Automating T1 evaluation for ^{1}H quantitative NMR

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NMR is a spectroscopic technique mostly known for its application in structural and dynamical studies. However, it also gives quantitative information and this potential has been exploited since many years.[1,2]

The key advantages of NMR in quantification are: high specificity, high reproducibility, high precision, high accuracy and speed. [1,2] Moreover, NMR can be easily automated thus allowing fast, safe and unbiased quantification of chemical compounds, pure as well as in mixtures, with a simple sample preparation and in most cases no matrix effects.

The quantification relies on the following equation that relates the integral area of a peak I_x with N_x (number of nuclei generating the peak under investigation):

$$I_x = K_s N_x \qquad (1)$$

where K_s is a constant that depends on the NMR spectrometer hardware.[1] Therefore, upon calibration of K_s , NMR gives absolute quantitative results.

Calibration can be achieved with a known standard that is dissolved together with the analyte (internal standard method) [3] or measured in an independent experiment (external standard method).[4,5] The two setup have specific advantages. For instance, quantification with external standard method does not contaminate the analyte and some spectroscopic parameters (i.e. number of scans or relaxation delay) are optimized on each experiment. On the other hand, internal standard method is known to give more accurate results.[6]

Acquisition of quantitative ^{1}H NMR spectra requires special care in the sample preparation and experimental setup. Probably the most critical parameter is the relaxation delay.[7] After each excitation pulse, the magnetization relaxes back to the equilibrium following an exponential decay

$$M_z = M_0 (1 - e^{-t/T1}) \qquad (2)$$

where M_z is the magnetization recovered after time t, M_0 is the magnetization at the equilibrium and T1 is the time constant of the relaxation process. This process is spin dependent, i.e. each signal peak is characterized by a different T1 constant. T1 depends on many different factors, such as spin chemical environment, temperature, concentration, solvent, etc.

To allow a recovery of 99.9% after a 90° excitation pulse, corresponding to a maximum uncertainty of 0.1%, one should consider a relaxation delay of 7*T1. When considering a recovery of 99%, corresponding to a minimum uncertainty of 1%, the recovery delay should be set equal to 5*T1.[4] In the case of 1H NMR spectra in solution, T1 ranges from few hundreds of milliseconds to tens of seconds. If T1 is not known, one should set the relaxation delays considering the most unfavorable situation, that is very long T1 and therefore very long relaxation delays. This leads to long experimental time.

To optimize the usage of the experimental time with no compromise in terms of performance, T1 must be measured, or at least evaluated, on each sample. This can be easily achieved by running a T1 inversion recovery experiment (T1ir) prior to the qNMR spectrum acquisition. In the Bruker library one can call for the parameter set "PROTONT1". In this experiment the fraction of the recovered magnetization after each one of the arrayed delays is measured, then the T1 is calculated according to the equation (2). One should note that the D1 used during the measurements of the proton T1 ($D1_{eval}$) is also important. If it is shorter than 3*T1, the evaluation of the relaxation times may not be correct.
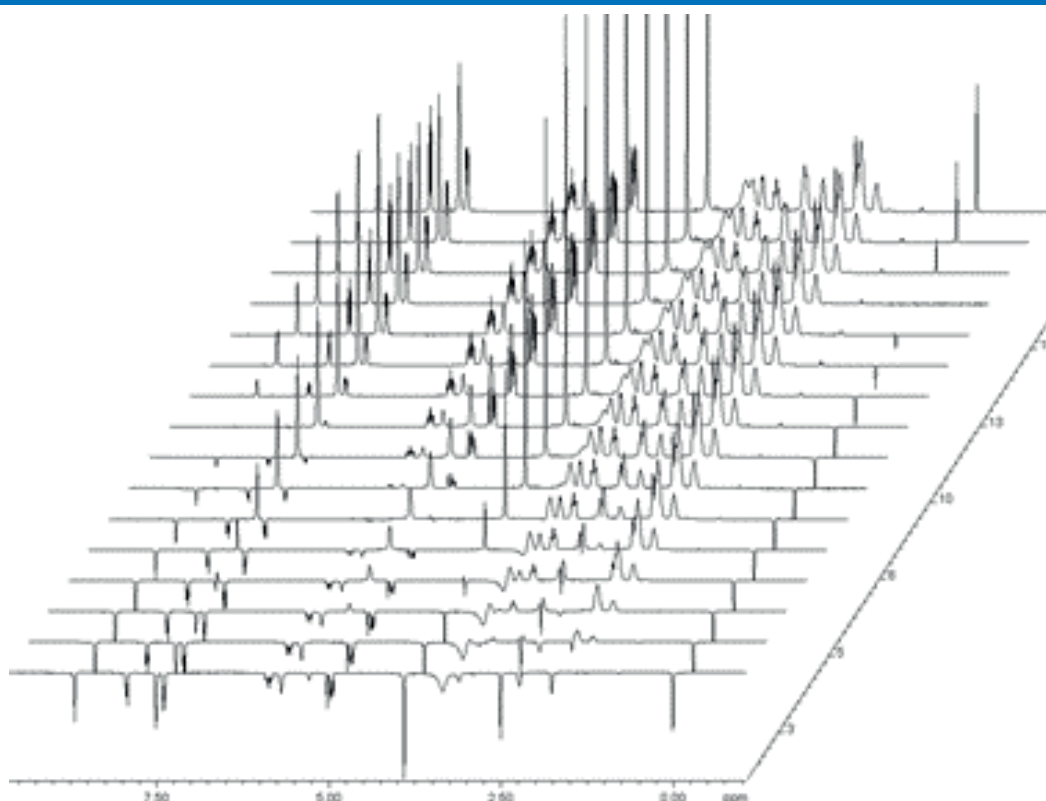

