

NMR Training Course

9th September 2021
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quantitative NMR

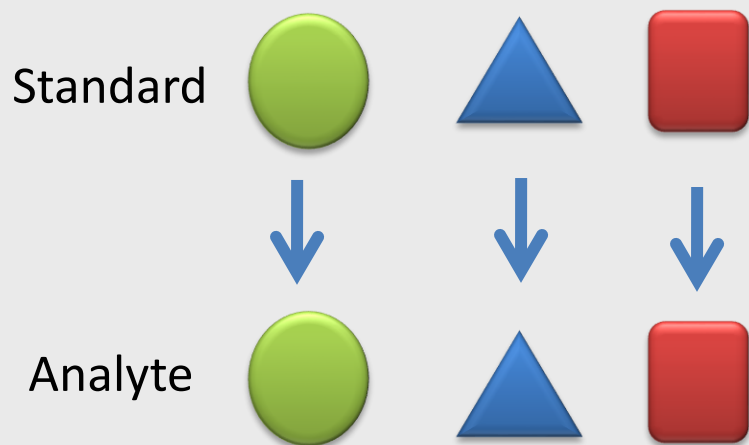
Quantitative analysis by chromatography and NMR

Chromatography

Detects molecules

Detection by UV/VIS absorbance, refractive index, fluorescence, etc.

Object = Molecule



- ◆ Requires **specific reference material**
- ◆ The instrument must be calibrated by recording calibration curve

DETECTION

STANDARD

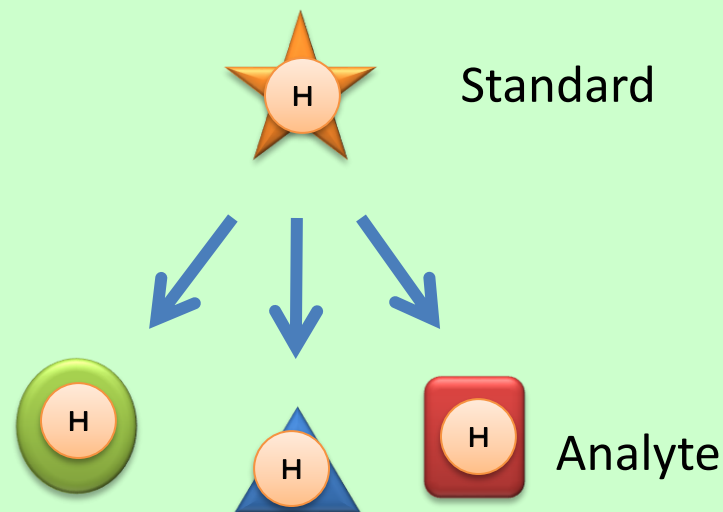
FEATURES

NMR spectroscopy

Detects NMR-active nuclei

One molecule is usually characterized by several (many) peaks

Object = Nucleus (^1H , ^{19}F)

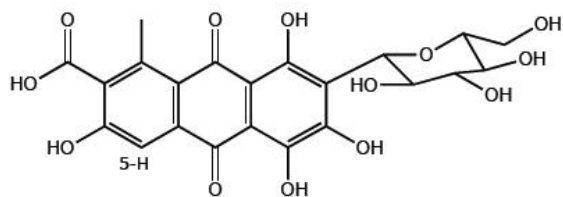


- ◆ **Standard is non-specific**
- ◆ Most qNMR methods do not use calibration curve

Case study

Figure1

Comparison of Commercial Carminic Acid Reagents (results)



carminic acid (CA)

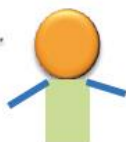
Cochineal extract:

A red pigment obtained by drying scale insects (superfamily Coccoidea) and extracting with water or ethanol. The main component is carminic acid.

No SI- traceable reference material for quantitative analysis exists

Grade	Maker	Catalog purity	qNMR purity (%) : as potassium salt trihydrate
(Reagent)	A	>70%(HPLC)	25.3%
(Reagent)	B	>95% (Spectrophotometric)	92.9%
(Reagent)	C	Not listed	81.1%
(Reagent)	D	Not listed	80.8%
(Reagent)	E	95%	91.6%
(Reagent)	F	70~90%	86.5%

I'll use A



Mr. A



Analyte



Ms. B

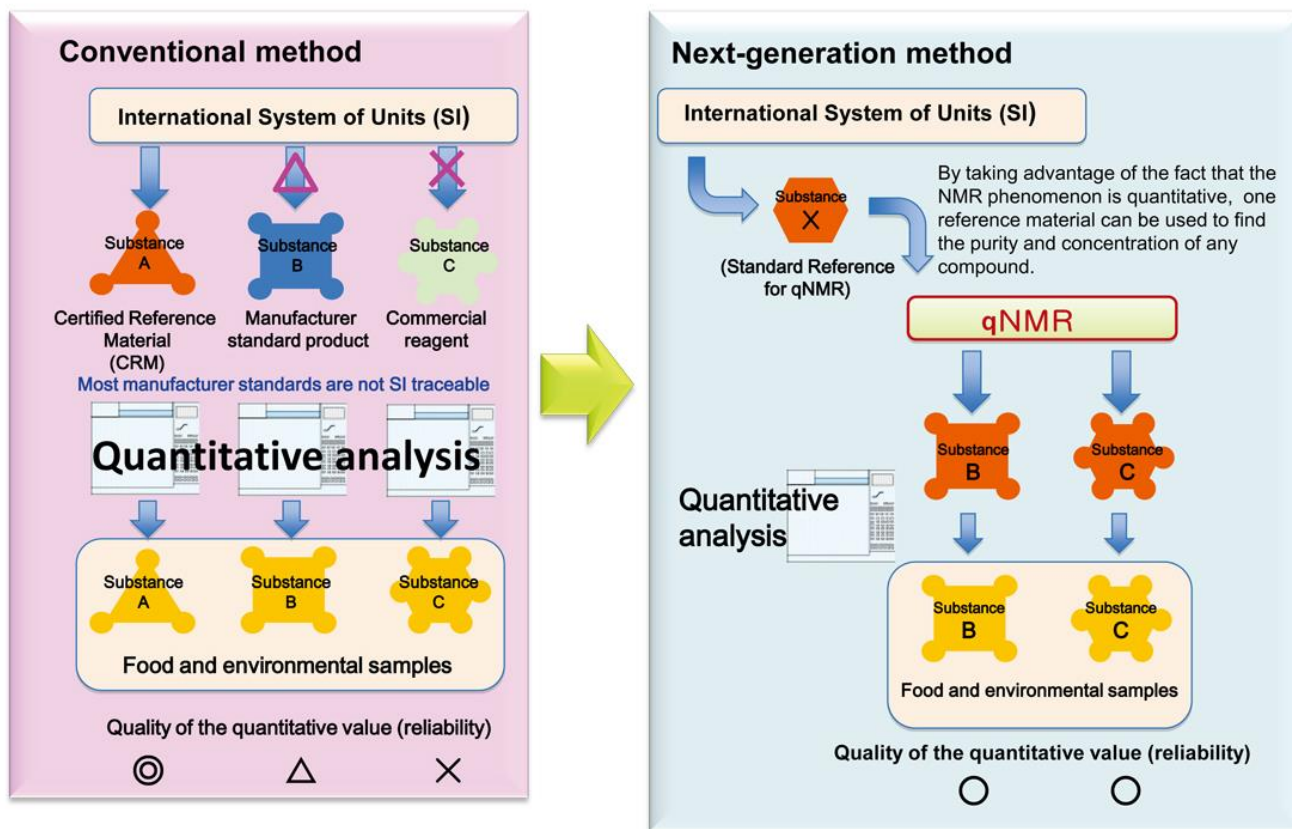
I'll use F



Correction
can be applied

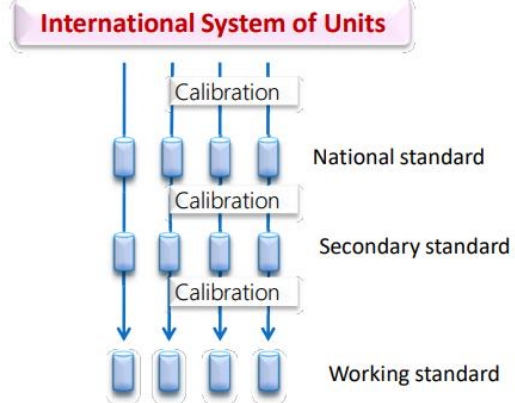
Traceability

Figure2
Future Image of Quantitative Analysis of Organic Compounds



Metrological Traceability

In the case of a reference material



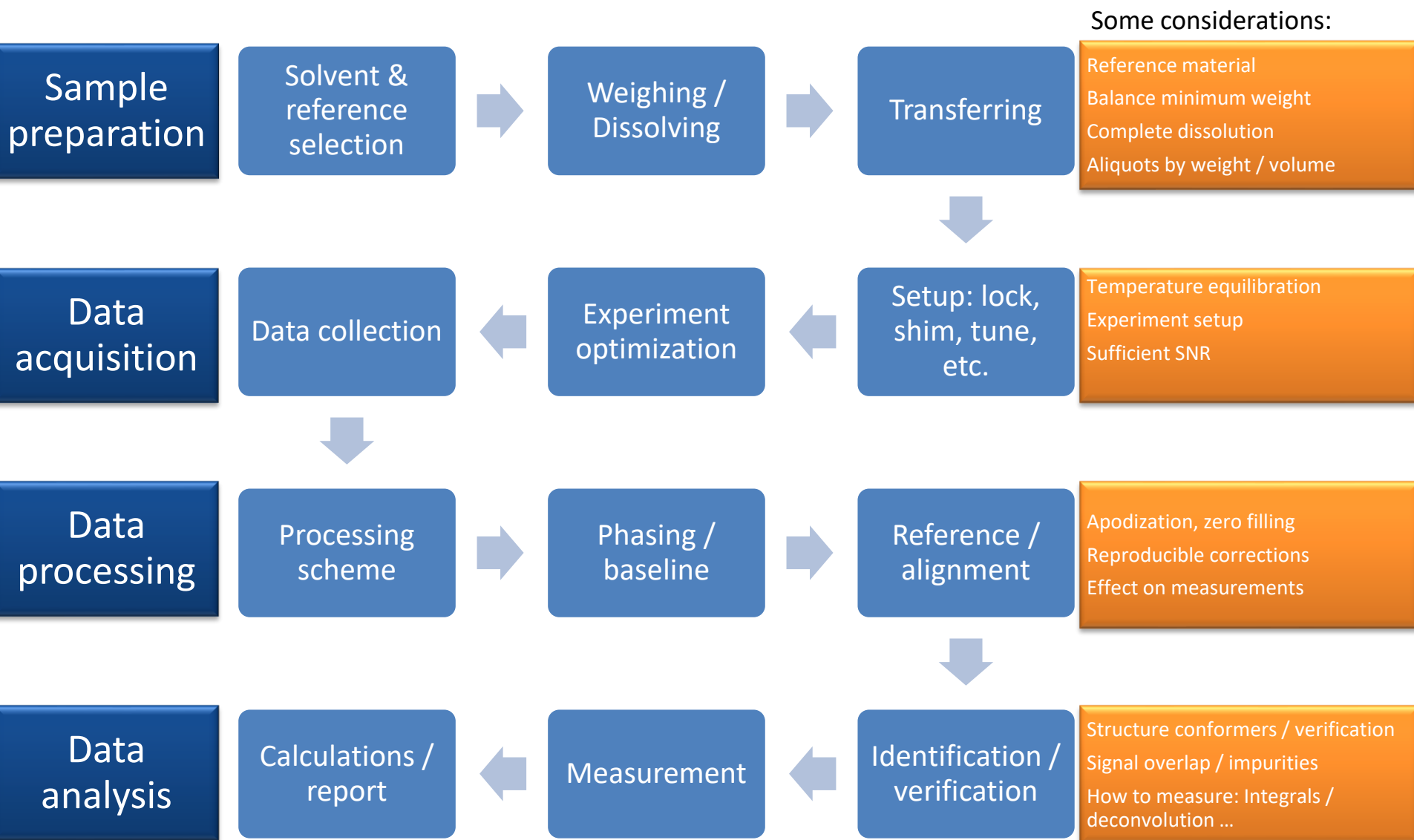
Measurement results can be traced back to a reference standard (usually, a national standard material) through an unbroken chain of calibrations.

https://www.jeol.co.jp/en/products/nmr/qnmr_nl/qnmr_issue007.html

https://www.jeol.co.jp/en/products/nmr/qnmr_nl/qnmr_issue005.html

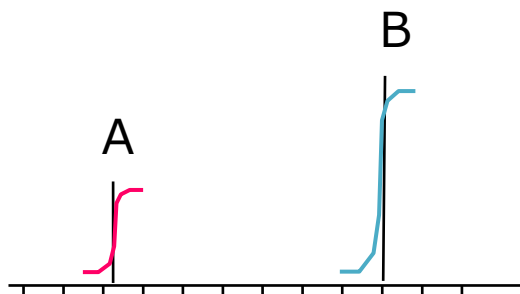
<https://www.sigmaaldrich.com/MA/fr/applications/analytical-chemistry/calibration-qualification-and-validation>

What is involved in qNMR?



Quantitative analysis by NMR spectroscopy

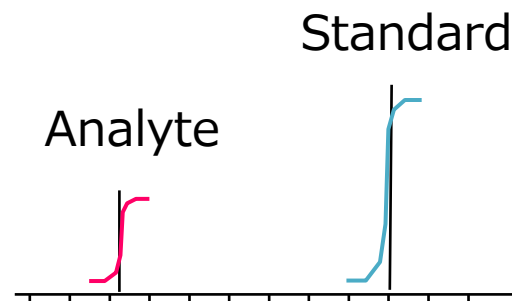
Relative quantification



Q: What is the **ratio** between signal A and signal B in my sample?

Signals A and B may represent two signals in one compound or two signals in two compounds

Absolute quantification



Q: What is the **concentration/amount** of analyte?

Standard compound of known concentration/amount is added

Relative quantification in NMR spectroscopy

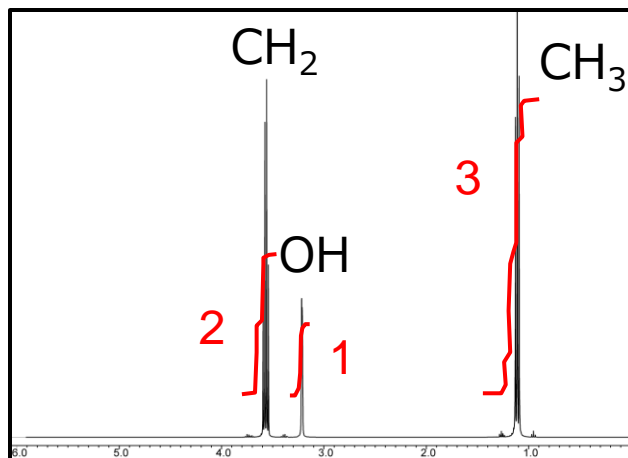
The proportionality between the integral intensity I and the number of protons N under quantitative conditions:

$$\frac{I_i}{I_j} = \frac{N_i}{N_j}$$

I : Integral intensity

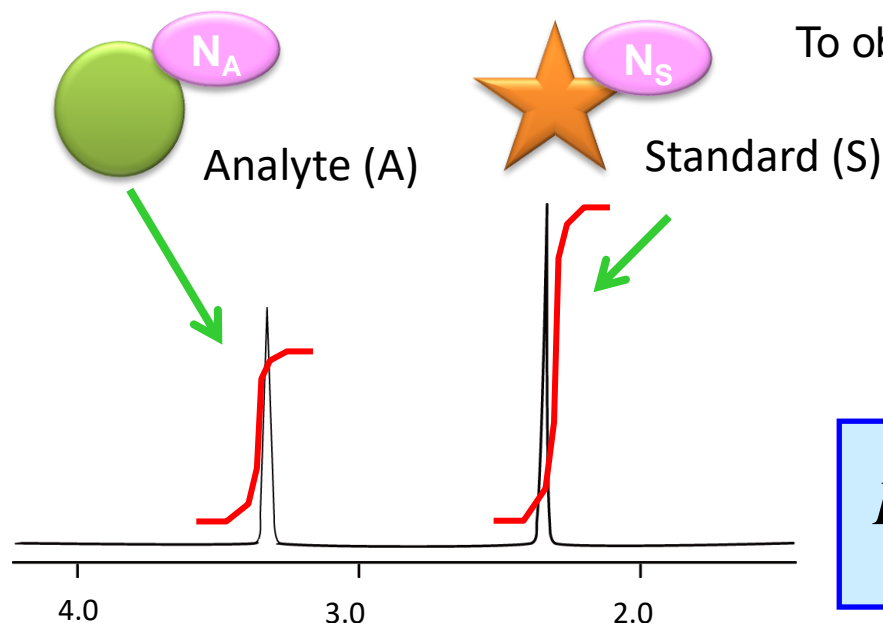
N : Number of protons

^1H spectrum of ethanol



Absolute quantification in NMR spectroscopy

Suitable for purity evaluation of reference substances



To obtain concentration:

$$c_A = \frac{N_S c_S I_A}{N_A I_S}$$

I : Integral intensity
N : Number of protons
c : Molar concentration
m : Weight
M : Molecular mass
P : Purity
A : Analyte
S : Standard

To obtain purity ***P*** of analyte A:

$$P_A = \frac{I_A}{I_S} \times \frac{N_S}{N_A} \times \frac{m_S}{m_A} \times \frac{M_A}{M_S} \times P_S$$

- ◆ qNMR does not use reference substance identical with analyte
- ◆ qNMR can measure concentration in ***mole*** and determine purity as mass fraction

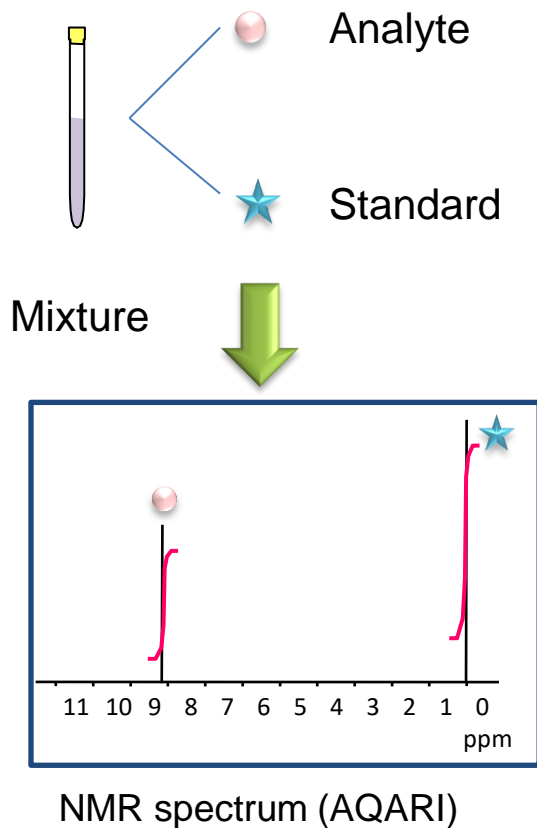
qNMR is qualified as a primary method under the following conditions:

