



Understand(s)
your compounds

qNMR: setting up a CCF

Mike Bernstein
VP R&D, Mestrelab Research

Intro

- You will need a spectrum of the concentration reference sample recorded on the spectrometer you will usually use
- The probe should be tuned and matched
- Use your typical acquisition conditions
 - the pulse repetition rate should not too rapid

Processing

- Zero fill once
- Window function
 - Exponential multiplication ca. 0.3 Hz
- Baseline correction (if using “sum” integrals)
 - Bernstein polynomials, Order 3
- Phase: best possible if “sum” integrals
- Auto Multiplets or Auto Assignments done

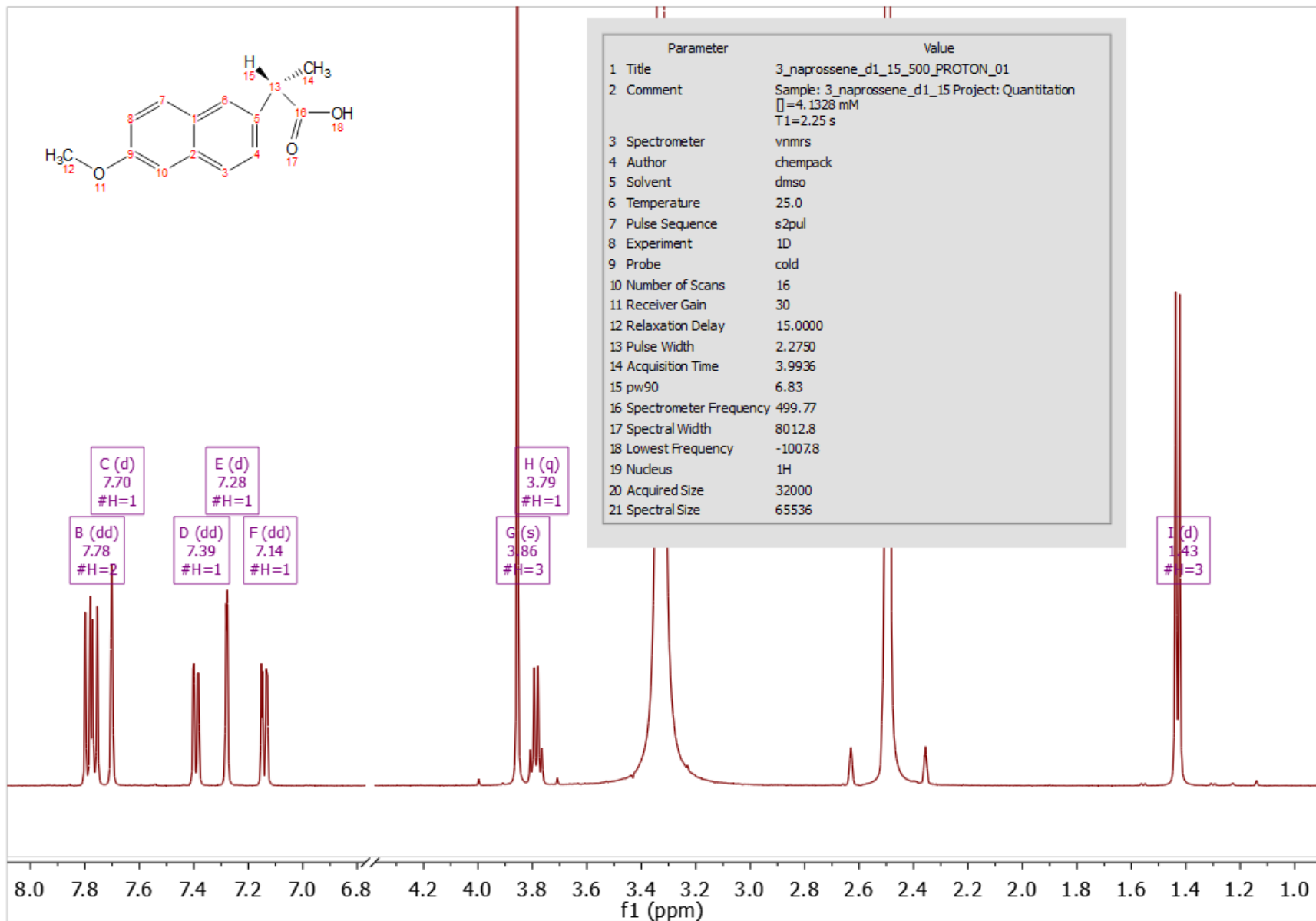
Integration method

Determine a CCF for the method you use

- “Sum”: for simpler molecules and when the most accurate results are needed
- GSD: for most cases, assuming shimming is good, etc.
- Line fitting: can improve GSD, but not good for crowded spectra

Top GSD tip: Use “High resolution” and 5 fitting cycles (Peak Picking > Options)

4.13 mM naproxen in DMSO-d6



qNMR Concentration: 1st settings

NB The CCF value at this stage is a total guess

For this example

The screenshot shows the 'Settings' dialog box with the 'Concentration' tab selected. The 'Concentration Method' is set to 'Internal Reference'. Under 'Nudides of Reference', the 'Concentration Conversion Factor' is selected, with a value of '1.0000000000' and units of 'mM'. A red box highlights the CCF value, with a red arrow pointing to it and the text 'CCF "seed" value'. A blue box highlights the 'Parameters for Reference' section, which includes fields for Receiver Gain (30.00), Number Of Scans (16), 90° Pulse (6.83), DIGMOD (0), Pulse Program (s2pul), Instrument Name (vnmrs), and Probe Name (cold). A 'Library' button is at the bottom right. The 'OK' and 'Cancel' buttons are at the bottom.

