-shapes and response factors to the model. Once analyzed at one field strength, the models can be used at any field strength.

The field-independent adaptive spectral libraries (FIASL) can be applied to analyses of complex mixtures by using targeted recipes, which contain prior knowledge about the abundance and spectral properties of certain type samples (example: Urine). Because QMSA is tolerant to chemical shift variations, these are some problems for very complex mixtures.

The holistic QMSA (hQMSA) means the principle that combining N analyses provides more information than N independent analyses. For example, when the correlations of chemical shift variations are obtained from spectra where they are well-defined, they can be used for those where they are defined poorly.

Computation time is not a bottleneck in QMSA: even a very large number of spin systems can be compressed so that, for example, calculation of the NMR spectrum of cholesterol or urine model of 1001 spin particles from 214 compounds takes only a couple of seconds or less in multithreading.

Only 112/214 metabolites had more than 90% purity signal and could be quantitated without considering signal overlap. QMSA takes signal overlap into account and allows quantitation of 180/214 metabolites within 20% confidence limits. Combining analyses of different type of spectra (like pure shift and dispersion line-shape) seems to make almost all the metabolites analyzable. The qQMSA is a metric method and if one accepts biases of a few per cent, arising from the measurement techniques (e.g., T2-editing), there is no need for validation and calibration of the method.

BIPM: Advancements in qNMR Standardization

Gustavo Martos, *Organic Programme, BIPM*

BIPM is the intergovernmental organization responsible for the implementation of the International System of Units (SI). A recent landmark was the redefinition in 2019 of the SI base units in terms of natural constants. The kilogram and the mole are now defined in terms of fixed values for the Planck and Avogadro constants, respectively.

qNMR is taking an increasingly important role in the activities of National Metrology Institutes (NMI) in organic analysis and recent comparisons of purity assignment between NMIs have demonstrated it can yield results consistent with those obtained by mass balance approaches.

qNMR is potentially a primary ratio method where the ratio of the response of an unknown to the response of a standard for the same quantity – in the case of ^{1}H qNMR, a standard for moles of ^{1}H atoms – allows the assignment of purity values potentially traceable to the SI.

BIPM, through collaboration with NMIJ/AIST, has validated a suite of standards which can address the full needs of a qNMR lab. Desired characteristics for each standard include stable solid of high purity (> 995 mg/g) assigned with small associated uncertainty (< 2 mg/g), ease of handling for gravimetry, non-volatile, non-hygroscopic, not subject to electrostatic effects, soluble (> 2 mg/g) and stable in desired solvent(s).

The main components contributing to the uncertainty associated with a qNMR result arise from the trueness and precision of the signal integral ratio, the uncertainty of the purity of the standard and uncertainties associated with gravimetric operations. The latter may become major contributors for systems under optimized measurement conditions where small sample sizes are used.

Guidelines for the use of each compound making up the calibrator suite are available for open-access download from the BIPM website. Each includes recommendations for performing qNMR, worked examples of their application and description of limitations associated with their use.

Multinuclear NMR, Chirality, and Holistic Quality Analysis

Bernd Diehl, *CEO, Spectral Service*

Glycerylphosphorylcholine (GPC) is not a drug, but it is widely used as a dietary supplement and other applications. Therefore, rigor in its determination is warranted. The analysis of GPC represents a challenge for classical chromatographic methods: it is not optically active as it contains no double bonds.

Quantification must be possible in very small amounts as presented in formulations. The stereochemistry of GPC is also very important as synthetic methods produce preferentially the D stereoisomer. Determination of GPC content can be conducted by phosphorus NMR. But the enantiomeric purity of GPC still represents a challenge.

GPC from natural sources, namely from vegetable or animal lecithin, always shows the natural stereochemistry. Two methods, including chemical / enzymatic treatment and derivatization with chiral reagents, discriminate the L and D forms in NMR spectra down to 0.1% detection limit.