- proper operation
- SI-traceable reference substance (CRM)



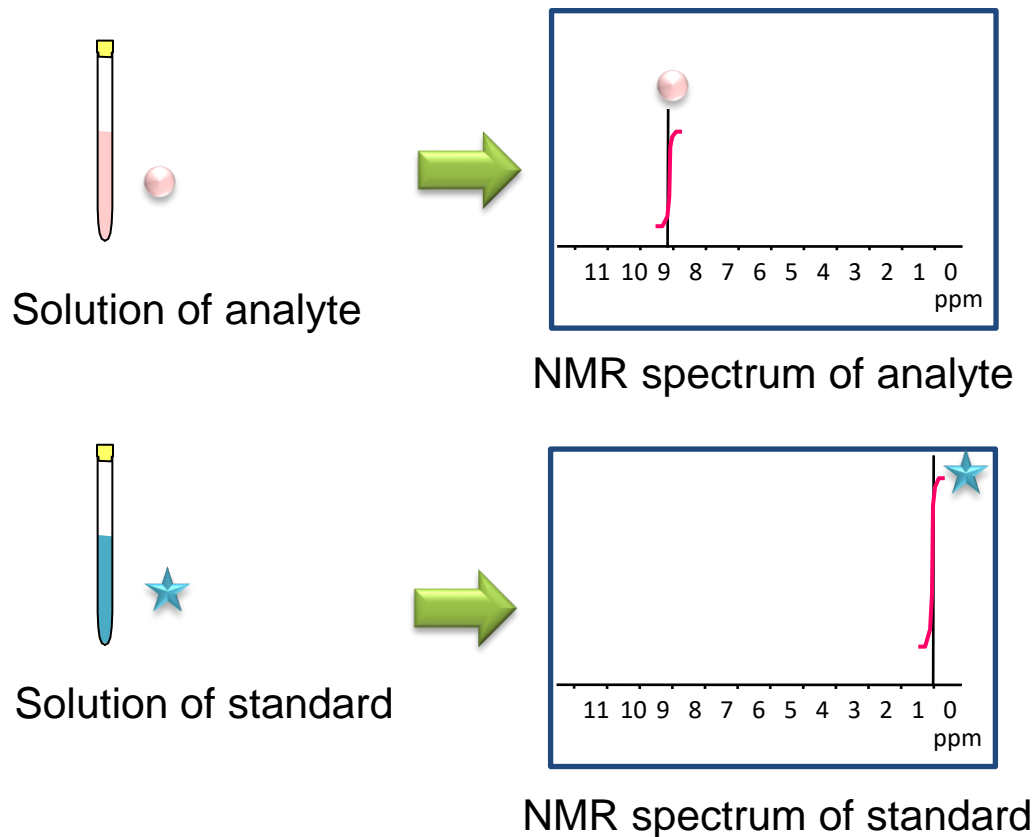
qNMR methods using internal and external standard

Internal standard method



One NMR sample
Comparison within one spectrum

External standard method (PULCON)



Two NMR samples
Comparison between two spectra

Internal vs external quantification

qNMR

Sample preparation

NMR measurement

Processing/Analysis/
Confirmation

Preparation of the balance
Measure the minimum weight of the balance.

Preparation of the balance
Measure the minimum weight of the balance.

Preparation of sample for quantitative NMR
Weigh out the reference material and the analyte using a micro- or ultramicro-balance. Completely dissolve both the reference material and the sample in the selected solvent. As a basic procedure, make 3 samples for each lot.

Preparation of sample for quantitative NMR
Separately weigh out the reference material and the analyte using a micro- or ultramicro-balance. Completely dissolve the reference material in a solvent to make the reference solution. Completely dissolve the sample in a solvent to make the sample solution.

Quantitative NMR measurement
Set the sample solution and perform quantitative measurements. As a basic procedure, make measurements 3 times for each of the 3 samples, non-consecutively. (total of 9 data acquisitions)

Calibration
Set the reference solution and specify the temperature. Then, pulse width setting and measurement using the qNMR conditions is performed. If necessary, create a calibration curve.

Processing
The acquired data is processed and the integrated values of the target signals are extracted.

Quantitative NMR measurement
Set the sample solution and specify the temperature. Then, pulse width setting and measurement using the qNMR conditions is performed.

Analysis
The integrated values and weighted values are loaded into the calculation program and the purity and concentration are calculated.

Processing
The acquired data is processed and the integrated values of the target signals are extracted.

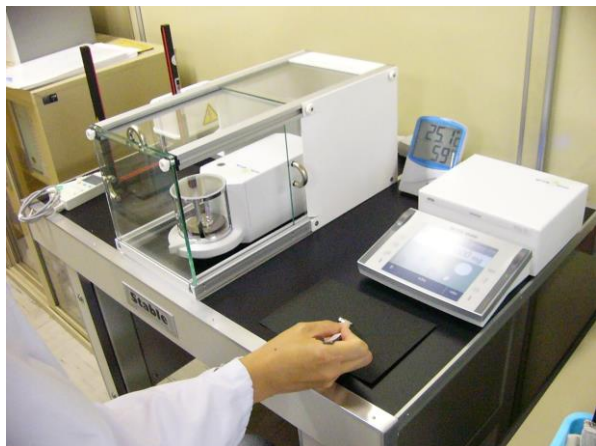
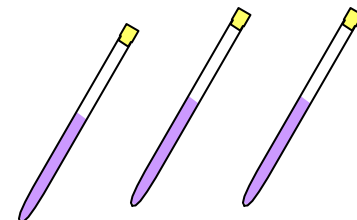
Confirmation
Check the data and results using various software programs.

Analysis
The integrated values and weighted values are loaded into the calculation program and the purity and concentration are calculated.

Confirmation
Check the data and results using various software programs.

Accurate and precise weighing

To ensure high accuracy and precision, the analyte and standard are weighed on an ultra-microbalance. The definition of minimum sample weight (W_{\min}) has been adopted from the United States Pharmacopeia (USP). Three samples are prepared for qNMR measurement and measured in triplicates.



Ultra-microbalance Mettler Toledo XP2U

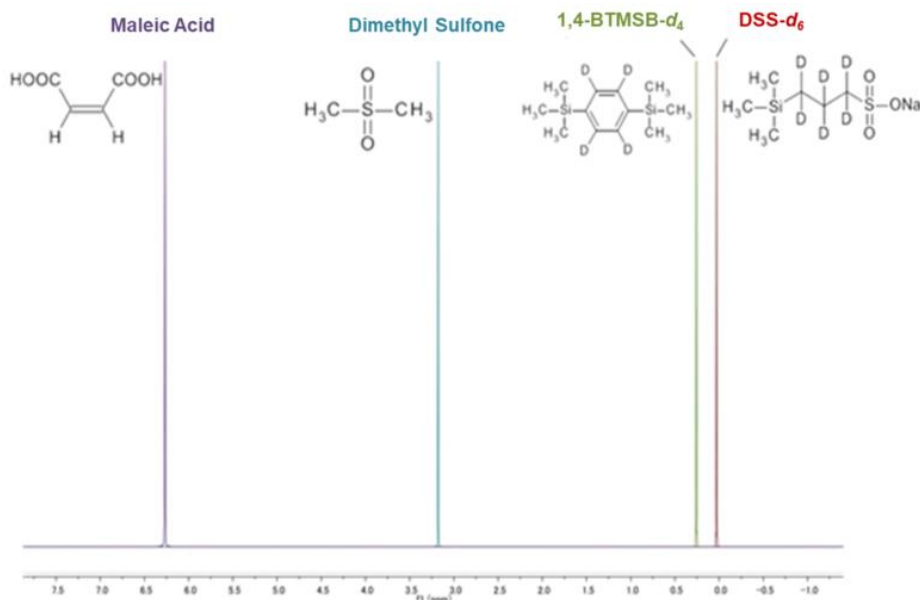


A substance in an aluminum weighing boat
in a vial

Analyte:	> 1 mg
Standard:	> 1 mg
Solvent:	1 mL

Choice of internal standard

- ✓ Stable and soluble in the solvent
- ✓ No signal overlaps with signals of the analyte and solvent
- ✓ No interactions with the analyte and solvent
- ✓ High and assured purity (CRM)



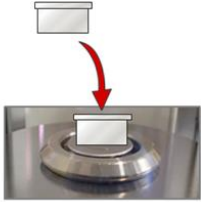
Information and CRMs:

<https://labchem-wako.fujifilm.com/europe/category/00622.html>

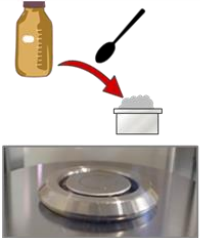
<https://www.sigmaaldrich.com/MA/fr/applications/analytical-chemistry/calibration-qualification-and-validation>

Weighing internal standard

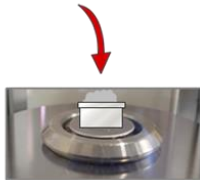
Step 1
Weigh the tare



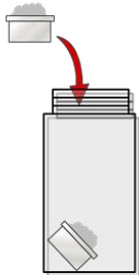
Step 2
Remove the tare from the weighing pan and put the sample into the tare with a spatula



Step 3
Weigh the tare with the sample (i.e., tare + sample)



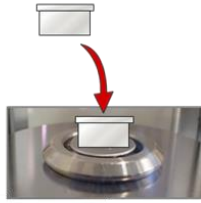
Step 4
Put tare+sample into a vial




The masses of the sample and the calibration standard are calculated by the following formula

- Sample = (tare+sample) - tare
- Calibration standard = (tare + calibration standard) - tare

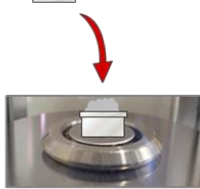
Step 5
Weigh the tare



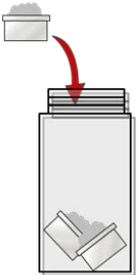
Step 6
Remove the tare from the weighing pan and put the calibration standard into the tare with a spatula



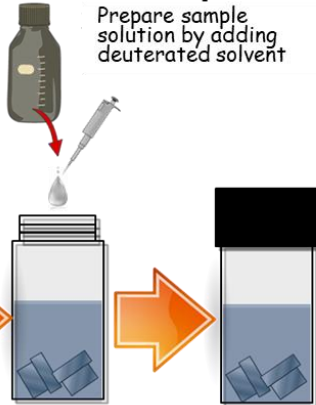
Step 7
Weigh the tare with the calibration standard (i.e., tare + calibration standard)



Step 8
Put tare+calibration standard into a vial



Step 9
Prepare sample solution by adding deuterated solvent



$$W_{\min} = \sigma \times 2000$$

W_{\min} : Minimum Weight

σ : Standard deviation calculated with ten repeated measurements



Measurement example of Minimum Weight

Type of balance	Minimum Weight (W_{\min})
Semi-micro balance (readability: 0.01 mg)	13.9 mg
Micro balance (readability: 0.001 mg)	2.8 mg
Ultra-micro balance (readability: 0.0001 mg)	0.2 mg

Volume measurements

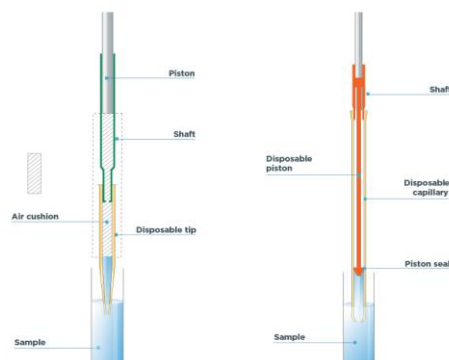
Generally pipettes are less precise than analytical balances

Consider liquids viscosity, temperature, interactions with pipette

Manual vs electronic, air displacement vs positive displacement, etc.

Air-Displacement Pipettes

- Recommended for aqueous samples and for general laboratory work.
- Always have a cushion of air (dead volume) between the pipette piston and the liquid sample.
- The piston is integrated into the lower part of the pipette.



Positive-Displacement Pipettes

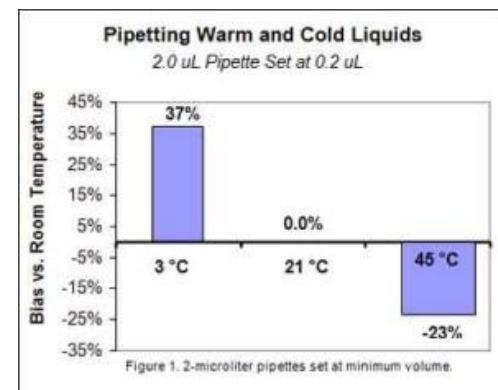
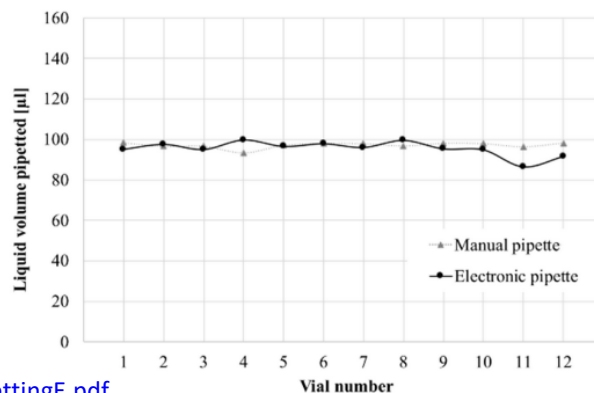
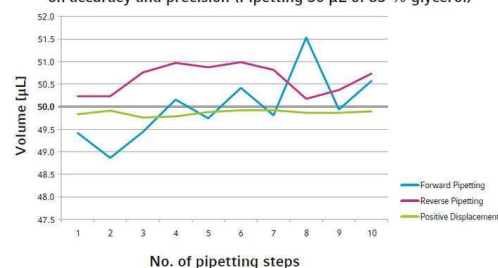
- Recommended for problem samples (viscous, dense, volatile, radioactive, corrosive, contaminating, hot and cold).
- Direct contact of the piston with the sample (no air cushion).
- The disposable piston is part of the tip (not integrated into the pipette).

Cat. No.	Volume Range (calibration)	Volume Range (functional)†	Increments	Accuracy (%)†	Precision (%)†	Color Code
46200000	1–10µL, micro	0.5–10µL	0.01µL	3.5 to 1.0±	3.0 to 0.5	Pink
46200100	1–10µL	0.5–10µL	0.01µL	7.0 to 1.0±	6.0 to 0.5	Yellow
46200200	5–50µL, micro	2.5–50µL	0.1µL	3.0 to 0.8±	2.5 to 0.3	Turquoise
46200300	5–50µL	2.5–50µL	0.1µL	3.0 to 0.8±	2.5 to 0.3	Yellow
46200400	10–100µL	5–100µL	0.1µL	3.0 to 0.8±	1.0 to 0.2	Yellow
46200500	30–300µL	15–300µL	1µL	3.0 to 0.6±	0.7 to 0.2	Orange
46200600	100–1000µL	50–1000µL	1µL	3.0 to 0.6±	0.6 to 0.2	Blue
46200700	0.5–5mL	0.25–5mL	0.01mL	3.0 to 0.6±	0.8 to 0.2	Green
46200800	1–10mL	0.5–10mL	0.01mL	3.0 to 0.6±	0.8 to 0.2	Red

†Factory calibration limits achieved under strictly controlled conditions (ISO 8655).

‡The Functional volume range indicates the volume range that the pipette can cover in the Stepper, Sequential Stepper and Sequential Aspirate functions.

