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Spinsolve

q-HSQC for Sensitivity-Enhanced ^{13}C Quantitation

Reduce the quantification time of ^{13}C **1000** times by including polarization transfer and indirect detection

The most common approach to quantifying materials by NMR utilizes ^{1}H NMR spectra, which combines high intrinsic sensitivity with straightforward, inherently quantitative signal integrals. In complex formulations and reaction mixtures, however, ^{1}H spectra often suffer from peak overlap, which limits the accuracy to quantitate the different components. A way to minimize overlap is to measure ^{13}C spectra. The ^{13}C chemical shift spreading is far wider than that of ^{1}H, and, with ^{1}H decoupling, ^{13}C resonances appear as simple singlets, which greatly simplifies assignment and integration. The drawback is sensitivity: the low natural abundance of ^{13}C (≈1.1%) and its typically long T_1 relaxation times make direct ^{13}C acquisition time-consuming and, for many samples, impractical. Polarization transfer addresses this limitation by using polarization from abundant ^{1}H spins to boost ^{13}C signal-to-noise. Schemes such as NOE, INEPT/DEPT, and related variants can yield up to ~4-fold increases in apparent ^{13}C sensitivity and allow repetition delays to be governed by the shorter ^{1}H T_1 rather than the longer ^{13}C T_1, accelerating data collection. The common perception, however, is that polarization-transfer experiments are not quantitative because the transfer efficiencies vary across different carbon sites and molecular environments, so direct comparison of peak areas to an internal standard no longer reports concentration in a site-independent way. We have recently shown that this limitation can be overcome. Under fixed acquisition conditions, transferred ^{13}C signal amplitudes remain proportional to analyte concentration; by calibrating each target signal against external standards containing known concentrations, one obtains linear response curves that restore quantitative accuracy without sacrificing the sensitivity benefits of polarization transfer. In this application note, we extended the approach to the more sensitive two-dimensional HSQC sequence, where polarization is transferred back to ^{1}H for detection, leveraging ^{1}H sensitivity with an up to 32-fold increase in SNR relative to the ^{13}C inverse-gated experiment. This method also benefits from shorter measurement times due to the shorter ^{1}H T_1 enhancing the sensitivity of ^{13}C detection for quantitative measurements in scenarios where conventional ^{1}H quantitation is compromised by spectral overlap.

Table 1 summarizes the advantages and disadvantages of the three approaches.

Experiment	Advantages	Disadvantages
^{13}C{^{1}H} via inverse gated decoupling	- Quantitative – single calibration - High signal discrimination	- Very low sensitivity, - Slow accumulation due to long ^{13}C T_1
^{13}C{^{1}H} DEPT	- Higher sensitivity – x4 compared with ^{13}C{^{1}H} - High signal discrimination - Fast accumulation with ^{1}H T_1	- External calibration for each component
^{1}H,^{13}C HSQC	- 2D method with high signal discrimination - Up to 32x sensitivity enhancement compared to inverse gated ^{13}C{^{1}H} - Fast accumulation with ^{1}H T_1	- Lower resolution along the ^{13}C dimension - External calibration for each component

Table 1. Advantages and disadvantages of different qNMR approaches.

Experimental Setup

Experiments have been performed on a Spinsolve 90 ULTRA Multi-X with ^{13}C available on the X-channel. Spectra were acquired for samples containing 10, 20, 50, 100, 200, 500 and 1000 mM lidocaine in $CDCl_3$. For a fair comparison, experimental parameters were set to yield equal acquisition times for DEPT and HSQC, i.e. an HSQC with 128 increments and 4 scans x 2 (for phase-sensitive detection) would be the equivalent of a DEPT with 1024 scans. DEPT spectra were acquired as DEPT-45. The HSQCs were acquired with an echo-antiecho sequence. Repetition times of 2, 5 and 10 seconds were assessed, with 5 s being a good compromise for experiment time and quantitative measurement condition. For the analysis, the singlet for the aromatic CH_3 groups at 2.3 / 18.7 ppm was integrated, with horizontal traces extracted and integrated in the HSQC and direct integration in the DEPT.

