Nuclear Magnetic Resonance Research Resource

Performing qNMR Experiments on the AV-300

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The AV-300 NMR spectrometer has been calibrated such that ^{1}H and ^{31}P quantitative NMR (qNMR) experiments can be performed **without** the need for an internal calibration standard. This novel approach is straight-forward and robust, ultimately producing integral areas that are scaled to yield directly mM concentration units. The accuracy of the technique relies on a consistent spectrometer response between the calibration experiment and your qNMR experiment(s) and so a rigid protocol is essential. Certain NMR parameters that you may be accustomed to changing routinely may now need to stay fixed to ensure an accurate quantification. What follows is a step-by-step procedure for accurate qNMR experiments s well as several practical considerations.

qNMR Acquisition

Tips & Tricks
- Since population differences (and therefore signal areas) are a function of temperature, you might consider as part of your protocol waiting 5 minutes for thermal equilibrium to establish immediately after inserting your sample into the magnet.
- Consider using the same model of NMR tube for your qNMR studies to minimize differences in volume within the sensitive region of the coil.

Acquisition Protocol
(a) Ensure the temperature controller is on with the temperature set to 300 K (as it should be by default).
(b) Insert your sample into the magnet and lock and shim as usual.
(c) Click on NEW EXP and enter the information for your new data set. It is very important that you select "1d_qnmrH" for proton and 1d_qnmrP for phosphorus in the Experiment drop-down list.
(d) Enter the command "**atma exact**" and wait for probe tuning to complete.
(e) To calibrate your 90° pulse, perform the following:
   i. For ^{1}H qNMR only: Enter the command "**pulsecal**"
   ii. For 31P qNMR only: Enter the command "**pulsecal sn p31**"
(f) The default number of transients for both nuclei is 16. You are free to change this number to suit the signal-to-noise requirements of your sample. To do so, enter the command "**ns**" and input the desired number.
(g) The default relaxation delay is 30 s for proton and 45 s for phosphorus. Although this parameter is adjustable either up or down, changes should only be made considering available T_1 data. To change it, enter the command "**d1**" and input the desired number in seconds.
(h) DO NOT ADJUST DE, DIGMOD, PULPROG, TD, SW, AQ, or RG and DO NOT ISSUE THE COMMANDS: gpro, getprosol, rga
(i) Start the experiment by typing "zg" in the command line

qNMR Processing

Tips & Tricks
- To quantify 99% of your signal area, integrals should cover at least 20x the line width in each direction. For example, for a proton signal that is 0.5 Hz wide at half height, your integral limits should be at minimum +/- 10 Hz which corresponds to +/- 0.033 ppm.
- Remember that for protons bonded to carbon, 1.1% of your area is found in the ^{13}C satellites. If you include the satellites for one peak, include them in all (be consistent).
- Whenever possible, use singlets instead of multiplets for quantification. Avoid broad lines and resonances involved in exchange.

Please note that since the version of TopSpin installed on the spectrometer is now dated, two sets of instructions are provided here for qNMR analysis. The first set is for performing the analysis directly on the spectrometer using TopSpin 2.1. The second set applies to TopSpin 3.5.7 (academic version) on a data station.

qNMR Analysis Directly on the Spectrometer Using TopSpin 2.1
(a) When the experiment has completed, type "**ef**" in the command line to exponential multiply and Fourier transform.
(b) Accurate phasing is essential! Issue the command "**apk0**" and then increase the vertical expansion to ensure accurate phasing. Manually adjust the phasing if necessary.
(c) Also crucial is a flat baseline free of any dc offset. To correct the baseline, issue the command "**abs n**" and then examine the baseline carefully, correcting any problems.
(d) Enter the integration subroutine and Integrate the signal(s) that you wish to quantify. Click on Save and Return when done.
(e) Type the command "**quant**" which will convert all integral values to mM concentration values and by default place a synthetic 10 mM signal at -0.5 ppm. You can control the synthetic peak by using the quant command with arguments: "quant intensity position" with intensity in mM units and position in ppm.
(f) Remember that the integral values after running quant are in mM of nuclei giving rise to the corresponding signal. The concentration of your compound would then be obtained by normalizing it according to the number of nuclei.

qNMR Analysis on a Data Station Using the Academic Version of TopSpin 3.5.7
In order to perform the qNMR analysis on your data station, you must have a copy of the relevant qNMR calibration experiment. I have placed copies of the most recent experiments on the NMR[3] server. You can find these under the nmr3_user Documents folder in "…/nmrdata/qNMR Calibrations AV300". Copy these to your local folder where you store NMR data and then proceed as follows:

(a) Perform items (a), (b), (c) and (d) as for the TopSpin 2.1 protocol.
(b) Within the integration subroutine, once you have integrated all the signals of interest, select them all by clicking the Select/Deselect All Regions icon (see the red arrow in the Figure on the right).
(c) With your mouse hovering over any of the selected integrals, right-click and from the context-sensitive menu that opens, choose Eretic → Calculate Concentration.
(d) A table entitled "Eretic2 Quantification" opens as shown in the Figure below. Some of the entries in this table are optional but others are essential. It is essential that the "Reference Dataset" entry specify the calibration dataset you copied from the NMR[3] server. If it does not, click on the … and browse for the "eretic" file stored under the pdata folder. Also, it is crucial that the column entitled "Number of
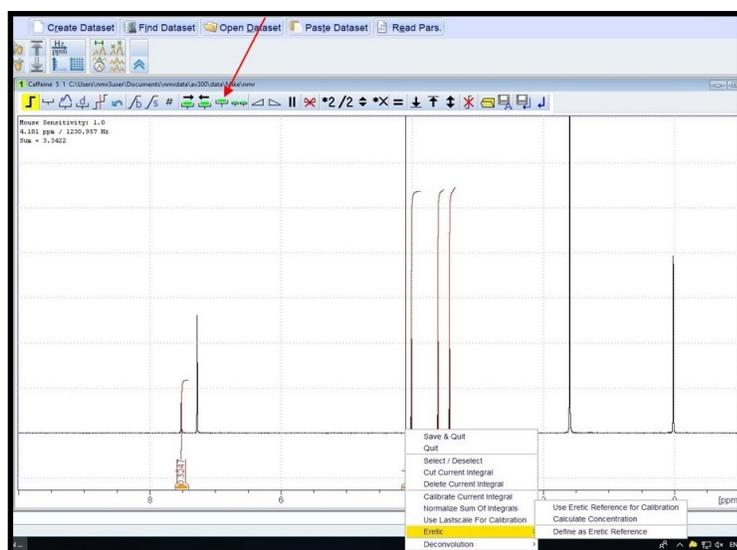
Atoms' is accurate. For each integral that you defined, enter in this column the number of nuclei that the integral corresponds to (i.e. specify 3 for a CH_3 proton integral). Optionally, you can populate the "Molecule Name" column, the "Molar Mass" column, as well as the Sample Volume field. Click ok.

(e) After clicking ok, the concentration results appear directly in the spectrum along with the average result in the upper right of the display. If you specified a volume and a molar mass, the mass of the compound is your NMR tube is also specified.

Optional Step: Including a Synthetic Signal in Your Spectrum: Although not necessary for the method to work, it is possible to include a synthetic quantitative signal into your NMR spectrum. To do so, select the ProcPars tab and then click on the **E** button. From here, you specify EPOS [ppm] for the position of your synthetic signal and ELW [Hz] for the width. Then click on the **A** button to add the synthetic signal. It should be mentioned that the integral value of this signal is weighted by the P90, NS and RG ratios between your quantified spectrum and the calibration spectrum.