Air-cushion pipette: Viscous liquids have a negative effect on accuracy and precision (Pipetting 50 µL of 85 % glycerol)



<https://gb.gilson.com/pub/media/docs/GuideToPipettingE.pdf>

<https://handling-solutions.eppendorf.com/liquid-handling/faqs/>

<https://www.eppendorf.com/MY-en/liquid-guide/>

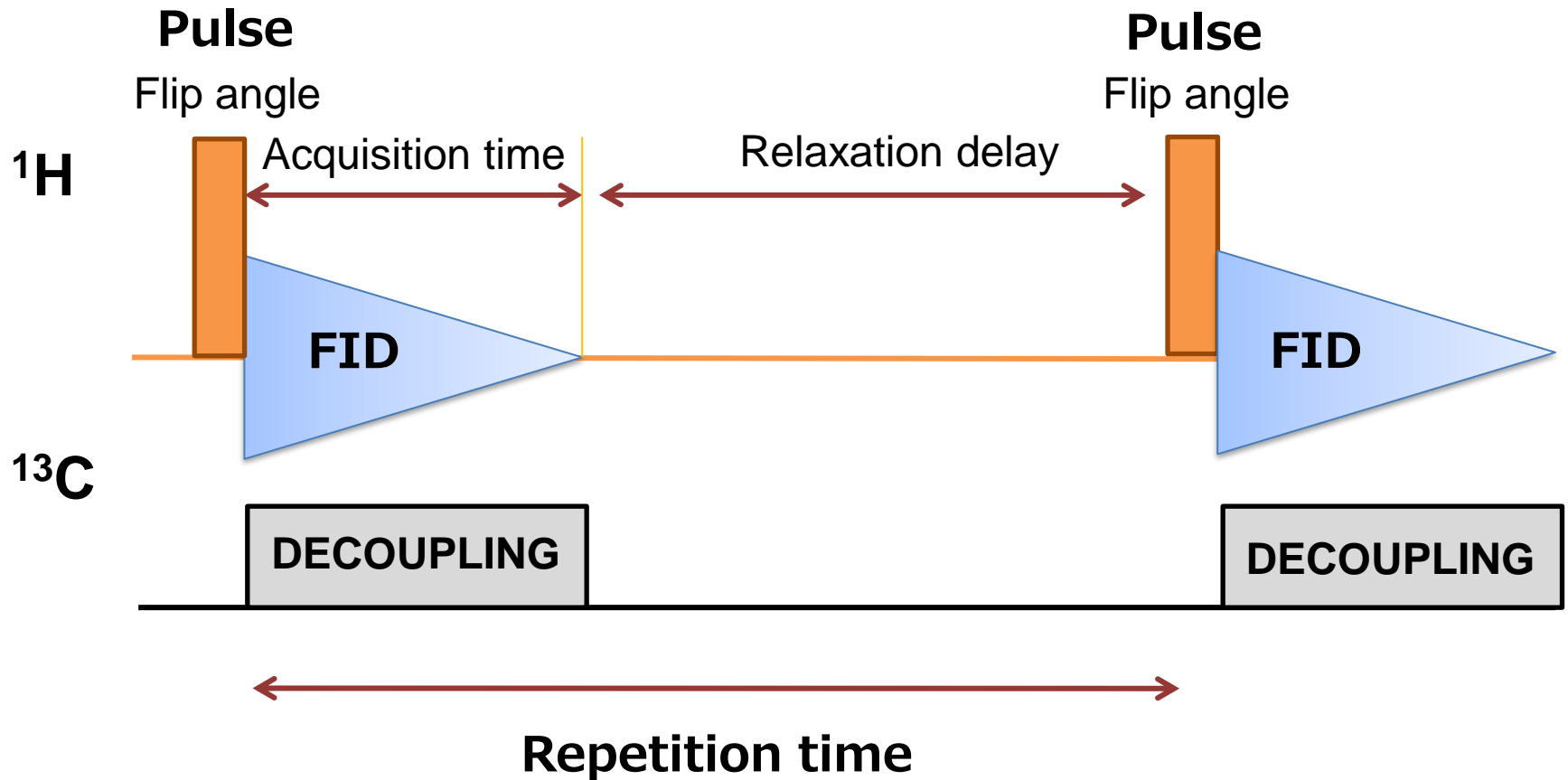
https://www.artel.co/learning_center/in-the-lab-pipettes/

<https://doi.org/10.3390/en1102567>

<https://www.pipettipfinder.com/Topics/Why-We-Dont-List-Pipettor-Accuracy.aspx>

<https://www.integra-biosciences.com/china/en/blog/article/everything-you-need-know-about-pipettes>

qNMR measurement



Acquisition time + relaxation delay = repetition time

Conditions used in qNMR measurement

The acquisition parameters have been optimized to obtain accurate quantitative information from ^1H spectrum

Parameter	Value
Pulse flip angle	90°
Digital resolution	$< 0.25 \text{ Hz}$
Repetition time	$> 60 \text{ sec } (7 * T_1)$
Acquisition time	$> 3 \text{ sec } (> 5 * T_2)$
Number of scans	$\text{SNR} > 150$ ($< 1\%$ error)
Dummy scans	2
Spinning	OFF
^{13}C decoupling	(ON)

SNR

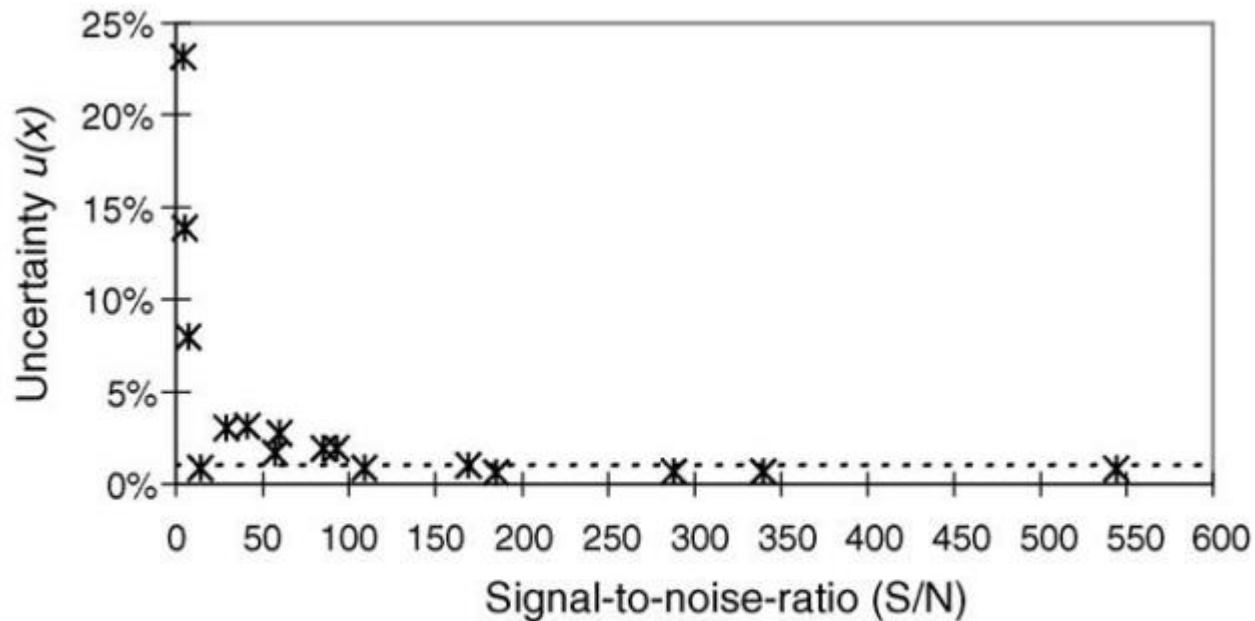


Fig. 4. S/N and its influence to the uncertainty of qNMR. Dotted line represents an uncertainty level of 1% relative.

<https://doi.org/10.1016/j.jpba.2005.01.043>

SNR

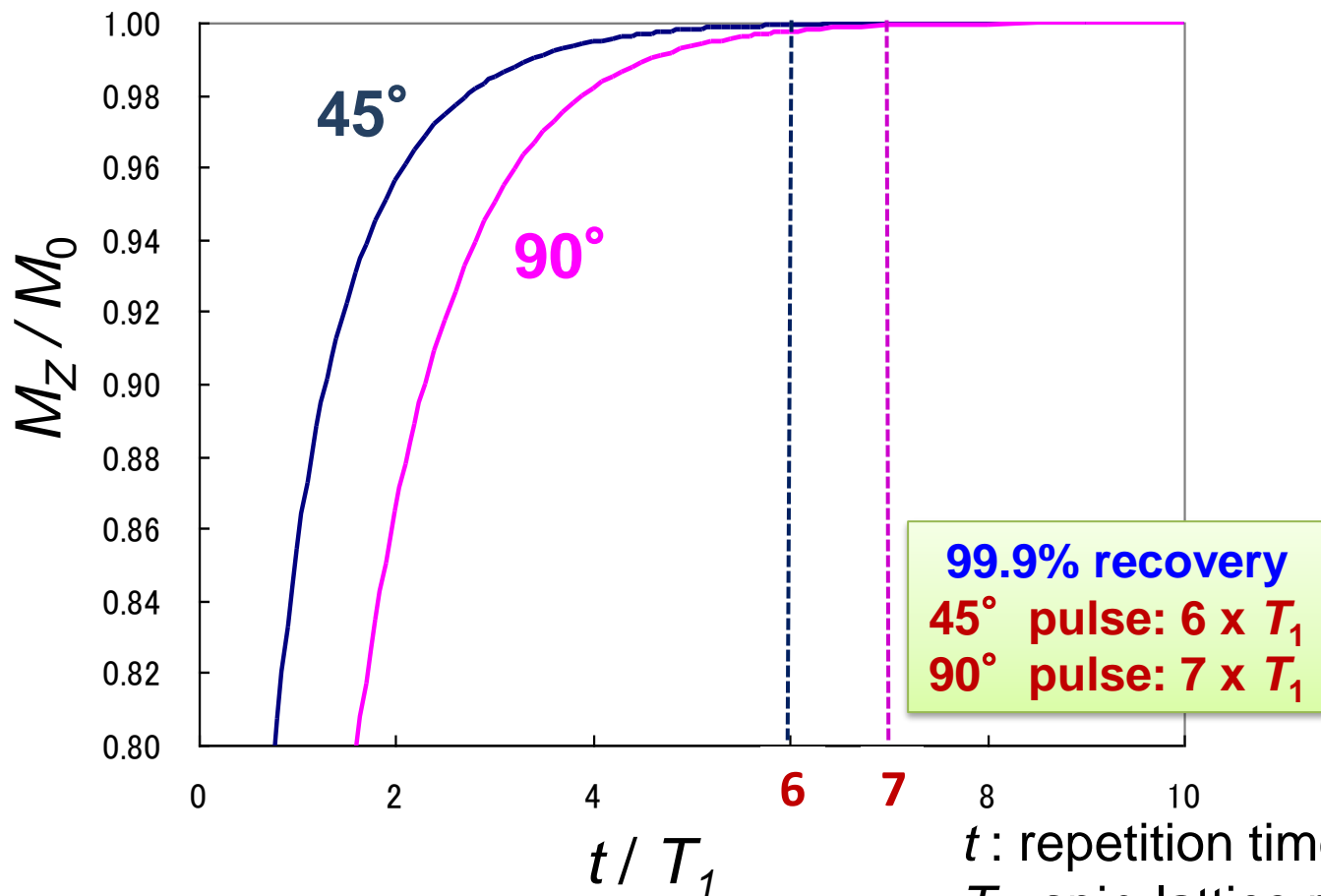
- LOD: Limit of Detection is 3:1 for every signal from the molecule!
- LOQ: Limit of Quantification depends on confidence level of results

S/N	Relative Measurement Uncertainty in %*
30	10
60	3
150	1
400	0.5
1200	0.1
10000	0.07
20000	0.05

<http://dx.doi.org/10.13140/RG.2.1.1244.3689>

Pulse flip angle and relaxation time

Recovery of magnetization M_Z



Error of 5°

$$\sin(90)=1$$

$$\sin(85)=0.996$$

$$\sin(95)=0.996$$

$$\sin(45)=0.707$$

$$\sin(40)=0.643$$

$$\sin(50)=0.766$$

99.9% recovery

45° pulse: 6 x T_1

90° pulse: 7 x T_1

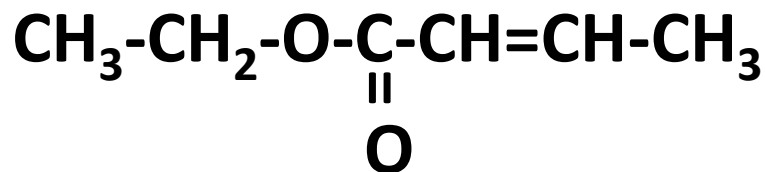
t : repetition time

T_1 : spin-lattice relaxation time

$$M_z = M_0 \left[1 - (1 - \cos \vartheta) e^{\frac{-t}{T_1}} \right]$$

[https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Quantitative_NMR_\(Larive_and_Korir\)/02%3A_Practical_Aspects_of_Q-NMR/2.05%3A_Effects_of_Tip_Angle_in_Quantitative_NMR_Experiments](https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Quantitative_NMR_(Larive_and_Korir)/02%3A_Practical_Aspects_of_Q-NMR/2.05%3A_Effects_of_Tip_Angle_in_Quantitative_NMR_Experiments)

