The qPackage

Guidebook for Participants

October 6 & 7, 2016
USP Headquarters, Rockville (MD)

USP U.S. Pharmacopeial Convention Global Expertise Trusted Standards Improved Health

version 20161004

CENAPT

Agenda

Day One

Thursday, October 6, 2016 – Spalding Auditorium

8:00 a.m.	Registration & Coffee	
8:30 a.m.	Administrative Matters	Renee Stake
	Welcome and Opening Remarks	
8:40 a.m.	Welcome; qNMR in Furthering the USP Mission	Dr. Jaap Venema
	qNMR Background & Perspectives (Moderator - Anton Bzhelyansky)	
8:50 a.m.	qNMR Research through the Decades	Dr. Guido Pauli
9:25 a.m.	qNMR in Complex Matrices	Dr. Bernd Diehl
10:00 a.m.	qNMR and Pharmacopeial Analysis	Dr. Gabriel Giancaspro
10:20 a.m.	*Morning break*	
	qNMR at USP (Moderator - Torsten Schönberger)	
10:50 a.m.	NMR in *USP-NF*: History, Chapters, Monographs	Dr. Edmond Biba
11:10 a.m.	USP Reference Standard Development Process	Dr. Shiow Wey
11:30 a.m.	qNMR in RS Characterization, a Case Study	Dr. Arunima Pola
11:45 a.m.	Overview of qNMR Analysis at USP	Dr. Sitaram Bhavaraju
12:05 p.m.	*Lunch*	
	qNMR at Large (Moderator - James Hook)	
1:15 p.m.	Implementation of qNMR in Japanese Pharmacopoeia	Dr. Yukihiro Goda
1:45 p.m.	qNMR through the Regulatory Lens	Dr. Kang Chen
2:15 p.m.	Low-Field qNMR: Traditional and Chemometric Approaches	Dr. John Edwards
2:45 p.m.	SS-qNMR: Practice and Promise	Dr. Eric Munson
3:15 p.m.	Social Mixer	
	Small Molecule NMR in Pharmacy	
4:30 p.m.	Keynote *Evolving NMR Capabilities – The Impact on What is Possible*	Dr. Gary Martin
5:15 p.m.	Day One Wrap-Up	Dr. Pauli / Dr. Giancaspro
5:30 p.m.	Adjourn	
6:00 p.m.	*Dinner*	

Agenda

Day Two

Friday, October 7, 2016 – Briggs & Parker, Marshall and Wiley Rooms

8:00 a.m.	*Registration & Coffee*	
8:30 a.m.	Welcome Remarks	Dr. Giancaspro / Dr. Pauli
8:45 a.m.	Administrative matters, Introductions, "Charge for the day"	all
9:00 a.m.	**Flash Presentations & Flash Discussion of qNMR Theory (T)**	assigned presenters, all
9:00 a.m.	qNMR Theory T1: Acquisition & Processing	all
9:20 a.m.	qNMR Theory T2: Metrology	all
9:40 a.m.	qNMR Theory T3: Challenges & Applications	all
10:00 a.m.	Further Discussion of Identified High-Priority Theory Topics	all
10:30 a.m.	*Morning break*	
11:00 a.m.	**Flash Presentations & Flash Discussion of qNMR Practice (P)**	assigned presenters, all
11:00 a.m.	qNMR Practice P1: Qualitative & Quantitative Definitions	all
11:20 a.m.	qNMR Practice P2: Measurement, Evaluation & Documentations	all
11:40 a.m.	qNMR Practice P3: Education & Outreach	all
12:00 a.m.	Further Discussion of Identified High-Priority Practice Topics	all
12:30 p.m.	*Lunch*	
1:30 p.m.	**Workgroup I: Theory-to-Practice Perspective**	all
	Workgroup II: Practice-to-Application Perspective	all
3:15 p.m.	*Afternoon break*	
3:45 p.m.	Presentation of Workgroup Summaries	all
4:45 p.m.	Discussion	all
5:15 p.m.	Day Two Wrap-Up	Dr. Pauli / Dr. Giancaspro
5:30 p.m.	Adjourn	

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About

The qPackage was prepared by:

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Confidentiality Statement

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Licensing Information

1. How to Prepare Yourself for the Discussions on Day 2?

Get into the q before you arrive!

Participants are kindly requested to prepare themselves as follows:

- **STEP 1 – READ.**
  Familiarize yourself with the entire q Package

  - The materials give you a jump start as they already reflect input from all participants received via the qNMR Left Hemisphere Challenge
  - For each section of the program, discussions will specifically address certain **Key Progress Topics** and overall revolve around the **Big qNMR Questions**; see **2. Overview of Morning Workshops and Afternoon Workgroups** (p. 7)
    - **Section 4. Key Topics & Issues for Progress:** Participant Contributions Grouped for Flash Presentations & Discussions (p. 15 ff) contains the participants' priority topics, grouped by qNMR **theory (T)**, **practice (P)**, and **implementation (I)**
    - **NOTE:** assigned flash presenters will frequently differ from the **[contributor]** of the topic, for two reasons: (a) to get diverse views and opinions on the topics, and (b) to distribute contributions evenly across all participants.
    - **Section 3. The Big qNMR Questions** (p.10 ff) contains all the questions we eventually seek to answer; we will have to pick wisely and focus on a few

- **STEP 2 – PRODUCE**
  Generate flash presentations and **prepare flash statements** for **your assigned section of** the **Key Progress of the morning sessions**, compiled on p. 15 ff
  - Make **single-slide, max. two-slide PPTs** for each major topic you want to address
  - Plan for a **1-minute, max. 2-minute flash presentation**
  - Email PPTs to anb@usp.org and gfp@uic.edu and bring them on a USB memory stick

- **STEP 3** – **PLAN AHEAD**
  Be ready for making succinct contributions to focused discussions
  - Time will be of the essence ("Time flies when you are having fun"). Just for perspective: if we jointly manage to distribute the time we have over all participants equally, each person will have a total of ~10-12 minutes of everybody's attention.

2. Overview of Morning Workshops and Afternoon Workgroups

qNMR Theory (T) – Friday Morning 9:00-10:30 Workshop

Goal & Charge to the Group of Participants. Assess the **current status of qNMR methods and concepts** in terms of **(i)** qNMR's fundamental capabilities and limitations predicated by physics, chemistry, hardware design; **(ii)** its analytical concepts and (mis)perceptions; **(iii)** its use in different occupational contexts, the expectations associated with it and overlooked opportunities.

T1 THEORY – ACQUISITION & PROCESSING

- **Key Progress Topics** for flash presentation and discussion: page 15
- **Big qNMR Questions** for flash presentation and discussion: page 10
- **Flash Presenters:**
  - *Matthias Niemitz*
  - *Bernd Diehl*
  - *Toru Miura*
  - *Patrick Hays*
  - *Christina Szabo*
  - *Kristie Adams*

T2 THEORY – METROLOGY

- **Key Progress Topics** for flash presentation and discussion: page 15
- **Big qNMR Questions** for flash presentation and discussion: page 10
- **Flash Presenters:**
  - *John Warren*
  - *Charlotte Corbett*
  - *Yukihiro Goda*
  - *James Hook*
  - *Michael Nelson*
  - *Torsten Schönberger*

T3 THEORY – CHALLENGES & APPLICATIONS

- **Key Progress Topics** for flash presentation and discussion: page 16
- **Big qNMR Questions** for flash presentation and discussion: page 11
- **Flash Presenters:**
  - *Eric Munson*
  - *Hector Robert*
  - *Pearse McCarron*
  - *Guido Pauli*
  - *Michael Bernstein*
  - *John Edwards*

qNMR Practice (P) – Friday Morning 11:00-12:30 Workshop

Goal & Charge to the Group of Participants. Assess the **current status of qNMR practice** in terms of **(i)** prevalent and contrasting industrial/academic/pharmacopeial/forensic uses of the technique; **(ii)** the prospect of formulating a unified general approach to qNMR practice despite evidently different analytical goals; **(iii)** the need to build a strong foundation and propagate the technique into wider practice through education, elaboration of standard(ized) procedures, adaptation to (low-field) hardware, and creation of a sustainable qNMR ecosystem.