Distinction of synthetic material is possible due to the natural asymmetric distribution of the ^{13}C isotopes in the glycerol or choline structural part. The ^{13}C NMR spectroscopy gives an accurate proof of origin using multivariate data evaluation. Internal standards for quantitation should follow SISSRS: Selectivity, Inertness, Solubility, Sufficient Resolution, Relaxed signals, Sensitivity.

Proficiency Testing to Improve Your qNMR Laboratory Performance

Markus Obkircher, *Head of Reference Materials R&D, Sigma-Aldrich*

The implementation of qNMR in new application fields brought along more complex molecules and systems that required additional classes of qNMR Certified Reference Materials (CRM), based on different NMR active nuclei, namely phosphorous and fluorine. With the rise of qNMR in the pharmaceutical industry and testing laboratories, there is a strong need for proficiency testing and inter-laboratory comparison studies in that field. Unfortunately, only a very limited number of providers is currently available.

Therefore, a need for a completely new qNMR proficiency testing scheme was set up using Millipore Sigma's in-house developed qNMR standards that did not only involve ^{1}HqNMR, but also ^{19}F and ^{31}P qNMR measurements. From the Millipore Sigma's existing qNMR portfolio of ^{1}H, ^{31}P and ^{19}F qNMR CRMs, that includes not only neat materials but also ready to use standards in solution, 4 substances were selected for the interlaboratory comparison study: trimethoxybenzene (TMXB) for ^{1}H qNMR, 2,4-dichlorobenzotrifluoride (DCBTF) for ^{19}F qNMR, triphenylphosphate (TPP) for ^{31}P qNMR, as well as 2-nitro-4-(trifluoromethyl) benzyldimethyl phosphate) (FHP) as a multi-nuclei substance.

The participants in the interlaboratory comparison were selectively invited and chosen from metrological institutes, pharmaceutical companies and testing labs in order to have representative subgroups for a detailed analysis. The statistical analysis was performed in collaboration with Quo Data, Germany, including a comparison of different evaluation methods such as mean values, Hampel means or uncertainty-weighted Hampel means. While the NMR equipment seemed to have only a minimal effect on the outcome of the measurement, it could be demonstrated that the use of a suitable balance was one of the main bias contributors. If the weighing step was performed on ultra-micro balances with at least five-digit readability much lower errors were obtained.

Amongst the participated groups, proficiency levels in the ^{1}H qNMR measurements was higher than in the experiments that involved ^{31}P and even more ^{19}F qNMR. In the latter case, parameters like spectral width and transmitter offset had a strong influence on precision and accuracy. For participating in similar qNMR proficiency testing rounds, the lab/person can register and order through the Millipore Sigma PT platform (supelco-pt.com) or email markus.obkircher@merckgroup.com. In this Proficiency Testing scheme, the participant determines the mass fraction of dimethyl sulfone (PT material: PE5000-100MG) by ^{1}H qNMR with maleic acid as internal standard in D_2O.

Good Weighing Practice for Accurate qNMR Sample Preparation

Klaus Fritsch, *Manager Compliance, Mettler-Toledo*

Weighing is very important for accurate qNMR results as it can contribute significantly to the measurement uncertainty in the qNMR process during sample and standard preparation. When used for quantitative analysis, USP sets clear requirements for weighing in its General Chapter <41>: “Balances”. It stipulates the use of a calibrated balance and defines repeatability and accuracy tests that the balance needs to accomplish, both with an acceptance criterion of 0.10 %. Calibration is the foundation of accurate weighing as it determines the measurement uncertainty of the instrument: a weighing device is accurate when it meets the user’s process and quality requirements, i.e. when its measurement uncertainty is smaller than the respective process tolerance. The absolute measurement uncertainty of the balance increases linearly with the load on the pan. The relative measurement uncertainty (i.e., the absolute measurement uncertainty divided by the load) increases as the sample mass decreases. The minimum weight is defined as the smallest net sample mass that needs to be weighed in order to achieve a relative measurement uncertainty smaller than the weighing process tolerance. In simple words, if the smallest net weight that the user wants to weigh (user requirement) is larger than the minimum weight (property of the balance), the relative measurement uncertainty of the weighing process meets the weighing tolerance requirement.

