

Measuring Concentrations of Samples in Protonated Solvents

You can use NMR to determine the concentration of a solute using the ERETIC2 protocol (**HQ** = ^1H quantitation). While less common, you can also do this with an unlocked spectrometer in a protonated solvent (**noD** NMR) while using the large ^1H signal of the solvent for shimming. This **noD-HQ** technique allows you to estimate concentrations of solutes in protonated solvents as long as there is at least one easily identifiable peak that is well separated from the protonated solvent peaks.

Precise NMR Method for Titering Organometal Reagents, Jakhar, et. al., Org. Lett., 2021, 23, 4945-4948; <https://doi.org/10.1021/acs.orglett.1c01006>

Instructions for Bruker

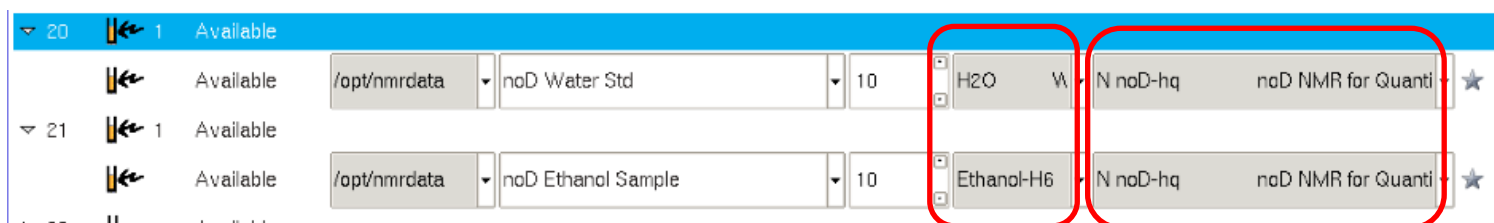
1. Sample Prep

- One standard sample of regular (protonated) DI water
- One or more samples to analyze (e.g., organometallics in protonated solvents)
- All samples must be in identical NMR tubes (i.e., style/quality, wall thickness, same manufacturer, etc.)
- All samples must have the same solvent height (e.g., use 0.60 mL)
- Note: for reactive samples, it's safer to use medium or thick walled NMR tubes instead of standard thin walled NMR tubes.

2. Collecting Data

Place samples in the sample tray, give them meaningful names, and do the following for each sample:

- Choose the correct *protonated solvent* or choose *None* if your protonated solvent isn't listed. (You can also simply choose *None* for any protonated solvent.)
- Choose the **noD-hq** protocol
- Submit your samples
- Note: for reactive samples, it's important that you remain in the lab to monitor your samples and then remove them as soon as they are finished running. It's not safe for other students to handle your samples if they contain reactive mixtures.



3. Processing Data

You must setup the water reference correctly in order for the concentration calculation to work on your other sample(s).

- First, process the data from the *water standard sample*
 - Using the auto-processing seems to work fine. However, you can process it manually, if you prefer, using the commands **efp** ↪ then **apk** ↪ then **abs** ↪
 - Note: due to clipping artifacts, there might be a small negative deviation at the base of the peak. This artifact has an area < 1% of the large H₂O peak, so it can be safely ignored.
 - Enter integration mode. The water peak should already be integrated; if not, or if you want to adjust it, integrate the *entire* water peak – make sure you include the tails of this broad peak; i.e., increasing the vertical scale can help you see where the tails of the peak end.
 - Right click on the integration region, choose *Eretic*, then choose *Define as Eretic Reference*
 - Set the *concentration* to **55410** mM (this is the molar concentration of water at 20 °C and the lab is typically ~21 °C)
 - Set the *number of atoms* to **2** (H₂O has two equiv. protons)
 - Click *OK* and then *exit and save* the integration mode.
- Next, process each of your other samples
 - Using the auto-processing seems to work fine, However, you can process it manually, if you prefer, using the commands **efp** ↪ then **apk** ↪ then **abs n** ↪ (you might need to adjust the phasing manually if apk isn't good enough).
 - Note: due to clipping artifacts, there might be a small negative deviations in the baseline; these can usually be ignore. If they happen to overlap with a peak of interest, it will decrease the accuracy of the measurement.
 - Enter integration mode and clear/delete any auto-integrated peaks.
 - Choose a peak to integrate that is well removed from any solvent peaks and that is easily identifiable (i.e., you know how many equiv. protons are at that position).
 - Note: you can integrate multiple peaks, if desired, and the values from each peak will be averaged. Methyl groups or other aliphatic protons in flexible sidechains tend to give the most accurate results due to their faster relaxation times. Rigid protons like those on aromatic rings or pi systems will typically give results that are *lower* than reality due to their longer relaxation times.
 - Right click on the integration region, choose *Eretic*, then choose *Calculate Concentration*.
 - Define the *number of atoms* for this region (or for each region that you integrated. if you integrated multiple peaks).
 - Click *OK* and you will see the concentration displayed above each integral region and an average value at the top if you integrated multiple regions.
 - After noting the concentration, *save and exit* the integration mode.