P1 PRACTICE – QUALITATIVE & QUANTITATIVE DEFINITIONS

- **Key Progress Topics** for flash presentation and discussion: page 17
- **Big qNMR Questions** for flash presentation and discussion: page 13
- **Flash Presenters:**
  - *Kristie Adams*
  - *Christina Szabo*
  - *Guido Pauli*
  - *Torsten Schönberger*
  - *Patrick Hays*
  - *Matthias Niemitz*

P2 PRACTICE – MEASUREMENT, EVALUATION & DOCUMENTATION

- **Key Progress Topics** for flash presentation and discussion: page 17
- **Big qNMR Questions** for flash presentation and discussion: page 13
- **Flash Presenters:**
  - *Bernd Diehl*
  - *Michael Nelson*
  - *Kang Chen*
  - *Takako Suematsu*
  - *Charlotte Corbett*
  - *Michael Bernstein*

P3 PRACTICE – EDUCATION & OUTREACH

- **Key Progress Topics** for flash presentation and discussion: page 18
- **Big qNMR Questions** for flash presentation and discussion: page 14
- **Flash Presenters:**
  - *Yukihiro Goda*
  - *Charlotte Simmler*
  - *Elina Zailer*
  - *James Hook*
  - *Hector Robert*
  - *Eric Munson*

qNMR Implementation (I) – Friday Afternoon Workgroups & Joint Summaries

Goal & Charge to the Participants Working in Two Groups. Address the overarching question which qNMR methods are ready for immediate implementation in industry, in metrology, in pharmacopoeias, in academic and biomedical research? How can they be implemented most rapidly and efficiently?

I1 IMPLEMENTAION – THEORY-TO-PRACTICE PERSPECTIVE

- Which qNMR experiments should be implemented as standard methods? Lesser level of detail, keeping big picture in mind?
- Which processing methods should be implemented as standards methods? Which standard methods exist, which need to be implemented?
- What are reasonable precision and accuracy aims? Address LOD, LOQ considerations?
- Which instruments should be used by whom? What are the cost considerations?

- **Workgroup Facilitators:** *Bernd Diehl, Matthias Niemitz*
- **Workgroup Members:** *Torsten Schönberger, Toru Miura, Eric Munson, Patrick Hays, Hector Robert, Michael Nelson, Takako Suematsu, James Hook, Kang Chen, Charlotte Simmler*

I2 IMPLEMENTAION – PRACTICE-TO-APPLICATION PERSPECTIVE

- How can qNMR be implemented for a broader base of processing only users, who interpret qNMR data but are not concerned with acquisition? Broadening the user base like in LC and MS?
- How important is qNMR's analytical orthogonality? Essential vs. nice vs. overkill? Impact on health product and science?
- How can qNMR compete with gold standard LC *et al.*? Are cost considerations prohibitive, neutral, or in favor for qNMR?
- What reporting standards and tools exist? Which ones are needed (badly)? Which standards and tools should be developed first and open-sourced?

- **Workgroup Facilitators:** *Kristie Adams, Guido Pauli*
- **Workgroup Members:** *Pearse McCarron, Yukihiro Goda, Christina Szabo, Charlotte Corbett, Michael Bernstein, John Edwards, John Warren, Gary Martin, Daron Freedberg, Elina Zailer*

3. The Big qNMR Questions

The following are key questions which the Summit seeks to answer.

3.1 Big Questions - qNMR Theory

T1 THEORY QUESTIONS: ACQUISITION & PROCESSING

Acquisition of 1D qHNMR Data

- How to decide about the acquisition parameters for quantitative conditions?
- How to decide about instrumentation incl. low vs. high-end/-field?
- Can universal acquisition parameters be formulated, or is there a need to customize them for each analyte?
- Are the published qNMR acquisition models more or less harmonic, or are there major differences that are justified?
- Is there consensus regarding relaxation delays in 1D qHNMR? Can one-fits-it-all recommendations be made, or is a range of recommendations necessary?

qNMR Instrumentation

- Who has used magnets of different field strength for the same analyte and registered any noticeable effect on quantitation?
- Are all NMR instruments suitable for qNMR? Who has data to document the suitability of older vs. modern NMR instruments?

T2 THEORY QUESTIONS: METROLOGY

qNMR Unlimited

- What is achievable by qNMR? At what effort & cost?
- What accuracy and precision is needed & practical? In pharmacopoeias – metrology - industry - academia - biomedical research?

qNMR Validation

- Which system suitability tests should be followed for qNMR? Which documented procedures exist and are used in your lab?
- Are system suitability tests universal?
- Is there a need to establish specialized validation guidance for quantitative NMR analysis? If so, what are the specialized key considerations?
- Is it necessary to reconcile qNMR validation with a typical ICH-type validation protocol?
- Which conventional validation parameters are sensible to implement for qNMR, and how should they be modified/interpreted to make the most sense?
- What conventional validation tests are inapplicable in qNMR validation framework?
- What tests are unique to qNMR and crucial in performing validation?
- How can the validation acceptance criteria be defined and justified?
- How important is it to establish traceability in qNMR? How important is it in pharmacopoeias – industry (chem./pharm./natural products) – regulatory – academia? How important is it for the to-be-established levels of qNMR, e.g., metrology/pharmacopoeia – general precision – research/production?

Round Robin qNMR

- What are the key outcomes of documented (published and unpublished but documented/available) qNMR round robin tests?
- What should be the targets/aims of future round robin tests?
- How can future round robin tests be organized and completed? In the real vs. ideal world.
- Is there value in "Online Test"? E.g., shared data for download to assess community performance in qNMR analysis and interpretation. Who provides the resources?

Pharmacopoeial qNMR

- How many replicates should be performed for pharmacopoeial purity assignment?
- How important is the use of different instruments, different operators, or different acquisition parameters to achieve a desirable degree of certainty in pharmacopoeial purity analysis?
- Is it necessary to conduct pharmacopoeial purity assessment as a collaboration among several institutions, or can it generally be confined to the same organization?
- Should monographs that rely on qNMR-characterized reference standards always use qNMR as an assay method?
- Is there any logical contradiction in using qNMR-characterized reference standards in pharmacopeial chromatographic procedures?
- What justifies a significant expansion of qNMR as a pharmacopeial analytical methodology in the next few years?
- Does qNMR have the potential to replace chromatographic techniques as the primary means of pharmacopoeial analysis?
- Should existing monographs currently using LC procedures be revised to include qNMR or replace LC with qNMR analyses? If so, is there a particular category of monographs to be targeted for this process? What may guide such a decision?
- How do you see the future of pharmacopoeial reference standard characterization? Would you envision, eventually, that the majority of reference standards will undergo qNMR analysis as a routine procedure, or this should not be expected in the near future? Remote future?

T3 THEORY QUESTIONS: CHALLENGES & APPLICATIONS

Complex Samples

- What capabilities does qNMR have to determine individual constituents in a multicomponent mixture, e.g., a botanical extract?
- Do you have any examples of this type of qNMR analysis? Something in the works?
- 1D vs. 2D qNMR: what determined the limit of 1D qNMR? How useful is 2D qNMR and what are the main challenges in its implementation?

qNMR Orthogonality

- What can qNMR see that other methods don't? Is it orthogonal or just complementary?
- What can other methods see that qNMR is blind for?
- Who currently uses qNMR for what application? For which reasons? How can qNMR be in wider use in the fields where it is suitable but just not used for various reasons?
- What drives the costs? How do they compare to other analytical methods?

Picking qNMR Battles

- Which analytes are most suitable for qNMR characterization and why?

