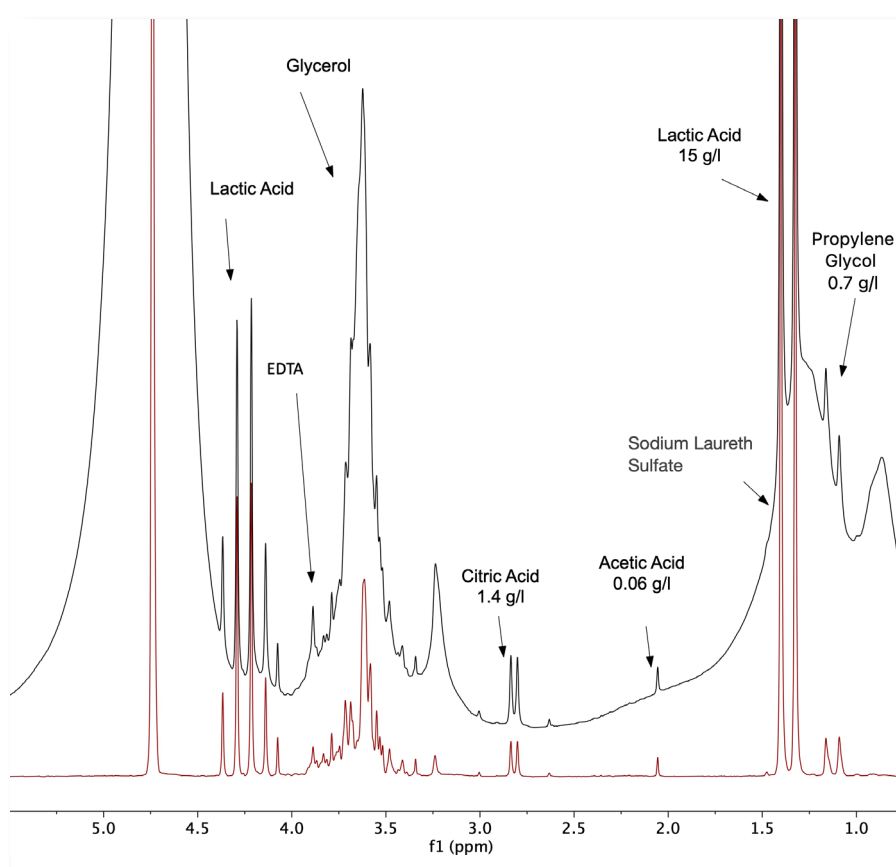# Unmask the NMR spectra of small molecules in complex formulations

The NMR signals of small molecules present in complex formulations are often hidden by the large signals of regular solvents and the broad resonances of larger molecules. Learn in this application note how to unmask the spectra of these small molecules by combining efficient solvent suppression with relaxation filters. The new WET-CPMG sequence available with the solvent suppression package delivered with the Spinsolve ULTRA models is a robust method that combines a WET solvent suppression module with a CPMG echo-train sequence to introduce a T_2 relaxation filter before the signal acquisition. As the signal of the large molecules decays much faster than the one of the small molecules, the large molecules can be filtered by properly setting the duration of the CPMG sequence.