The screenshot shows the 'Integration' tab of the 'Settings' dialog box. The 'Integration Method' is set to 'Multiplet Integration Method: Peaks with GSD Peak Picking'. The 'Multiplication Factor' is set to '15'. A tip is displayed: 'Tip: GSD Peak Picking and Peaks calculation method for Multiplet Integrals are advised for accurate quantitation. These settings are available in Peak Picking Options and Multiplet Analysis Options.'

Simply...

The screenshot shows the 'Options' tab of the 'Settings' dialog box. Under 'General Options', 'Autoselected Multiplets' is set to '3' and 'Concentration Average Calculation' is set to 'Simple Average'. The 'Calculate Area Purity' checkbox is checked. Under 'Autoselection Method', 'Minimum RMSD' is selected. A note at the bottom right says 'Choose multiplets ranked using the Multiplet Au'.

Spectrometer properties

Calculating the real CCF

The screenshot shows the 'Concentration' window with a table of NMR data. The 'Concentration Average' field is highlighted with a red box and contains the value 439.5310 mM. The table below shows the following data:

	Multiplet	Autoselected	Shift	Range	Hs	Abs. Integral	Concentration	SNR
1	<input checked="" type="checkbox"/> D(dd)	1	7.39	7.42..7.36	1	441.7909	441.7909	866.59...
2	<input checked="" type="checkbox"/> E(d)	1	7.28	7.30..7.26	1	436.4301	436.4301	1392.3...
3	<input checked="" type="checkbox"/> H(q)	1	3.79	3.83..3.75	1	440.3721	440.3721	921.63...
4	<input type="checkbox"/> A(s)	0	12.31	12.33..12.29	1	372.8643	372.8643	52.3260
5	<input type="checkbox"/> B(dd)	0	7.78	7.82..7.73	2	916.8165	458.4082	1474.3...
6	<input type="checkbox"/> C(d)	0	7.70	7.72..7.68	1	420.9719	420.9719	1319.5...
7	<input type="checkbox"/> F(dd)	0	7.14	7.17..7.11	1	466.3539	466.3539	909.24...
8	<input type="checkbox"/> G(s)	0	3.86	3.88..3.84	3	1226.9292	408.9764	10049....
9	<input type="checkbox"/> I(d)	0	1.43	1.46..1.40	3	1274.3280	424.7760	3940.5...