- Which analytes should generally be considered unsuitable?
- In your experience: which reference standards have been selected for characterization (purity assignment) by qNMR? What considerations guided the selection? Is it necessary to thoroughly evaluate the prospective analytes using other techniques, *e.g.*, chromatographic, prior to commencing the qNMR characterization?
- What are the examples of cases where qNMR has "come to the analytical rescue"; *i.e.*, qNMR was successful in producing insights or results that other analytical methods could not achieve?
- Is qNMR currently being used as a "method of last resort", *i.e.*, qNMR is considered only after all other (available) methods have been tried without success, or for long-term analytical problems for which the scientific community has no other solutions? If so, how can this be changed?

3.2 Big Questions – qNMR Practice

P1 PRACTICE QUESTIONS: QUALITATIVE & QUANTITATIVE DEFINITIONS

qNMR Sampling

- Which general sample preparation protocols exist for (pharmaceutical) qNMR?

Actual qNMR Quantitative Measurements

- What is known about integration as quantitative measurement in qNMR: accuracy – precision – reproducibility – documentation?
- Which quantitative measurements other than integration are used/suitable?

Global qNMR

- What and who should drive the development of qNMR: pharmacopoeia – industry (chem./pharm./natural products) – regulatory – academia?
- Are the needs of the four main sectors (pharmacopoeia – industry – regulatory – academia) different enough to justify different approaches?
- Should all qNMR methods be harmonized completely, or is there justification for establishing different levels of qNMR, e.g., in three-tiered schemes such as metrology/pharmacopoeia – general precision – research/production levels or metrology – development – research grades?

Calibrants vs. Standards?

- Is there agreement that materials for quantitative calibration should be called calibrants, rather than standards? Acknowledging that NMR already has reference standards for the chemical shift (TMS et al.)?
- Are Internal **C**alibrants (ICs) the gold standard? What about **E**xternal **C**alibration (**EC**)? What about combined **E**xternal-**C**alibration+ **I**nternal **C**alibration (ECIC)?
- Internal Calibrants (ICs): What determines the choice of the IC? Is it done by trial-and-error, or a deliberate decision? Is the same IC used for all measurements, or are ICs customized in each case? If certain ICs are considered unsuitable, what factors contribute to this decision? If more than one IC is tried with the same sample, are substantial disagreements observed, and if yes, how can they be resolved?
- What is the best role for uncalibrated, 100% qHNMR?

P2 PRACTICE QUESTIONS: MEASUREMENT, EVALUATION & DOCUMENTATION

qNMR Documentation

- How should qNMR analyses be documented?
- Should there be different guidelines for qNMR in pharmacopoeia – industry (chem./pharm./natural products) – regulatory – academia? To match to-be-established different levels of qNMR, e.g., research grade – development grade – metrological grade?

qNMR Resources

- Should there be a qNMR master document? If so, what format: handbook – textbook – article (Series) – online publication?
- What is the value of sharing qNMR relevant methods and information?
- What are the best formats for the sharing of qNMR data (raw, processed) and resources (protocols, experimental settings, processing protocols, evaluation protocols)? Should they be open source? If so, how can this be achieved?

Beyond qNMR

- What do you do with your qNMR data? Does it get centrally stored / organized / catalogued / archived, or does it reside within individual laboratories?
- Do you contemplate having a centralized repository for the data?
- Do you consider making the data freely available to the scientific community?
- Do you contemplate any form of "electronic reference standards" being developed and issued that involve qNMR?

P3 PRACTICE QUESTIONS: EDUCATION & OUTREACH

qNMR Training

- What are the training requirements for a qNMR operator?
- What are the training requirements for a qNMR data analyst?
- What resources are available for qNMR training?
- What new resources should be developed for qNMR training? Who should/can/is willing to develop them?

qNMR Community

- Does it make sense that the current qNMR practitioners establish a more formal qNMR community? Workgroup – Network – Society? Is the organizational overhead of such a community justified? If so, how can it be established and sustained?
- Should there be something like "qNMR School"? Your vision for the *real* world?

Global qNMR Implementation & Acceptance

- Is there more enthusiasm about the technique from pharmacopoeia – industry (chem./pharm./natural products) – regulatory – academia?
- How do your customers/communities accept your qNMR products or vision?
- Is qNMR embraced as an approach, or do your peers demand "tested and proven" chromatographic procedures instead?
- What obstacles have to be overcome when first introducing qNMR based products: qNMR based monographs (pharmacopoeia) – reference material dossiers (industry) – qNMR research results (academia)?
- Are the regulatory inspectors receiving adequate training in reviewing qNMR-generated data?

4. Key Topics & Issues for Progress: Participant Contributions Grouped for Flash Presentations & Discussions

Quest from the *qNMR Left Hemisphere Challenge*. What short **topics or issues, that, once resolved will permit major progress in qNMR** methodology adoption in laboratories worldwide? Provide one or two that you find most important.

4.1 Key Progress Topics - qNMR Theory

NOTE: assigned flash presenters will frequently differ from the **[contributor]** of the topic, for two reasons: (a) to get diverse views and opinions on the topics, and (b) to distribute contributions evenly across all participants.

T1 THEORY TOPICS: ACQUISITION & PROCESSING

- **Topic T1: Enhance Procedures and Transparency of Processing Workflow.** Pre-Processing (baseline, phase, etc. **[Bernd Diehl]**; achieve better definitions of what happens between acquisition of the raw qNMR data and the extraction of the quantitative information **[Guido Pauli]**
- **Topic T2: Calibration Strategies** for non-standard qNMR (e.g., troublesome ^{1}H and HSQC) **[John Warren]**
- **Topic T3: Support Low-field qNMR.** Specifically for benchtop NMR, develop and make available approved traceable standards, accepted OQ/PQ methods **[Hector Robert]**
- **Topic T4: qNMR for Heteronuclei** such as ^{19}F, ^{31}P, and ^{13}C **[Bernd Diehl]**
- **Topic T5:** *[w/Grain of Salt]* **Myths vs. Reality.** Can the equation 5 x T1 = B x S be resolved for a standard recycle delay, S, if the magnetic field, B, is known? **[Brian Killday & Guido Pauli]**

T2 THEORY TOPICS: METROLOGY

- **Topic T6: qNMR as Pharmacopeial Analytical Methodology.** JP has already engaged the research aimed at acceptance of qNMR for determination of the purity of synthetic drugs instead of mass balance method. **[Yukihiro Goda]**
- **Topic T7: Acceptance by Regulatory Agencies.** For pharmaceutical applications, the acceptance of qNMR by regulatory authorities and recognition of this acceptance within pharm. companies is crucial. I am trying to teach qNMR to Japanese regulatory authorities. **[Yukihiro Goda]** Greater inclusion of qNMR techniques by worldwide governmental, regulatory and pharmacopeial bodies, regardless of industry influence and stereotypes. **[Kristie Adams]**
- **Topic T8: Validate Excitation Profiles.** Solve impact of excitation profile on signal intensity. For simple 1D-qHNMR, I observed and quantified this influence; it leads to deviation in per mill range. I cannot find anything clear in the literature. It is sometimes mentioned, but never quantified. Ideas are needed how to avoid this (small) error. **[Torsten Schönberger]**
- **Topic T9: Improved Measurement Uncertainty** [John Warren]
- **Topic T10: Control Costs.** The costs of upfront investment and cryogen maintenance of high-field NMR systems are prohibitive for many small institutions, state or local government laboratories.

