Accurate CSI Quantification for Targeted Profiling

Colin Vitols, Pascal Mercier, Isabelle McNutt-Holland

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Accurately quantifying metabolites using targeted profiling in Chenomx NMR Suite requires using a chemical shape and shift indicator (CSI) of known concentration. The accuracy of the CSI concentration thus has a significant influence on quantification accuracy using this technique. Measuring ratios of the CSI peak area with that of a second, commercially available standard under optimal acquisition conditions offers a simple method of accurately measuring the concentration of the CSI.

Introduction

Quantifying metabolites absolutely using targeted profiling in Chenomx NMR Suite requires using a chemical shape and shift indicator (CSI) of known concentration, usually 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) or 3-(trimethylsilyl)propionic acid (TSP).

This note describes a method for preparing and determining the concentration of a DSS solution for use as a CSI with Chenomx NMR Suite using 1D-^{1}H NMR spectroscopy. The method requires a second commercially available standard compound of certified concentration.

An appropriate commercially available compound should:

- be soluble in the solvent of choice
- be chemically stable
- have at least one reference signal that:
  - does not overlap with signals from DSS or TSP
  - is not subject to proton exchange with the solvent
  - is not affected by solvent suppression techniques (where applicable)

The following example describes the experimental steps and NMR methods used by Chenomx to prepare and quantify DSS solutions using a commercially available sodium acetate NMR standard.

Sample Preparation

We combine the chosen NMR reference standard (50 mM sodium acetate in 99% D_2O, 500 µL) with the DSS solution to be quantified (~5 mM DSS in D_2O, 500 µL) and vortex for 30 seconds. We then transfer 600 µL of the resulting solution to an NMR tube for analysis. An sodium acetate NMR reference standard is available from ISOTEC, a member of the Sigma-Aldrich group, product #613053.

Data Acquisition

The 1D-^{1}H NMR peak area ratio between the methyl signals of DSS and acetate allows us to determine the concentration of DSS. The NMR experiment must allow for complete longitudinal relaxation (T_1) of both components. At 500 MHz, the T_1 relaxation times of the methyl groups are 3.4 seconds for DSS and 6.1 seconds for acetate (see Measuring T_1 Relaxation Times). Details of the pulse sequence used to acquire the quantitative experiment appear in Figure 1. Briefly, the pulse sequence consists of a 2 second recycle delay, then a single 1 µs hard pulse followed by an 8 second detection period.

Higher field strengths may require longer delays to ensure complete longitudinal relaxation. Using a shorter 1-µs pulse instead of a full 90° pulse reduces the signal-to-noise ratio in each scan, but allows a shorter recycle delay (more scans)

over the duration of the experiment. The 90° pulse would require approximately 40 seconds (5 x T_1) of recycle delay for accurate quantification and complete longitudinal relaxation.

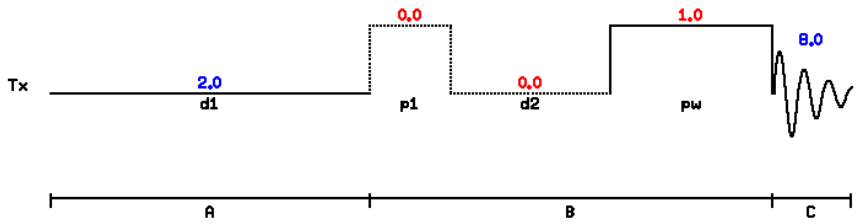

Figure 1. 500 MHz 1D-^{1}H NMR pulse sequence used to determine DSS concentration in aqueous solution (D_2O). Numbers in blue indicate timings in seconds (s); red indicates timings in microseconds (µs).

We zero-fill and Fourier-transform the acquired free induction decay (fid) with no windowing function, and manually phase and baseline correct the resulting 1D-^{1}H NMR spectrum. We perform baseline correction using VNMRj (Varian) and an in-house modified version of the `hregions` macro that excludes applying autophasing. Sodium acetate produces a simple ^{1}H-NMR spectrum consisting of a single peak near 1.91 ppm, locating it away from the water resonance, and not overlapped with DSS (see Figure 2).

