Rapid Estimation of Repetition Time (T_r) for Quantitative Nuclear Magnetic Resonance

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ABSTRACT

Despite significant advances, many scientists within the Nuclear Magnetic Resonance (NMR) community continue to express reservations about the capability of NMR for quantitative analysis. A major source of this skepticism stems from the gap between standard NMR acquisition settings (e.g., default relaxation delay, D1 as 1 s) and those required for quantitative NMR (qNMR), with one critical requirement being the determination of longitudinal relaxation times (T_1). This illustrates that standard NMR experiments often cannot achieve complete spin relaxation. However, by taking advantage of this phenomenon, it may provide a simple and straightforward approach to estimate the appropriate D1 value for qNMR parameter setup. So, the present study explores an alternative approach that simplifies the process of D1 estimation, connects standard NMR setting with repetition delays (T_r) and avoids T_1 determination in qNMR applications. Specifically, this study applies a single-exponential decay regression model to predict optimal T_r, using only a minimal set of 1D standard NMR experiments. With just three datasets acquired at varying D1 values, the proposed approach accurately estimates T_r values corresponding to nearly maximal (99.99%) signal response intensity. Experimental validation across six chemical compounds and three solvents confirmed the robustness of the approach. The predicted T_r values consistently fell within a "sweet spot" range, approximately 7 - 10 times the measured T_1, which is sufficient for reliable quantitative analysis. Additionally, this study shows that such T_r prediction not only reduces the technical barrier for users but also helps them understand the importance of qNMR acquisition settings, thereby streamlining qNMR workflows.

Keywords: Quantitative NMR, T_1 determination, T_r prediction

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INTRODUCTION

In NMR, accurate quantitation relies on ensuring complete spin relaxation between scans, typically achieved by setting repetition time (T_r) to typically no less than $5 \times T_1$. Currently, precise determination of T_1 is essential for robust qNMR method development. The inversion recovery experiment, based on a 180°–τ–90° pulse sequence (**Fig. 1**), has long served as the standard method. This technique, developed in the 1950s following the theoretical work of Bloembergen, Purcell, and Pound, captures the full dynamic recovery of longitudinal magnetization, from $-M_0$ to $+M_0$, and remains essential for physical characterization and pulse sequence development.[1,2]

As such, the focus in practical qNMR workflows is not on accurately measuring T_1, but rather on determining a reliable T_r that ensures complete spin relaxation between scans. The present study introduces an efficient, application-driven approach: signal response intensities (i.e., absolute integral values or under peak area) are collected at various T_r values

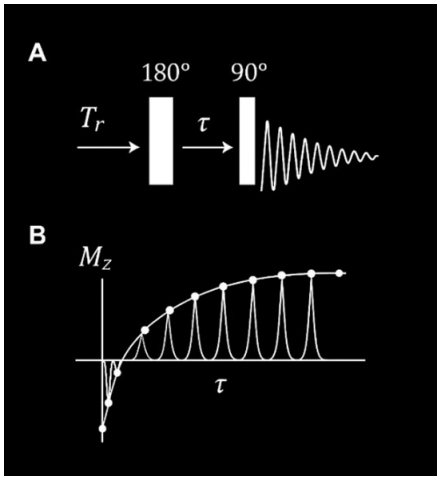

Fig. 1. Pulse sequence (A) and corresponding exponential-recovery fitting curve (B) for the inversion-recovery experiment used to determine T_1. (B) shows the dependence of longitudinal magnetization M_z.

under fixed experimental conditions and fitted into a single-exponential recovery model. Rather than measuring T_1, this proposed approach directly predicts proper T_r value, by establishing the relationship between factors of T_r and signal response intensity. The single-exponential recovery model for T_r estimation is fundamentally different from the inversion recovery model used for T_1 measurement. Designed to be user-friendly, especially for all NMR scientists, this proposed approach avoids understanding and practice of phase-sensitive detection and inversion pulses, reduces data acquisition effort, and enables rapid T_r estimation. A supporting Python script has been provided (**Supporting Information I**) to further facilitate the widespread application and validation of this method.

EXPERIMENTAL

Materials and Reagents

Test samples were all purchased from National Institutes for Food and Drug Control (Beijing, China), including aripiprazole, repaglinide, diclofenac sodium, donepezil impurity C, trazodone hydrochloride impurity B, vitamin B6 (pyridoxine hydrochloride). Deuterated solvents and NMR tubes were obtained from Sigma Aldrich (St. Louis, MO, USA). All NMR solvents, such as DMSO-d_6, D_2O, and acetone-d_6, contained 99.9% deuteration (D).