There is little to do with regard to upfront cost (especially in recent times), though advances in technology are making NMR systems more cryogen-efficient. **[Mike Nelson]**

T3 THEORY TOPICS: CHALLENGES & APPLICATIONS

- **Topic T11: Working with Mixtures.** Our work entails mixtures, so methods, such as quantitative HSQC or faster ^{13}C would aid purity determination for samples with too much overlap in the proton spectrum. **[Charlotte Corbett]**
- **Topic T12: Make qNMR Work with Minimal Human Interaction.** Simplified or automated sample preparation, acquisition and processing. Software that would allow a novice to easily process the spectrum for purity results with minimal effort and NMR knowledge, as well as setup methods for automated processing of compounds commonly observed. **[Charlotte Corbett]**
- **Topic T13: Progress in the Low-field Instrument Segment.** Improved data quality and automated software tools would dramatically boost the adoption of qNMR methodologies worldwide. **[Mike Nelson]**
- **Topic T14: Implement into Chemical Synthesis.** Increase the uptake of qNMR in the Experimental Sections of synthetic publication/work. What can be done to increase awareness and improve uptake? **[Mike Bernstein**; referring to *J. Med. Chem. 57*, 9220 (**2014**) as exemplary/first step**]**
- **Topic T15: High-throughput qNMR.** Can qNMR analysis be automatic when it is repetitive and unique each time? **[Mike Bernstein]**

4.2 Key Progress Topics - qNMR Practice

NOTE: assigned flash presenters will frequently differ from the **[contributor]** of the topic, for two reasons: (a) to get diverse views and opinions on the topics, and (b) to distribute contributions evenly across all participants.

P1 PRACTICE TOPICS: QUALITATIVE & QUANTITATIVE DEFINITIONS

- **Topic P1: Establish Standard Protocol.** Such a standard protocol should include not only NMR measurement, but also sample preparation and data processing. It might be most difficult to define data processing parameters for qNMR. **[Takako Suematsu]**
- **Topic P2: Establish 5. Highly Generic qHNMR** Framework (Draft)**.** Based on the input received and our own research, there is a need for a consensus-driven definition of a highly generic qHNMR workflow. Importantly, such a framework should **accommodate all types of qHNMR analyses**
  - See the draft in section ***5. Highly Generic qHNMR*** Framework (Draft) *on page19*, which evolved from the *qNMR Left Hemisphere Challenge* **[Anton Bzhelyansky & Guido Pauli]**

- **Topic P3: Nomenclature.** There also is a need for unified qNMR terminology, especially for key aspects such as: **(i)** calibration/reference standards (e.g., IC vs. IS); **(ii)** naming of the method and its variants (e.g. qHNMR vs QNMR vs. quant ^{1}H NMR etc.); but also **(iii)** pulse/experiment related (e.g., D1 vs d1 vs RD) **(iv)** qNMR workflow related (pre-processing vs. post-processing vs. post-acquisition processing) **[Kristie Adams & Guido Pauli]**
- **Topic P4:** *[w/Grain of Salt]* **The Five Commandments of qNMR.** Are five sufficient? Adequate?
  - Does **SISSR** (✂) help make qNMR cutting edge?
    1. **S**electivity sufficient
    2. **I**nertness (no reaction; no qNMR-method induced Residual Complexity)
    3. **S**olubility unimpeded (target and calibrant fully soluble)
    4. **S**/N sufficient
    5. **R**elaxation sufficient
  - "Sufficient" in the sense of "sufficient (for the **desired General qNMR Levels 2A1/2/3**; see section ***5. Highly Generic qHNMR*** Framework (Draft) *on p. 19* **[Bernd Diehl & Guido Pauli]**

P2 PRACTICE TOPICS: MEASUREMENT, EVALUATION & DOCUMENTATION

- **Topic P5: Simplicity.** Put qNMR software on all NMRs that is able to have ANYONE (not just an NMR spectroscopist) perform qNMR. The software needs to automate all the usual things that produce good qNMR purity results with low (acceptable) uncertainty levels. We have 300 general chemists running qNMR on a regular basis on all kinds of organic molecules because we have software that takes manual manipulation of the spectrum out of the hands of the chemist. Before we had this software only a handful of chemists used NMR at all for ID, and maybe one or two used it for qNMR. Chemists now go to qNMR over chromatography because of how easy the preparation is and how fast it is. **[Patrick Hays]**
- **Topic P6: Simple Validation of Relaxation Status.** Proposed use of an additional final 90° pulse as quick and highly practical means of assessing the relaxation status of every qNMR measurement in daily work. Can also be used as system suitability test (SST) for qNMR. **[Bernd Diehl]**

- **Topic P7: Dealing with Signal Overlap.** Spectral overlap is a key issue in qHNMR and a universal concern that exists in all samples (highly pure, mixtures, crudes) to various degrees. Unified strategies are needed to **(a)** deal with unavoidable overlap, and **(b)** to reduce/overcome overlap [**Bernd Diehl & Matthias Niemitz & Guido Pauli]**
- **Topic P8: Public Data Sharing.** Open access to quality qNMR assignments and data is critical for spreading the use of qNMR. After all, qNMR is always also a qualitative technique, which means that qNMR data has significant structural value. **[Matthias Niemitz, Hector Robert, Guido Pauli]**

P3 PRACTICE TOPICS: EDUCATION & OUTREACH

- **Topic P9: Education & Accessibility.** (a) Put benchtop NMRs (or bigger) in all colleges and universities. (b) Train professors and teachers how to perform and teach qNMR. (c) If they have access to simple programs for performing qNMR, then it is plug-and-play. **[Patrick Hays]**
- **Topic P10: Education of Decision Makers.** Get chromatographers out of positions of making decisions about all quantitation for an organization (or at least put some NMR people on these panels/boards). I find that the organizations where qNMR is stifled are those places where managers had bad experiences with NMR (or no experience at all) and no knowledge of its capabilities. In my organization, the push-back to NMR, let alone qNMR, was from supervisors and lab directors whose experience with NMR was poor or even terrible "when they were on the bench as a chemist". "We could not do that when I was a chemist" was a common comment on qNMR by these people. **[Patrick Hays]**
- **Topic P11: Support of General Education.** Independent and supported communication (not by instrumentation manufacturers/vendors) of the benefits of NMR for quantitative analysis. In particular, the benefits of linearity, specificity, speed, ease-of-use, low operating costs with low-field. This communication should reach out to a broad audience of regulators, users, and decision makers; beyond the researchers and first adopters. **[Hector Robert]**
- **Topic P12: Education.** NMR heretofore has not been a prominent quantitative technique amongst much of the analytical chemistry community. Some of the mystique of this method is associated with a lack of history and broad NMR expertise amongst classical quantitative chemical analysis. This is changing as time continues and qNMR gains more traction. Guidance and encouragement for qNMR will go a long way to make it a more widely adopted methodology. **[Mike Nelson]**
- **Topic P12: Education.** I believe one key issue is educating users of other analytical techniques about the advantages of qNMR such as being non-destructive, linear with varied types of compounds, and being a high throughput technique with the ability to simultaneously quantitate numerous components. **[Brian Killday]**
- **Topic P13: Consensus and Team Building.** PANIC Validation Workshop Group is busy with establishing a unified mission to "Provide education and awareness in NMR validation through fostering communication concerns and progress in the community." **[Torsten Schönberger]**

5. Highly Generic qHNMR Framework (Draft)

(Proposal for P1 – PRACTICE and Other Discussions)

Evolving from the *qNMR Left Hemisphere Challenge* The following generic procedure was compiled by considering all responses received (see section *6. Generic qHNMR* Sketch below), including numerous other communications with Summit participants. We also added points resulting from our own research. The following draft consolidates all contributed aspects of a qHNMR procedure and is aimed at providing a **Universal Framework for qHNMR Analysis**.

Guido Pauli

A **generalized, modular qHNMR Kit** consisting of **four major steps**
Can be developed into practical building sets for typical applications, e.g., grouped by the **desired General qNMR Levels 2A1/2/3**
Potential to develop into generalized qHNMR blueprint document?

STEP 1. QUALITATIVE DEFINITION ("Quantify what?")

Define the target analyte(s) and characterize them qualitatively:

1A. Identify the structural parameters incl. MW of the target analyte(s), the sample matrix, and potential interferences

1B. Acquire/validate the NMR spectra (1D/2D) of the target analyte(s)

1C. Obtain/make robust spectral assignments (including ^{1}H for qHNMR)

STEP 2. QUANTITATIVE DEFINITION ("Quantify how?")