NMR Results & Discussion

Fig. 1 shows the structure of lidocaine as well as representative DEPT and HSQC spectra as well as a vertical HSQC trace, with signals used for quantitation and respective signal-to-noise ratios (SNR) highlighted in blue. For the DEPT the limit of detection is around 23 mM, while for the HSQC it is ~3 mM.

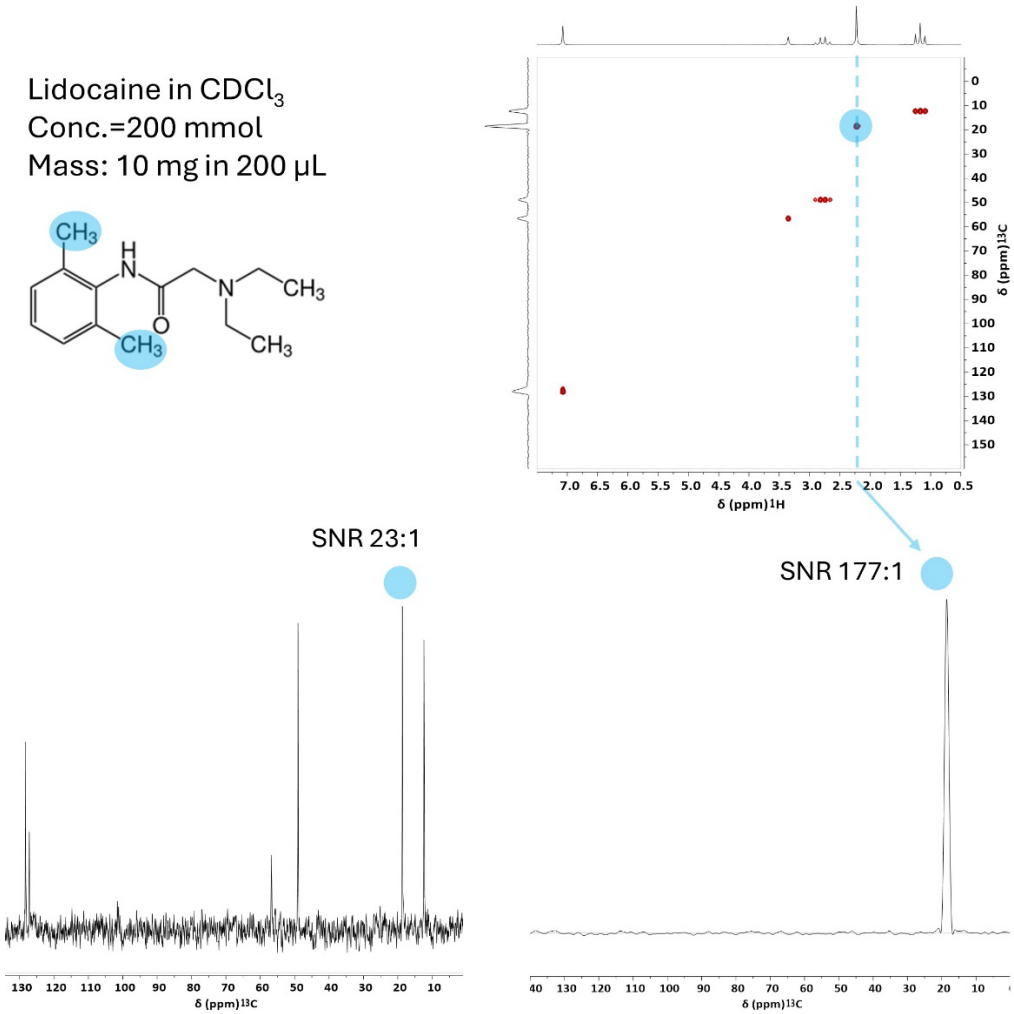

Figure 1. Structure of lidocaine (top left), HSQC (top right), ^{13}C DEPT-45 (bottom left) and vertical traces of HSQC (bottom right) for the aromatic CH_3 groups of lidocaine (200 mM in $CDCl_3$). Signals used for quantitation and their respective SNR are highlighted in blue.