Integration under semi-quantitative and quantitative conditions

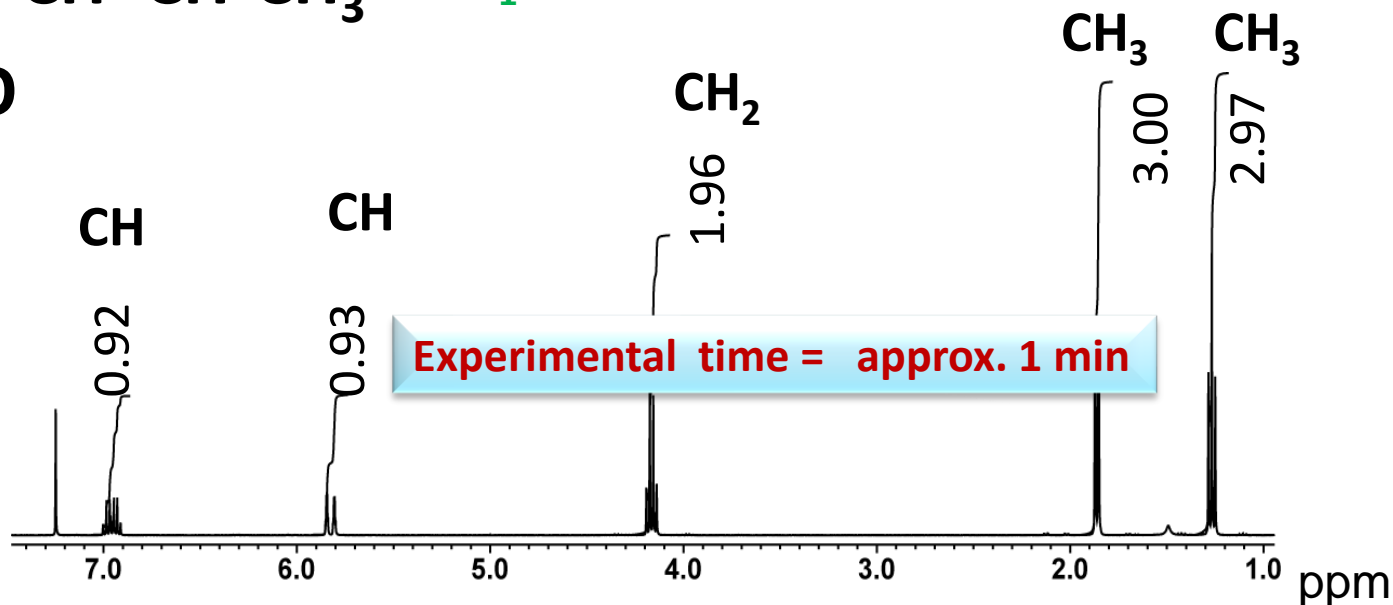


$T_1 = 3.6 - 5.9 \text{ sec}$

Semi-quantitative

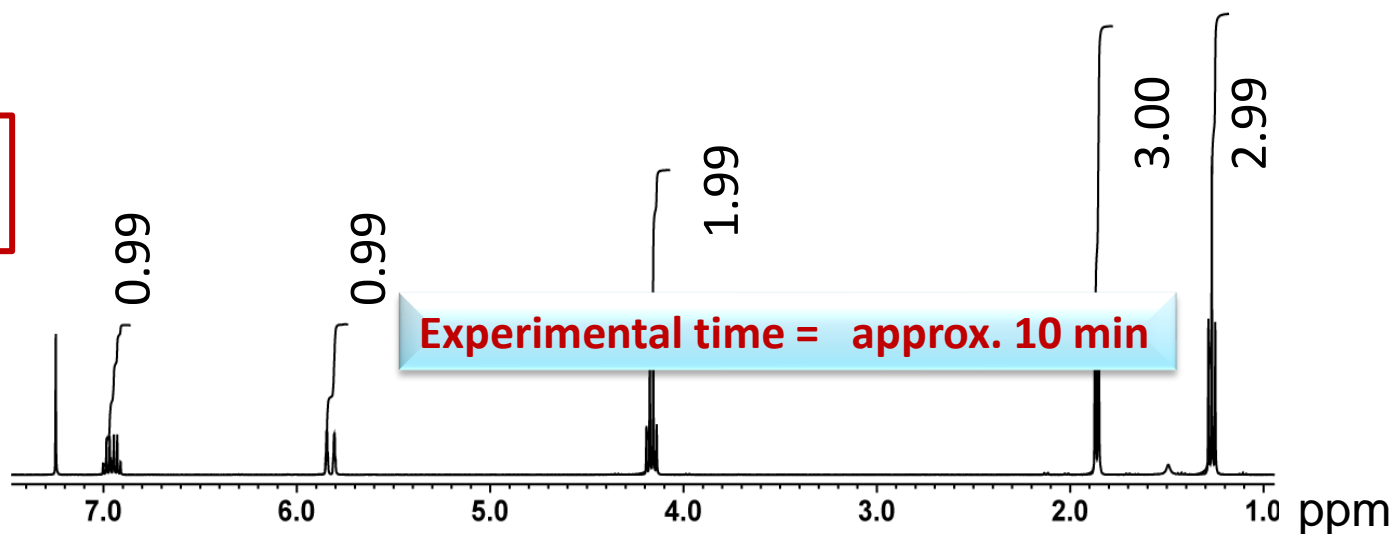
Flip angle: 45°
Repetition time: 7 sec

8 scans



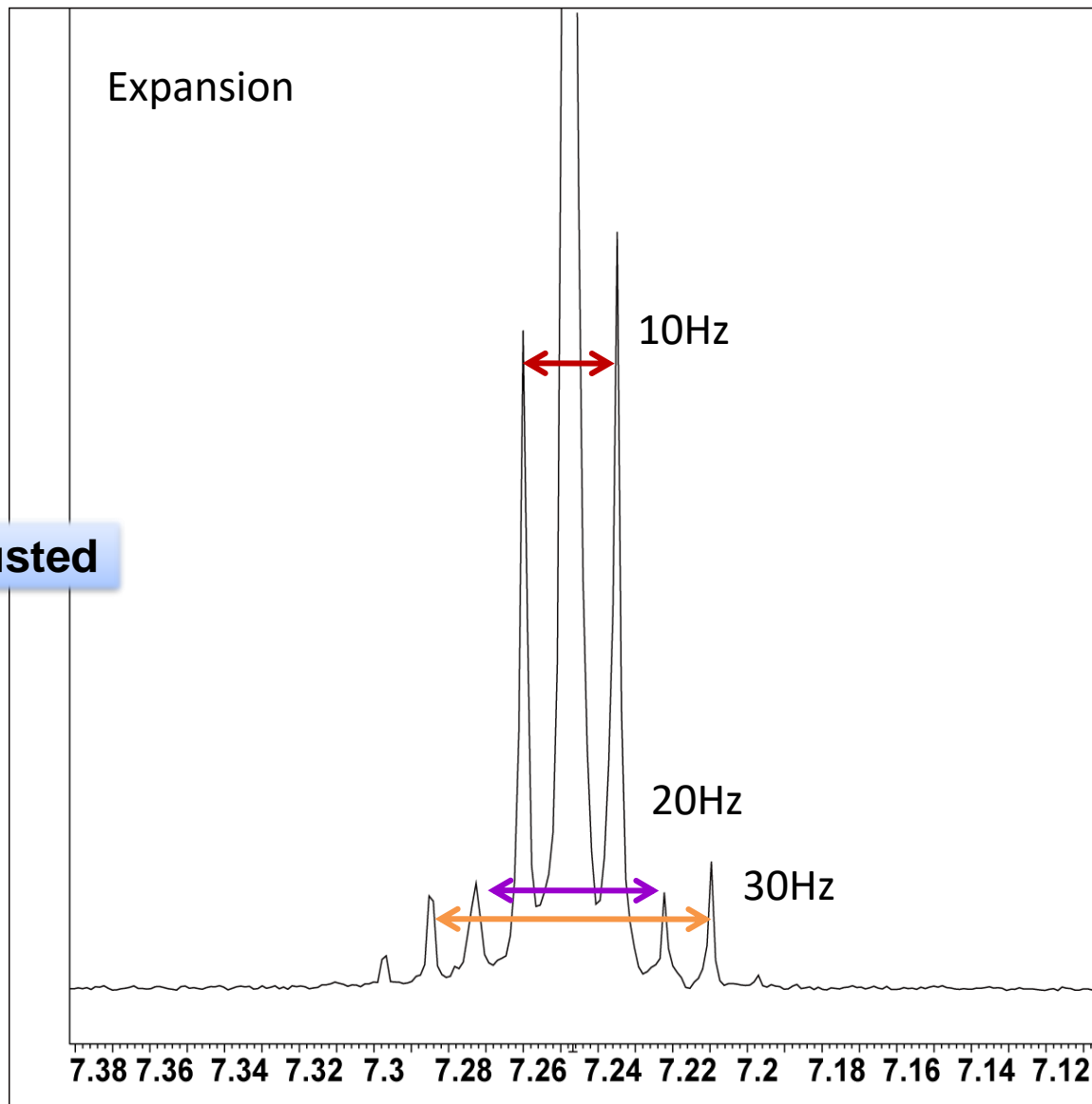
Quantitative

Flip angle: 90°
Repetition time: 64 sec



Spinning side bands

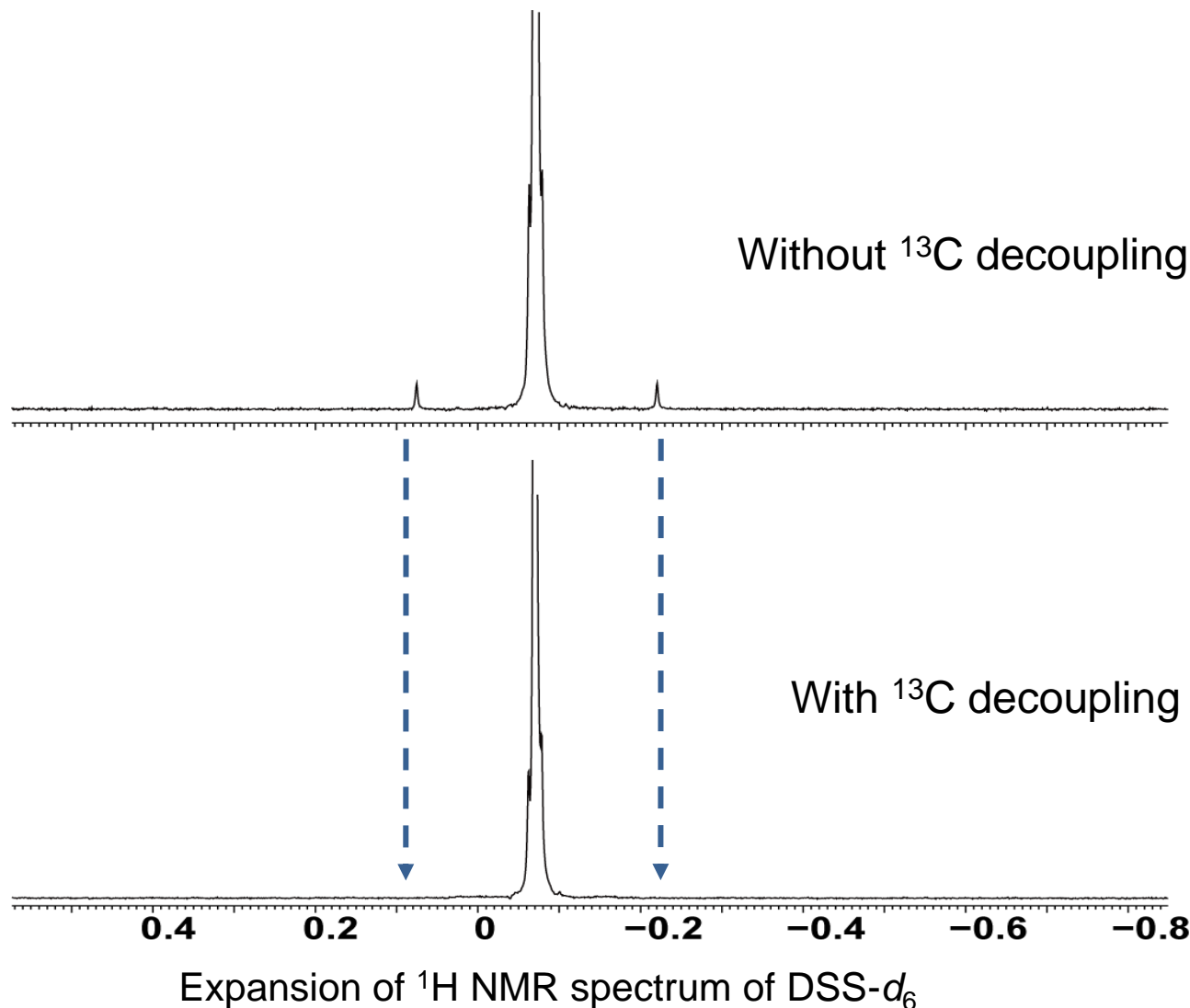
^1H -NMR spectrum of chloroform spinning at 5 Hz



If radial shims are misadjusted

^{13}C broadband decoupling

^{13}C decoupling eliminates ^{13}C satellites (1.1% of main signal)

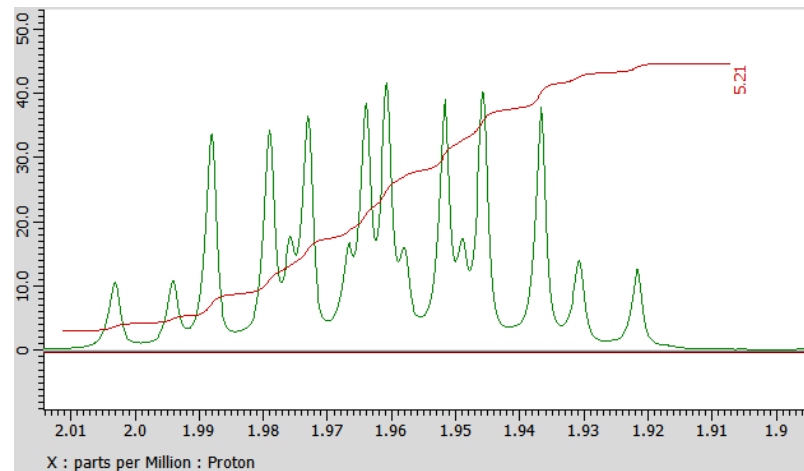
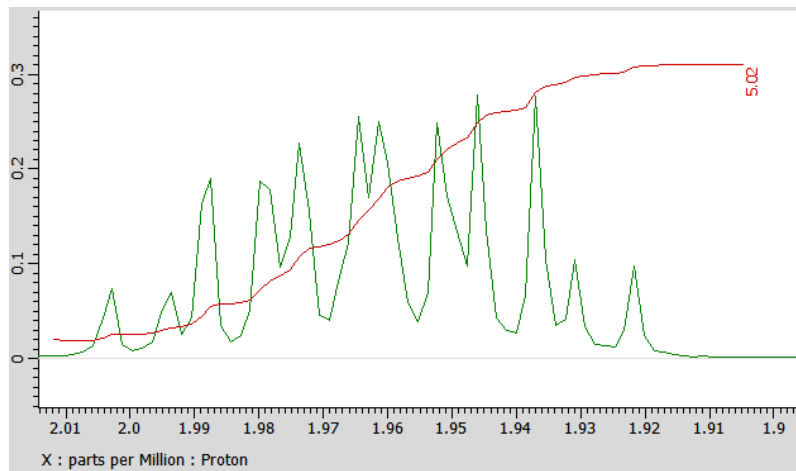


Resolution

1s

vs

5s



- 1s acquisition time does not provide enough resolution to resolve multiplets
- Integrals are less accurate due to this loss of information
- It can be compensated with additional zero-filling, but this may not resolve additional signals beyond 2x zero-filling
- At least 4[s] acquisition time is needed

Data processing and analysis

Accurate and precise qNMR analysis requires:

- ◆ 3 repetitions per each sample
- ◆ Each peak defined by several points (zero filling)
- ◆ Adequate phase and baseline correction
- ◆ Accurate integration with sufficient width and with the same margins for each region (e.g. 50Hz), peak deconvolution will be necessary for overlapped peaks
- ◆ Appropriate calculation of concentration/purity of analyte

Other nuclei

Spectral windows can be very broad for nuclei other than ^1H , consider the effects of:

- Receiver (ECZ is linear, older consoles not so much)
- Tuning (physical limitation, qNMR may require retuning at different offsets)
- Excitation profile (1[us], <2% error across 160kHz)
- Background signal (Deconvolution, LP, tube material, Depth pulse sequence,...)
- $T_1(^{109}\text{Ag}$ in AgNO_3 is $\sim 10\text{min}$), relaxation agents reduce it
- ^{19}F : <https://doi.org/10.1002/cmr.a.21422>

Fluorine excitation (hard pulse in a ECZ-S 400)



13C qNMR setup

- Quantitative 13C measurements are time-consuming
- It is thus important to optimize the total measurement time. Measure the T1s of your signals, at least once for the type of compounds studied.
- Use saturation recovery sat_recovery_dec.jxp, this experiment, unlike inversion recovery, does not need a very long relaxation delay (which cannot be set correctly unless you already know an estimate of the T1s)
 - Use as many scans to get an SNR of 50 in 13C
 - Set relaxation_delay to 1[s]
 - Set *x_sat_time* to **0.04[s]** and *x_sat_atn* to **xatn + 10[dB]**
 - Ensure this is typed correctly, otherwise probe may be destroyed
 - Array tau_interval, e.g. y_acq {10[s], 0.2[s], 0.5[s], 1[s], 2[s], 5[s], 10[s], 40[s]}
 - Use curve analysis Sat. Recovery fitting (or decay analysis processing) to evaluate T1s
- Use 5 times the longest T1 as the repetition time (relaxation_delay+x_acq_time) of the 13C qNMR experiment
 - Or focus only on a signal of the compound of interest, one with the shortest t1 and strong intensity (more carbons)

x_atn	79[dB]	xatn
x_sat_time	40.0[ms]	
x_sat_atn	89[dB]	xatn + 10[dB]

13C qNMR setup, scans

- Achieving enough SNR to achieve uncertainty criteria can be the most challenging aspect of 13C qNMR
- Depending on the application we can settle with an SNR around 50, but ideally SNR should reach 150. There is no major gain by increasing the SNR beyond this, without taking great care of other parameters
- As a rule of thumb, if no signals are discernible after 4 scans, acquiring a quantitative spectrum is not feasible with that sample concentration/probe
- SNR scaling up with the number of scans:

SNR	Uncertainty in %
30	10
60	3
150	1
400	0.5
1200	0.1

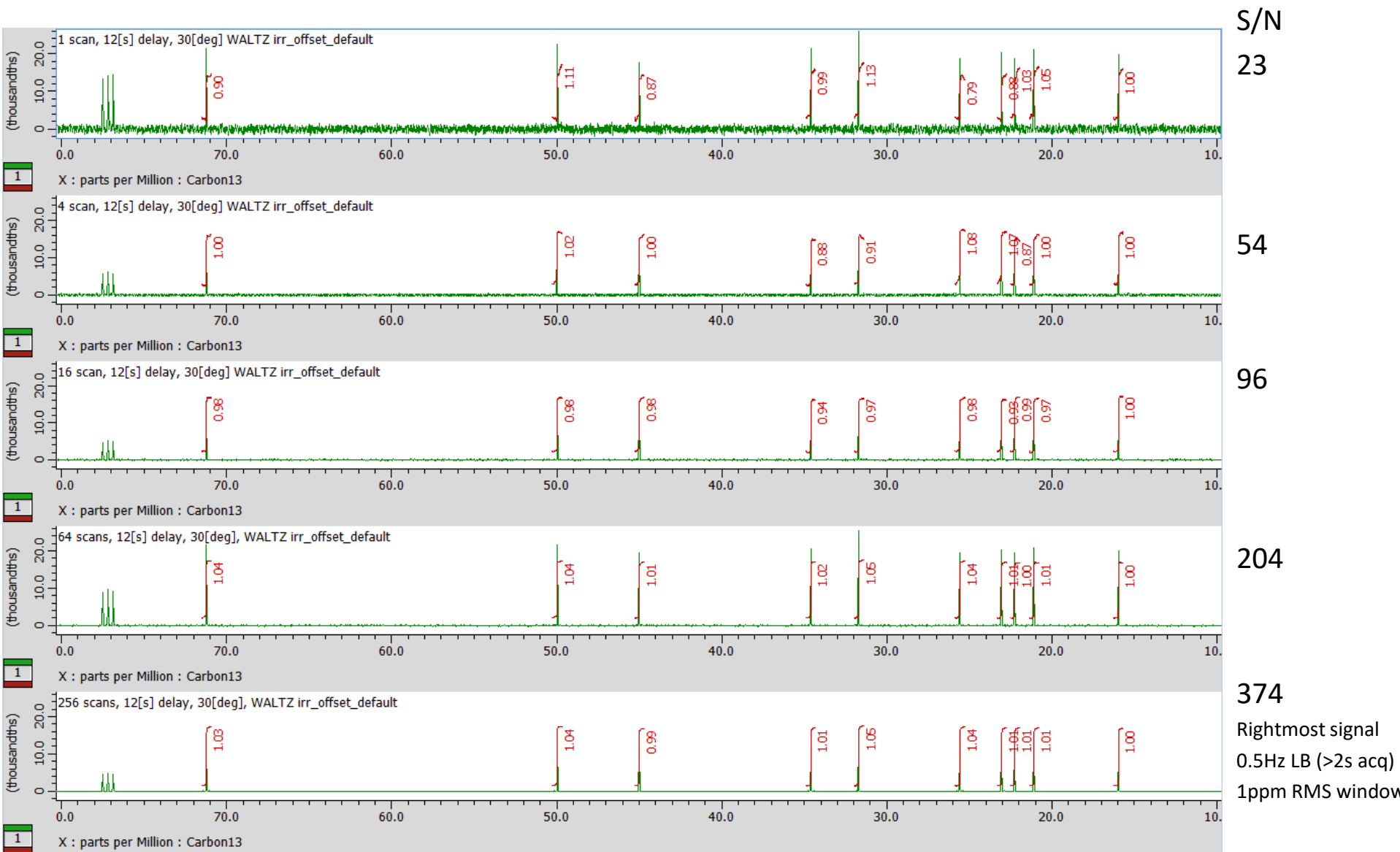
<http://dx.doi.org/10.13140/RG.2.1.1244.3689>

SNR	Scans
10	4
40	64
160	1024
640	16384

SNR	Scans
5	4
20	64
80	1024
320	16384

SNR	Scans
2	4
8	64
32	1024
128	16384

^{13}C qNMR (menthol 30%) scans



^{13}C qNMR setup, relaxation delay

- Magnetization recovers as per the following equation

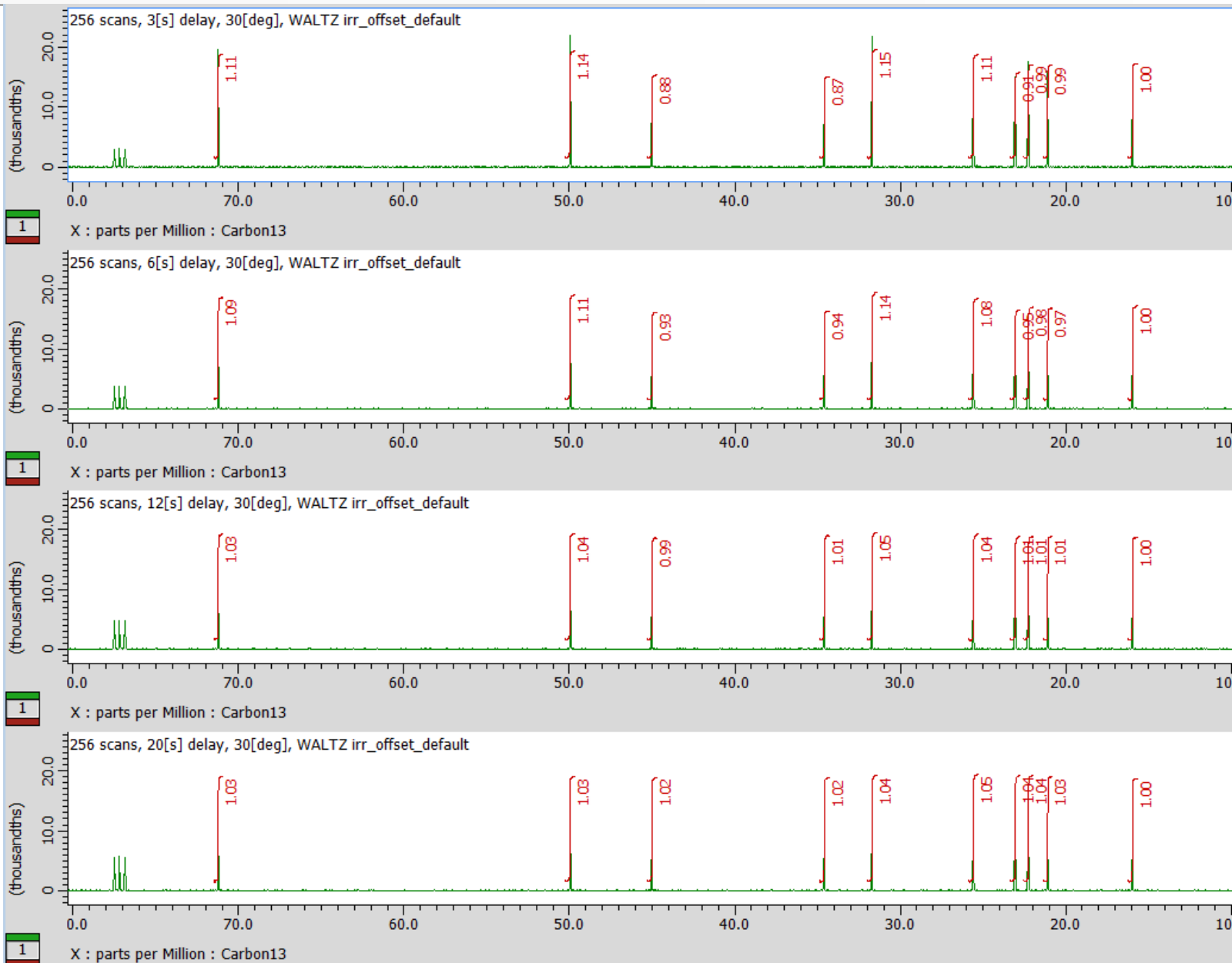
$$M_z = M_0[1 - (1 - \cos \vartheta)e^{\frac{-t}{T_1}}]$$

