Spinsolve 80 ULTRA

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Solvent suppression: the key to measure your samples every time you need while they are synthesized

Figure 1. Spinsolve NMR spectrometer used in the chemistry lab for quick analyses of products. The typical presence of protonated solvents in the sample requires the use of solvent suppression and carbon decoupling methods to acquire the spectra of analytes of interest.

NMR spectroscopy in the chemistry lab

Benchtop NMR spectrometers have been developed to work in the chemistry lab, where chemists can use them to quickly analyze their samples as they are produced. These compact NMR systems can be simply installed on the chemistry bench or directly inside the fume hood to identify or quantify products in real time, as your synthesis proceeds. A key challenge that appears when samples need to be measured as they come out from the process comes from the that the products are typically dissolved in regular protonated solvents. For fast sample analysis it is important to minimize sample preparation. For example, the tedious workup required to exchange solvents is not desired. To acquire NMR spectra in the presence of protonated solvents in the sample requires the implementation of methods able to attenuate the large solvent signals, which can easily be up to four orders of magnitude larger than the signals of the products. The solution known from high-field NMR is to apply solvent suppression methods that selectively attenuate the solvent signals before the NMR signal is acquired. However, as these methods are based on selective frequency excitation, they can only work efficiently if the magnetic field generated by the magnet is highly homogeneous. In a homogeneous magnetic field all nuclei of a particular chemical group have the same resonance frequency and can be efficiently attenuated when they are excited at the center frequency of the signal. To meet the required standards Magritek introduced the Spinsolve ULTRA models. These models are the only benchtop spectrometers that deliver a homogeneity comparable to the standards of superconducting magnets, where the line-shape is specified at 50%, 0.55% and 0.11% of the peak height.

Efficient solvent suppression

To demonstrate the performance of the WET solvent suppression method implemented on a Spinsolve ULTRA spectrometer, a sample of histidine dissolved at a concentration of 10 millimolar in neat water was used. Neat water has a ^{1}H concentration of 110 Molar (two ^{1}H per molecule) contributing to a single peak. The full-scale and zoomed spectra (Fig. 2a and 2b) show the comparison between the standard spectrum (black) and the one obtained with solvent suppression (red). Taking into account that the solute has a concentration that is four orders of magnitude smaller than the solvent, a large vertical zoom is required to see the signals of histidine (see Fig. 2b). In the standard spectrum the signals of histidine (Fig. 2b black) appear mounted on the tails of the water peak, which covers a big fraction of the spectrum and makes quantification almost impossible. Note that the sample does not require any D_2O as Spinsolve benchtop NMR spectrometers are equipped with an external hardware lock system that works independent of the sample being measured.