Calibration is usually carried out by an accredited calibration laboratory once a or twice a year and ensures, among other things, the traceability of weighing results to the SI. Routine tests are carried out by the user between calibrations and constitute of a reduced set of testing in order to verify that the instrument continuously performs according to the weighing process requirements. Automatic adjustment of the balance sensitivity by built-in weights improves the balance accuracy and allows for reducing user routine testing. The combination of calibration, routine testing and adjustment by built-in weights are the basis for any quality management system of weighing instruments.

Automation of weighing allows for the reduction of minimum weight due to the exclusion of environmental influences and mitigates handling errors that are difficult-to-impossible to assess. Accurate dosing of liquids can also be accomplished by gravimetric methods to obtain accurate concentrations, i.e. solutions are obtained with a concentration stated in mg/g instead of mg/mL The usage of gravimetric sample and standard preparation by automation of dosing for both the solid and the liquid components allow for an improvement of the qNMR accuracy.

QbD principles to the Development of qNMR Methods

Christoph Freudenberger, *NMR Application Scientist, Bruker Biospin*

Quality by Design (QbD) is defined as a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. In the context of analytical method development (aQbD) risk assessment is one of the key elements which includes a detailed study of the method workflow (e.g. Ishikawa’s diagram)

and FMEA analysis to define the analytical design space. From the FMEA analysis, critical method parameters can be identified and suitable test methods can be designed to test and control the said parameters.

As an example, we present a test for the performance of quantitative ^{1}H-NMR spectra acquired with inverse gated ^{13}C composite pulse decoupling (CPD). The test is using a mixture of tetrachloronitrobenzene (TCNB) and trimethoxybenzene (TMXB), which is referred as qPQ-CRM (Merck Millipore). Quantitative {^{13}C}^{1}H-NMR spectra have been acquired with systematic variation of the decoupler offset and power. Each spectrum was individually processed, and the analyte signals were integrated. By plotting the integral ratios against the decoupler offset, it was possible to identify an operational range for the decoupler offset of up to 158 ppm within which the method uncertainty is not increased. In addition, it has been shown that this operational range is robust with respect to the ^{13}C pulse power within ± 1.5dB.

The best performance was found by applying adiabatic bi-level decoupling based on the P5M4 super-cycle using a smooth-chirp pulse. By scaling the adiabatic RF bandwidth, the performance can be tailored to the requirements of the method with respect to RF power to avoid unnecessary sample heating. The method was tested with various probes and at different field strengths.

The resulting test can be performed in the context of operational and performance qualification of and enables the control of the parameters "^{13}C decoupler bandwidth" and "^{13}C decoupler pulse calibration". A similar test method for aQbD control of the 1H excitation bandwidth is in progress.

General Rules for Quantitative NMR spectroscopy (JIS K 0138:2018)

Toru Miura, *Research Chemist, FUJIFILM Wako Pure Chemical Corporation*

In 2005, the experts interested in qNMR voluntarily gathered to begin the cooperative research in order to allow qNMR to achieve metrological traceability. In 2009, the Japan Ministry of Economy, Trade and Industry supported the cooperative research on ^{1}H qNMR methodology. In 2011, the first stipulation based on 1H qNMR methodology was introduced into Japan's Specifications and Standards for Food Additives 8th Edition. It was adopted for the purity determination of fludioxonil analytical standard used for chromatographic analysis. In 2014, the Japanese Pharmacopoeia admitted ^{1}H qNMR methodology into General Information. Over the next few years up to the present, the assays using ^{1}H qNMR for analytical standard substances derived from the natural sources have been added. In 2018, the Japanese industrial Standard standardized ^{1}H qNMR: JIS K 8073, benzoic acid (reagent) individual standard, and JIS K 0138, general rules for quantitative nuclear magnetic resonance spectroscopy. JIS K 0138 includes terms and definitions, apparatus, and preparation for ^{1}H qNMR etc., and serves as a practical standard guide for NMR beginners. It describes the important techniques such as handling hydroscopic substances and setting key parameters for qNMR measurement to accurately quantify the values of analytes. ^{1}H NMR operating conditions recommended in JIS K 0138 are as follows:

Apparatus:	^{1}H resonance frequency of not less than 300 MHz
Target nucleus:	^{1}H
Digital resolution:	0.25 Hz or lower
Measuring spectrum range:	20 ppm or greater, including between −5 ppm and 15 ppm
Spinning:	off
Pulse angle:	90°
^{13}C decoupling:	on
Delay time:	at least 60 seconds
Scans:	8 or more
Dummy scans:	2 or more
Temperature:	temperature controlled between 20°C and 30°C

Inter-laboratory testing showed that the method falls within the uncertainty of the test substance with a certified value of 99.9% +/- 0.6%.

Progress in Proposal of an ISO Standard for Purity Assessment by qNMR

Takeshi Saito, *Chief of the Metrological Information, NMIJ*

Development of ISO standard for qNMR methodology for purity determination. The ISO/TC 34 (technical committee for food products) was chosen to propose this standardization as this TC has many standards that can improve the quality of the food materials. During the ISO/TC34 meeting at Washington DC in 2018, proposal to submit a new work item proposal (NWIP) to the TC was discussed. It was accepted and agreed to launch a ballot for the work item once the proposal draft became ready, TC34 also requested to create a liaison with ISO/REMCO (ISO committee on reference materials). In April 2019, the draft NWIP on "Quantitative nuclear magnetic resonance spectroscopy — Purity determination of organic compounds used for foods and food products — General requirements" was submitted and is currently under balloting in ISO/TC 34. When the ballot was approved, a new working group convened by Dr. Saito, JISC (Japanese Industrial Standards Committee, Japan), would be formed directly under TC34. For the information, the reference numbers of the proposal are ISO/TC34 N 2078 for the acceptance of NWIP submission, ISO/NP 24583 for the submitted draft, and the proposer is JISC. The original intention was to make the JIS (Japanese Industrial Standard) K0138:2018 to transfers and recognized as an ISO standard. The TC34 secretariat is at AFNOR (Association française de normalisation, France) and twin secretariat is at ABNT (Associação Brasileira de Normas Técnicas, Brazil). The intention of creation of ISO standard is to make internationally acceptable general guidelines for using qNMR with ^{1}H nucleus to determine the purity of foods and food products (including food related substances such as food additives, pesticides, natural toxins, and functional compounds). The contents of the documents and the proposed revised contents were shown during the meeting.

USP General Chapters <761> and <1761> – Pertinent qNMR Topics

Yang Liu, *Scientific Fellow, Research & Innovation, USP*

Initially, Dr. Yang Liu introduced the reason why qNMR is relevant to USP objectives. One of the main USP goals is to establish quality specifications, such as identify, strength, and purity of medicines, dietary supplements, and food. The qNMR spectrometry can assess all of these three quality specifications. This is beneficial for providing all quality control scientists with the established and accepted quantification methods. The ultimate intent is that the public has access to safe, quality medicines.

Historically, USP has consistently pushed the application of quantitative technologies, such as HPLC. Dr. Liu showed the chronology of HPLC applications between industries and USP. This demonstrates that it is necessary for USP to lead and guide qNMR applications and standard development. To move this forward, USP is going to update current NMR related general chapters <761> and <1761> shortly. This will be the first step toward fulfilling the needs of the industry.

At the end, Dr. Liu described the USP vision of qNMR applications, e.g., benchtop (q)NMR and qNMR digitization. This will reduce cost of qNMR operation and open the door for (q)NMR novices. To achieve this, some major challenges will have to be addressed, such as user friendliness and automation. In addition, public availability of raw data will play an important role in the future of qNMR's reach into laboratories around the world.