- There is no major gain by increasing the repetition time beyond 5 T_1 , without taking great care of other parameters
- Uncertainty as per pulse width and repetition time:

Uncertainty in %	Repetition time for 90 deg (T_1 times)	Repetition time for 60 deg (T_1 times)	Repetition time for 30 deg (T_1 times)
10	2.3	1.6	0.3
5	3.0	2.3	1.0
1	4.6	3.9	2.6
0.5	5.3	4.6	3.3
0.1	6.9	6.2	4.9

^{13}C qNMR (menthol 30%) relaxation delay

T_1 s
1.7-2.3[s]



13C qNMR setup, pulse width

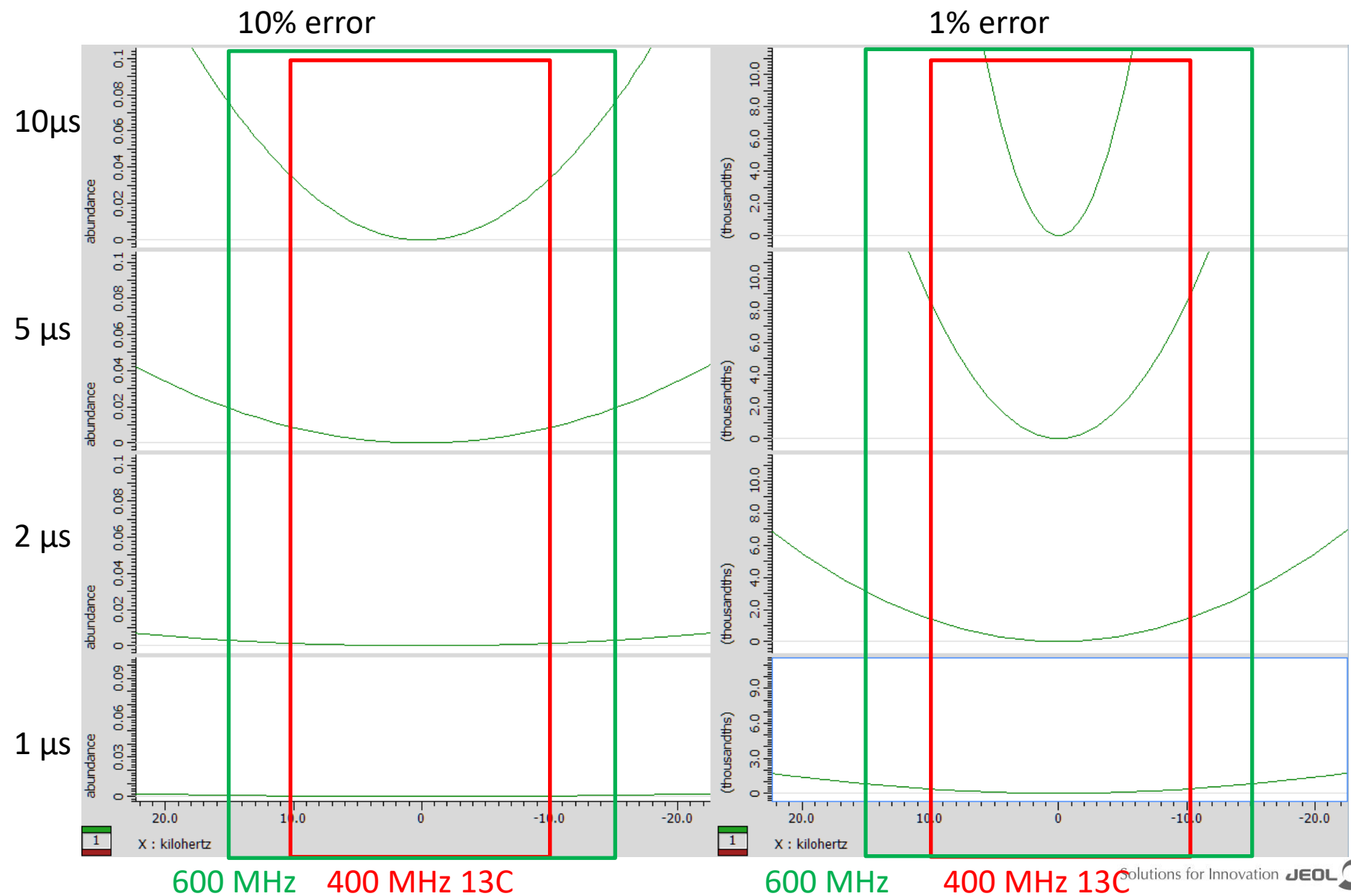
There are different considerations when choosing the pulse flip angle / pulse width:

- Excitation profile for <1% error
 - <5.5us @ 400MHz
 - <4.3us @ 500MHz
 - <3.6us @ 600MHz
- SNR per unit time when using the optimum repetition time for target uncertainty level (sine flip angle/ rep. time)

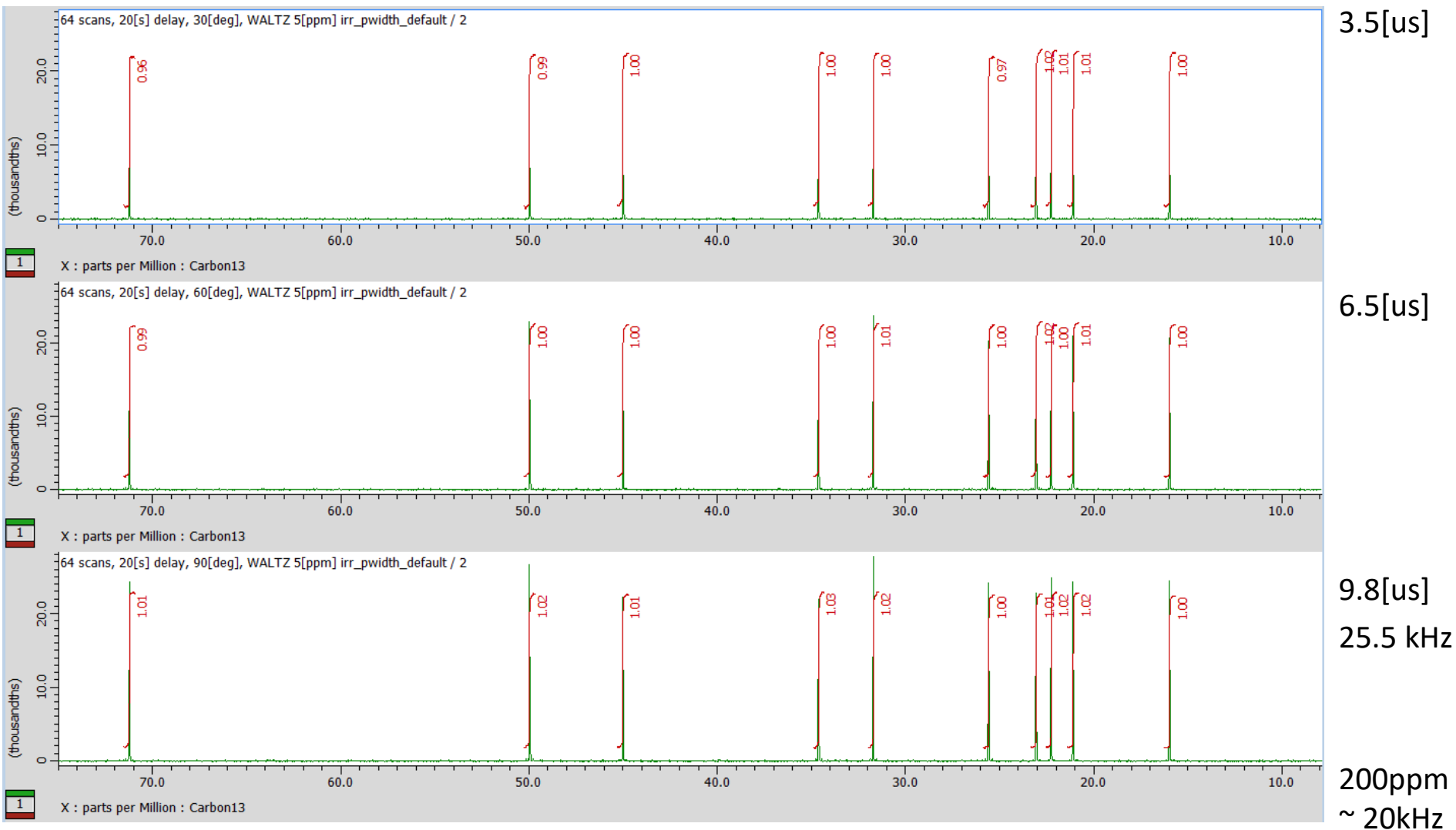
Uncertainty in %	SNR/time for 90 deg	SNR/time for 60 deg	SNR/time for 30 deg
10	1.00	1.24	3.94
5	0.77	0.87	1.17
1	0.50	0.51	0.44
0.5	0.43	0.43	0.35
0.1	0.33	0.32	0.24

- Reproducibility for external quantitation

^{13}C qNMR setup, excitation profile



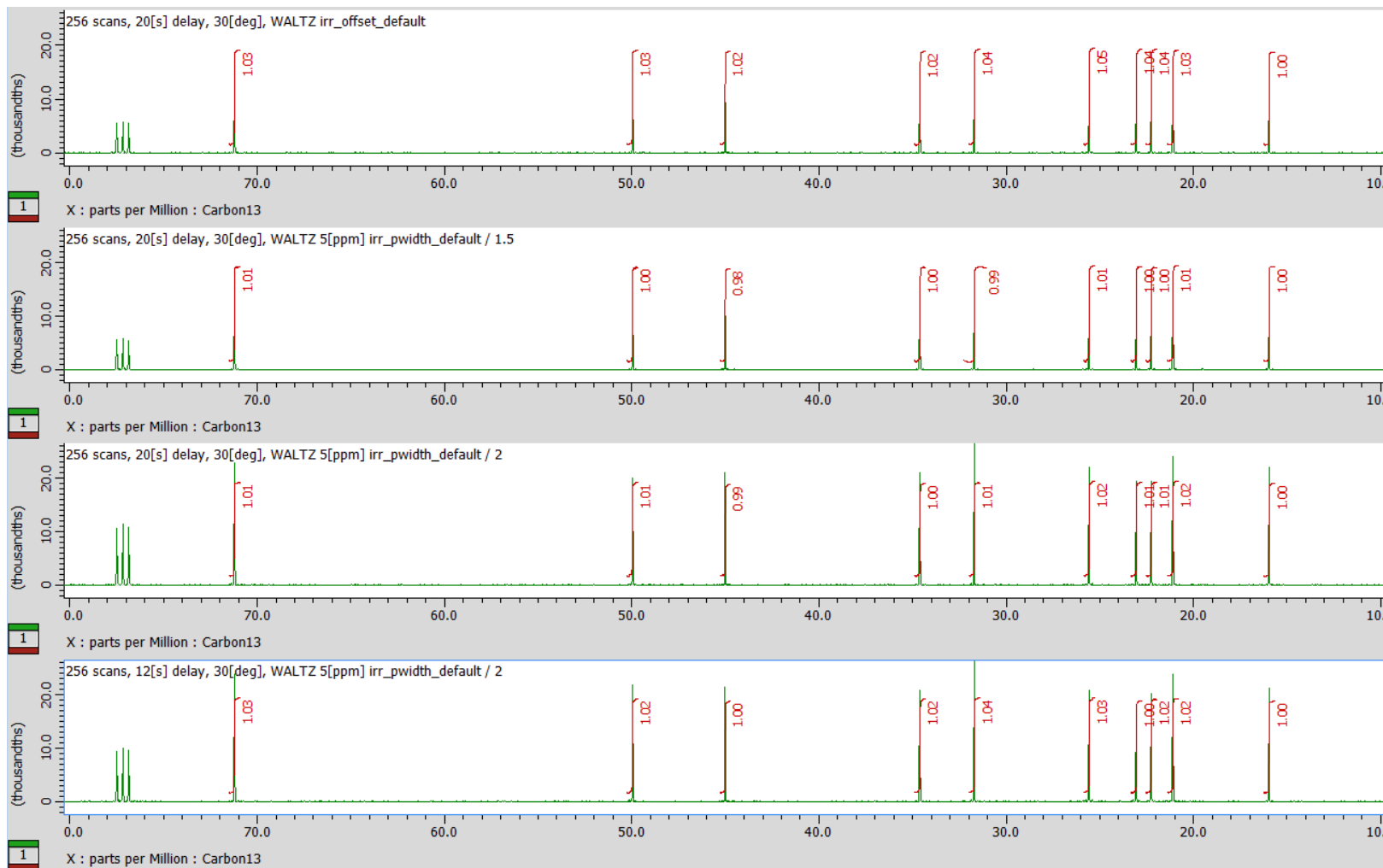
^{13}C qNMR (menthol 30%) pulse width



¹³C qNMR setup, decoupling

- The ¹H decoupling is an important source of error
 - NOE effects are negligible with no decoupling during the long relaxation delay
- The decoupling modulation used affects both the effective bandwidth and artifacts. WALTZ generally provides spectra with low level of artifacts
- WALTZ64 is the default decoupling in Delta, no significant differences observed with WALTZ65 (available with Delta 6.0)
- Default decoupling pulse width insufficient to achieve 1% uncertainty. Set irr_pwidth to irr_pwidth_default/1.5 to improve the effective bandwidth
 - Ensure that irr_atn_dec is at least 18[dB] higher than the power level for the square pulse for Proton in the probe tool. Otherwise probe may be damaged
 - Approximately equivalent to 85us @ 400MHz, 67us @ 500MHz, 55us @ 600MHz
- Adiabatic decoupling could potentially provide better results (<https://doi.org/10.1016/j.jmr.2006.11.007>), but this has not been observed in our measurements and no other publication has been found using it
- Bilevel decoupling could help reduce the decoupling power requirements for higher field systems (<https://doi.org/10.1021/acs.analchem.0c03753>), this has not been tested yet in our measurements

13C qNMR (menthol 30%) decoupling bandwidth



400 MHz
10ppm[4kHz]
115[us]
2.17 kHz

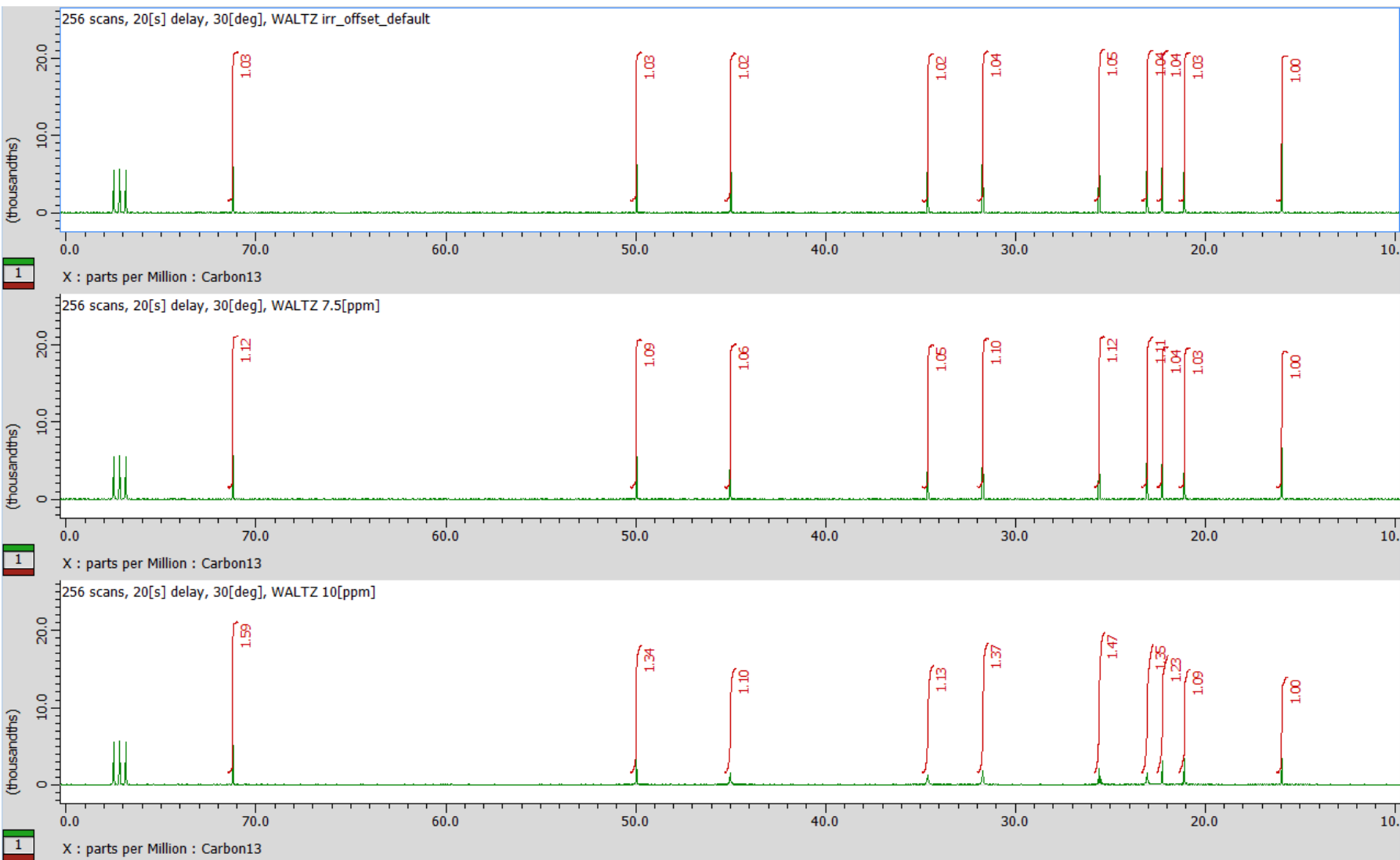
76.67[us]
3.26 kHz

57.5[us]
4.35[kHz]