Determine the overall aims and conditions of the qNMR analysis:

2A. General Level: determine desired/adequate (un)certainty level

2A1. Metrology/pharmacopoeial level:
98.76% major – 0.12% minor– 5 determined and 4 significant figures

2A2. General precision level
98.7% major – 0.1(2)% minor– 4 determined and 3 significant figures

2A3. Research and chem./pharm. production type:
98(.7)% major – 0.1(2)% minor– 3 determined and 2 significant figs

2B. General Class of Quantitative Measurement: determine HOW the quantitative numbers will be derived from the spectra (typically one method, but can be combination); address/manage signal overlap

2B1. Peak Height
2B2. Integration
2B3. Fitted Deconvolution
2B4. Quantum-mechanical Deconvolution

2C. General Type of Calibration will depend on the sample (abundant, precious, unique; costs) and practical circumstances (use of the results, desired (un)certainty level, lab environment specific parameters)

2C1. Internal Calibration (IC)
2C2. External Calibration (EC)
2C3. Combined External and Internal Calibration (ECIC)

STEP 3. MEASUREMENT

Obtain the raw qNMR data using specified conditions:

3A. Validate and calibrate (for EC and ECIC) instrument and balance for desired accuracy and precision

3B. Prepare the samples

3C. Acquire the qNMR spectra, including repetitions for statistics; parameters and validity in line with choices 2A/B/C. General Level/Class/Type

STEP 4. EVALUATION & DOCUMENTATION

Evaluate and document the qNMR data and results:

4A. Process the qNMR spectra; procedures and validity in line with choices 2A/B/C. General Level/Class/Type

4B. Computation of quantitative measurement values and final results

4C. Document essential information from all Steps 1-3 in unified formats, both as documents and electronically

4D. Make documentation publicly available to the extent possible, using a sustainable storage solution

6. Generic qHNMR Sketch

Quest from the *qNMR Left Hemisphere Challenge*. How do you envision a **very generic ^{1}H qNMR procedure** that would sufficiently **instruct an experienced and thorough scientist** in conducting purity determination of an unknown non-hygroscopic organic compound? Provide a sketch in as little as 4 but not more than 10 concise steps. Include parameters/considerations that you consider essential for the analytical outcome.

6.1 Contributed qHNMR Procedures

Patrick Hays

1. Fit for purpose: know what qNMR question you are trying to answer, what uncertainty limit is desired, what sample you are dealing with. With this information you can determine the steps required to ensure good results.
2. Acquisition Parameters and their significance: solvent, internal standard, T1 relaxation and pulse delay (d1), acquisition time (at), spectral width, filters used and their impact (uniform response throughout spectra width), decouple C13 or not (probe type and are there any artifacts produced by decoupling), delay in place to allow thorough thermal equilibration of the sample in the probe,
3. Acquisition problems: shimming, solubility, broad line widths, insufficient temperature equilibration of sample in probe
4. Processing spectra: phasing, baseline correction, which peaks to integrate and where to place integral setpoints, use of various peak area methods (integration, deconvolution and other techniques)
5. Processing problems: when is a spectrum out of phase, not baseline corrected properly, integrated properly
6. Sample problems: degradation of sample in solvent/internal standard, exchangeable protons, “multiple form” compounds (amide shifting, keto-enol tautomers, fused ring tertiary amines ion-paired with acids that produce two signals due to acid alpha and beta to the amine nitrogen, mixtures of these, etc.)
7. How to verify the accuracy and uncertainty of a qNMR measurement: value and significance of multiple purity values, what the other components are in the solution and where their signals are and can you subtract their integrals from the analyte integrals being used for qNMR, validation procedures

Charlotte Corbett

1. Determine the appropriate solvent for the unknown; If nothing is known about the substance then try CD3OD as most compounds are soluble in methanol, and using a solvent that exchanges with labile protons sometimes produces a spectrum with a flatter baseline by eliminating signals from labile protons (sometimes these can be broad peaks, especially those from OH groups).
2. Accurately weigh at least 1 mg of unknown substance and at least 1 mg of quantitative reference material, such as 1,4-BTMSB-d_4. (The quantitative reference material can be omitted, if using a synthesized pulse)
3. Add ~2mL of solvent and mix thoroughly (sonicate if necessary)
4. Transfer 1 mL to a 5 mm NMR tube. If insoluble materials are present, then add 1 mL of solvent and mix thoroughly, transfer 1 mL of this solution to a second NMR tube. [gfp: volumes correct??]

	5. Acquire NMR spectrum using one experiment for all samples: **(i)** delay between pulses is long enough to be greater than 5xT1 of any quantitative reference material used; **(ii)** spectral width is wide enough, so uniform response is received between -0.5 and 10 ppm; **(iii)** non-spinning (no spinning side bands to interfere); **(iv)** 90 degree pulse width to maximize sensitivity; **(v)** At least 5 second acquisition time for FID collection. 6. Integrate analyte and quantitative reference material peaks. If signal to noise is too low to accurately determine integral set points, then reacquire the spectrum with more scans or re-prepare using more material. It would be helpful here to have a method that acquired 1 scan first, processed, and then determined how many scans to collect automatically and did so. 7. Spectral Processing: **(i)** Flat baseline; (**ii)** Appropriate phasing; **(iii)** Integrate analyte and quantitative reference material peaks; **(iv)** Calculate purity based upon (**iv-1**) Integral values of sample and quantitative reference material; (**iv-2**) sample and quantitative reference material weights and molecular weights; (**iv-3**) purity of quantitative reference material; (**iv-4**) number of hydrogens represented by each integral 8. If insoluble materials are present, the purity results of the two tubes should match. If the results do not match, re-prepare samples using more solvent. 9. Re-acquire a spectrum after some time (hours or days) to confirm stability of the solution.
Takako Suematsu	1. Inspection of experimental conditions: **(i)** Assignment of spectrum; **(ii)** Stability of sample; **(iii)** Select internal standard, solvent, concentration. 2. Sample Preparation: **(i)** Prepare the balance for weighing, determine minimum weight; **(ii)** Weigh standard and analyte sample on a micro – or ultramicro-balance; fully dissolve them in selected solvent; prepare a lot of three samples. 3. Measurement: repeat 3 times per sample for total of nine acquired data sets. 4. Data Processing: data are processed and integral values for target signals are determined 5. Analysis: Calculate purity and concentration from sample weight and integral values; confirm results and data using corresponding software
Torsten Schönberger	1. Sample preparation: **(i)** I concentrate on internal standard method; use reliable reference material suitable for the solvent used, consider uncertainty level for purity; **(ii)** weighing of sample and reference in vial or in NMR tube, select balance according to intended uncertainty level of analysis; **(iii)** ensure that analyte + reference are completely dissolved (ultrasonic treatment as needed); if by-products remain undissolved, use duplicates with different weightings 2. Acquisition: select recycle delay depending on longest T1!!! (5 or 7 times, depending on uncertainty level); use 90° pulse for best S/N 3. Processing: use only “soft” apodization function (e.g., lb=0.2Hz); ensure accurate phasing (if necessary, manual); ensure accurate baseline correction 4. Evaluation: integrate signals consistently (regarding regions) without covering signals from by-products 5. General: make sure that analyzed compounds are not degraded up to the measurement, *e.g.*, by duplicate measurement at different times after preparation

John Warren

1. Choose solvent/standard: select a suitable solvent and internal standard based on solubility, mutual chemical inertness and resolution of NMR signals
2. Check for interferences from solvent/standard: run ^{1}H NMR of both sample and standard separately in the solvent to ensure that neither contains any significant peaks that interfere with chosen signals for quantitation.
3. Check that analyte peak is resolved from impurities: no significant impurities in the sample give signals that fall within the chemical shift range of the analyte signal (2Ds, changes in solvent etc.).
4. Determine relaxation times for the chosen signals (unless a large default relaxation time is to be chosen).
5. Gravimetrically prepare analytical solutions using sufficient material of both sample and internal standard to ensure appropriate measurement uncertainty.
6. Determine number of repeats/replicates for desired measurement uncertainty
7. Run ^{1}H NMR spectra ensuring appropriate relaxation delay, resolution and signal-to-noise.
8. Process spectra – ensure spectra are well phased and that an appropriate baseline correction is applied – this should normally be manually applied to limited regions of the spectra.
9. Integrate spectra – ensure integration ranges for the signals from sample and internal standard are consistently applied (with or without ^{13}C satellites, 70 x half width etc.).