Figure 1: stacked plot of a series of T1ir experiments acquired as pseudo 2D

Once T1 of the peaks in the spectra are determined and the longest value ($T1_{MAX}$) is known , the relaxation delay (D1) can be calculated as follows:

$$D1 = 5 * T1_{MAX} - AQ \qquad (3)$$
or
$$D1 = 7 * T1_{MAX} - AQ \qquad (4)$$

according to the required uncertainty.

Here we will discuss the python script eval_t1: a new, automatic routine that, launched from a dataset for the acquisition of 1H NMR spectrum, measures T1ir experiment in a different dataset, calculates the T1 values, extracts the longest $T1_{MAX}$ and sets the D1 in the original 1H qNMR experiment according to equation (4). Further, this routine allows remeasuring the T1 if the $D1_{eval}$ during the first run of the T1ir experiment was not sufficiently long. The script is available from Topspin4.1.4 and can be called in ICONNMR by setting "au_zg_qNMR t1" as AUNM in a PROTON parameter set.

Experimental setup

To check experimentally the importance of $D1_{eval}$, a series of T1 measurements were conducted with different $D1_{eval}$ values.

The behavior of the eval_t1 script has been tested on spectra measured on an Avance NEO NMR spectrometer at 400 MHz with a BBFO probe. The ^1H NMR experiment was set with 8 scans, SW of 20 ppm, AQ of 4 sec, O1P of 4 ppm. The pulseprogam for the acquisition was zg and the 90deg pulse (duration and power) was taken from the prosol table.

Results and discussion

For an accurate measurement of T1 with an inversion recovery experiment a sufficiently long $D1_{eval}$ should be used. For this reason a series of exploratory T1 experiments with increasing $D1_{eval}$ was measured. The results are shown in Table 1.

Table 1

sample	$D1_{eval}$ = 12s $T1_{max}$ (sec)	$D1_{eval}$ = 25s $T1_{max}$ (sec)	$D1_{eval}$ = 50s $T1_{max}$ (sec)
10% Ethylbenzene in CDCl3	6.66	8.13	8.36
0.1% Ethylbenzene in CDCl3	6.78	10.37	13.84
200mM maleic acid in DMSO-d6	2.46	2.55	2.55
50mM maleic acid and 50mM antipyrine in DMSO-d6	5.85	6.05	6.07
70mM maleic acid and 70mM antipyrine in DMSO-d6	5.87	5.95	5.89
250mM sucrose in D20	2.45	2.41	2.41
50mM quinine in DMSO	6.60	8.42	8.97

Table 1: longest T1 measured in a series of samples indicated using the indicated $D1_{eval}$

From table 1, it can be concluded that for a correct measurement of T1, a $D1_{eval}$ that is roughly 3 times larger than the expected T1 is required. Since a priori T1 is not known, the eval_t1 script runs a first T1 measurement with $D1_{eval}$ = 24sec. If the result is a T1 longer than 8 sec, a second run is performed. The $D1_{eval}$ of this optional second run is set as 3 times the longest T1 measured in the first run, but with an upper limit of 240sec.

