

May 2022 NMR Topic of the Month: Quantitative NMR

What are the requirements for doing quantitative NMR?

The actual quantitative experiment is rarely anything more exotic than pulse-acquire, but a great deal of thought and setup is required to make a pulse-acquire experiment quantitative.

Sample Preparation and Conditions

This is mission critical, there's no point in trying to do quantitative NMR on a junk sample. This means the synthesis/purification, sample handling, sample tube, *et cetera* all has to be good. During the experiment the sample needs to be temperature regulated, and kept in as consistent an environment as possible.

Relaxation Delay

Following a pulse, the magnetization returns to equilibrium through relaxation. You need to wait after your acquisition pulse for the magnetization to recover almost entirely. The spin-lattice relaxation is the hold-up, and it is an exponential decay ($\exp[-t/T_1]$). Therefore, following a $\frac{\pi}{2}$ -pulse, to get 99% of your magnetization back you need to wait $5T_1$ as $1 - \exp[-5T_1/T_1] = 1 - \exp[-5] \approx 0.993$. This is the total time between scans, so (essentially) acquisition plus relaxation delay times, but you first need to determine the sample's T_1 's.

Excitation Profile

You really should use a full $\frac{\pi}{2}$ -pulse that is calibrated on your sample. Even then, the pulse will only be true on the carrier frequency, so you have to compensate for offset. Yes, you could use a different flip-angle, but the math, time required, and ease of optimization favors using a $\frac{\pi}{2}$ -pulse. Furthermore, the excitation profile of decoupling must be taken into consideration; as, more often than not, you will need to decouple - even a proton for natural abundance carbon couplings.

Signal-to-Noise

Use the ADC to its fullest, allowing the signal to be defined by as many bits as possible. Furthermore, you must meet quantitative signal-to-noise benchmarks, which are frequency and accuracy dependent. To meet these benchmarks you need to be well shimmed (do not spin the sample) and acquire good FID transients to achieve what you need. Remember: the signal-to-noise improves as the square-root of the number of scans - See November 2021's topic.

Processing

Most processing techniques are fair to use, but be careful when applying nonlinear frequency-dependent methods. For example, simple linear baseline correction is often essential, but higher-order baseline corrections could sabotage your results. Regardless, the spectrum must be integrated; and, ideally, each integral should be centered on a single peak and extend some 25 times the linewidth (FWHM) in both directions. An integral of this width accounts for 99.0% of the area of the peak. The integrals need to be properly phased and consistent. There are several methods for the deconvolution of peaks, should peak areas overlap: choose wisely, apply fairly, and explain fully. There is software to facilitate processing.

References

1. S.K. Bharti and R. Roy, *Trends Anal. Chem.* **35**, 5-26 (2012).
2. Sigma Aldrich, Quantitative NMR - Technical Details and TraceCERT® Certified Reference Materials, www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/marketing/global/documents/101/854/qnmr-brochure-rjo.pdf
3. L. Griffiths and A.M. Irving, *Analyst* **123**(5), 1061-1068 (1998).
4. T. Saito, S. Nakaie, M. Kinoshita, T. Ihara, S. Kinugasa, A. Nomura, T. Maeda, *Metrologia* **41**(3), 213-218 (2004).
5. E. Alvarado, Practical Guide for Quantitative 1D NMR Integration, lsa.umich.edu/content/dam/chem-assets/chem-docs/Quantitative_NMR.pdf