Sample Preparation

Prior to preparing the sample solution, the samples were taken out of storage and placed in a desiccator with a desiccant (e.g., silica gel) to equilibrate with an ambient temperature. A test sample solution was prepared under ambient temperature and 20–60% of relative humidity.

The sample preparation process began with weighing approximately 10-20 mg test sample to conveniently achieve an adequate signal-to-noise ratio and transferring it to a 2-mL vial. Subsequently, 0.75 mL of deuterated solvent (**Table 1**) was added using a gas-tight syringe and added to the same vial. Finally, the solution was transferred into a 5-mm NMR tube.

Table 1. Measured T_1 values of selected compounds in different solvents

Chemical	Solvent	Measured T_1 (s)
Aripiprazole	DMSO-d_6	2.0
Repaglinide	DMSO-d_6	2.0
Diclofenac sodium	DMSO-d_6	2.4
Donepezil impurity C	DMSO-d_6	2.6
Trazodone hydrochloride impurity B	Acetone-d_6	4.8
Vitamin B_6	D_2O	4.8

Instrumentation, Data Acquisition, and Data Analysis

The ^{1}H qNMR experiments and inversion recovery experiments were implemented using high-field NMR instrument, a Bruker AVANCE 500 spectrometer equipped with a 5-mm BBO/cryoprobe probe, and the Topspin software (version 3.6.5).

Regarding measurement of T_1 values, inversion recovery experiments were assayed using an automatic sequence[1,2] available on NMR spectrometers.

While T_r prediction experiments required use of acquisition time (AQ) of 4 s and D1 values of 0.1 s, 1 s, and 10 s, respectively. Receiver gain was constant for all experiments.

For data analysis, the spectral width was adjusted to capture the entire spectral region. All spectra were processed using standard Fourier transform with exponential line broadening (typically 0.3 Hz) prior to integration. All data were the absolute integration values, instead of the normalized integration values.

Models of Inversion Recovery and Saturation Recovery

Inversion recovery model for measured T_1

As shown in **Fig. 1**, the inversion recovery technique involves a 180° inversion pulse that flips the magnetization, followed by a variable delay time (τ) to allow partial relaxation. A 90° readout pulse is then applied, and the resulting signal response follows an exponential recovery equation (a.k.a., the inversion recovery of longitudinal magnetization,[3] described as **Equation 1**), enabling the measurement of relaxation dynamics. Data is fitted to this equation to determine T_1.

$$M_z(\tau) = M_0\left(1 - 2e^{-\tau/T_1}\right) \qquad \textbf{(Equation 1)}$$

where $M_z(\tau)$ is the longitudinal magnetization at time, M_0 is the equilibrium magnetization, T_1 is the longitudinal relaxation time, and $2e^{-\tau/T_1}$ represents the exponential recovery process.

Procedurally, each identified peak or pattern must first be processed to obtain its individual single T_1 value. Only after all single T_1 results are calculated can the maximum T_1 value be correctly identified. The reported T_1 value for a given sample typically corresponds to the signal response with the longest T_1 observed in the NMR spectrum.

Saturation recovery model for T_r estimation

Single-exponential decay regression

To estimate the relaxation behavior, a single-exponential decay model was applied using nonlinear least squares regression. The fitting was performed with the curve_fit() function from the SciPy library in Python, which internally implements the Levenberg-Marquardt algorithm. This approach allows for accurate modeling of maximum amplitude behavior using minimal data, typically as few as three standard ^{1}H NMR experiments