The variable delay lists used by eval_t1 are set to 0.1, 0.5, 4 and 16 sec for the 1st run and 0.1, 1, 8, 60 sec for the 2nd run. These lists are adapted to the maximum T1 expected for the two runs, which is up to 8 sec for the first run,and between 8 to 30 sec for the second run. The chosen delays allow optimal sampling of an exponential recovery, with 2 points with negative intensities, one around zero crossing and a strongly positive one. With these parameters, the time needed for the acquisition of the first run is 2 min. The duration of the second, follow-up run depends on the $D1_{eval}$ calculated, but it can never exceed 7 min and 13 sec. Examples of fitting curves of the same peak acquired during the first and the second run of the T1 inversion recovery measurements are shown in Figure 2.
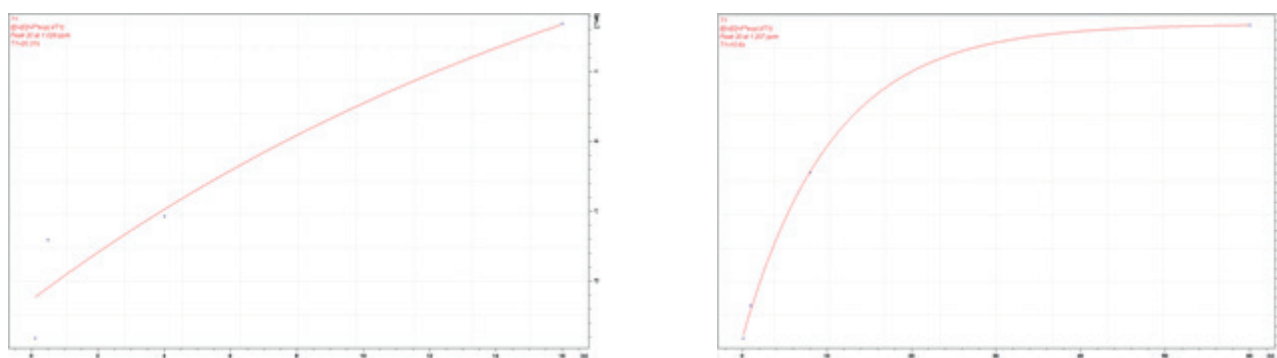
Figure 2

Figure 2: On the left side, fitting of the T1 inversion recovery curve measured with $D1_{eval}$ of 24 sec, on the right side, fitting of the T1 inversion recovery curve measured with $D1_{eval}$ of 60 sec, as used in the second run of the eval_t1 method.

Based on the T1$_{max}$ measured with optimized D1$_{eval}$ and variable delay list, a series of quantitative ^{1}H NMR spectra were setup and measured twice in full automation using the eval_t1 script. The D1 values set by the script are shown in Table 2. This data shows that the script provides well reproducible results and that the second T1 inversion recovery measurement is correctly acquired samples whose spectra contain signals with longer T1 values. The script is robust and can also be safely used for mixtures and for potency determination. The shortest D1 set by the script eval_t1 is 2 sec, while the longest D1 is limited to 240s. An example of a ^{1}H qNMR spectrum with signal integration done in full automation is shown in Figure 3. In this example, the integrals were set by the sigreg command, without manual adjustments.
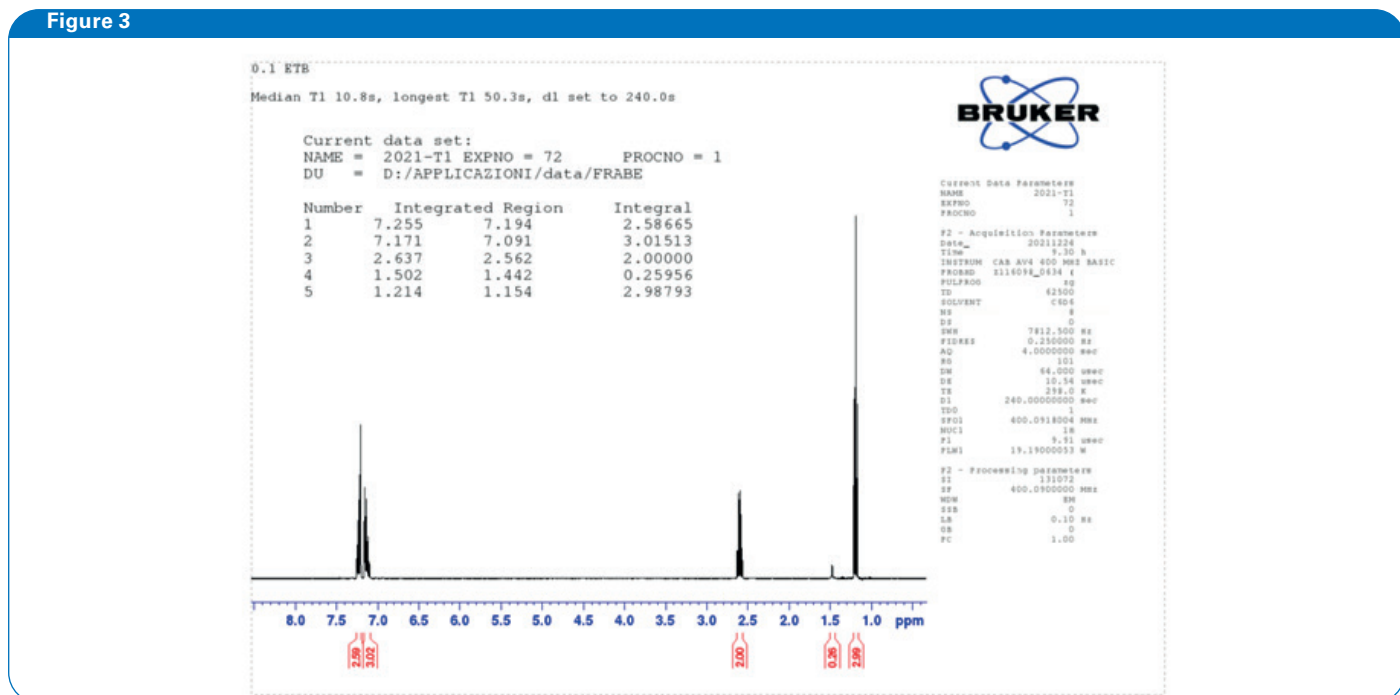
Figure 3

