April 2024 NMR Topic of the Month: ACS qNMR

Does the ACS have guidelines for doing qNMR?

Why yes, it does. In addition to all of the spectroscopic requirements mentioned last month, there are additional conditions that must be met for a ^{1}H experiment to be considered quantitative. To aid researchers the *Journal of Medicinal Chemistry* (and similar) have a set of general guidelines taken largely from a paper by G.F. Pauli, *et alia* (2) for the purposes of determining purity.

What are these guidelines?

The journal has taken conditions and parameters from the aforementioned paper, but sometimes they do not match up. For example, the guidelines ask for a spectral width of 30 ppm acquired with 4 seconds of acquisition time divided into 64k data points, but that simply isn't possible as 64k data points makes up only about 2.75 seconds of acquisition time with a dwell time that corresponds to 30 ppm. What's important is to make the 4 second mark, so the guidelines are interpreted in this way. Besides, with zero-filling the mismatch in number of points will be largely irrelevant.

A. Sample Preparation
   Samples in 5 mm tubes should have a target concentration between 13-40 mM and be 600 μL. Samples in 3 mm tubes should have a target concentration between 24-71 mM and be 170 μL. Samples should be either flame sealed or capped and wrapped with PTFE and paraffin tapes. Do not forget to include an internal calibrant!
B. NMR Spectrum Setup
   The only (easily) acceptable sequence is 90° single pulse-acquire without decoupling. Using this sequence, samples should be run at 25℃ with 4 steady-state scans, 4 seconds of acquisition time, a carrier at 7.5 ppm, a spectral width of 30 ppm, and a relaxation time of 60 seconds. If the spectrum is collected on a room temperature probe, a total of 64 transients should be collected and will require *circa* 85 minutes to acquire. If a cold probe is used, a total of 16 transients should be collected and will require *circa* 25 minutes to acquire.
C. NMR Hardware Setup
   There are a significant number of parameters that should be calibrated and documented. The pre-acquisition delay, 90° pulse parameters, tuning characteristics, and temperature regulation amongst them.
D. Processing and Integration
   There are only two approved types of apodization: exponential (lb=0.1 Hz) and Lorentzian-Gaussian (lb=-0.3 Hz, gb=0.05). The data should be zero-filled to 256k real points. Phasing may be adjusted manually, but any baseline correction should only be done with a 5th order polynomial.
E. Purity and Quantification Calculations
   In general, you must be able to integrate the internal calibrant, contaminants, and target(s) accurately. This is not necessarily trivial, review the requirements noted in our May 2022 Topic of the Month: Quantitative NMR.

There are some allowances for sample (eg: there is a 10° pulse version) and instrumentation conditions, but these are the base rules.

Why are there so many rules for qNMR?

Mostly because far too many people labor under the delusion that every ^{1}H NMR is quantitative, and that simply isn't so. Again, consider our May 2022 Topic of the Month: Quantitative NMR brief, to make good quantitative measurements by NMR is not easy.

Why can't I just use the solvent as the internal calibrant?

The solvent does have known purity and deuteration percentages. So provided the solvent was used immediately from an ampule under proper conditions, you could use the solvent peak. Provided, of course, that it has a separately integrable signal that has fully relaxed between acquisitions, which could well not be the case.

Do I have to integrate everything?

No. But you have to integrate everything that you *can* properly integrate that is due to target, contaminant, or calibrant. Then all of those numbers should all be used to determine the average results for each source.

How challenging are these guidelines?

Most of these rules just require planning and attention to meet. The journal even provides a step-by-step workflow section and examples in their release.

A. Sample Preparation
   The concentrations detailed above are for the quantitative target, so make enough and prepare the sample properly. As noted in last month's Topic of the Month: ACS NMR, reviewers can quickly qualitatively double-check you by comparing the target and solvent peaks.
B. NMR Spectrum Setup
   The best way to be sure you are complying with all the qNMR requirements here is to run the system manually.
C. NMR Hardware Setup
   Many of these parameters have been set up for you and are documented, just ask the NMR staff for help. But the 90° pulse must be calibrated using your sample. The value in the probe file/prosol should be very close, but the requirements mandate that it be verified using your sample.
D. Processing and Integration
   Follow the rules and you'll be alright. If in doubt: do less, not more to the data. If you cannot comply with the requirements fully, you need to be honest about it and adjust your claims/errors appropriately. For example: the qNMR acquisition may not use broadband decoupling, so you may need to integrate the ^{13}C satellites. But if there just isn't enough frequency space to accommodate the 25 times linewidth rule you cannot. In a concentration calculation not including the ^{13}C satellites could cost the accuracy up to 1.1%, depending on the sample.
E. Purity and Quantification Calculations
   There are well-known equations for these results. What you need to do is see to it that you can determine all the variables with minimal headache. For example: choose an internal calibrant that is accurate, to scale with your target, and separated in frequency from your other signals.

The NMR staff can help you with all but the first guideline, but it is without a doubt the most important. There's no point in attempting a quantitative NMR on a poorly made sample. And that means everything about the sample, including the tube (make sure it's new and rated for the instrument on which the experiments are done)!

Notice anything odd about the journals' examples?

Yeah, several things are not quite right in the examples.

1. The targets' concentrations are too low because (assuming they used 99.9% deuterated DMSO-d_6) the solvent peak should only be 14.1 mM, but that DMSO-d_5 signal towers over the target peaks.
2. The $DMSO_2$ internal calibrant is too concentrated for the target in example 2.
3. The integration regions are suspiciously small for the accuracies they are claiming. In the examples all the signals arising from ^{13}C couplings are avoided, which is acceptable for these samples.
4. They also failed to integrate the (prominent) water contaminant peak in the examples, but they may have excused it as being part of the solvent (which you do not have to integrate).

References

1. The American Chemical Society (www.acs.org), specifically the Author Guidelines of the *Journal of Medicinal Chemistry* (https://publish.acs.org/publish/author_guidelines?coden=jmcmar).
2. Pauli, G. F.; Chen, S.-N.; Simmler, C.; Lankin, D. C.; Gödecke, T.; Jaki, B. U.; J. Friesen, B.; McAlpine, J. B.; Napolitano, J. G.. Importance of Purity Evaluation and the Potential of Quantitative ^1H NMR as a Purity Assay. *J. Med. Chem.* 2014, **57**(22), 9220-9231.
3. May 2022's Topic of the Month: Quantitative NMR (https://nmr.tamu.edu/Tidbits.php).
4. March 2024's Topic of the Month: ACS NMR (https://nmr.tamu.edu/Tidbits.php).