The quantification of the ingredients of a complex formulation by NMR spectroscopy is sometimes limited by the overlapping of the signals of the multiple compounds present in the product. The situation is particularly complicated when the sample contains molecules with high molecular weight. Macromolecules have broad resonances that cover large fractions of the spectrum and mask the signals of smaller molecules, making quantitative measurements highly inaccurate. Example of such complex mixtures are household and cosmetic products, like shampoos, detergents, or soaps. These products contain ingredients with specific functions, like surfactants, perfumes, dyes, thickeners, humidifiers, and preservatives, among others, typically dissolved in water or a mixture of alcohols. Figure 1 shows the ^{1}H spectrum of a typical liquid soap. The first challenge that needs to be faced comes from the fact that the massive signal of the water overlaps with some of the signals of interest.
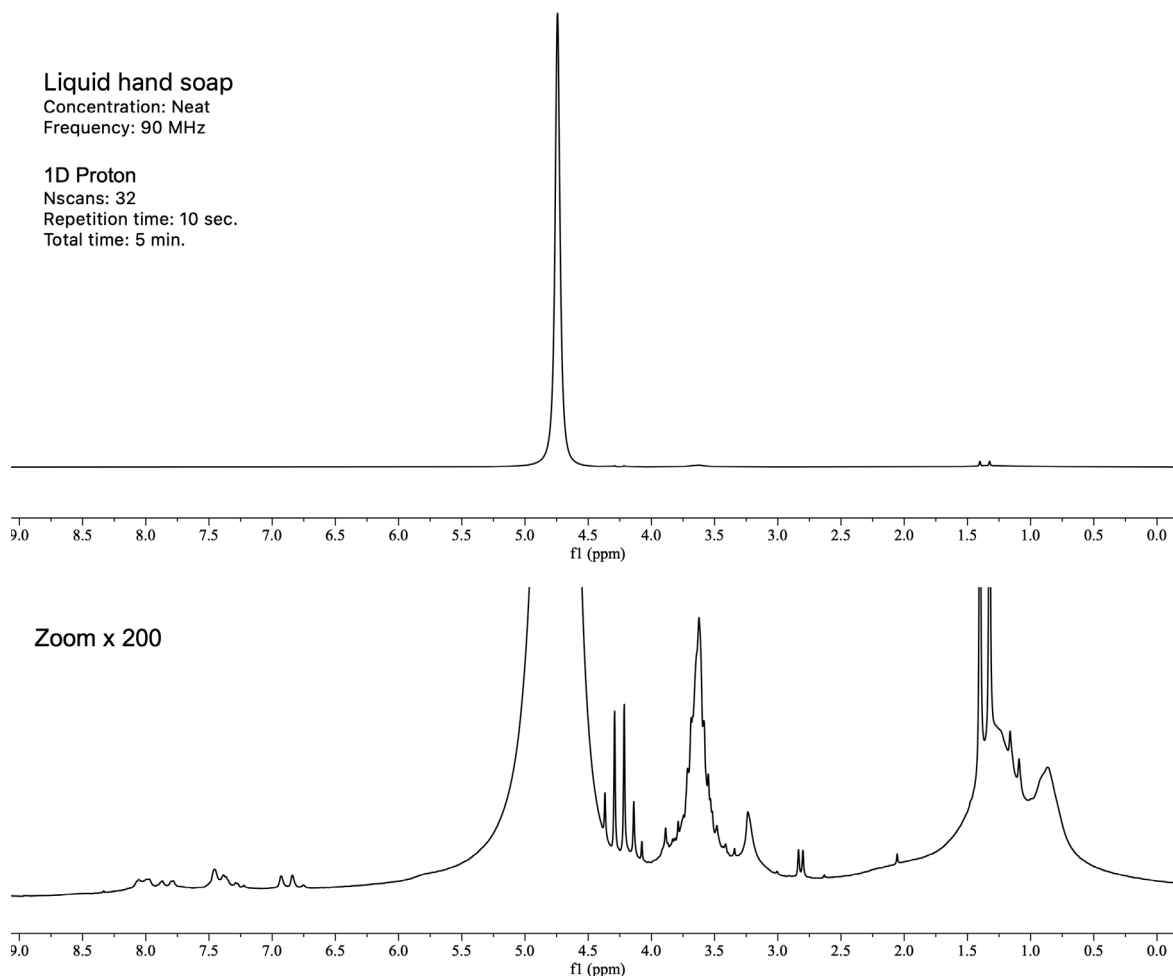

Figure 1: Spectrum of a neat hand liquid soap measured on a Spinsolve 90 ULTRA without any sample preparation. The spectrum was acquired averaging 32 scans in a total time of 5 minutes. At full scale, the water signal appears as the main component and the rest of the ingredients have signals at least two orders of magnitude smaller. A zoom x200 of the spectrum shows the intensity of signals arising from the multiple components present in the sample.