Audience comment (Lucy Botros): About standard-setting, all USP chapters are published in the Pharmacopeial Forum and are open for comment and revision before becoming official.

qNMR in a Contract Development and Manufacturing Organization

Dan Sørensen, *Analytical Team Leader, Alphora Research Inc.*

Speaker did not provide permission to distribute extended talk details

- Implementation of qNMR for development and manufacture of drug substances (API)
- Highlight of applications of qNMR throughout the drug development process
- The advantage of qNMR accuracy, power and speed
- Obstacles to the use of qNMR as a universal method
- Regulatory expectations of qualified instruments and procedures

Panel Discussion
qNMR 2019 Progress and Next Steps

Invited experts for panel discussion

- BD: Bernd Diehl
- CC: Charlotte Corbett
- DS: Dan Sørensen
- GFP: Guido Pauli (Moderator)
- GM: Gustavo Martos
- JN: Jose Napolitano
- KF: Klaus Fritsch
- MO: Markus Obkircher

Questions for qNMR Summit Panel Discussion

- THE NEAR-FUTURE
    - qNMR Validation: Who is going to write a globally acceptable document?
    - qNMR Economics: Is qNMR more costly than the 'gold standard' LC analysis?
    - qNMR as the Challenger: How soon will qNMR replace the 'gold standard' LC analysis?
    - qNMR for Everybody: Do we have what we need? Or are we waiting for the perfect benchtop instrument, or suitable software, or both?
- THE FUTURE OF THE FUTURE
    - Balance vs. qNMR: Is qNMR a relative primary or a primary method? What makes qNMR a RELATIVE primary method? Is this written in stone? Does qNMR validation require balances?
    - Is the Kibble balance an NMR spectrometer?
    - Who & what decides what the future role of qNMR is in chemical measurements?

Question #1: qNMR Validation: Who is going to write a globally acceptable document?

GFP (Moderator): Is this a universal requirement?

MO: There are several committees who are working on harmonization of global documents, among others – use and validation of qNMR. These requirements should be aligned and globally accepted and validation of qNMR measurements should apply to a range of standards for different uses.

MO: Critical attributes of qNMR standards and certified reference materials are:

- Extensive characterization of the materials including chromatographic purity
- Long-term and accelerated stability studies for estimation of expiry dates
- Homogeneity studies (between and within bottles) for a consistency of certified value over all units of a batch
- Traceability to SI units through primary calibration materials from National Metrology Institutes (NMIs)

GM: There are general validation requirements for CRMs, as those described in ISO 17034. The usefulness of the standard can be based, among other factors, on the region of the spectrum that is of interest.

GFP (Moderator): Is absolute purity needed? Is high purity required for a reference standard?

MO: Determination of absolute purity is important, otherwise testing results cannot be used quantitatively since they come with a potential bias. If the measurement is performed for an identification or more qualitative result, absolute purity is not necessary. High purity may not be a requirement for a reference standard, it is more important that determination of certified value is accurate. However, higher purities assure better commutability between different analytical methods – e.g., when the product is characterized by qNMR but with an intended use in chromatography.

GFP: Does this mean standards can be cheap as they do not need to be extremely pure?

JN: If the absolute purity of the standard is lower, it becomes important what else it consists of, i.e., what the impurities are. Peak overlap would become a problem, if the purity is low and/or if the composition influences stability.

GFP (Moderator): Does ISO 17034 specify whether or not the CRM has to be of high purity?

MO: ISO 17034 does not specify purity levels – it is more important that the certified reference material is fit for purpose. By using one of the qNMR standards of Merck's toolbox in-house reference materials can be created that fulfill the individual demands of the customers SOPs.

GFP (Moderator): How to validate the NMR spectrum? High purity is not just more convenient, but can help validate the method? Are we testing the validity of quantum mechanical theory or validity of NMR spectrometer and operator?