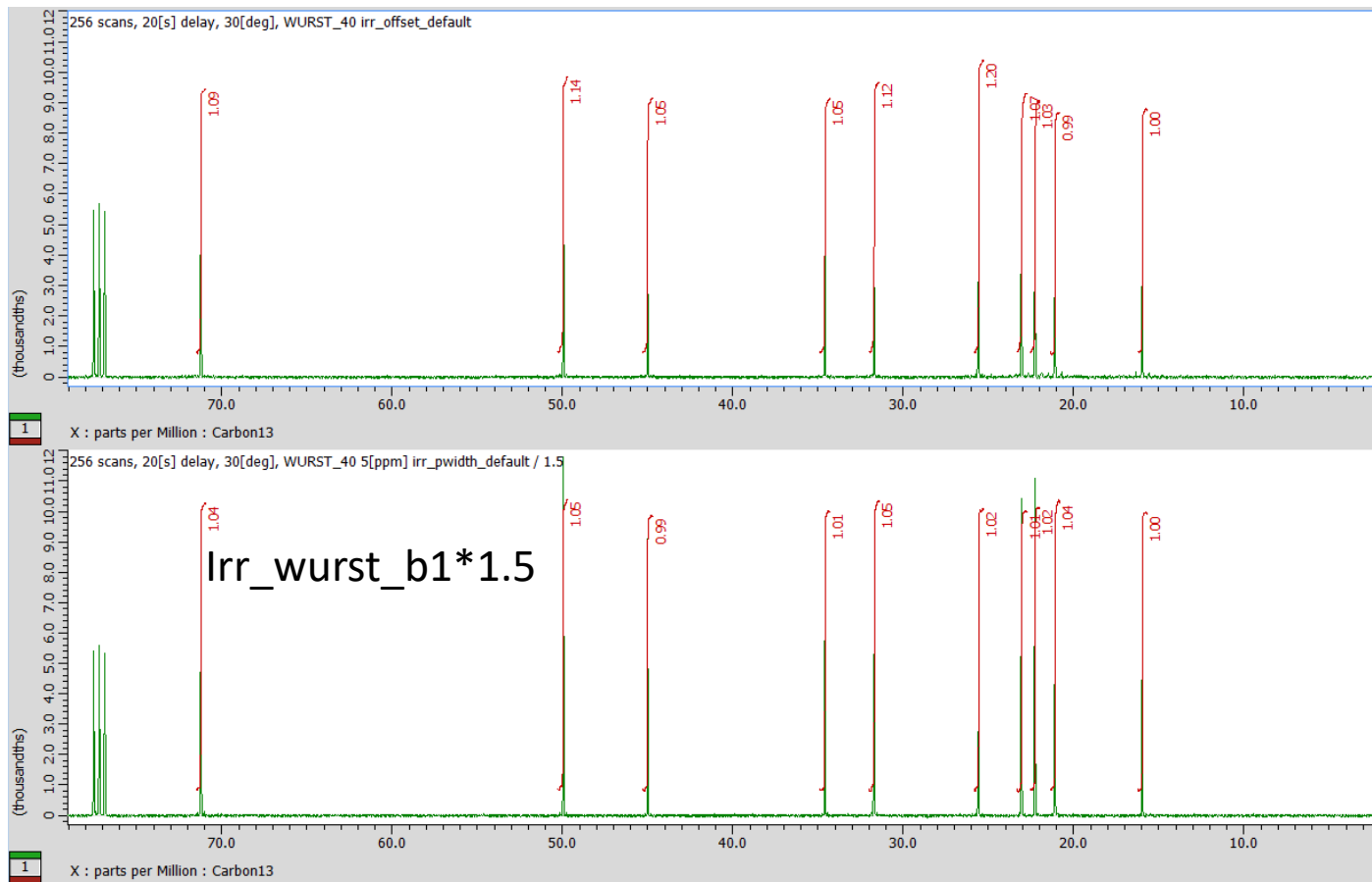
57.5[us]
4.35[kHz]
12s relaxation

WALTZ bandwidth $\sim 2 \times 90$ pulse power
(85us@400, 67us@500, 55us@600 to get pulse power covering 150% of bandwidth)

^{13}C qNMR (menthol 30%) decoupling offset



¹³C qNMR (menthol 30%) adiabatic decoupling



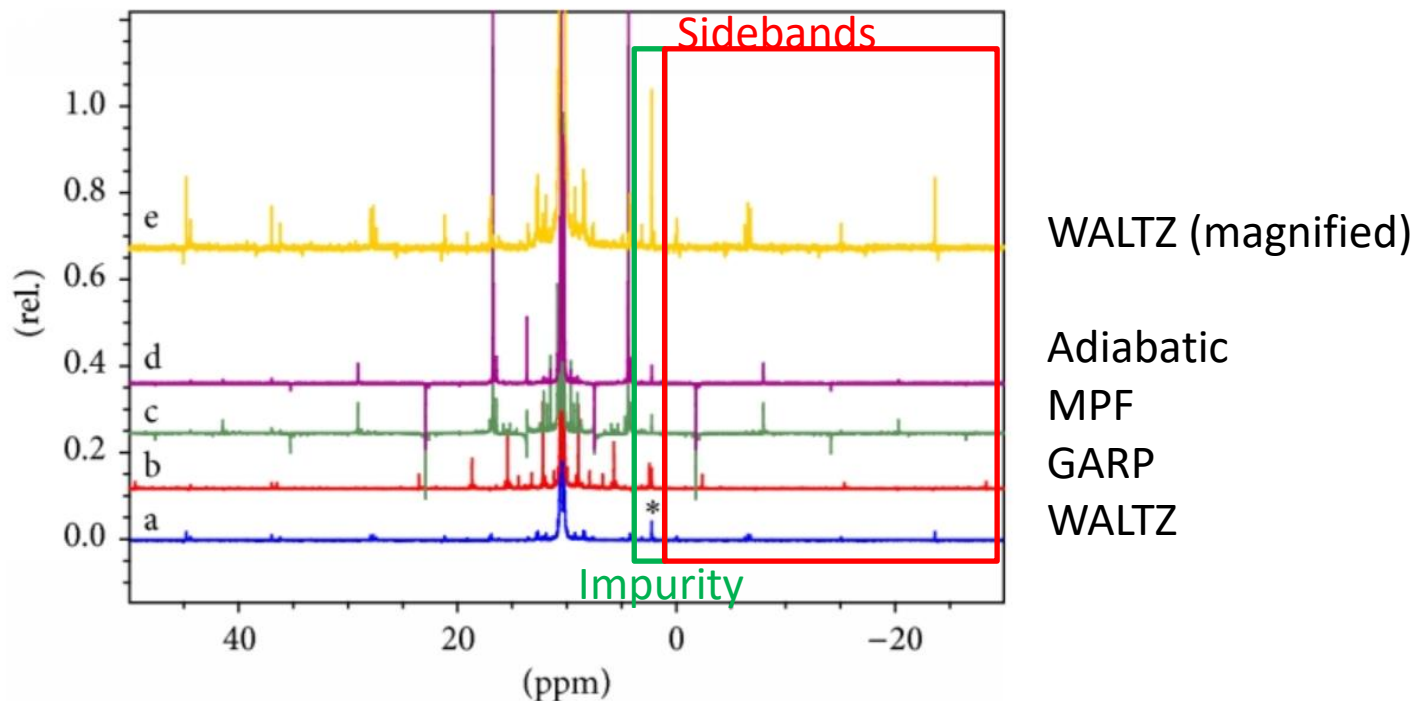
The homonuclear couplings are known to interfere with the decoupling process and can seriously compromise the quality of the spectra

Complete homonuclear decoupling is achieved only if the sweep bandwidth is twice the chemical shift difference between spins A and X

[https://doi.org/10.1016/S0076-6879\(02\)38216-8](https://doi.org/10.1016/S0076-6879(02)38216-8)

Decoupling sidebands

The sidebands in all cases apart from the spectrum acquired under WALTZ decoupling conditions completely override the signal of a minor impurity (~3%) at 2.24 ppm



The ^{31}P NMR spectrum of $\text{HP}(=\text{O})(\text{OCH}_2\text{CH}_3)_2$ indicates the resultant sidebands with decoupling of ^1H by (a) CPD decoupling using WALTZ (PW, 60 μs ; PL, 10 dB; , 1.33 Hz; DB, 10 kHz; SDB, 5 kHz); (b) CPD decoupling using GARP (PW, 60 μs ; PL, 9.4 dB; , 1.35 Hz; DB, 19 kHz; SDB, 7 kHz); (c) MPF decoupling (PW, 1 ms; PL, 11.3 dB; , 1.32 Hz; DB, 28 kHz; SDB, 7 kHz); (d) APD using CHIRP (PW, 1 ms; PL, 5.8 dB; , 1.47 Hz; DB, 44 kHz; SDB, 15 kHz); and (e) the same as (a) but with higher amplification to highlight the sidebands. The signal of a minor impurity (~3%) at 2.24 ppm is indicated with an asterisk.

Note: all spectra within this figure, and similarly for spectra within the following figures, have been acquired with the same number of scans and receiver gain and processed in exactly the same way with identical line broadening and amplification unless explicitly stated otherwise. Legend: PW, pulse width (length); PL, power level; , linewidth (width at half-height); DB, decoupling bandwidth; and SDB, sideband decoupling bandwidth.

<https://doi.org/10.1155/2014/289638>

13C qNMR setup, alternatives

- There are a number of experiments claiming to be better alternatives for 13C NMR quantitation
- These can be useful to compare samples of the same type, but due to the relaxation delays during the pulse sequences and the signal dependency on 1H coupling these are not inherently quantitative experiments
- QPOMMIE has been tried to evaluate whether quantification is possible

Q-POMMIE



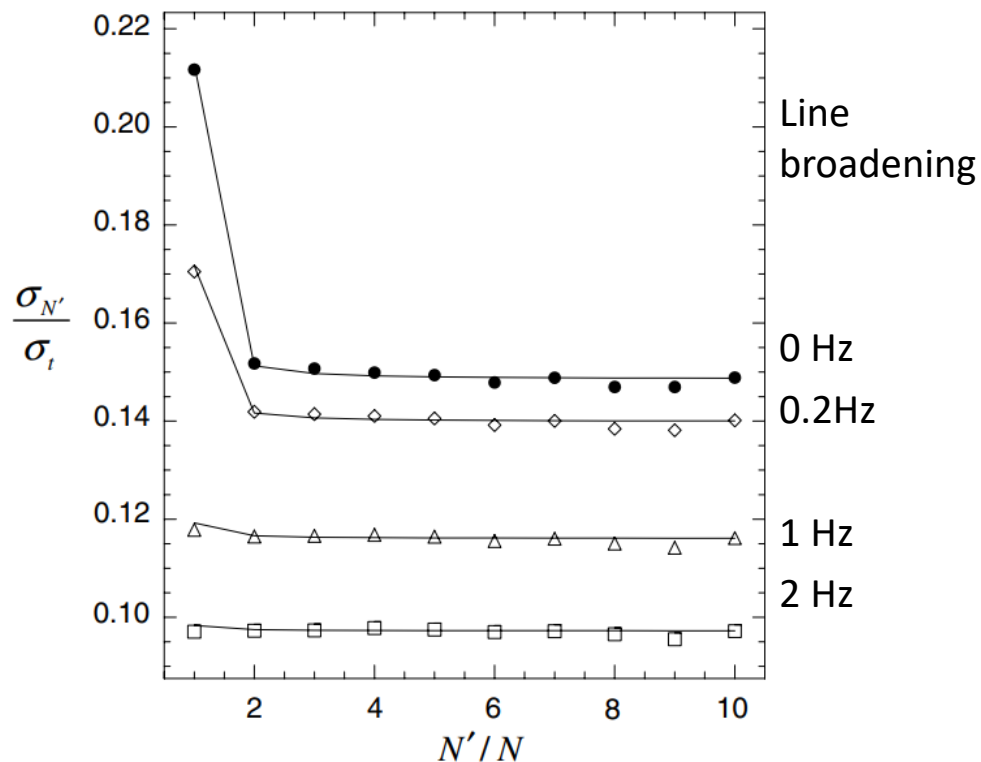
13C qNMR setup, processing

- Use a matched filter to maximize SNR (exponential line broadening equal to peak linewidth)
- For cold probes, FID prediction (blip_cld) may be needed, typically two predicted points are sufficient to obtain flat baselines
- Set at least 2 times zero filling
- Baseline noise is typically considerable
 - The default Delta integration does a local baseline correction, where the baseline is considered to be at the edges of the integral. This leads to substantial error when integrating spectra with low SNR
 - Reset the slope and offset when integrating spectra with low SNR, like 13C spectra.
 - The default behavior can be changed in menu Options>Preferences, Geometry tab: Adjust Integral Slope/Offset
 - Use the simplest possible baseline correction (such as polynomial order 3)
 - Alternatively increase the number of points averaged to more than 11. The default behavior can be changed in menu Options>Preferences, Geometry tab: Integral Averaging Points. This is less accurate than zero slope and offset

Zero-filling and line-broadening

- 1-time zero filling is needed (2 times zerofill in Delta definition)
- Zero-filling further will improve results when integrating peaks with different linewidths*

- Further zero-filling also increases the importance of noise integral affecting quantification precision



* Different papers reach different conclusions (inconclusive results from this dataset)

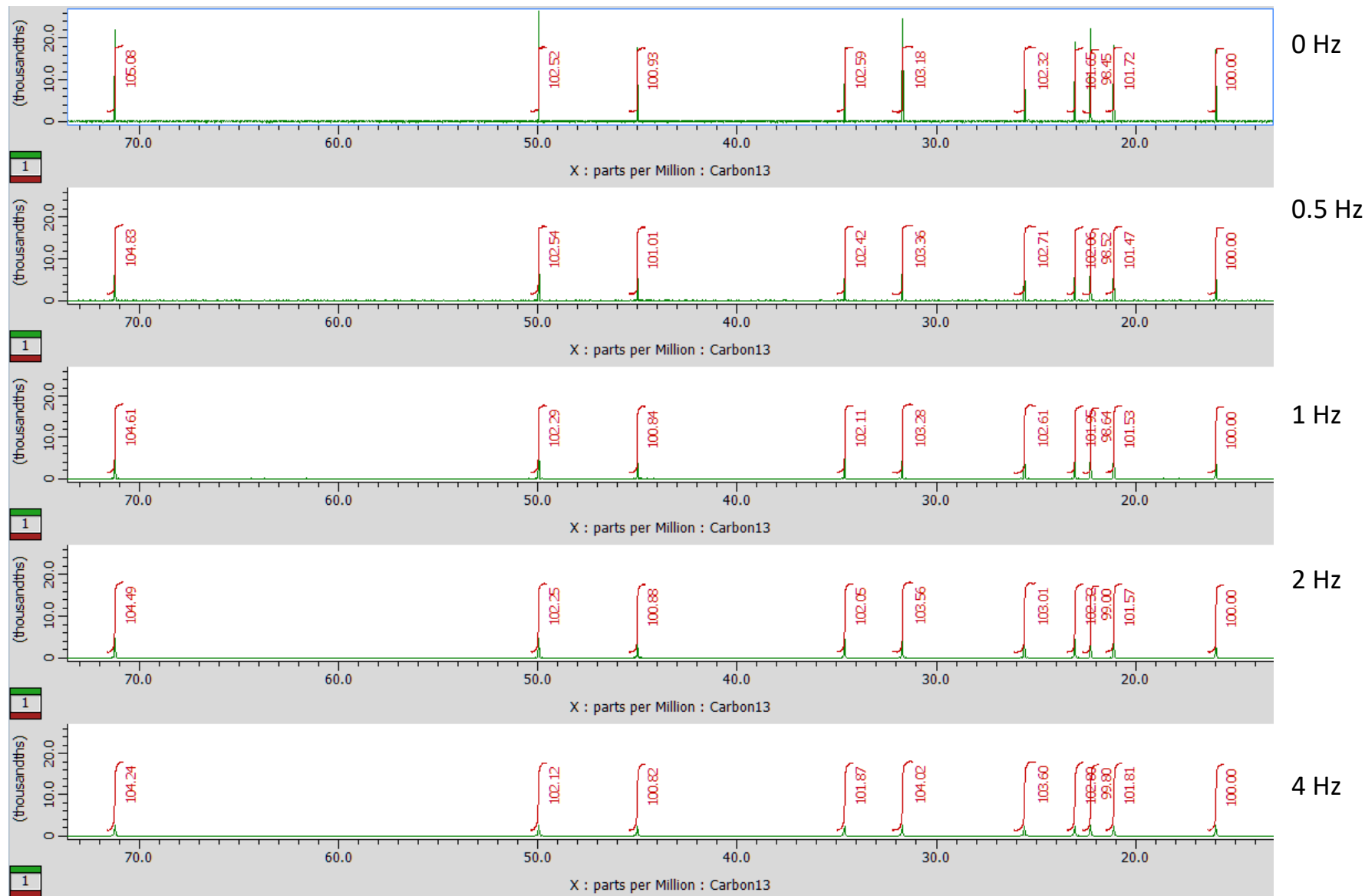
<https://doi.org/10.1016/j.jmr.2006.06.026>

[https://doi.org/10.1016/0022-2364\(89\)90115-7](https://doi.org/10.1016/0022-2364(89)90115-7)

<https://doi.org/10.1021/acs.analchem.1c00407>

<https://doi.org/10.1039/C3AY26106A> parameters choice may lead to better results with no zero-filling

