NMR - SOFTWARE

Streamlining NMR Spectrometer Usage - A Use-Case of SmartDriveNMR for Pharmaceutical Industry

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Innovation with Integrity

Abstract

Optimization of costly Nuclear Magnetic Resonance (NMR) spectrometer acquisition time while maintaining short turnaround times are of peculiar importance for analytical services, usually dealing with large number of samples. For structural analysis, non-uniform sampling was a significant milestone to limit acquisition time. However, without prior scouting analysis, even advanced users may fail to select appropriate acquisition parameters, such as NS or the amount of sampling points. This can result in low-quality data and wasted spectrometer time.

With the introduction of a tool like SmartDriveNMR, a fail-safe on-the-fly optimization of NMR acquisition parameters for structural analysis in full automation is now possible without altering spectral quality.

This application note describes how Evotec ID (Lyon) has implemented SmartDriveNMR in its routine structural analysis workflows, leading to a significant increase in instrument capacity.

Evotec ID

Evotec ID is a life science company specialized in research, development and manufacturing of highly effective therapeutics. With 5,000 employees, Evotec ID is established in Europe and in the US.

Based in Lyon, Evotec ID hosts a medicinal chemists team focused on anti-infective drug discovery, as well as an analytical platform equipped with Liquid Chromatography-Mass Spectrometry (LC-MS) systems and NMR spectrometers which provides support services to chemists for both open access and expert analysis. Within this platform, NMR is mainly used for structure validation or structure elucidation of small organic molecules.

NMR for Structural Analysis at Evotec ID

As a support for the local chemists, the main goal of the analytical team is to provide high quality NMR data while maintaining a short turnaround time (TAT) and the highest instrument availability. Therefore, the question of ensuring the required spectral quality while avoiding over-use of spectrometer time is of the utmost importance.

NMR structural analyzes at Evotec ID are usually performed using 1 to 2 mg of organic molecules with molar weight ranging from 200 to 800 Da. Half of these molecules exhibit fluxional behavior and/or have conformers. About 120 samples per month are being analyzed, based on the following set of experiments: 1D ^{1}H, HSQC, HMBC, COSY, and most of the time 1D $^{13}C\{^{1}H\}$. 2D experiments are recorded with non-uniform sampling, and using the settings described in table 1. The estimated duration of a sample analysis is 67 minutes. The analytical team strives to ensure that the TAT is less than one day from sample submission to report delivery.

Experiment	NS	TD(F1)	%NUS	Duration (min)
^{1}H	8	/	/	1
HSQC	2	256	25	4
HMBC	4	512	40	24
COSY	1	256	100	8
$^{13}C\{^{1}H\}$	512	/	/	30

Table 1: NMR parameters used for manual data acquisition

So far, structural analysis by NMR were conducted using two main strategies described below:

- "2 steps" workflow, where a preliminary 1D ^{1}H experiment is recorded to evaluate the quality of the sample through several factors including concentration, line shape, among others. This scout experiment enables to accurately set up some key acquisition parameters for the 2D experiments like number of scans (NS) and sampling density (%NUS). This strategy reduces the risk of failure or missing a crucial 2D experiment but generates delay and waste of human and machine time.
- "1 step" workflow, where a full set of experiments is recorded "at risk", without sample-based optimization of key parameters (NS and %NUS). Consequently, the main drawback of this strategy is in most of the cases an over-use of machine time.

Both workflows suffer from non-optimal use of human and machine time. With the aim of improving the management of human and spectrometer resources, the analytical team at Evotec ID has decided to implement SmartDriveNMR software on one of the NMR systems of the platform.

SmartDriveNMR

SmartDriveNMR is a software that fully automates the optimization of 1D and 2D NMR experiment acquisition, routinely used in structure elucidation. Through its algorithm, the software sets the appropriate number of scans for each NMR experiment defined in a user portfolio to achieve a user-defined signal-to-noise ratio. Additionally, for 2D experiments, the algorithm determines the optimized number of increments (TDF1) needed to generate high-quality data in minimal time. To complete these tasks, SmartDriveNMR extensively utilizes non-uniform sampling and performs a two-level optimization of sampling density and number of scans. An initial value is proposed for NS and %NUS based on a preliminary 1D ^{1}H experiment, with these values being refined on-the-fly during data acquisition.

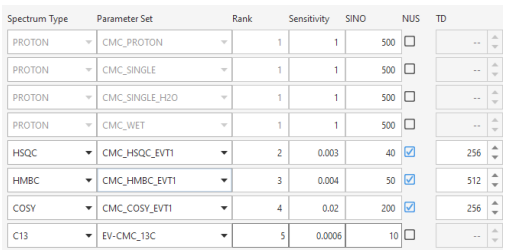
To evaluate the usefulness of SmartDriveNMR in optimizing NMR acquisition time, this software has been implemented on the 500 MHz system of the analytical platform. The spectrometer is equipped with an AVIII HD console and a 5 mm Prodigy BB(F)O cryoprobe and is controlled using TopSpin 3.6.5. Details of SmartDriveNMR setup (operation mode, maximum experimental time, experiments portfolio…) as well as key acquisition parameters values are described below and in table 2 respectively:

- Operation mode: MAXperiment (all experiments that fit into the given time will be carried out with optimized parameters)
- Maximum experimental time: 120 minutes
- Experiment portfolio: ^{1}H/HSQC/HMBC/COSY/^{13}C{^{1}H}
- No structure file
- No quantification

Experiment	Minimal NS	TD(F1)	Signal-to-Noise Criteria
^{1}H	1	/	500
HSQC	2	256	40
HMBC	4	512	50
COSY	1	256	200
^{13}C{^{1}H}	256	/	10

Table 2 Default SmartDriveNMR Evotec ID portfolio

In addition to these settings, an initial set-up may be required and, when necessary, should be done stepwise. Especially, the "sensitivity" parameter, which is purely empirical, plays a crucial role in the optimization process. Default sensitivity values are defined for each of the NMR experiments, these values can be adjusted based on trials and errors (see figure 1 below). SmartDriveNMR relies on various scripts (AU and Python), which can also be tailored to user samples for optimal data acquisition.



Spectrum Type	Parameter Set	Rank	Sensitivity	SINO	NUS	TD
PROTON	CMC_PROTON	1	1	500	☐	--
PROTON	CMC_SINGLE	1	1	500	☐	--
PROTON	CMC_SINGLE_H2O	1	1	500	☐	--
PROTON	CMC_WET	1	1	500	☐	--
HSQC	CMC_HSQC_EVT1	2	0.003	40	☑	256
HMBC	CMC_HMBC_EVT1	3	0.004	50	☑	512
COSY	CMC_COSY_EVT1	4	0.02	200	☑	256
C13	EV-CMC_13C	5	0.0006	10	☐	--

Figure 1: Final setting of Evotec ID portfolio (sensitivity, signal to noise, TD(F1))

SmartDriveNMR Benefits

SmartDriveNMR has been executed on a pool of 1,400 samples. Optimization run time on the full set of samples is shown in Figure 2.

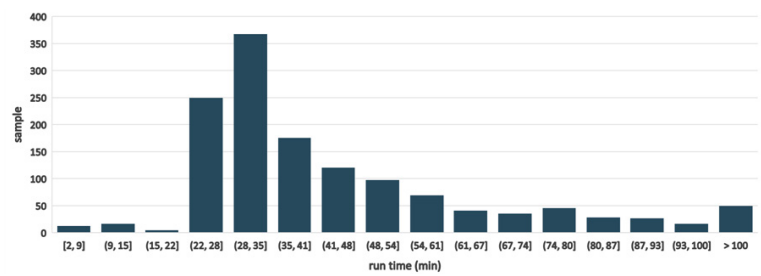


Figure 2: SmartDriveNMR optimization run time for the pool of samples used in the study

After careful analysis of this series of runs, several observations can be made.

First, the "peak tailing" profile of the run time is in accordance with the diversity of samples that have been submitted to the software analysis engine.

Inspection of the run time values indicate that SmartDriveNMR has been successfully executed on 80% of the samples. When run time was whether too long or too short, which happened for 20% of the analyzes, the corresponding samples were classified as "challenging". In that case, optimization failed, due to too low concentration or broad resonances in the 1D ^{1}H NMR spectrum. For these "challenging" samples, using SmartDriveNMR does not bring significant improvement compared to "classical" workflows, but still avoids wasting of human and machine time that could be observed with the "1 step" strategy.

In addition, calculating the average and median run times from previous analyses yielded 45 and 35 minutes, respectively. Compared to the 67 minutes required for NMR data recording using the classical approach (see Table 1), this represents a two-fold increase in instrumental capacity.

An in-depth analysis of the SmartDriveNMR runs can provide a better understanding of this 2-fold factor increase. Figures 3,4,5 and 6 illustrate the statistics of the NS and %NUS optimizations obtained from the SmartDriveNMR runs.

In figure 3 the distribution of the optimized NS values for the three different 2D experiments is shown for the entire test set (1,400 samples). The initial NS value set by SmartDriveNMR algorithm based on the 1D ^{1}H preliminary experiment is in general not modified by the on-the-fly optimization. This means that the signal to noise criteria and sensitivity parameters are well suited to the sample concentration used in this study.

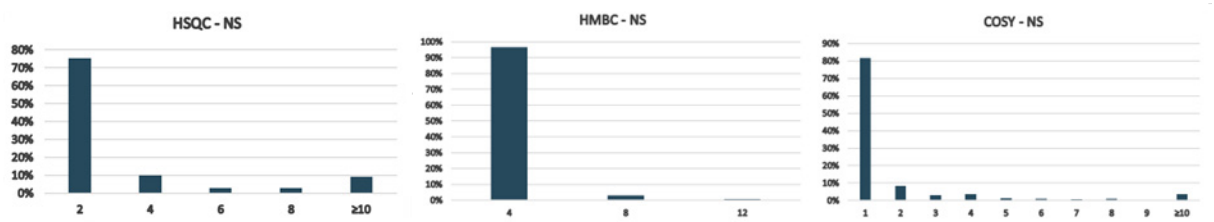


Figure 3: Optimization of number of scans (NS) for 2D experiments in portfolio

Figure 4 shows the %NUS optimization results for the HSQC experiment. The SmartDriveNMR algorithm estimated a wide range of sampling densities across the diverse sample pool, demonstrating the software's adaptability. The significant machine time savings mainly stem from the low sampling density (23%) achieved through on-the-fly optimization. Manually adjusting this parameter is complex and must be done for each experiment and sample. Automating this process with SmartDriveNMR is a key strength of the software.