BD: Yes, it can be mainly an operator test. Deviation comes more from the user than from the instrument or the reference standard. If during measurement the deviation is lower, it could be due to the operator. Reference standards can also validate the operator and the instrument.

GFP: *High purity standards are multipurpose: they can also help validate the qNMR method.*

Comment from the audience: According to ICH Q2, you always have to validate parameters. It doesn't go away.

DS: You need to validate the parameters irrespective of the technique. Specificity is important.

KF: Traceability and measurement uncertainty are becoming increasingly important for validation, and it helps industrial applications to become more robust.

GFP (Moderator): What about stability, for example if we are looking at a terpene such as cymene?

MO: Yes, demonstrating stability is very important because it has an influence on the purity over time.
Question from the audience (Rasika Phansalkar): How is residual solvent quantitated?
MO: It is possible to quantify residual solvent in a substance similar to perform qNMR with another compound. For the production of certified reference materials, it is important to assess the potential purity changes (residual solvents or hygroscopicity) and include this in the uncertainty value.
Question from the audience: What is the difference between CRM and RM in this conversation?
DS: qNMR use for producing a certified reference material (CRM). A CRM is characterized by a metrologically traceable instrument with expression of uncertainty. But a reference material (RM) does not need to be SI traceable. We normally consider accuracy and precision, but the concept of trueness is being included in the evolving terminology.

GFP (Moderator): For words such as 'standard', 'IC', 'EC', 'reference': do we agree on their definitions?
DS: The best way to define them is to reference a document that gives the definition (e.g., ISO). The International vocabulary of metrology exists, with the definitions of RM, CRM etc.
MO: The naming of standards is not consistent. ISO defines reference materials and certified reference materials, USP General Chapter <716> uses internal reference standard and external reference standard. The definitions do not match entirely, but in either case confidence levels must be linked to uncertainties.
GFP (Moderator): As per the Supplemental Information data submitted to journals, the purity of natural products is around 80%. And some are of 50%. Who decides what is fit for what product?
DS: It depends on whether they have well-characterized impurities. As long as the substances are fully characterized, they may meet the requirements.
KF: It should be determined by the user what is fit for purpose. The user should decide the values of uncertainty which are acceptable for his process.
DS: It is hard to calculate and express uncertainty. Guidelines should be more practical.
GFP: Instead of setting target purity, just share your data so others can reproduce the value later.

Question #2: qNMR Economics: Is qNMR more costly than 'gold standard' LC analysis?

GFP (Moderator): Next point. Economics: Is qNMR more costly than LC?
DS: Since qNMR gives mass fraction directly, we should compare with not only the costs of LC, LC-MS but also other techniques like KF, ROI, *etc*. Method development and the time it requires should be considered in the "costs," and is particularly important for new molecules without existing techniques, because qNMR can be more rapidly developed for the new molecules for going from R&D to implementation.
BD: We do not have to separate R&D and GMP. The number of samples is critical to justify an NMR instrument. If you have enough samples, qNMR becomes cheaper. At Spectral Service, all our three high-field instruments are in GMP and R&D can be done on all of them. It would cost around $10-15 only if instrument and solvent costs are included but it increases when human resource cost is included. If we have a greater number of samples, the cost is further reduced.

GFP (Moderator): How does DEA look at the cost factor?

CC: NMR is mostly used for quantitation, apart from various other purposes, such as for structure elucidation and qualitative identification. For the DEA scope, it is key whether we can always get reportable quantitative results. The challenge is to get a reportable number from NMR analysis. Right now, we cannot always provide that. So, we use other techniques. This makes it difficult to estimate the cost of qNMR applications at DEA.