Line-broadening

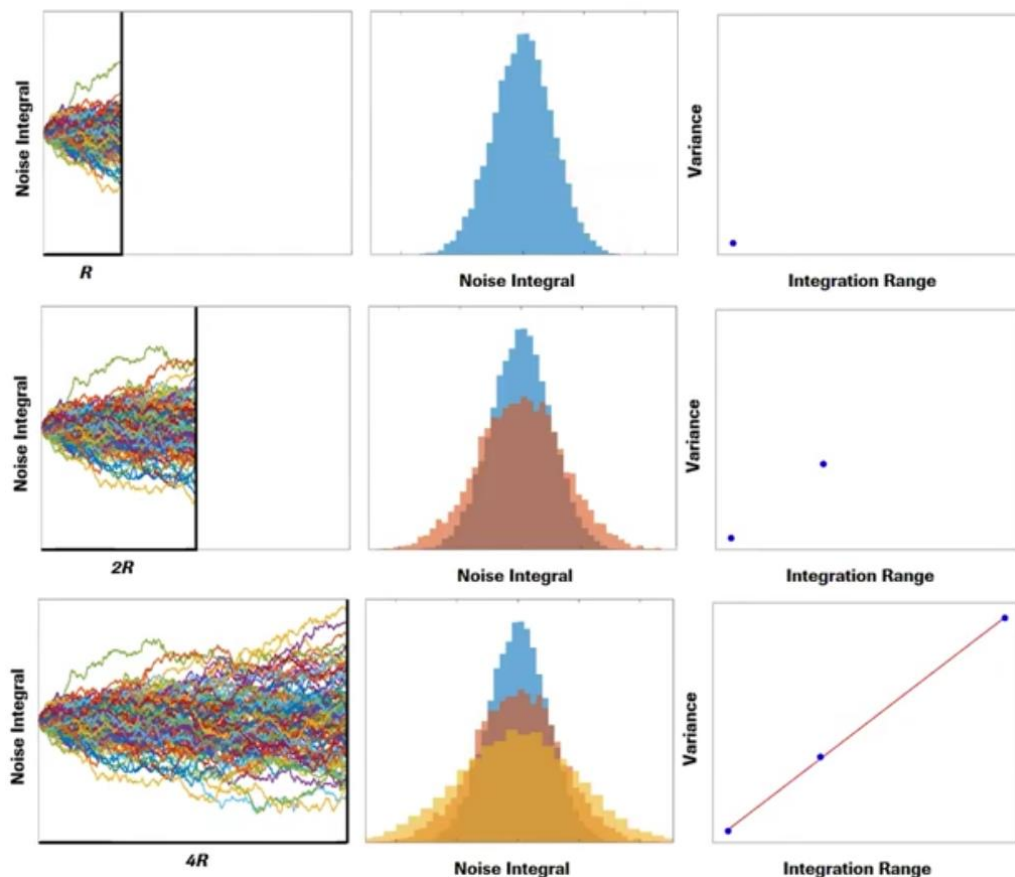


Integral regions

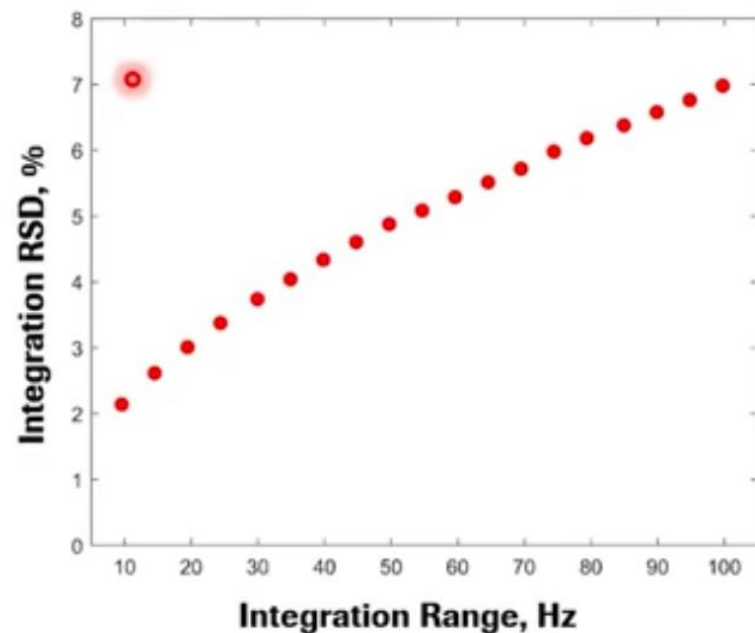
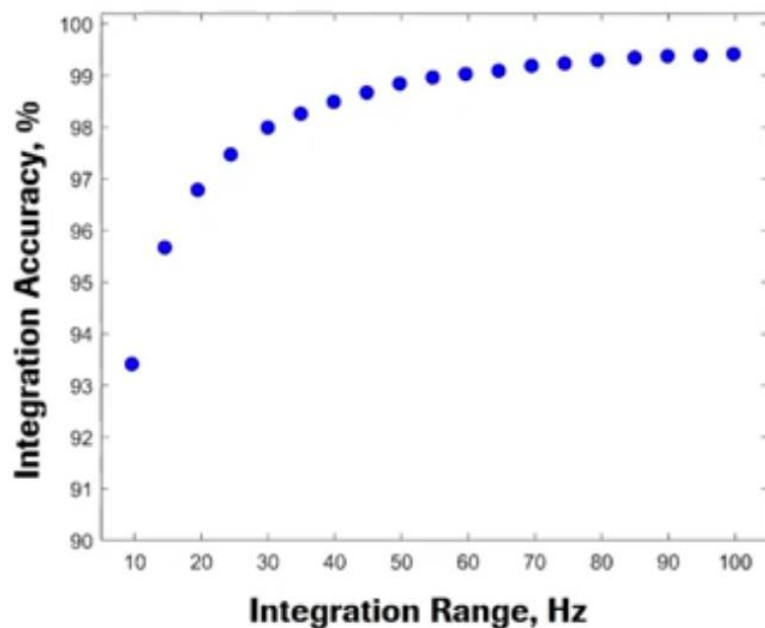
- Increasing integral regions improves accuracy, as we integrate a wider region of the peak, but it decreases precision due to the integrating more noise region.
- For lower SNR data integral regions should be smaller

“While an increase in the integration range results in improved accuracy, the precision decreases because the variance of the integral of white Gaussian noise is a Wiener process and thus increases linearly with range.”

<https://doi.org/10.1021/acs.analchem.1c00407>
<https://www.youtube.com/watch?v=7VYE-W28VYg>



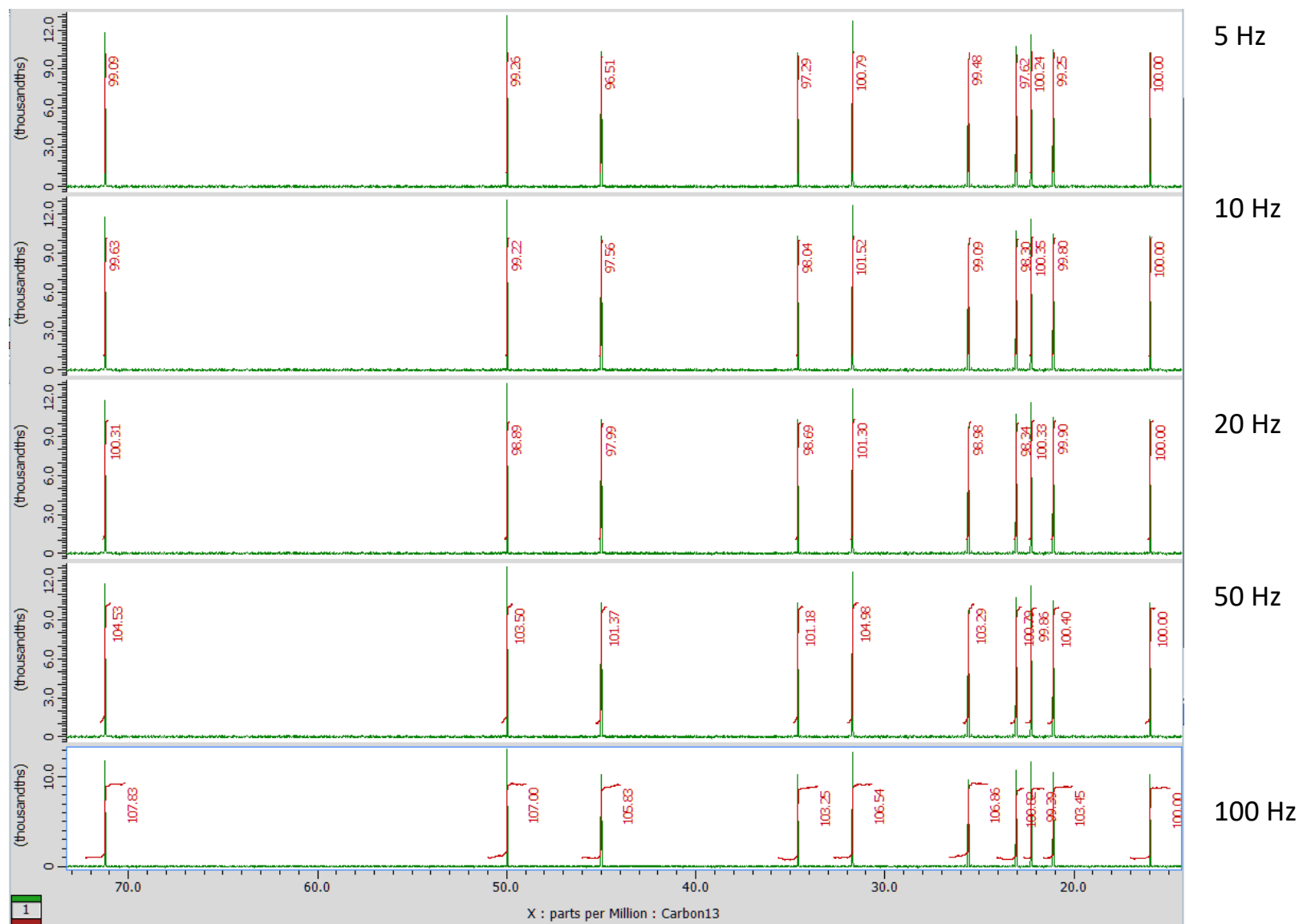
So the smaller the integral region the better?



Lorentzian Singlet
FWHM = 1 Hz
SNR = 25

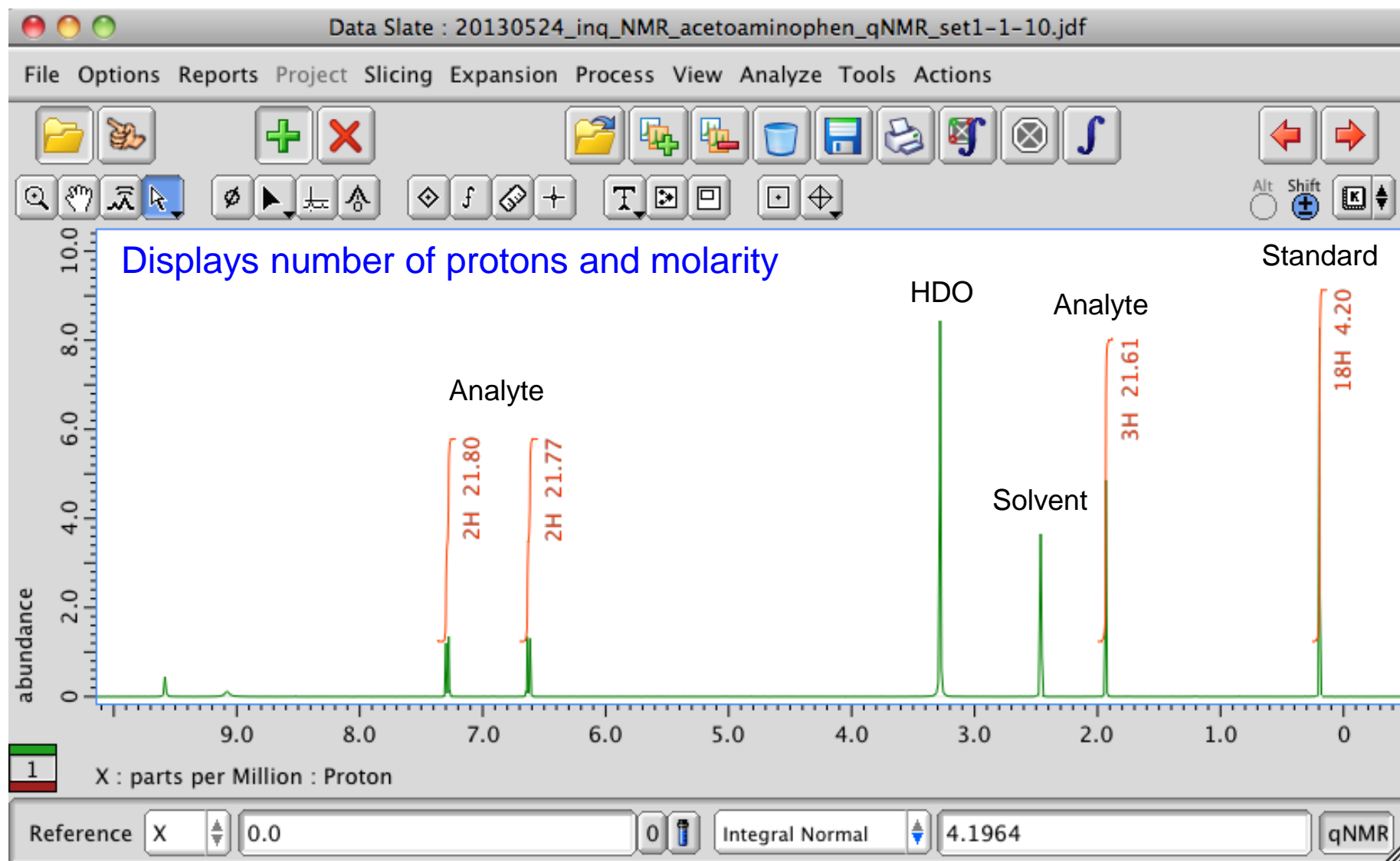
- Smaller integral region : higher precision, lower accuracy
- Note that the absolute value of an integral is not important, we compare the integral of the analyte of interest with the reference integral
- Both analyte peak and reference peak must therefore have similar linewidths to improve accuracy (consider different integral width if different linewidths)
- Line broadening attenuates linewidth differences