Brian Killday

I am making the assumption that by "conducting purity determination of an unknown non-hygroscopic organic compound", the unknown entity is the purity of the organic compound and not the structure of it. The structure, molecular weight and assigned ^{1}H-NMR spectrum of the compound must be known/determined to assess its purity via qHNMR. This information is needed in the pulcon quantitative method to know the number of equivalent nuclei per signal integrated. I am also assuming in this particular case that this compound is fully soluble in an appropriate NMR solvent rather than a complex matrix requiring extraction, in which case extraction efficiencies would need to be taken into account.

1. Before running analytes on the NMR instrument, especially when using an external standard, appropriate system suitability tests should be performed on known calibration standards to confirm that the spectrometer meets required specifications such as lineshape, resolution, and sensitivity for the nucleus observed.
2. An appropriate amount of the compound should be accurately weighed and dissolved in an accurately measured amount of solvent (such as a deuterated NMR solvent).
3. A qHNMR spectrum of the sample should be obtained utilizing a validated method. If utilizing an external standard and the pulcon method, the same pulse sequence should be used for both. The 90-degree pulse of the analyte should be calibrated and enough transients acquired to obtain an adequate signal-to-noise ratio as specified in the method. The recycle delay should typically be >5*T1 of the analyte signal.

4. The spectra should be processed as defined in the validated method, utilizing the appropriate line broadening and baseline correction, with quantitation of the analyte using the procedures defined in the method.
5. The actual concentration of analyte determined in step 4 is then compared to the theoretical concentration of compound prepared in step 2 to determine the purity.

Toru Miura

We usually conduct the internal calibration qNMR method.
1. Decide the structure of target compound which is related to molecular weight and the number of ^{1}H of target compound. Both of them are used for calculation of qNMR purity determination.
2. Decide the Certified Reference Material and D-solvent which should be separated from target signals sufficiently.
3. Decide the sample solution concentration which is related to SNR.
4. Decide the weight of the sample and CRM, the volume of D-solvent (the weight should be more than Minimum Weight of USP).
5. Decide the qNMR parameter sets as follows: **(i)** PD (90°is better); **(ii)** SW (when not using digital filtering, chose more than or equal to 100 ppm; when using digital filtering, approximately 20 ppm is OK); **(iii)** FID size; **(iv)** ^{13}C decoupling (we usually use MPF8 because it is most efficient); acquisition time (more than or equal to 4 s is better because of preventing FID truncation); **(v)** number of transients (if possible, signal-to-noise ratio >1000 is better); **(vi)** relaxation delay (we usually set up 10 times of the longest T1 of all signals)
6. Confirm the stability of sample solution in analysis time
7. Conduct sample preparation (We usually prepare 3 sample solutions of which data is used for confirmation of precision and we usually use ultra-micro balance for minimize the amount of sample, CRM and D-solvent)
8. Conduct NMR measurement (we usually repeat 3 times measurement of which data is used for confirmation of precision)
9. Conduct data process as follows: (**i**) Window Function (OFF is better); (ii) FFT; (iii) Phase correction (Manual is better); (iv) Baseline correction (this parameter would mostly influence the analytical value and actually I am not sure what type we should choose); (**v**) Integration (Integral range should be sufficiently broad)

Matthias Niemitz

[points raised in addition to outline of a general method]
1. Address or manage (and document) signal overlap as it can leads to under-determination.
2. Employ quantum mechanics-based methods to adequately consider concealed liens and non-first order effects.

Bernd Diehl

As add-on to all proposed general qNMR workflows: add advanced integration procedure using an Automated Integration procedure (e.g., Matlab script)
1. Pre-processing
2. Data Import
3. Peak-picking
4. Selection of signals (IC, target analytes)

Yukihiro Goda

1. Selection of peak most suitable for integration, considering impurity noise and sensitivity by normal NMR.
2. Selection of proper deuterated solvent and proper internal standard.
3. Weighing sample and selected internal standard (by Ultra-microbalance, if possible)
4. Automated qNMR measurement by optimized condition as shown below.

Further Considerations

Water vs. Water. We have learned that "hygroscopic property" of compound is very important factor for the qNMR purity assignment. Because, if the compound has "hygroscopic property", the purity value determined by qNMR is changeable during the second weighing time when the humidity is different from that of the first weighing time for qNMR determination. *First weighing time*: weighing before qNMR measurement to determine the purity of the compound. *Second weighing time*: weighing after qNMR measurement to use the compound for HPLC or other relative quantification method. If the compound has "hygroscopic property" (such as saikosaponin b2 or ginsenoside Rb1), the compound must be delivered after qNMR determination not with purity information but with absolute amount information, because the compound purity is easily variable, depending on the humidity of atmosphere. Crystal water itself does not disturb qNMR measurement, determination of purity and use of the determined purity value after qNMR, unless the water easily disappear under normal experimental room condition.

Dichotomy of Herbal Pharmacopoeial qNMR. The monograph indicates use of HPLC as assay method. But, the monograph also prescribe the compound of which the purity was determined by qNMR is used as reference standard of the HPLC quantification and the purity value is utilized for calculation of the content of the marker compound in herbal medicine. Some people, especially old chromatographers, do not understand qNMR is the SI-traceable primary ratio method, and the primary ratio method does not need the certified standard of the analyze compound. Also, they do not understand that qNMR does not need validation data which need to chromatographic methods, such as accuracy data using the reference standard of the analyze compound and range of linearity.

Role of qNMR in Pharmacopoeial Monographs. At first, purity determination of JP reference standards. Then, use of qNMR instead of assay of synthetic compound may be allowed. Accumulation of scientific data and efforts that help stakeholders understanding the rationales will definitely guide this decision.

Purpose of qNMR. For the purity determination of marker compounds used for HPLC quantification, qNMR is the method of choice. Although qNMR works well for botanical extracts, JP does not apply qNMR for marker quantification yet.

Particular Values for Herbal Analysis. In the field of herbal medicines, most of JP customers well understand qNMR, because they know chromatographic qualification data by using a reagent as reference standard sometimes indicate different value depending on the purity of the reagent. They experienced some trouble because of the use of different reference standard and they understand use of qNMR determined purity value completely resolves it. qNMR also has a merit to reduce the effort of completing purification of natural compounds as