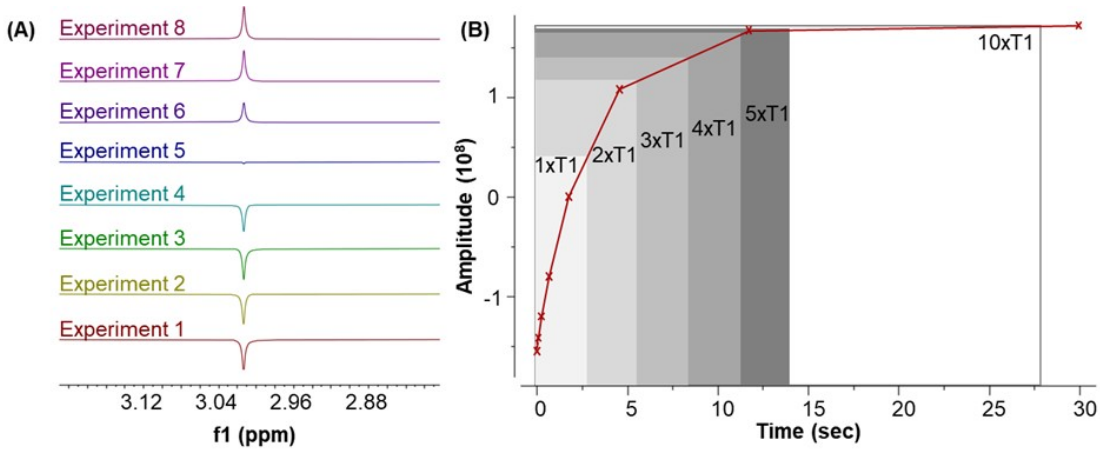

Fig. 2. (A) inversion recovery experiment of methyl sulfonyl methane for T_1 determination; (B) an exponential recovery fitting curve, displaying the relationship between delay time, τ (see **Fig. 1**) and T_1 value. In the experiment, the T_1 value of this solution was 2.8 s. The figure refers to the book chapter, *Quantitative NMR in Quality Control, in Quality Control of Chinese Medicines-Strategies and Methods. Springer Nature: 2023.*[4]

using different T_r setups. The model can effectively simulate the shape of a saturation recovery profile, providing a T_r value at the amplitude, 99.99% of I_{max} ($I_{max}^{99.99\%}$).

Given the nature of a single-exponential decay fitting for T_r prediction, it can be described as:

$$I(T_r) = I_0\left(1 - e^{-T_r/T_1}\right) \qquad \textbf{(Equation 2)}$$

where $I(T_r)$ is the signal response intensity at the specified repetition time T_r, I_0 is the amplitude, and e^{-T_r/T_1} describes the exponential recovery process.

T_r Estimation Using AI Tool – ChatGPT 4o As An Example

The T_r can be efficiently estimated using ChatGPT 4o through the following workflow:

Step 1: Provide the Python script (available in **Supporting Information I**) to the AI tool. The script is designed to perform single-exponential decay fitting using a limited set of T_r values and corresponding signal response intensities.

Step 2: Input the command prompt, e.g., "*Perform single-exponential decay fitting and report the estimated 99.99% of maximum intensity (I_{max})*". The AI tool will process the input and execute the fitting algorithm accordingly. The result should be the same as **Fig. S1**.

Step 3: Input the measured datasets of a test sample, i.e., measured absolute integral values and corresponding T_r values.

Step 4: Based on the fitted model, the AI tool can provide estimated T_r values required to achieve $I_{max}^{99.99\%}$ for the qNMR procedure.

[Note 1: Different signal responses in an NMR spectrum can exhibit variations in predicted T_r values due to individual relaxation behavior. In this study, the longest predicted T_r value was used, against the T_r value derived from measured T_1 value. This ensures that the selected T_r reflects the near-complete relaxation condition specific to all signals.]

[Note 2: This approach enables direct T_r prediction for both the target compound signal response(s) and the internal calibrant signal response(s), thereby ensuring quantitative results.]

Step 5: The resulting T_r allows the selection of appropriate D1 settings for a quantitative analysis, where the D1 value used in quantitation should be no less than the value from the resulting T_r – AQ.

^{1}H Quantitative NMR Determination

The analytes were dissolved in appropriate deuterated solvents, to ensure complete solubility. ^{1}H qNMR experiments were carried out on selected compounds. All experiments were carried out at 25 °C.

The acquisition parameters were optimized for each compound to ensure accurate quantification. In particular, the T_r was predicted using the single-exponential decay model fitting based on three datasets. For all experiments, the AQ was fixed at 4 s. The predicted D1 values were used in ^{1}H qNMR experiments. The number of scans (NS) was typically set to four or more. Signal integration was performed after appropriate processing parameters (see section **Instrumentation, Data Acquisition, and Data Analysis**).