Figure 3: ^{1}H qNMR spectrum of 0.1% Ethylbenzene in CDCl3, with signal integrals measured in full automation. The integral regions were chosen automatically with the "sigreg" command

Table 2

Sample	1st or 2nd run of T1ir	D1 (in sec) for qNMR experiment
10% etylbenzene- Bruker standard	1st	46.4 ; 51.7
Formamide-Bruker standard	1st	28.2 ; 28.8
Triphenyl Phosphate- Bruker standard	2nd	67.5; 66.6
Trifluoro Toluene-Bruker standard	2nd	197.2; 194.7
3% Chloroform- Bruker standard	2nd	240; 240
ASTM- Bruker standard	1st	2.0 ; 2.0
0.1% ethylbenzene- Bruker standard	2nd	240; 240
Mixture 4.3mg maleic acid + 9mg flurbiprofen in DMSO-d6	1st	15.9; 16.0
Mixture 6.7 mg maleic acid + 13.8 mg flurbiprofen in DMSO-d6	1st	16.8; 16.5
Dexamethasone 21-phosphate 80mM in D2O	1st	9.4; 10.0
Olive oil in CDCl3	1st	20.8; 21.3
α-pinene (50 μl), 1,7-octadiene (50 μl), 1-methylnaphthalene (50 μl) in 0.6 ml C$_6$D$_6$	2nd	145.1; 146
Ibuprofen in DMSO 20 mM	1st	3.4 ; 3.4
Quinine in DMSO 50 mM	1st	13.3 ; 13.1
Quinine in DMSO 1 mM	1st	13.3
Quinine in DMSO 0.5mM	1st	7.3

Table 2. D1 set by eval_t1 in a series of samples. All measurements have been run twice to check the reproducibility of the method. Both results are shown. In the table it is indicated if the second T1 inversion recovery run was performed or not.

The comparison between the D1 set for quinine samples at different concentrations shows that the 0.5mmol solution results in a considerably shorter D1. The reason for this is that in low concentrated samples, the sensitivity obtained with a single scan in the inversion recovery experiment is not sufficient to detect all the relevant peaks using the default dynamic range of 10 set by the script. This parameter can be edited and for instance be adjusted to a higher value of 200, according to the expected sensitivity, by setting "au_zg_qNMR t1 -drange 200" as AUNM in the parameter set. As can be seen in the Table 3 below, with a higher dynamic range the D1 is measured more correctly.

Table 3

Quininine in DMSO	drange value	1st or 2nd run	D1 (in sec)
50m mmol	10 (default)	1st	13.3 ; 13.1
1 mmol	10 (default)	1st	13.3
0.5 mmol	10 (default)	1st	7.3
0.5 mmol	200	1st	10.4

Table 3. D1 set by eval_t1 in a series of samples of quinine in DMSO-d6 with different concentration

Conclusion

It has been shown that accurate and automatic T1 calculation is feasible in automation using IconNMR and the python script eval_t1. This approach implies a two step process that aims to optimize measurement time with no compromise in terms of accuracy. The fitted T1 curves are available for manual analysis to check if the D1 values are correctly estimated.

The script is in python programming language and can be customized by the user to cover specific needs such as diluted sample, special T1 ranges, different flip angle, etc. These options are described in the help menu that can be called with the "eval_t1 -h" command.

The script is available from Topspin 4.1.4.

References

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