The solvent signals can be efficiently suppressed in the Spinsolve ULTRA models by applying a WET sequence. Figure 2 shows a comparison of the spectra collected with and without solvent suppression. The large attenuation achieved on the solvent signal removes the overlapping between the water peak and the neighbor resonances. This can be observed, for example, in the aromatic region and at the position of the quartet of the lactic acid. However, we can quickly identify several broad signals (mainly the signals of the large molecules, like the surfactants) that all over the aliphatic region overlap with several narrower signals that correspond to the smaller molecules present in the sample.
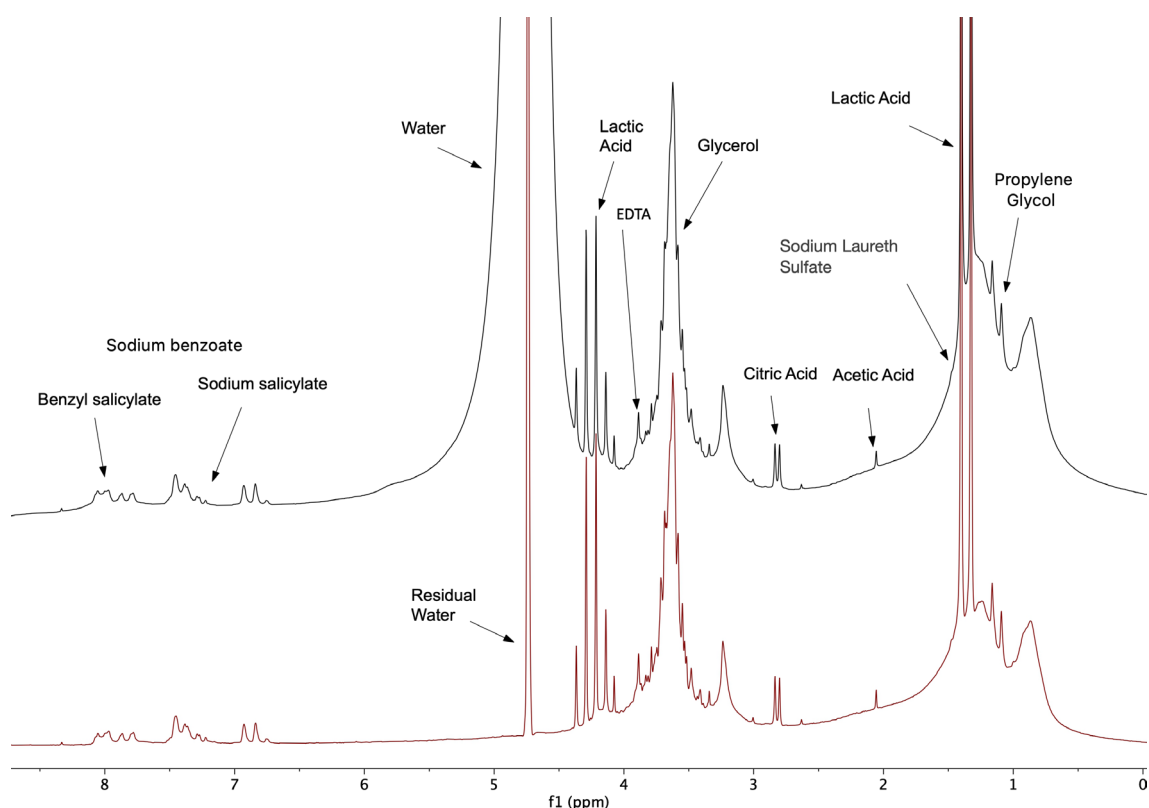
Figure 2: Comparison of the spectra collected with (red) and without (black) solvent suppression. The large solvent peak can be efficiently attenuated by applying a WET sequence that selectively excites the signal of the water at 4.74 ppm. While this method helps cleaning the spectrum around the solvent peak, it can be observed that the red spectrum shows important signal overlapping.