RESULTS AND DISCUSSION

Nowadays, T_1 values are measured by inversion recovery experiments. As shown in **Fig. 2**, the relationship between the factors of delay time (τ) and signal response intensity was established across eight datasets, enabling the calculation of T_1 and the determination of required T_r. Since T_1 characterizes the return of the magnetization to its equilibrium along the z-axis after being perturbed, there is an intrinsic correlation between signal response intensity and the time domain parameter (i.e., directly with T_1 and correlated with T_r). Following this line of reasoning, the present study explored if a correlation between signal intensity and the T_r can be directly established using a standard 1D NMR experiment.

In a standard 1D NMR experiment, a scan or pulse sequence contains the pulse (P1, μs), acquisition delay (DE, μs), acquisition time (AQ, s), and relaxation delay (D1, s). Therefore, the time domain parameter, T_r typically refers to AQ + D1. In 1D (e.g., ^{1}H) NMR practice, D1 is often set to a default value (e.g., 1 s), which is usually insufficient to achieve full relaxation delay. As a result, the measured signal response intensities may not reflect true equilibrium conditions, leading to inaccurate quantitative measurement. This highlights a fundamental distinction

Table 2. Artificial signal response intensities at different time points (T_r) for three mock-up datasets

	Artificial signal response intensity		
Time (T_r, s)	X (dataset 1)	Y (dataset 2)	Z (dataset 3)
0.1	10	10	10
1.0	80	96	50
10.0	99.9	99.6	96

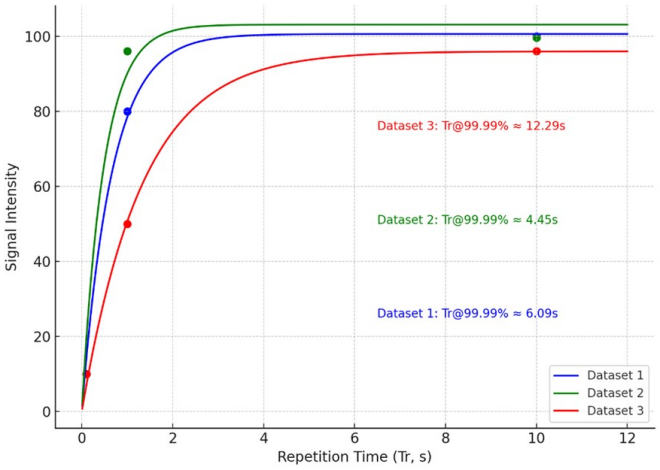

Fig. 3. Single-exponential decay regression fits of simulated NMR signal response intensities across three datasets

between standard NMR and qNMR, one that chemometric tools are particularly well suited to detect. Therefore, this study exploits this distinction by varying D1 values while keeping the AQ constant. For example, increasing D1 from 0 to 10 seconds or more produces measurable variations in signal response intensity that reflect different extents of longitudinal relaxation. By systematically collecting signal responses at multiple D1 values, a correlation between T_r and signal response intensity can be constructed. Applying a single-exponential decay regression to these data enables curve fitting and estimation of the optimal T_r corresponding to the (almost) fully relaxed signal state (i.e., $I_{max}^{99.99\%}$). More discussion can be found in section ***Estimated T_r at $I_{max}^{99.99\%}$ for quantitative analysis***.

Selection of Regression Models

Prior to conducting the NMR experiment, we constructed three sets of mock-up datasets (**Table 2**) to rapidly screen different regression models using ChatGPT-4o, in order to identify those best suited for curve fitting of the correlation between T_r and signal response intensity. This approach enables prompt and systematic evaluation of regression models under uniform, controlled conditions, while eliminating the need for data acquisition, analysis, and cleaning. In particular, artificial integration values were generated for three fixed repetition times (0.1 s, 1 s, and 10 s), which were designated as X, Y, and Z, respectively. These values served as synthetic input-output pairs, as shown in **Table 2**, to evaluate model behavior in a scenario mimicking real experimental acquisition.

To identify effective saturation recovery model for predicting appropriate T_r values in qNMR, three types of regression models were evaluated, including (i) single-exponential decay regression model, which is the classical model widely used for relaxation analysis; (ii) logistic regression model, which is used for datasets exhibiting saturation effects; and (iii) linear regression model as a negative control. By inputting the same mock-up dataset into each modeling approach, their performance can be assessed and understood.