Integral regions width

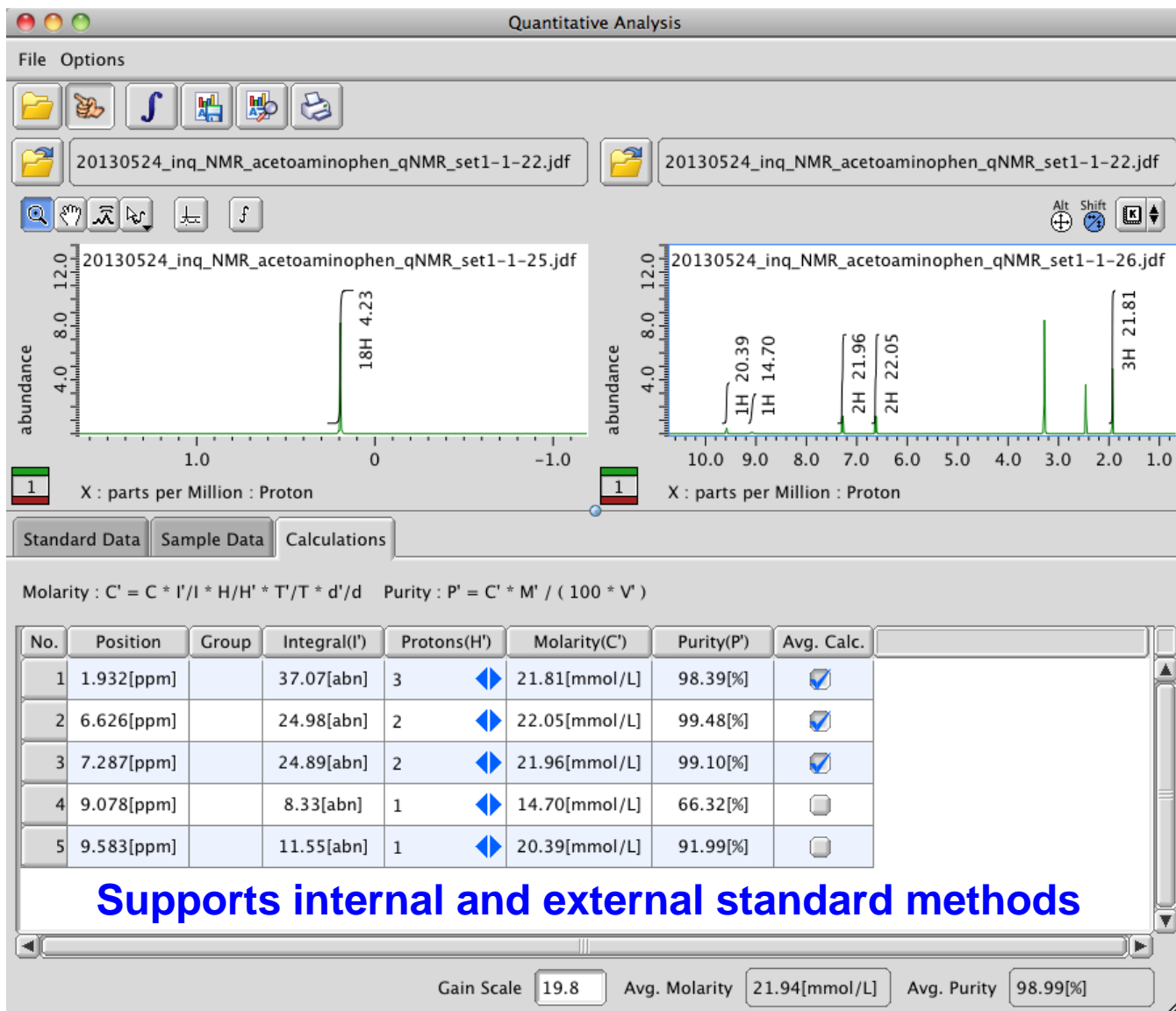


qNMR in Delta

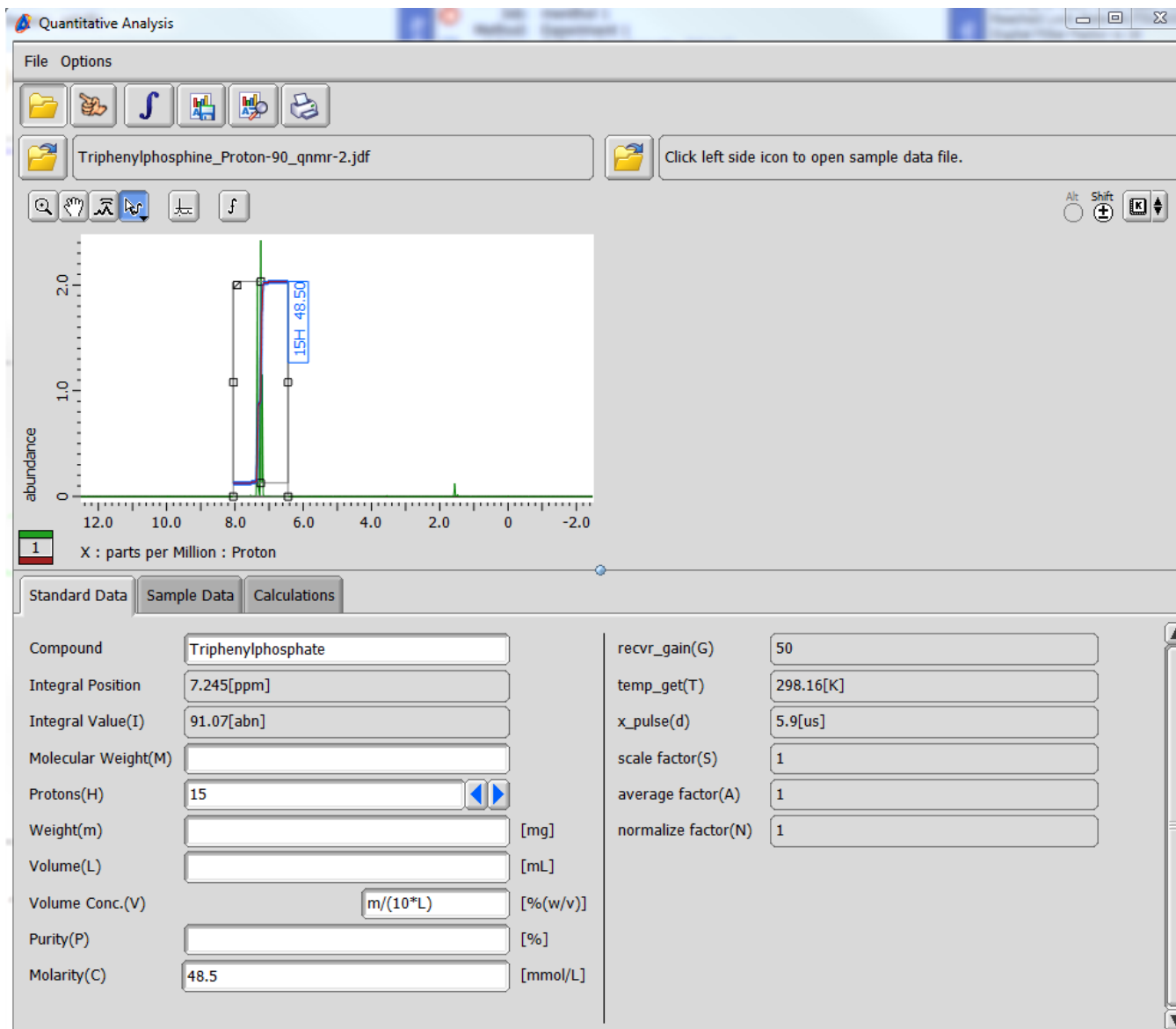
Data processing and analysis – Delta software for qNMR



Data processing and analysis – Delta software for qNMR

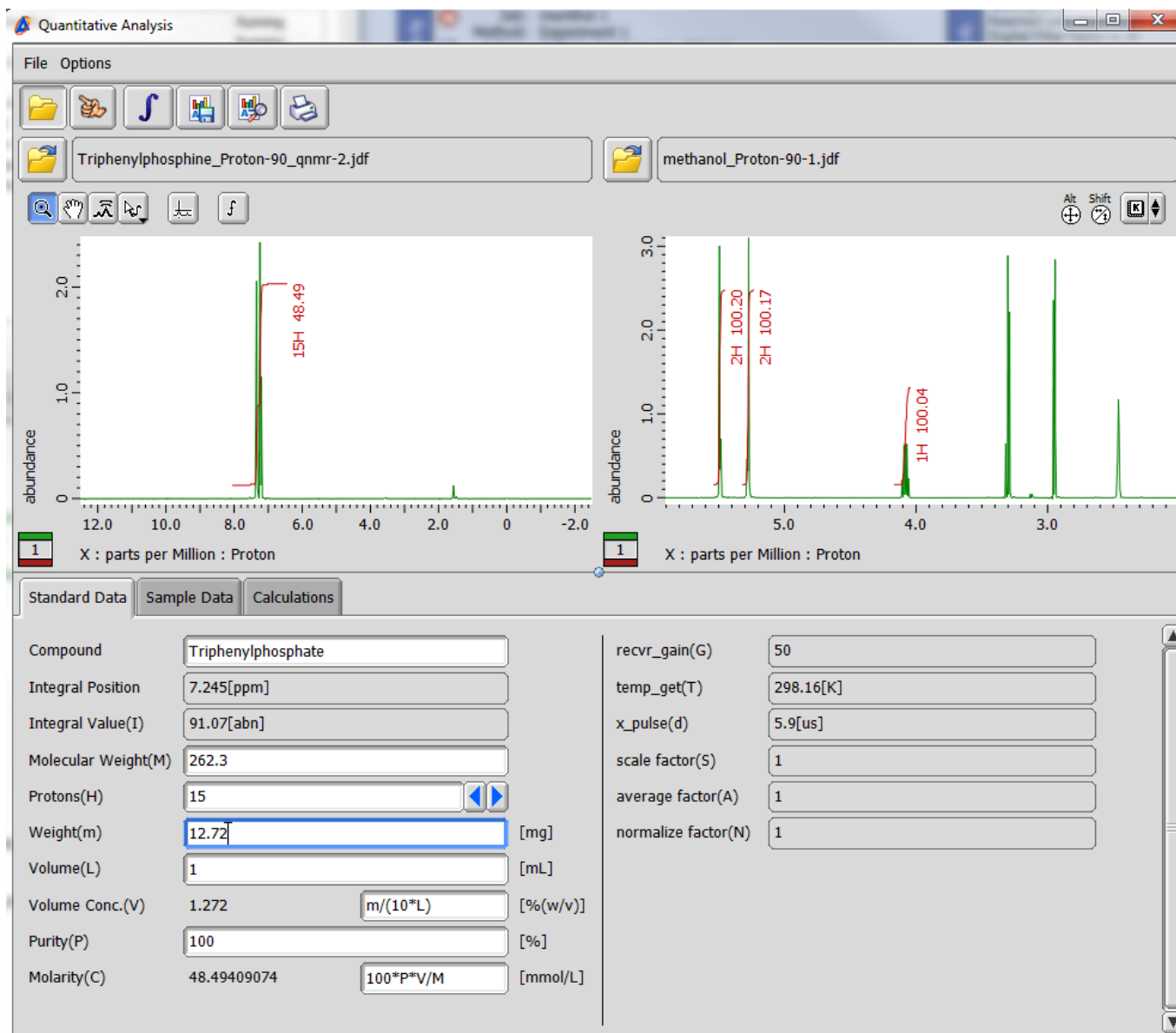


Data processing and analysis – Delta software for qNMR



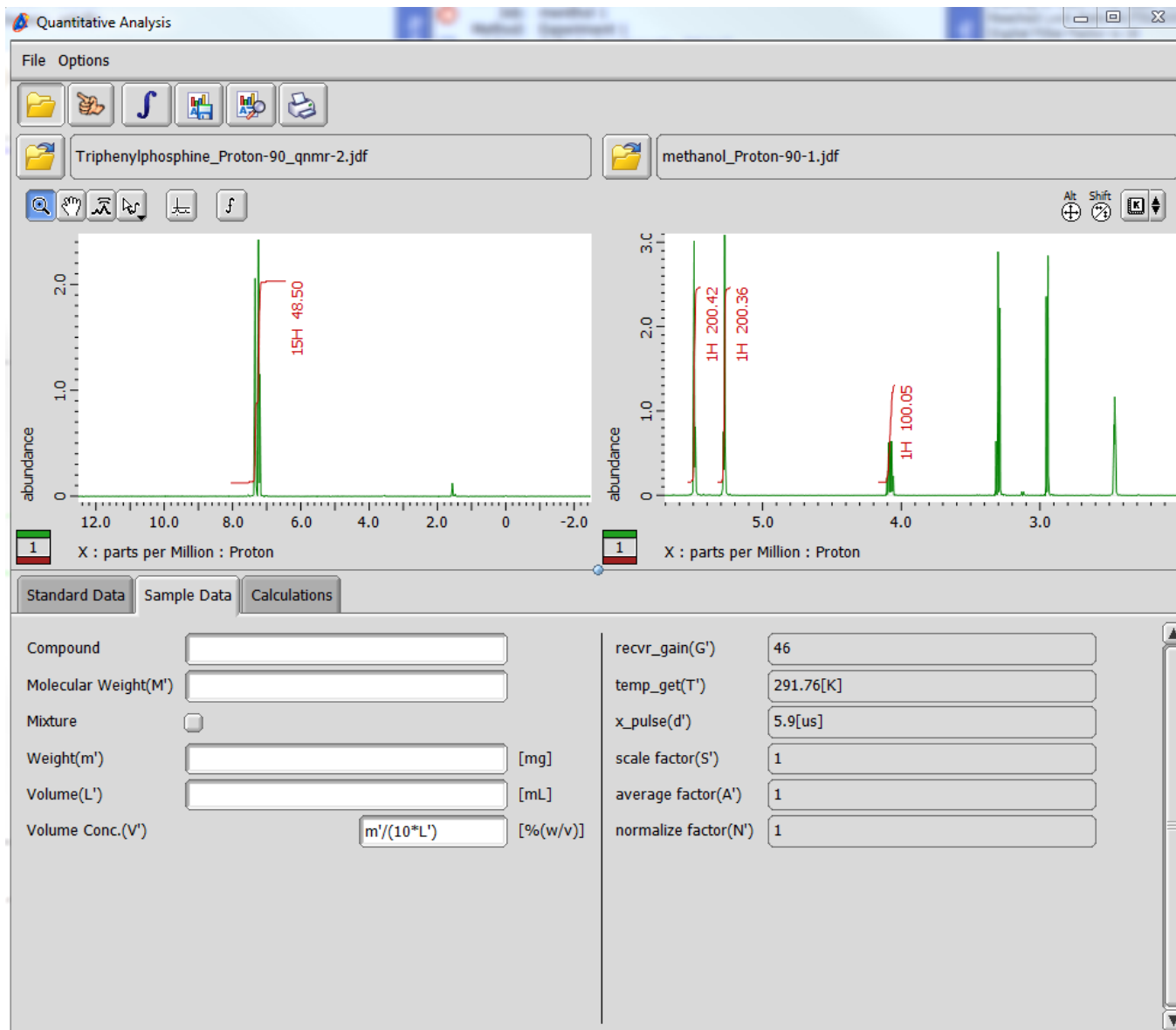
Input for
standard

Data processing and analysis – Delta software for qNMR



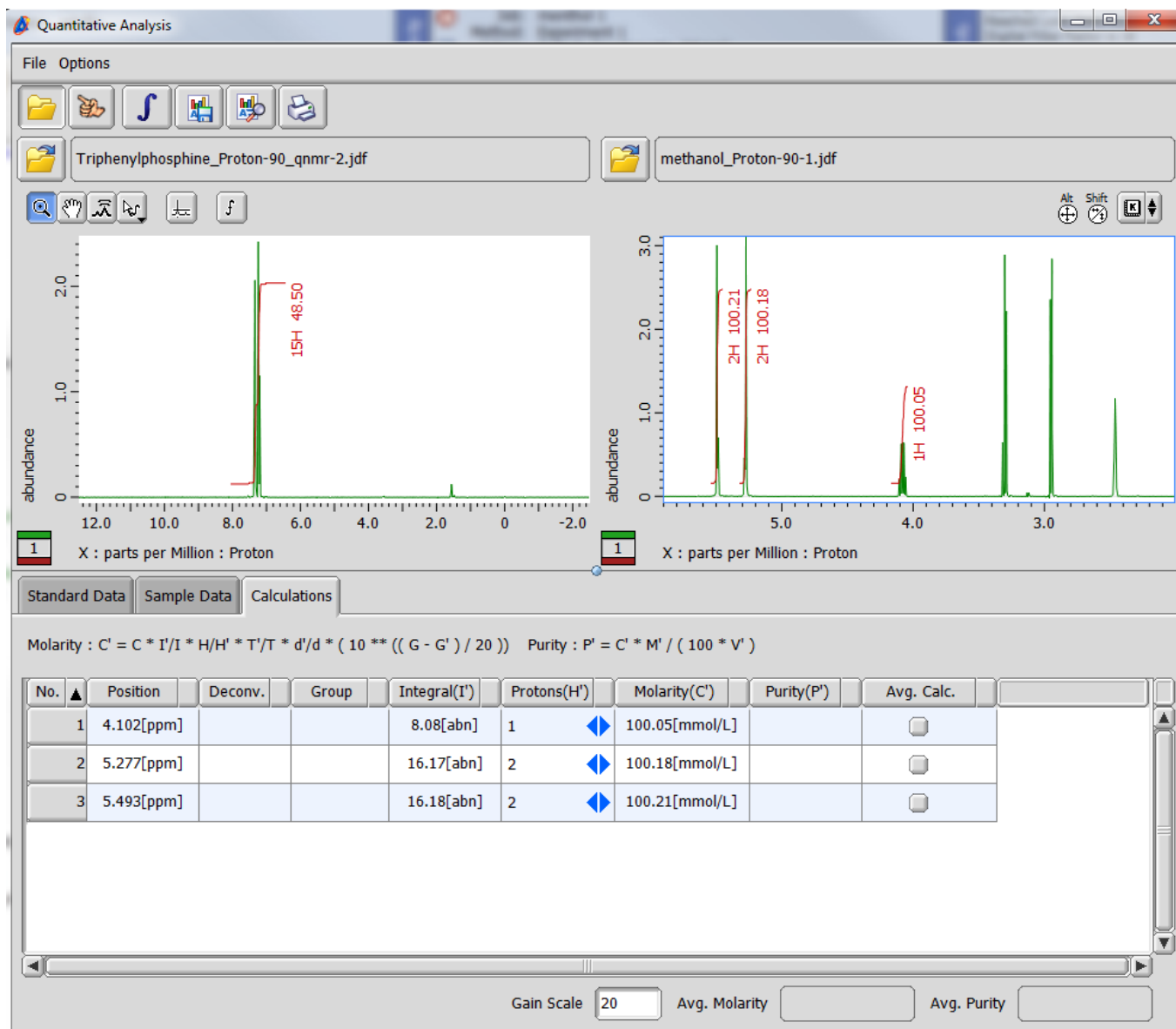
Alternative
Input for
standard
for purity
calculation

Data processing and analysis – Delta software for qNMR



**Automated
Calculation
for Sample data**

Data processing and analysis – Delta software for qNMR



Setting number of protons

Resources

1) Our qNMR webpage has access to several resources:

https://www.jeol.co.jp/en/products/nmr/qnmr_index.html

including a note on traceability for qNMR:

<https://www.jeol.co.jp/en/applications/detail/1171.html>

2) The Japanese legislation describing the methodology for this:

https://filex.jeol.com/filex/153668483730426/qNMR_Japanese_Pharmacopeia_english.pdf

3) Chapter “Analytical Standards Purity Determination Using Quantitative Nuclear Magnetic Resonance”, available from:

https://link.springer.com/chapter/10.1007/978-981-10-3421-3_20

which you can preview from:

https://books.google.ie/books?id=VIxkDgAAQBAJ&pg=PA275&lpg=PA275&dq=doi:10.1007/978-981-10-3421-3_20&source=bl&ots=74FGNFTX_G&sig=pHZ8zc7eSF02FGwXeFgsbZNuD9Y&hl=en&sa=X&ved=2ahUKEwjIz_LsrPdAhVNdcAKHeiMDfQQ6AEwAHoECAIQAQ#v=onepage&q=doi%3A10.1007%2F978-981-10-3421-3_20&f=false

4) Chapter “Applications of Quantitative ^1H NMR in Food- Related Analysis”

<http://www.eurekaselect.com/141180/chapter/applications-of-quantitative-1h-nmr-in-food-related-analysi>

Thank you

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- <http://www.jeol.co.jp/en/>
(Products -> NMR)
 - Description of our products
 - Free processing software
 - Free natural products database
 - Application notes
 - Events
 - And more

- <http://nmrsupport.jeol.com/> (license)

The screenshot displays the JEOL website's NMR section. At the top, the JEOL logo is on the left, and navigation links for PRODUCTS, APPLICATIONS NOTES, SUPPORT, and ABOUT US are on the right. Below the navigation bar is a 'NEWS' section with a list of updates from 2018/08/24 to 2018/08/14. A banner for 'Important information for NMR users' is also present. The 'CASE STUDY' section features three images with captions: 'Synthetic Organic Chemistry Laboratory (Kobayashi Lab)', 'Analysis Center (CRL), Central Research Laboratories, DIC Corporation', and 'Toray Research Center, Inc. (Shiga)'. The 'PRODUCT LINEUP' section shows five categories: ECZS NMR spectrometer FT NMR, ECZS NMR spectrometer FT NMR, Delta NMR Software, Year Hold Magnet, and Magnet. At the bottom, there is a grid of eight links: 'NMR data processing software', 'NMR peripherals/consumables', 'User stories', 'NMR basic knowledge/history', 'CH-NMR-ND', 'Liquid/solid state NMR probes', 'NMR application note', and 'quantitative NMR'.

JEOL

PRODUCTS APPLICATIONS NOTES SUPPORT ABOUT US

NEWS

2018/08/24 Update: NMR Challenge – Moving into the future with an evolution of reliability

2018/08/22 Delta NMR Software "Delta Tips" (NMDT_0057 to NMDT_0058)

2018/08/14 ROYALPROBE HPX Application Example (2)

2018/08/14 ROYALPROBE HPX Application Example (1)

2018/08/14 ROYALPROBE HPX

Important information for NMR users

CASE STUDY

Synthetic Organic Chemistry Laboratory (Kobayashi Lab), Department of Chemistry, School of Science, University of Tokyo

Analysis Center (CRL), Central Research Laboratories, DIC Corporation

Toray Research Center, Inc. (Shiga)

PRODUCT LINEUP

ECZS NMR spectrometer FT NMR

ECZS NMR spectrometer FT NMR

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Magnet

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NMR application note

quantitative NMR