reference standards, because the purity value determined by qNMR is SI traceable and even though the purity is not enough (such as 85%), the determined (and calculated) content of the compound in herbal medicine by HPLC with the reference standard having purity determined by qNMR is reliable.
IC. We use BTMSB-d4 for normal organic deuterated solvent and DSS-d6 for DMSO-d6. The selection was done depending on the solubility of both compounds.
IC Suitability. When we use $CDCl_3$ as qNMR solvent, we observed both internal reference standard such as BTMSB-d4 and the measuring compound a little gradually decompose. So we must do qNMR measurement quickly.
Scalability, Accuracy, Precision. Summary of validation study (Hosoe, J., et al. (2010). Pharmaceutical and Medical Device Regulatory Science 41(12): 960-970.): Quantitative NMR (qNMR) qualifies as an absolute quantification method and is theoretically able to determine the purity of any compounds with SI-traceability. Therefore, we are trying to introduce the qNMR to the Japanese Pharmacopoeia for the specification of reagents using marker compounds of quantitative analyses of crude drugs. In this study, we performed validation studies of qNMR by using two chemical reagents (magnolol: MW 266.34; and geniposide: MW 388.37) in 5 independent laboratories. The weighing amount of each sample was 5 mg ± 10% and each participant made 3 sample solutions and the absolute purity of each sample was measured with qNMR by 3 times. The total average (the average of the participant average) ± SD of absolute quantification results on magnolol and geniposide were 98.97±0.19% and 96.09±0.28%, respectively. The data for magnolol and geniposide suggested that the variability by each NMR measurement (the average of all the SD of each sample average) and each sample liquid preparation (the average of all the SD of each participant average) were about 0.08% and 0.07% (magnolol), and 0.17% and 0.14% (geniposide), respectively. These data suggested the significant figure of the purity determined by qNMR was practically two-digits when the molecular weight of target reagent is around 300 and its weighing amount is about 10 mg.
Validation and ICH Context. We think that a typical ICH-type validation is not needed for qNMR, because qNMR is directly measure the purity (absolute amount) of targeted compound by using SI-traceable standard weight. Our stance on validation of qNMR is described in the section of general information for crude drugs, "Management of Instrument Performance for Quantitative NMR". (See the excerption below). Quantitative NMR used to determine the purity of reagents for the JP, is an internal standard method that analyte compound and SI traceable reference material in a NMR tube are measured at the same time. In this method, the number of nuclei is measured using NMR phenomenon, which means that the molar quantity of analyte compound in a sample solution is directly calibrated with a reference material. In the management of instrument performance for quantitative NMR measurements, it should be confirmed that integral value of the targeted signals can be determined correctly within the spectrum where the signals are measured (in general, 0 – 10 ppm). The important point here is not to include the signals derived from impure substances in the quantitative spectrum when integrate.

Therefore, to manage instrument performance, a high-purity compound of already known purity (determined by quantitative NMR and not less than 99.0% is preferable) should be used. In addition, signals derived from simpler spin system should be selected and integrated, and the ratio of theoretical number of nuclei among signals should be accurate (for example, when each of the two signals is derived from 1H, the ratio of the integrated values of the both is 0.995 – 1.005). Normally we use vinclozolin, which has the absolute purity value determined by ASNITE (http://www.nite.go.jp/en/iajapan/asnite/index.html) for the performance check of qNMR. Vinclozolin is very stable and its signals appears widely, i.e. from 1.5ppm to 7.5ppm in DMSO solution, which is another reasons why we selected this compound. [gfp: etacrynic acid selected for similar reasons; PCA 2001, dx.doi.org/10.1002/pca.760]

Hector Robert

Workflow with Internal Calibration (IC):
1. Sample Preparation: (i) Use traceable reference materials with documented purity (e.g., NIST); **(ii)** Weigh analyte and IC into standard NMR tubes; **(iii)** Add predetermined solvent volume (for constant sample volume/height); **(iv)** Sample temperature conditioning if required
2. Quantitative Acquisition: (i) Regulation of sample/probe temperature; **(ii)** B0 field/frequency regulation (locking); **(ii)** B0 homogeneity optimization (shimming); **(iii)** Select Pulse Program o Software-controlled parameters for adequate S/N and resolution: acquisition time, spectral window, read pulse flip angle; number of transients (scans); dummy scans; relaxation delay (based on prior knowledge of T1s of analyte and IC); **(iv)** Adjustment of acquisition parameters (e.g. post-pulse delays) to minimize/eliminate first-order phase error (to give flat baselines)
3. Quantitative Data Processing: (i) Zero filling; apodization (if appropriate), Fourier transformation of FID; (optional) reference deconvolution; phase correction; baseline correction; **(ii)** Identify the peak(s) for the target analyte, integral range, determine integral(s); **(iii)** Identify peaks of the IC, determine and assign integral
4. Calculations: (i) Determine molecular weights of the target analyte and IC; **(ii)** Determine the purity of the target analyte using equations
5. Reporting and Documentation: (i) Document all parameters relevant to the sample quantification, including parameters in Steps 1-4; **(ii)** Store and protect data

Kevin Millis

1. Determine Structure. This will be the most time consuming step. Start by using high-res LC-MS as well as ^{1}H-NMR and ^{13}C-NMR. May want to use a couple of different solvents for the ^{1}H-NMR to distinguish between exchangeable and non-exchangeable protons. Additional tests may be useful: melting point, FT-IR, polarimetry and chiral LC.
2. Determine solvent, qNMR reference standard and resonance(s) from compound for use in quantitation. Based on the previous work in step 1, choose an appropriate solvent such that mM concentrations can be achieved (if possible). Choose an appropriate qNMR reference standard: ideally gives rise to a

singlet that is separated but close to a resolved resonance from the organic compound. At least one baseline-resolved (or nearly baseline-resolved) resonance in the ^{1}H-NMR spectrum from the target compound. The number of equivalent protons must be known for the resonance(s) used. May consider changing pH, temperature or solvent to help obtain resolved resonances, while being careful not to degrade the compound by changing these parameters. In fact, should be on the look-out for signs of sample degradation throughout the procedure.

3. NMR Sample Preparation. Using the known MW of the compound and reference standard, weigh enough material to obtain concentrations in the mM range. The target concentration depends on the field of the magnet and time available for data acquisition, among other parameters. Also consider the viscosity of the final solution - the more viscous, the lower the spectral resolution. Probably 10-100 mM concentrations are optimal. Use enough internal standard to make an equimolar (in terms of protons) mixture of the IS and analyte. For example, if a single methyl group from the compound will be used, and DSS is used as the reference, then one would want to mix the compound with the standard in a 3:1 (compound: DSS) molar ratio. Use a calibrated, vibration-free balance. A micro-balance is preferable, however, an analytical balance can also be used, depending on the mass that is to be weighed out. Consider using an anti-static device, and an inert weighing boat. The mass of the boat should be measured. Add the appropriate deuterated solvent. May place the filled weighing boat into a small beaker or volumetric flask. Solvent should be added using a volumetric pipette and the entire solution weighed. The compounds should be completely dissolved (check!). Stir and sonicate if needed. Calculate the concentration of the reference standard using the volume of the solvent added and the mass of the solvent added (needs to know the density of the solvent at the appropriate temperature). The mass of the boat should be taken into account. This result is not so important but gives the user some idea as to how long one should acquire the data for. The most important is knowledge of the masses of the reference and target compounds in the "bulk" solution and complete dissolution of both substances. The chemical purity of the reference compound must be known.

4. Setting up the Spectrometer. Determine the transmitter offset, spectral width, FID size, 90 deg pulse width, number of transients and "inter-pulse" delay time for a 1D experiment. A 1D pulse sequence which filters away ^{13}C satellites may be performed for spectra where ^{13}C satellites present a problem. Pre-saturation of the residual solvent resonance should only be done when absolutely needed (e.g., when the signals from the compound are not digitized sufficiently and there is sufficient separation between the solvent resonance and the compound resonance of interest (~0.5 ppm or greater). The sample should not be spun. The magnet should be shimmed up well enough to obtain baseline resolution between the resonances of interest. The probe should be temperature controlled. **(i)** Transmitter offset should be in the middle of the spectrum; **(ii)** the spectral width should be 20 ppm. **(iii)** the FID size should be 64K; **(iv)** the 90 degree pulse width should be calculated using the 360 deg null; **(v)** the number

of transients should be enough to fulfill the phase cycle and enough to obtain SNRs >500 (RMS) for the resonances of interest. Usually 16 or 32 transients are enough, depending on the concentration, spectral features and field strength; **(vi)** the delay time must be sufficiently long to avoid signal saturation. The T1 Inversion Recovery experiment would be used to determine the T1 values for the resonances of interest, however, this experiment can take significant time which may be too long for a high through-put laboratory. Thus, one could use a 60 sec delay time (D1) which would be long enough for the vast majority of compounds. **(vii)** Two dummy scans should be used.

5. Acquiring the data. Once the parameters are set, the FID should be collected. The experiment should be run locked using the deuterated solvent.