Regarding the single-exponential regression model, **Equation 2** can be used to fit curve for T_r estimation once $I_{max}^{99.99\%}$ is identified. The single-exponential regression model is well-suited for describing the T_r curve and has clear physical significance. It requires only two parameters, i.e., signal response intensity and T_r, to perform the fitting. For estimation, the Levenberg-Marquardt algorithm offers a robust and efficient solution. As shown in **Fig. 3**, integration of a nonlinear regression approach and exponential decay fitting model can predict T_r at $I_{max}^{99.99\%}$ value. Moreover, single-exponential decay regression directly reflects the exponential recovery behavior of magnetization, preserving theoretical transparency. The Python script appears in **Supporting Information I**. To evaluate the quality of the model fits, we computed the Root Mean Square Error (RMSE) for each dataset. This metric quantifies the average deviation between the input data and the model-predicted values, thereby serving as a robust indicator of fitting accuracy. RMSE of X (dataset 1) was 0.58; Y (dataset 2) was 1.86 and Z (dataset 3) was 2.79. Among the three datasets, the fitting error increases in the following order: X < Y < Z. X exhibited excellent agreement with the model. The intensity rapidly approaches the saturation value within 10 s, and the small RMSE indicates that the data closely follows the ideal exponential growth. However, Z has the highest RMSE, primarily due to the gradual and less defined curvature. The signal intensities rise more slowly, making it harder for the model to anchor the fit without additional intermediate time points. The sparsity of data between 1 s and 10 s contributes to uncertainty in determining the exact shape of the exponential rise. This trend reflects the increasing difficulty in accurately modeling the relaxation process when (i) the signal growth is more gradual; (ii) the experimental points are less evenly distributed across the curvature of the exponential rise, and (iii) there is less resolution in the early part of the recovery curve. These results underscore the importance of carefully selecting D1 values when designing experiments for T_r estimation. If needed, the use of additional datasets (i.e., more than three) can help mitigate the challenges described above. Ideally, D1 values should span the full dynamic range of the exponential rise to ensure minimal fitting error and higher confidence in T_r estimates.

Logistic regression model was also evaluated to test if it can be integrated with the fitting curve. However, compared to the fitting curves that asymptotic rise to I_{max} in **Figs. 2** and **3**, the logistic regression curve exhibits Sigmoidal (S-curve) with inflection points, highlighting significant deviations in the fitting behavior. This does not match the observed monotonic relaxation trend and then causes poor fitness and especially misrepresents early data (data not shown). As negative control, extrapolation using linear regression methods is unreliable due to the inherent assumption of linearity, which fails to capture the nonlinear relationships present in the data. A preliminary evaluation using process simulation and uncertainty indicates that linear regression method leads to significantly higher uncertainty for prediction of T_r at $I_{max}^{99.99\%}$ (data not shown). The linear approach struggles particularly with extrapolated values, as it exhibits high sensitivity to individual data points and lacks the flexibility needed to adapt to the nonlinear dependence. It has

Table 3. T_r based on measured T_1 vs. predicted T_r at $I_{max}^{99.99\%}$ for compounds under various acquisition conditions

	Measured T_r (s)			Predicted T_r when AQ=4 s		Predicted T_r when AQ=3 s	
Chemical	5×T_1	7×T_1	10×T_1	[a] (s)	[b] (s)	[a] (s)	[b] (s)
Aripiprazole	10	14	19	17	17	17	17
Repaglinide	10	14	19	18	18	18	18
Diclofenac sodium	12	17	24	22	22	22	22
Donepezil impurity C	13	18	25	23	23	22	22
Trazodone hydrochloride impurity B	24	33	47	43	44	42	42
Vitamin B6	24	34	48	44	44	42	42

[a] denotes five datasets use D1 values of 0 s, 0.01 s, 0.1 s, 1 s and 10 s; [b] displays three datasets use D1 values of 0.1 s, 1 s and 10 s; Predicted T_r is based on $I_{max}^{99.99\%}$.

to be mentioned that machine learning methods were excluded in this study. Machine learning often does not perform well for prediction of T_r at $I_{max}^{99.99\%}$ under the proposed scenario, due to machine learning algorithms requiring large, diverse datasets to effectively train and generalize patterns. However, in estimation of T_r at $I_{max}^{99.99\%}$, especially when only three experiments (with different T_r values) are conducted per sample, the data volume is far too limited to support robust machine learning model training.

Experimental-based Model Evaluation

To validate the findings above, we conducted experimentation using chemicals in various solvent systems, each estimating different T_r values using single-exponential decay regression. The actual T_1 values were measured using inversion recovery experiments, providing a reliable reference for model evaluation.