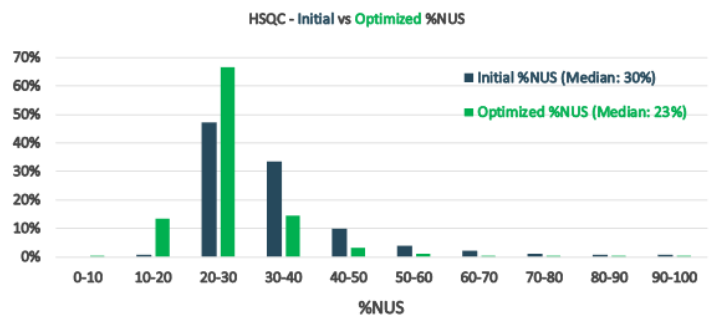


Figure 4: Initial and optimized sampling density obtained for HSQC experiment

Similar behavior is observed for COSY experiment, with an optimized median sampling density of 30% (data not shown).

Figure 5 illustrates the optimization of sampling density for the HMBC experiment. Similar to the HSQC experiment, the results reflect the diverse range of sample types used in this study. A significant discrepancy is evident between the median initial sampling density estimated by the algorithm (81%) and the median sampling density ultimately set following on-the-fly optimization (20%). The optimization of sampling density performed by SmartDriveNMR during data acquisition significantly reduces the experimental acquisition time for the HMBC experiment. This reduction plays a major role in doubling the instrumental capacity.

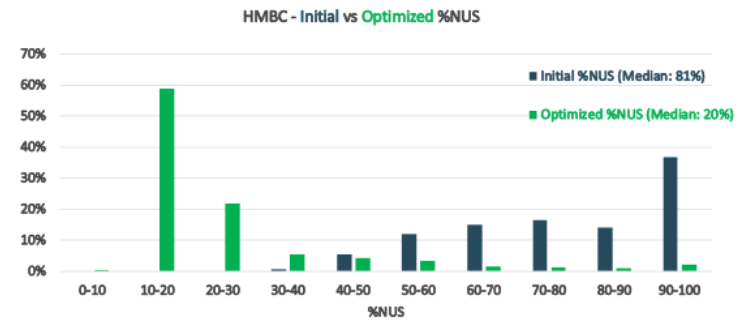


Figure 5: Initial and optimized sampling density obtained for HMBC experiment

Optimization of the number of scans for the $^{13}C\{^{1}H\}$ spectrum is shown in figure 6. Here again, the results indicated the great adaptability of the software to sample diversity, as a large distribution of the optimal NS value determined by SmartDriveNMR is observed. Interestingly, the proposed NS value undergoes significant changes during the on-the-fly optimization. The median NS value starts at 224 and decreases to 124 by the end of the optimization process. This drastically reduces the acquisition time for this experiment thus greatly contributes to improve the structural analysis workflow.

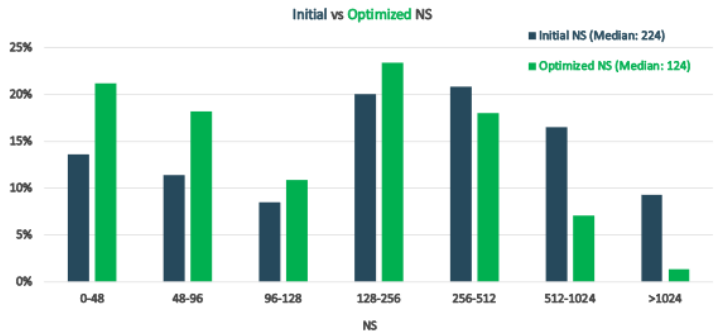


Figure 6: Optimization of the number of scans for $^{13}C\{^{1}H\}$ experiment

Conclusion

SmartDriveNMR has brought a very strong improvement of the NMR acquisition workflow for structural analysis at Evotec ID (Lyon).

With a two-fold reduction in experimental NMR data acquisition time, SmartDriveNMR enables to double the NMR instrumental capacity of the analytical platform while maintaining a high data quality. In addition, review of 1D ^{1}H scout experiment by an expert being no longer necessary, SmartDriveNMR opens the way to streamlining of sample submission while limiting errors (over-use of spectrometer, missing NMR experiments).

An initial setup may be required to come to optimal productivity, but the benefits obtained from this initial setup largely compensate the time spend for this task.

Finally, the flexibility of the current version of SmartDriveNMR allows deep customization and covers a wide range of possible use.

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