A powerful method, widely used in NMR-based metabolomics to remove the broad signals of the macromolecules, combines the WET solvent suppression module with a Carr-Purcell-Meiboom-Gill (CPMG) sequence that works as a T_2 filter applied before the signal acquisition (see Fig. 3) [1-3]. Such a combination requires a high solvent suppression performance to avoid the CPMG module generating imperfections in the region of the tails of the solvent peak.
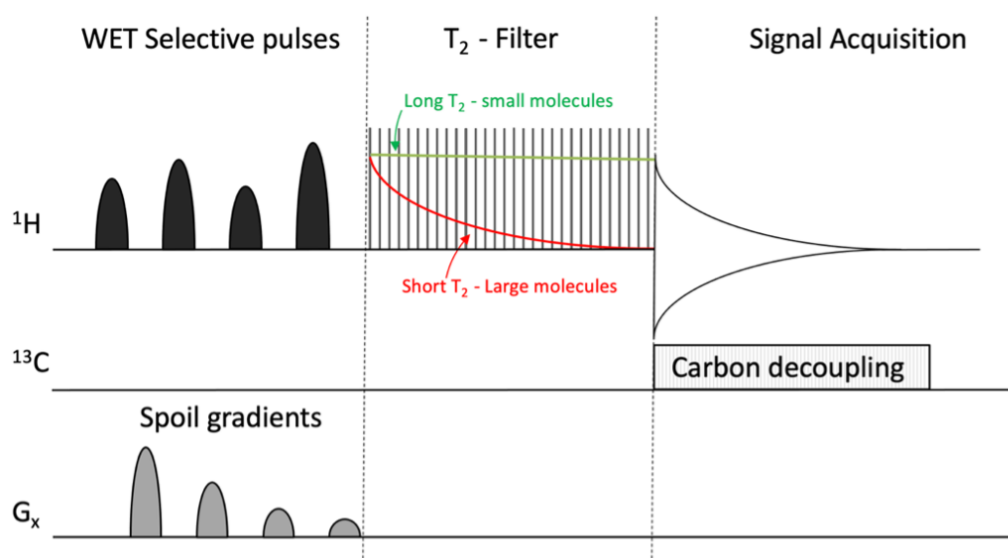

Figure 3: Pulse sequence combining a WET solvent suppression module with a CPMG T_2-filter sequence. By setting the duration of the CPMG echo-train, the fast-decaying signals from the larger molecules present in the sample (red exponential) can be eliminated while the signals of the smaller molecules, which have longer T_2s, remain almost un-affected to be acquired at the end of the filter period. The WET-CPMG sequence available with the Spinsolve ULTRA models offers the chance to suppress up to 3 solvent peaks and acquire the ^{1}H signal under carbon decoupling.