Estimated T_r at $I_{max}^{99.99\%}$ for quantitative analysis

Six distinct chemical compounds and three solvents were investigated in this study. The T_1 values of each sample solution were experimentally determined (**Table 1**), and the corresponding T_r values (**Table 3**) were predicted using the proposed single-exponential decay regression model. The key findings are as follows:

(i) Provided that sufficient digital resolution was maintained, AQ had minimal impact on prediction accuracy. Both 3 s and 4 s AQ settings produced comparable T_r estimates.

(ii) Predicted T_r values generally fell between 7 × and 10 × the measured T_1 values, supporting the conclusion that the quantitative accuracy exceeds 99.9%.[4]

(iii) Predictions based on five datasets (D1 = 0 s, 0.01 s, 0.1 s, 1 s, and 10 s) were consistent with those (**Table 3**) obtained using only three datasets. Therefore, three datasets, if selected properly, can reliably predict T_r values for quantitative analysis.

The findings strongly support the proposed hypothesis. The predicted T_r values aligned closely with those calculated from experimentally measured T_1 values, confirming the accuracy and reliability of the single-exponential decay regression approach. In all cases, predicted T_r values exceeded 7 × T_1, a widely accepted threshold for ensuring quantitative reliability in qNMR. This underscores the method's practical relevance and confirms that users can confidently set appropriate T_r (or D1) without relying on inversion recovery experiments.

Importantly, accurate T_r prediction can be achieved using only three 1D NMR experiments. Users can follow the stepwise procedure detailed in section **T_r Estimation Using AI Tool – ChatGPT 4o As An Example** or apply a Python-based script to directly estimate a suitable T_r value, enabling reliable quantitation. This streamlined approach simplifies qNMR method development and enhances its accessibility for routine and high-throughput applications.

Evaluation of predicted T_r values using qNMR spectra

Performance evaluation of the predicted T_r in quantitative analysis was based on the relative standard deviation (RSD) of absolute integrals for selected signal responses within the spectrum. Specifically, every sample solution was conducted in triplicate. For each experiment, the absolute integral values were normalized based on the number of equivalent ^{1}Hs, resulting in the integral-per-^{1}H values. These integral-per-^{1}H values were then used for RSD analysis. An example can be found in **Supporting Information II, Table S1**. Our acceptance criterion is that the overall RSD should be no more than 0.5%.

For the RSD analysis, the qNMR experiments were conducted as described in section **^{1}H Quantitative NMR Determination**. The selected signal responses were demonstrated in **Supporting Information III, Figs. S2-S7**. All RSD results can be found in **Table 4**. Each replicated results showed RSD values below 0.5%, indicating that the response of all selected signals had reached a state of full relaxation between scans. This confirms that in each run, the signals achieved complete relaxation.

Summary of Experimental Considerations

This hypothesized approach is feasible; however, several experimental parameters must be taken into account:

Acquisition time (AQ). To ensure proper digital resolution, the AQ (or data points) should remain sufficient and constant (e.g., 3 s or 4 s) as part of the T_r, while only the relaxation delay (D1) is varied in practice. The single-exponential decay regression model was then applied to experimental data acquired using D1 values of 0.1 s, 1s and 10 s, to estimate T_r value for quantitative analysis. It is important to note that the three-point D1 design proposed in this work is intended as a practical methodological framework rather than a rigid standard. For users with limited experience in relaxation analysis, these predefined settings offer a simple and robust starting point. Advanced users may further refine or adjust the number and distribution D1 (or T_r) values based on analyte-specific relaxation characteristics, analytical objectives, or instrument performance. This built-in flexibility supports broad applicability across diverse qNMR scenarios.

Table 4. RSD (%) of selected peak patterns in an NMR spectrum using the predicted D1 value of each chemical

Chemical	Experiment #1	Experiment #2	Experiment #3
Aripiprazole	0.33	0.37	0.34
Repaglinide	0.35	0.34	0.29
Diclofenac sodium	0.21	0.25	0.33
Donepezil impurity C	0.21	0.32	0.20
Trazodone hydrochloride impurity B	0.34	0.32	0.36
Vitamin B6	0.01	0.12	0.05

Receiver gain. It is critical to maintain consistent receiver gain across all NMR experiments in a test.

Signal response intensity. Since the objective is to assess the correlation between T_r and signal response intensity, it shall use absolute integral values, rather than normalized values, to ensure accurate curve fitting.