The theoretical maximum improvement in SNR over $^{13}C\{^{1}H\}$ is governed by the gyromagnetic ratios of ^{1}H and ^{13}C. For the DEPT experiment we get an improvement of 4 due to the polarization transfer from ^{1}H to ^{13}C:

$$\text{DEPT:} \qquad SNR \propto \frac{\gamma_H}{\gamma_C} \approx 4$$

For the HSQC with ^{1}H excitation and detection the improvement over DEPT is about eight-fold, or 32-fold over a 1D $^{13}C\{^{1}H\}$ inverse gated experiment:

$$\text{HSQC:} \qquad SNR \propto \left(\frac{\gamma_H}{\gamma_C}\right)^{\frac{3}{2}} \approx 8$$

The advantage of the HSQC lies in the significant reduction in measurement time, when compared to the $^{13}C\{^{1}H\}$ inverse gated experiment, a 32-fold higher SNR corresponding to a **1000-fold** shorter experiment time for the same concentration. The enhancement observed experimentally between DEPT and HSQC is about 7.7-fold, which is really close to the theoretical value. Figure 2 compares the concentration dependence of the DEPT and HSQC spectra for the aromatic CH_3 groups for the two signals. Both DEPT and HSQC signals yield the expected linear response as a function of concentration. However, thanks to the superior SNR of the HSQC a much smaller deviation from linearity is observed.

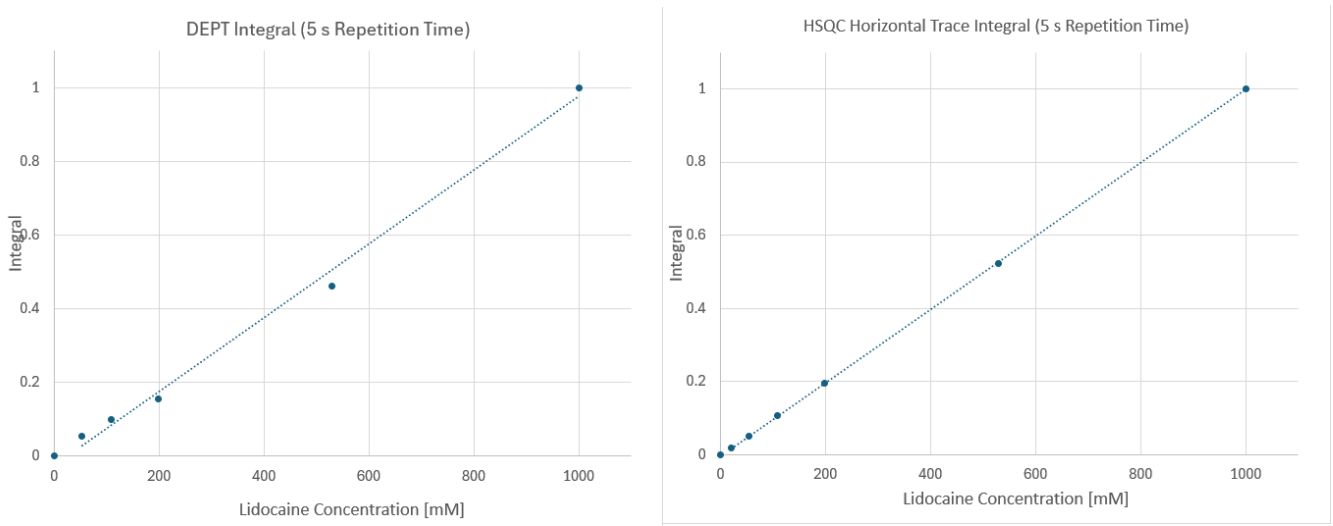

Figure 2. Concentration dependence of DEPT (left) and horizontal HSQC trace (right) integrals for the aromatic CH_3 singlet of lidocaine.

Conclusion

The data show that either DEPT or HSQC can be used for quantitation. However, the HSQC response is far superior in SNR, allowing extension of the concentration range at the lower end, and should be the preferred choice if suitable signals, preferably singlets can be identified. The SNR enhancement obtained with the HSQC makes it possible to reduce the measurement time by a factor of 64 compared to the time required by the DEPT sequence and a factor about 1000 compared to the quantitative inverse gated $^{13}C\{^{1}H\}$ experiment.