A CCF value of 1.0 results in a concentration of 439.5 mM

Correct CCF =

$$\frac{[\text{real}]}{[\text{apparent}]} * \text{“seed value”}$$

=

$$4.13 / 439.53 = \underline{\underline{0.0093964007}}$$

This value is inserted in the settings “Conversion Factor”

“Autoset” to calculate CCF

Using the spectrum of your concentration reference...

The screenshot shows the 'Settings' dialog box with the 'Concentration' tab selected. The 'Concentration Method' is set to 'Concentration Conversion Factor'. The 'Conversion Factor' is set to 0.0008420000 mM. A 'CCF Setup' dialog box is overlaid on top, with the following fields:

- Weighted Mass: 0
- Mol. Weight: 0
- Multiplet: nicotinic acid_1(dd)
- PL: 1

The 'OK' button in the 'CCF Setup' dialog is highlighted with a red box. A red arrow points from the 'Autoset' button in the main dialog to the 'CCF Setup' dialog, and another red arrow points from the 'OK' button in the 'CCF Setup' dialog back to the 'Autoset' button in the main dialog. The 'Autoset' button in the main dialog is also highlighted with a red box.

Checking it works

Concentration Conversion Factor

Conversion Factor: mM

The corrected OCF gives the correct concentration

Concentration

Paste Report Settings Autoselection Refresh

Concentration Average mM

RMSD(%):

Area %qNMR:

Line Fitting

	Multiplet	Autoselected	Shift	Range	Hs	Abs. Integral	Concentration	SNR
1	<input checked="" type="checkbox"/> D(dd)	1	7.39	7.42..7.36	1	441.7909	4.1512	866.59...
2	<input checked="" type="checkbox"/> E(d)	1	7.28	7.30..7.26	1	436.4301	4.1009	1392.3...
3	<input checked="" type="checkbox"/> H(q)	1	3.79	3.83..3.75	1	440.3721	4.1379	921.63...
4	<input type="checkbox"/> A(s)	0	12.31	12.33..12.29	1	372.8643	3.5036	52.3260
5	<input type="checkbox"/> B(dd)	0	7.78	7.82..7.73	2	916.8165	4.3074	1474.3...
6	<input type="checkbox"/> C(d)	0	7.70	7.72..7.68	1	420.9719	3.9556	1319.5...
7	<input type="checkbox"/> F(dd)	0	7.14	7.17..7.11	1	466.3539	4.3820	909.24...
8	<input type="checkbox"/> G(s)	0	3.86	3.88..3.84	3	1226.9292	3.8429	10049....
9	<input type="checkbox"/> I(d)	0	1.43	1.46..1.40	3	1274.3280	3.9914	3940.5...

Alerts

Saving the correct values to the Library

The screenshot shows the 'CCF Library' dialog box. The 'Parameters' section contains the following fields:

- Name: Naproxen
- Conversion Factor: 0.0093964007
- Receiver Gain for Reference: 30.00
- Number of Scans: 16
- 90° Pulse for Reference: 2.28
- Digmod: 0
- Pulse Program for Reference: s2pul
- Instrument Name: vnmrs
- Probe Name: cold

The 'Notes' section contains the text 'GSD integration'. Below the notes is a toolbar with icons for adding, deleting, and saving items. A mouse cursor is hovering over the 'Add item' icon (a blue plus sign).

Name	Conv. Factor	Receiver Gain	N. of Scans	90° Pulse	Digmod	Pulse Prog.	Instru
GSK sucrose	0.00265	11	4	8	1	zg	pt600
GSK_acetan...	0.000842	64	32	12	1	zg30	ag400
Naprossene	0.00453	30	16	0	0	s2pul	vnmrs

Buttons: OK, Cancel

1. Click to add to the table
2. Save the updated table

Last considerations

- You may want to repeat this “calibration” for your spectrometer from time-to-time
- Some may prefer to make 3 or more concentration standards to get a more accurate OCF, and to test the values
- Samples and spectra can be measured in multiples for higher accuracy and precision