Question from the audience: Economics of a bad answer also has an impact. A substance with 20% nickel in early discovery lab showed a clean chromatogram. The counterion showed purity that was off by 30%. A qNMR would have saved a lot of time. LC method requires a lot of time for method development of a new substance. However, qNMR gives a rapid answer. If GMP qualified NMR instruments are available, the speed of analysis increases as we can use NMR prior to LC. Partition between departments is important in the bigger picture.

Question from the audience (Joe Ray): Yes, many of my experiences also show cases that could only have been solved by NMR.

Question #3: qNMR as the challenger: How soon will qNMR replace 'gold standard' LC analysis?

GFP (Moderator): How soon will qNMR replace 'gold standard' LC analysis?

JN: Not anytime soon. We need to get out of our comfort zone to educate people. Education will be important, including it in curricula. It is not a competition between HPLC and NMR; both techniques can work together. Once a standard document on qNMR is produced, then this will become equally important among analytical users. If we need to determine the purity of substance, NMR is the choice technique; but if we need to separate the 0.1% impurity, then LC is the choice. We will not see validation the same way as LC. But it is a matter of time. Resurgence of low-field is significant; we have to reach out to the rest of the world.

Comment from the audience (Takeshi Saito): qNMR does not have a well-documented standard method like LC does. This is a disadvantage. People were excited at the beginning of NMR.

Question from the audience: Why think about replacing LC? It is better to find best applications for NMR that are more business-fulfilling. No LC *vs.* NMR. The real matter is what are we trying to answer with NMR.

Comment from the audience (Joe Ray): Right, first commercial NMR was bought by an oil company (TEXACO) for qNMR. It has been there all along. New qNMR takes quantitation to a new level.

Question #4: qNMR for Everybody: Do we have what we need? Or are we waiting for the perfect benchtop instrument, or suitable software, or both?

GFP (Moderator): Should qNMR be Spectroscopy or Spectrometry?

DS: Spectrometry may be a more accurate name.

BD: I agree, it should be spectrometry.

DS: An (NMR) spectrometer can do both spectroscopy and spectrometry.

BD: NMR (spectrometry) is advanced science, LC (analytics) is a routine "workers" technique.

GFP (Moderator): Does benchtop help to improve this situation?

DS: Benchtop opens the door to teaching people. If people understand what it is, they would come to qNMR applications.

JN: Anecdote: benchtop can attract people's attention. I got an experience on testing solvents in front of people in minutes. People actively crowded around to see what was happening.

CC: But for benchtops, we don't have everything we need yet. Benchtops are good for pure materials, but mixtures are challenging.

GFP (Moderator): What about software? Is qNMR automation helpful?

CC: We don't quite have all we need, but we are trying to figure it out.

DS: We need data integrity, data integrity, data integrity. I can't say it enough. There is no leniency with computerized systems. Everything has to be traceable.

GFP: Moving data across platforms is still very difficult.

DS: One advantage of LC is that its data is easy to transfer and use.

GFP: Is there a solution for NMR?

DS: Bruker is putting some efforts on this, but not much beyond.

Comment from the audience (Yang Liu): Benchtop plus automatic digital analysis is the future of qNMR. This way, everyone can use NMR without understanding how it works (like we use iPhones). With the development of technology, at some point, everybody might be able to use NMR without being an expert in the field.

Comment from the audience: It is also our duty to talk to people in other conferences and convince people that qNMR is not that hard.

Question #5: Balance vs. qNMR: Is qNMR a relative primary or a primary method? What makes qNMR a RELATIVE primary method? Is this written in stone? Does qNMR validation require balances?

GFP (Moderator): Is qNMR a relative primary or a primary method? How does the balance influence qNMR?

GM: We saw how the uncertainty from weighing can be comparable to the qNMR measurement uncertainty in certain cases. The error of a balance is negligible if used properly. qNMR is a primary ratio method. The user obtains the ratio of an analyte mass fraction to an IC mass fraction, that is, a ratio of purities. Coulometry is a primary direct method.

BD: Influence on qNMR will be distinct, as ones uses of balances with different ranges. The uncertainty in weighing also comes from handling. That is why NMR works better: you do not put your hand inside the magnet.