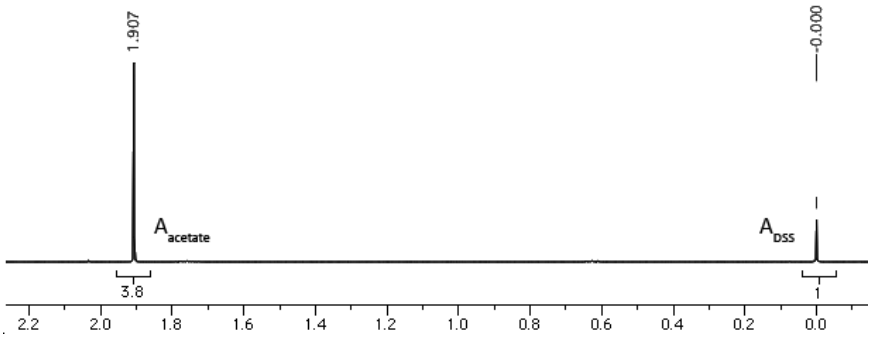

Figure 2. 500 MHz 1D-^{1}H NMR spectrum of sodium acetate (1.91 ppm, 25 mM) and DSS (0 ppm, 2.5 mM).

DSS Concentration Measurement

We integrate peak areas over a total width of 0.1 ppm centered at 0 ppm (A_{DSS}) and at 1.91 ppm ($A_{acetate}$), and calculate the concentration of DSS as:

$$[DSS] = [acetate] \times \frac{DSS}{k(acetate)}$$

where [acetate] is the known concentration of acetate in the sample, and k is the ratio of the number of protons of the DSS peak (9) with the number of protons of the acetate peak (3). For the current application, k=3.

Measuring T_1 Relaxation Times

We measure T_1 relaxation times at 500 MHz for the methyl groups of both acetate and DSS using the `T1meas` pulse sequence (Varian) at 25°C in D_2O. We array a total of ten "d2" time delays on an exponential curve from 0.5 to 10 seconds (see Figure 3).

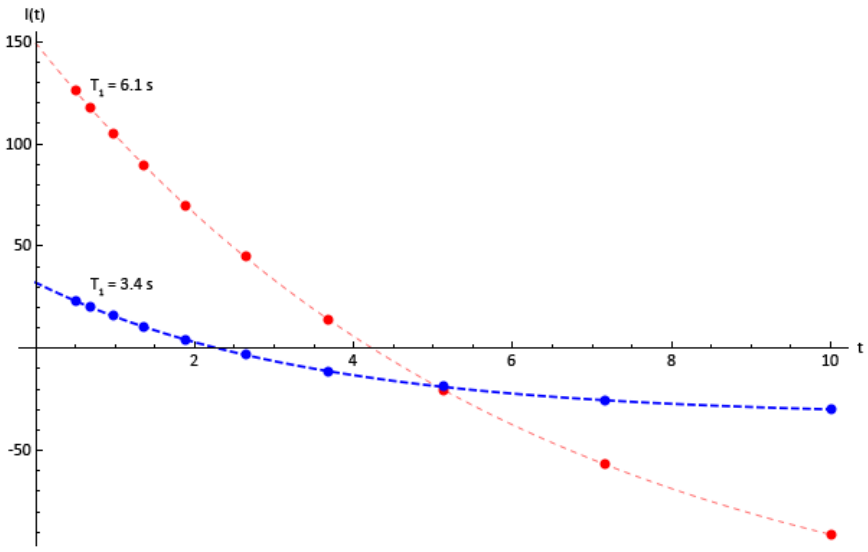

Figure 3. T_1 relaxation decay curves for the methyl groups of acetate (red) and DSS (blue) at 500 MHz and 25°C in D_2O.

We measure the intensity of the acetate and DSS methyl peaks automatically with the `dfp` and `t1` built-in macros (Varian), and fit the intensity decays using Mathematica to the following equation:

$$I(t) = [I(t) - I(0)]e^{-\frac{t}{T_1}} + I(0)$$