In this application note we demonstrate that a WET-CPMG sequence can be implemented on the Spinsolve ULTRA models to simplify the spectra of complex mixtures in a robust way. As the signals during the CPMG module decay faster (red) or slower (green), depending on the relaxation time T_2 of the molecules, the signal of the large molecules, with short T_2s, can be filtered by judiciously setting the duration of the CPMG. Figure 4 displays superimposed spectra collected for different durations of the CPMG module. The spectra clearly show how the broad signals get drastically attenuated as the filter time increases. For this sample, a filter time of 300 ms is sufficient to baseline resolve the narrow signals for accurate integration. During this short filter time, the signal of the small molecules remains almost unaffected and can be easily used for quantification.
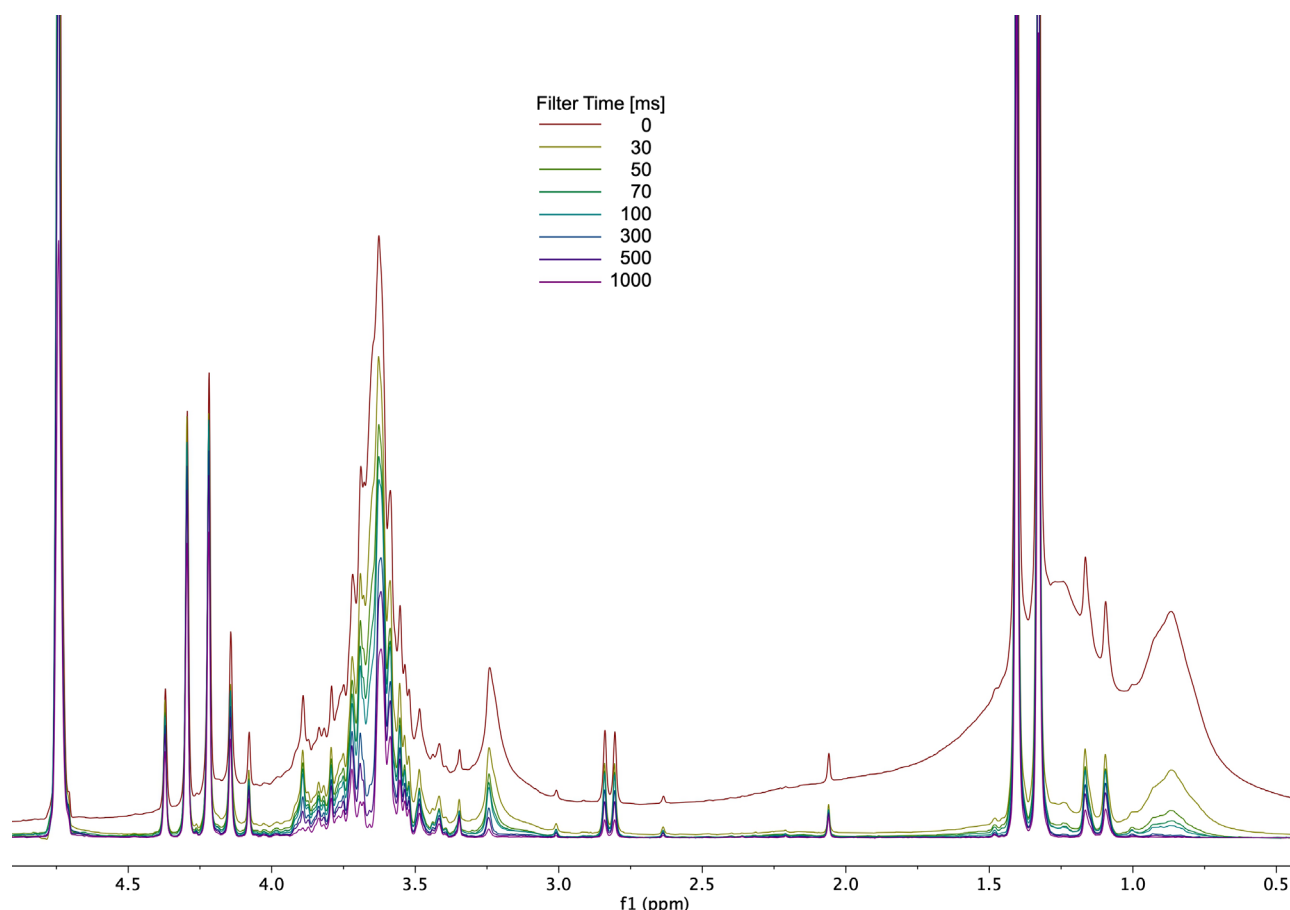

Figure 4: Spectra of a neat liquid soap sample acquired with the WET-CPMG sequence for filter times set to 0, 20, 50, 75, 100, 300, 500, and 1000 ms. A filter time of 300 ms (please indicate the color of the spectrum) leads to a very clean spectrum where most broad signals are eliminated. Each spectrum was collected averaging 32 scans acquired with a repetition time of 10 seconds, defining a total measurement time of about 5 minutes per spectrum. Solvent suppression was applied to attenuate the signal of the water and ^{13}C-decoupling was used to eliminate the satellites of lactic acid.