KF: Uncertainty is a very complex topic. The human factor is a major source of error. Even calibrated equipment still has variability from handling errors. So, automation becomes a good way to minimize error. Another possible improvement can come from proper user training.

DS: Is qNMR-only an end-purpose? In pharma, if you would like to know mass of product, the balance is still a practical reference point so far.

KF: User training is the first line of data quality control. But usually user training is the first to go away when budgets are cut, unless there is regulatory pressure.

Question #6: Is the Kibble balance an NMR spectrometer?

GFP (Moderator): As the Kibble balance was used to measure Plank's constant, and thereby the kilogram now, is the Kibble balance actually a type of qNMR spectrometer?

KF: No. It uses a quantum mechanical property/effect, but it is just a balance.

BD: Given the example: how much is the weight of a grain of sugar? In principle, it cannot be measured accurately by a balance at it is a very small quantity, but it can be placed in an NMR tube with some D_2O and calculated by qNMR. The Kibble balance can also not measure a grain of sugar.

KF: First of all, the Kibble balance was not invented to weigh a grain of sugar but to realize the unit of mass independent of artifacts. And actually, there is another big difference between weighing and qNMR: The purpose of weighing is to use the sample after the weighing step. If you put the sample in solvent for a qNMR measurement, the sample itself can't be used anymore afterwards. What you intend to do with the sample also determines how you will weigh or process it.

DS: Fentanyl, as an example: its dosage is really low and hard to quantify, so balance or NMR?

KF: The selection of the right weighing instrument is also very important. As an example, in hospitals, sometimes really small quantities of a few mg are measured on precision balances instead of analytical balances. This is frightening as the accuracy of precision balances is insufficient to weigh substances in the mg range.

CC: With NMR, you can easily quantify substances in samples with one test. For fentanyl analogues, one new (and typically illegal) compound can be produced per week. NMR can identify each and actually does not destroy the sample.

GFP: Yes, NMR can tell the weight of practically unweighable samples.

Question #7: Who & what decides what the future role of qNMR is in chemical measurements?

GFP (Moderator): How about the role of qNMR in future? Who or which factors will decide it, specifically in analytical chemistry?

BD: One key factor is the demand of the institutions, including governments, industries, and academia.

MO: It is important to have access to decision-making people to promote qNMR and show possible commutability between NMR and LC. In the case of the pharmaceutical industry these would be the Quality Assurance Managers.

CC: There is an increasing demand for purity determination because now it is possible to do so. The qNMR method validation will be a key. Validation includes:

> Proof that something is correct.
>
> The act of producing official documents that are legally acceptable and/or approved.

The feeling that other people approve of and accept the method.

Question from the audience: Are ^{31}P qNMR and ^{1}H qNMR orthogonal measurements?

DS: It is a good question...

MO: I would consider them as orthogonal even though the methodology is the same. GC and LC are considered often as orthogonal as well.

BD: In principle, they are all the same. We chop up the spectrum according to nuclei.

GFP: From H to P can be considered orthogonal, C and H less so for organic compounds; 1D and 2D NMR even less orthogonal.

DS: It is primarily a regulatory definition and expectation.

JN: Orthogonal methods should be testing through different properties that are independent from each other.

Concluding Remarks

- There is a need for a compendium of widely accepted qNMR terms and definitions for both practitioners and regulators regarding standards, references, calibrants, etc.
- Also, a compendium of techniques that people in different fields can easily adapt for their own purposes with a guide for answering “How do you determine when qNMR is the appropriate technique?” is needed. This would ideally include qNMR education, perhaps as an introduction to NMR in general. This is, in part, because some of what deters people from embracing NMR early on in their career is the complexity of structure elucidation that often requires years to understand.
- There is a need to make it clear that anyone can get a good NMR spectrum and that qNMR analysis of complex mixtures can be considerably more informative than any single analytical method alone.

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