Flip angle. This approach is not limited to the use of flip angle, e.g., 30° or 90°.

Number of scans (NS). It is also important to note that when the NS is set to 1, the influence of scan-to-scan relaxation recovery is effectively eliminated. This setup simulates a condition in which the T_r is effectively unlimited, rendering variations in D1 inconsequential. To verify this, preliminary experiments were conducted with NS = 1 across a range of D1 values. As anticipated, the signal response intensities remained consistent regardless of the D1 setting, confirming that full relaxation was achieved before each acquisition (data not shown). Therefore, in the final protocol, NS = 4 was selected to balance S/N with reasonable experimental time, while ensuring that incomplete relaxation could still be observed and modeled across D1 values (Note: S/N of at least 100 is generally required for acceptable quantitative analysis, and an S/N of 1000 or higher is ideal to ensure high-level accuracy and precision)[5]. This choice still allows for sufficient contrast between under-relaxed and "fully relaxed" conditions, facilitating reliable T_r-signal response intensity curve fitting. Users may increase the NS depending on sample concentration, sensitivity requirements, and instrument performance.

Predict T_r using $I_{max}^{99.99\%}$. This is an empirical value, established by comparing it against the measured T_r values. The 99.99% level of maximum amplitude was identified as a practical threshold, a "sweet spot" that optimally balances accuracy with experimental efficiency.

The T_r value used in a qNMR experiment. It should be no less than the predicted T_r value.

CONCLUSIONS

This study presents a practical and efficient approach for predicting T_r values in qNMR using a single-exponential decay regression model derived from a minimal set of 1D NMR experiments. By exploring the correlation between T_r and signal response intensity, we demonstrated that T_r behavior can be accurately modeled and predicted using either three or five selected data points. The resulting T_r estimates showed strong agreement with those obtained from conventional inversion recovery methods, with predicted T_r consistently exceeding the 7× T_1 threshold required for over 99.9% accuracy level of quantitation. The proposed approach has also been performed and verified by a different analyst, on different days, and using a different instrument. All results can be found in **Supporting Information IV**.

The major advantage of this method lies in its ease of use and understanding. On one hand, there is no need to perform inversion recovery experiments, making them accessible even to qNMR beginners. Compared to T_1 determination, this approach also offers a robust and efficient alternative, significantly avoiding specialized experimental setup and reducing experimental time and complexity, while maintaining high prediction accuracy. On the other hand, this method, analogous to inversion recovery experiment for T_1 determination, is efficient for a qNMR analysis, because it enables direct T_r prediction for a specific target compound signal response as well as the internal calibrant signal response, without the need to process multiple signals and to measure their integrals. As a result, the overall method development becomes more specific and purpose-built. Its simplicity and reproducibility make it particularly valuable for routine qNMR applications and method development. This approach can also objectively help NMR scientists who are unfamiliar with, or skeptical about qNMR to better understand the differences between the default settings of a standard NMR experiment and the parameter requirements specific to qNMR experiment.

Furthermore, integration with Python and/or AI tool enables automated prediction, lowering the technical barrier and promoting broader adoption of qNMR. Following the section of **T_r Estimation Using AI Tool – ChatGPT 4o As An Example**, some data were reassessed using ChatGPT 5, Copilot, Gemini and Claude, and all results were found to show no significant difference with those predicted by ChatGPT 4o. In addition, this methodology also lays the groundwork for future advancements, especially for nuclei such as ^{13}C with longer T_1 values, the approach may offer even greater benefits. Of course, in such cases, the requirement of longer D1 values demands require additional data points beyond 10 s, such as 20 s, 30 s or even 60 s, thereby ensuring the accuracy of the predicted T_r. Ultimately, this work illustrates the potential of AI-assisted tools to transform traditional analytical workflows, making NMR-based quantitation more accessible, scalable, and adaptable to diverse chemical environments.

DISCLAIMER

Certain commercial equipments, instruments, or materials are identified in this article to adequately specify the experimental procedure. Such identification does not imply approval, endorsement, or certification by USP or China's National Institutes for Food and Drug Control (NIFDC) of a particular brand or

product, nor does it imply that the equipment, instrument, or material is necessarily the best available for the purpose, or that any other brand or product was judged to be unsatisfactory or inadequate. The NMR raw data can be shared, as requested.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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SUPPLEMENTAL DOCUMENT

https://qnmrjournal.com/index.php/qNMR/article/view/7/5

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