The spectrum acquired with the WET-CPMG sequence using a filter time of 300 ms is shown in Fig. 5. The efficiency of the method to filter the signal of the larger molecules can be clearly appreciated in the aliphatic region, where the signals of citric acid, acetic acid, lactic acid, and propylene glycol are resolved without overlapping. These signals have been used to quantify the concentrations of these compounds in the product against an external standard where these compounds are dissolved at known concentrations. The results delivered by the quantification method are shown in the spectrum. While the content of lactic acid is 15 g/l, acetic acid is present at just 0.06 g/l. The high sensitivity of the Spinsolve NMR spectrometers makes it possible to achieve a remarkably low detection limit. For example, the detection limit of acetic acid in a measurement time of just 5 minutes is about 3 mg/l (3 ppm).
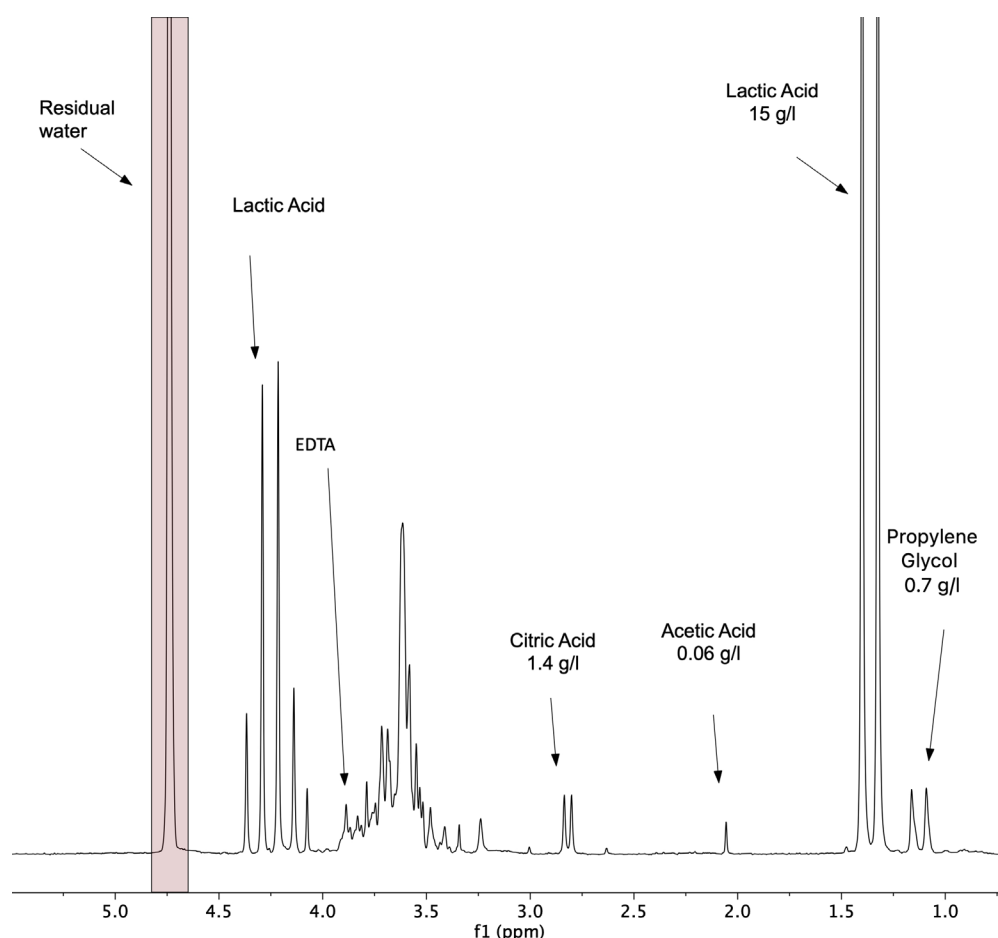
Figure 5: 1D spectrum of a liquid hand soap acquired with the WET-CPMG sequence using a filter time of 300 ms. The combination of the solvent suppression and the T_2 filter clearly helps to resolve the signals of several ingredients to accurately quantify them.

The WET-CPMG method is expected to offer great potential in a number of different fields like body fluids research, where metabolites need to be resolved in the presence of proteins. Another example is food science, where a variety of small molecules like acids, alcohols, and sugars need to be quantified in the presence of fat and proteins. Additionally, the T_2 filter sequence can be applied to screen chemical reaction products to identify contaminants, residuals of reagents or solvents, or impurities present in final products of medium to large molecular weight.

References

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