6. Processing the data. (i) The FID should be zero-filled one time. If high SNRs are obtained, the FID should not be apodized. A 0.1-0.3 Hz exponential decay can be used to apodize FIDs with lower SNRs. A greater amount of line broadening can be used as long as the line widths of the reference standard and compound are similar and that the resonances remain baseline-resolved; **(ii)** The FID should be Fourier transformed. The phasing should be done manually on a vertically-expanded spectrum; **(iii)** The baseline should be flattened using the spectrometer software. There should be zero intensity for integrals that cover parts of the spectrum which do not contain any resonances; **(iv)** The integrals should be cut such that either ^{13}C satellites are always included or always not included (assuming these are present in the spectrum). **(v)** The width of the integrals should be such that no signal intensity is lost (i.e., the outer portions of the integral should be completely flat); **(vi)** If the appropriate resonances are not baseline-resolved, then other methods to obtain integrals must be used.

Kristie Adams

1. Sample Preparation: (i) Weigh (±0.01 mg) sample directly into a quality NMR tube; record exact weights, volumes and dilution factors to ensure traceability. Document balance used and measurement accuracy. **(ii)** Select solvent appropriately, use a standard sample height. Record spectrum for solvent blank. **(iii)** Use a certified, traceable, well-characterized calibrant. Confirm purity of the calibrant using an additional method. Document everything.

2. Instrument hardware and data acquisition: (i) Ensure that the system is appropriate for use (system suitability), probe has been tuned and matched. Record probe type; **(ii)** Use 'single pulse' pulse sequence for data acquisition. Calibrate 90° pulse for sample. Run non-spinning. Ensure sample temperature is equilibrated and controlled ± 0.1 K before; **(iii)** Use appropriate relaxation delay, acquisition time, spectral window and transmitter offset. Acquire at least 64K data points, zero-fill to at least twice the number of data points.

3. Quantitative Data Processing: (i) Apply apodization functions (line broadening, exponential), baseline correction (polynomial with manual adjustment), phase manually, reference; **(ii)** Be sure of signal assignments. Choose appropriate (distinct, non-overlapping) signals for integration. Use a replicable method for integration. Correct integral areas for signal overlap as necessary; **(iii)** Make sure to use correct formulas for calculations.

6.2 Contributed Tabulated qHNMR Parameters

Takako Suematsu

Jeol Standard qHNMR Parameters

Parameter	1H-NMR Condition*	Quantitative Condition**	
Repetition Time* **	**7 sec**	**> T_1 × 7**	→ Theoretical improvement
Flip Angle	45°	90°	Improving accuracy of data processing
Number of Scan****	8	> S/N 200	
Resolution	approx. 0.45Hz	< 0.25 Hz	
Sample spinning	ON	OFF	
^{13}C Decoupling	OFF	ON	

* Default parameter setting on JEOL NMR instruments
** This condition was employed in Japanese Pharmacopoeia.
*** Acquisition time + relaxation delay = repetition time
**** In case of quantification, SNR of 100-200 is desirable

Yukihiro Goda

Description of JP is as follows:
Apparatus: An apparatus of nuclear magnetic resonance spectrum measurement having ^{1}H resonance frequency of not less than 400 MHz.
Target nucleus: ^{1}H. Digital resolution: 0.25 Hz or lower. (We normally use 0.25Hz)
Measuring spectrum range: 20 ppm or upper, including between -5 ppm and 15 ppm. (We normally use from -5ppm to 15ppm)
Spinning: off. Pulse angle: 90°. ^{13}C decoupling: on. Delay time: Repeating pulse waiting time is not less than 60 seconds. (We normally use 60 seconds as delay time.)
Integrating times: 8 or more times. Dummy scanning: 2 or more times.
Measuring temperature: A constant temperature between 20°C and 30°C.
We made 5 samples, meaning 5 independent weighings. Then we perform 3 independent qNMR measurements with each sample tube. Then, we use average value as purity.

Guido Pauli

Journal of Medicinal Chemistry *57*: 9220-9231 (**2014**);
dx.doi.org/10.1021/jm500734a

■ EXPERIMENTAL PARAMETERS & WORKFLOW

A. Sample Preparation

Samples are weighed (0.01 mg accuracy recommended) into 5-mm or 3-mm standard NMR tubes. If the nature of the sample precludes this approach, alternative methods of delivering the sample to the NMR tube (e.g., stock solutions) are suitable as long as the sample mass can be determined accurately. To facilitate shimming, a preset volume of solvent (see recommendations below) is added to achieve a constant solvent height, matched to or centered on the probe coil. To minimize evaporation and prevent moisture pickup, the tubes may be either sealed with a torch, or capped and wrapped with PTFE tape and subsequently with paraffin tape.

	5-mm tubes	3-mm tubes
Solvent Volume	600 µL	170 µL
Weight of Sample (for an approximate MW of 500 amu)	4 – 12 mg	2 – 6 mg

B. NMR Instrument/Software Controlled Parameters

Pulse Program: Single pulse, without carbon decoupling ('s2pul' [Agilent/Varian]; 'zg' with 90° pulse [Bruker]; "single pulse" [Jeol])

Spinning status: Non-spinning

Sample Temperature: 25 °C (298 K, regulated ± 0.1 K)

Acquired *Data Points*: 64 K[a]

Zero-Filling (SI or FN): To 256 K data points

Dummy scans: 4

Scans (NS or NT): The number of scans (transients) to be used depends on: (i) the sample mass and molecular weight (see A.); (ii) the type of probe [direct or indirect ^{1}H detection]; room temperature [RT] or cryogenic probe [CP]); (iii) the field strength, and (iv) the pulse width. The table summarizes recommended general conditions.

Pulse Width (P1 or PW)	90°		10°	
	RT	CP	RT	CP
Relaxation delay (D1)	60 s		0 s	
Acquisition time (AQ or AT)[a]	4 s		4 s	
Spectral Window (SW)[a,b]	~30 ppm		~30 ppm	
Transmitter Offset	7.5 ppm		7.5 ppm	
Number of Scans/Transients for 300–600 MHz	64	16	512	64
for 700 MHz and above	32	8	256	32

[a]At any given magnetic field, only two of the three parameters (data points, acquisition time, and spectral window) are independent. Their combination should be chosen to match the listed values as follows: acquisition time to match most closely; spectral window to be >~25ppm, acquired data points to be adjusted accordingly.

[b]This recommendation facilitates the achievement of a flat baseline. Smaller spectral windows can be employed provided that related parameters are adjusted accordingly.

The number of scans can be appropriately adjusted depending on factors (i)-(iii). For mass limited samples and molecules with significantly different molecular weights (e.g., <300 or >700 amu), the sensitivity of the measurement should be adjusted based on the molarity ratio, considering that the sensitivity is proportional to the square root of the relative number of scans/transients.

C. Hardware dependent parameters

Preacquisition Delay: Varies with instrument and probe (alpha [Agilent/Varian]; DE [Bruker]; delay [Jeol]); document the probe model and the preacquisition delay used.

90° pulse width: The value (P1 [Bruker]; PW(90) [Agilent/Varian]; pulse [Jeol]) depends on the instrument, probe, and NMR solvent. It should be calibrated and documented. The 90° pulse width can be calibrated by determination of the 360° pulse on the sample.

Tuning: The probe's frequency tune and impedance match must be optimized. Document that tuning and matching were performed.

Temperature: The probe temperature should be regulated within <0.1°C and documented.

D. Post-Acquisition Processing and Measurement of Integrals

The processing of 1D NMR data routinely uses some line broadening (LB) as an apodization (weighing) function, together with zero-filling (256 K). This can be used for qHNMR quantification as well. Application of Lorentzian-Gaussian (LB + GB) apodization together with zero-filling (to 256 K data points) may also be applied. Recommended values for these two processing conditions in qHNMR are as follows:

Processing Using Line Broadening: LB = 0.1 Hz

Processing Using Lorentzian-Gaussian: LB = –0.3 Hz, GB = 0.05

Zero Filling: To 256K real data points

Phasing: Manual phasing

Baseline correction: 5th order polynomial with manual adjustment as needed

The signals of interest to be used for the quantification are selected, integrated (quantitative measure), and both values (integral value and range [ppm/ppb]) documented for all the signals used for quantification.