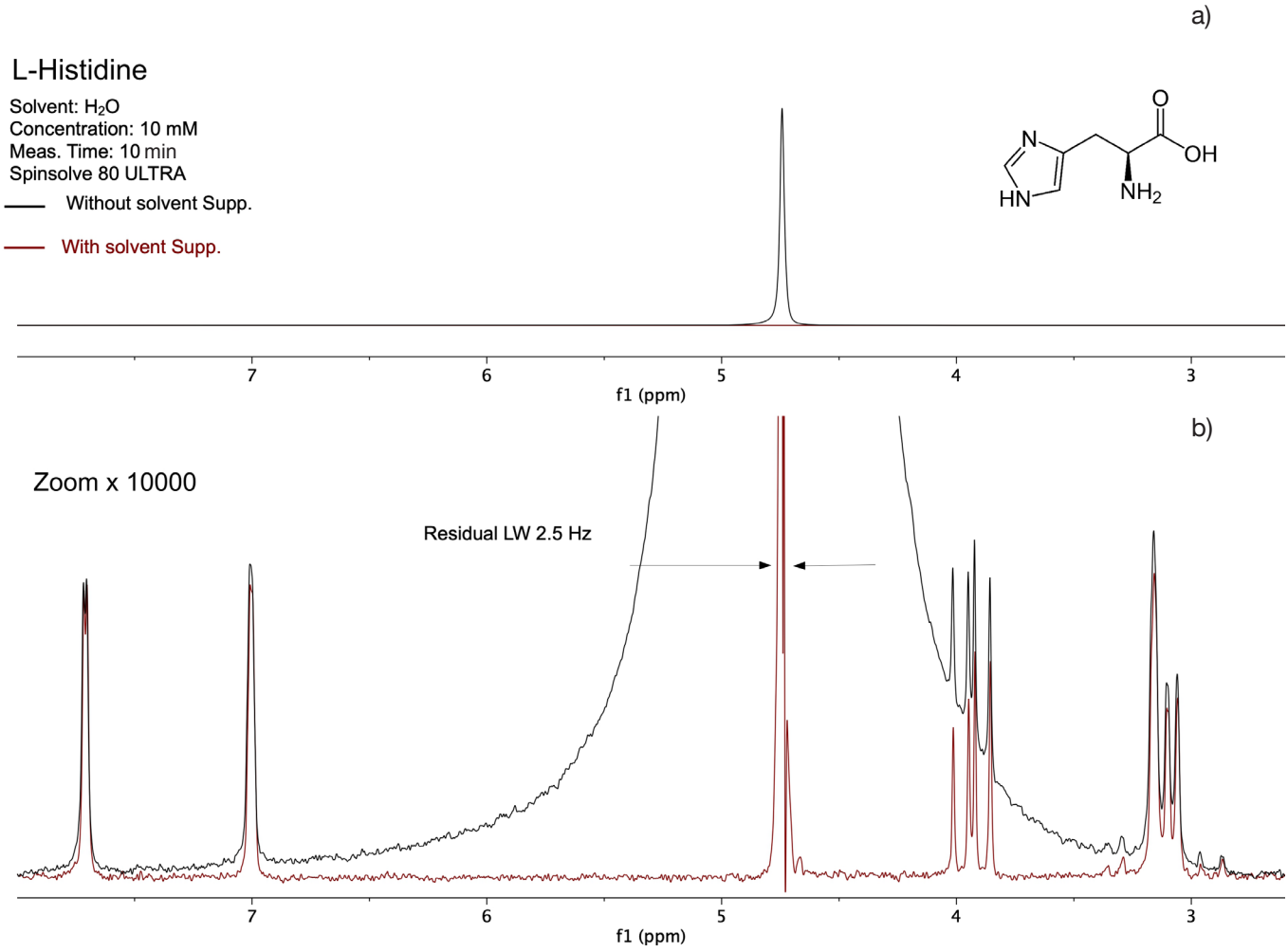

Figure 2. Spectra of histidine dissolved at a concentration of 10 mM in water acquired with a standard sequence (black) and using a WET solvent suppression sequence (red). a) Full scale spectra where only the water signal is visible, as it has a concentration of 55 Molar (effective 110 Molar of ^{1}H). b) The spectra from a) shown with a x 10000 vertical zoom. In this scale, it can be observed how the signals of histidine overlap with the tails of the huge water peak in the standard experiment (black), but can be all very well resolved on the spectrum acquired with a solvent suppression method (red). It can be observed that the water peak is attenuated to the point where the tails are efficiently removed without affecting the signals of histidine as they appear at resonance frequencies different than the one of water. The spectra in b) were acquired using 64 scans and a repetition time of 10 seconds on a Spinsolve 80 MHz Carbon ULTRA.

Although the water peak extends over a wide region in the spectrum, it can be strongly attenuated by applying a WET module at the central frequency of the peak. The spectrum acquired with solvent suppression is shown in red in Fig. 2a and 2b. In this experiment the water peak was attenuated a factor of about 1000, while the signals corresponding to histidine remained unaffected and can be accurately quantified.

As a second example, Fig. 3 shows the performance of the solvent suppression method on a 20 mM sample of sucrose dissolved in water. This sample is used as a standard for testing the selectivity of the solvent suppression to saturate the water peak without affecting the signal of the anomeric protons at 5.4 ppm. While this peak completely overlaps with the tail of the water peak in the standard measurement (black), it is fully resolved on the spectrum measured with solvent suppression (red).

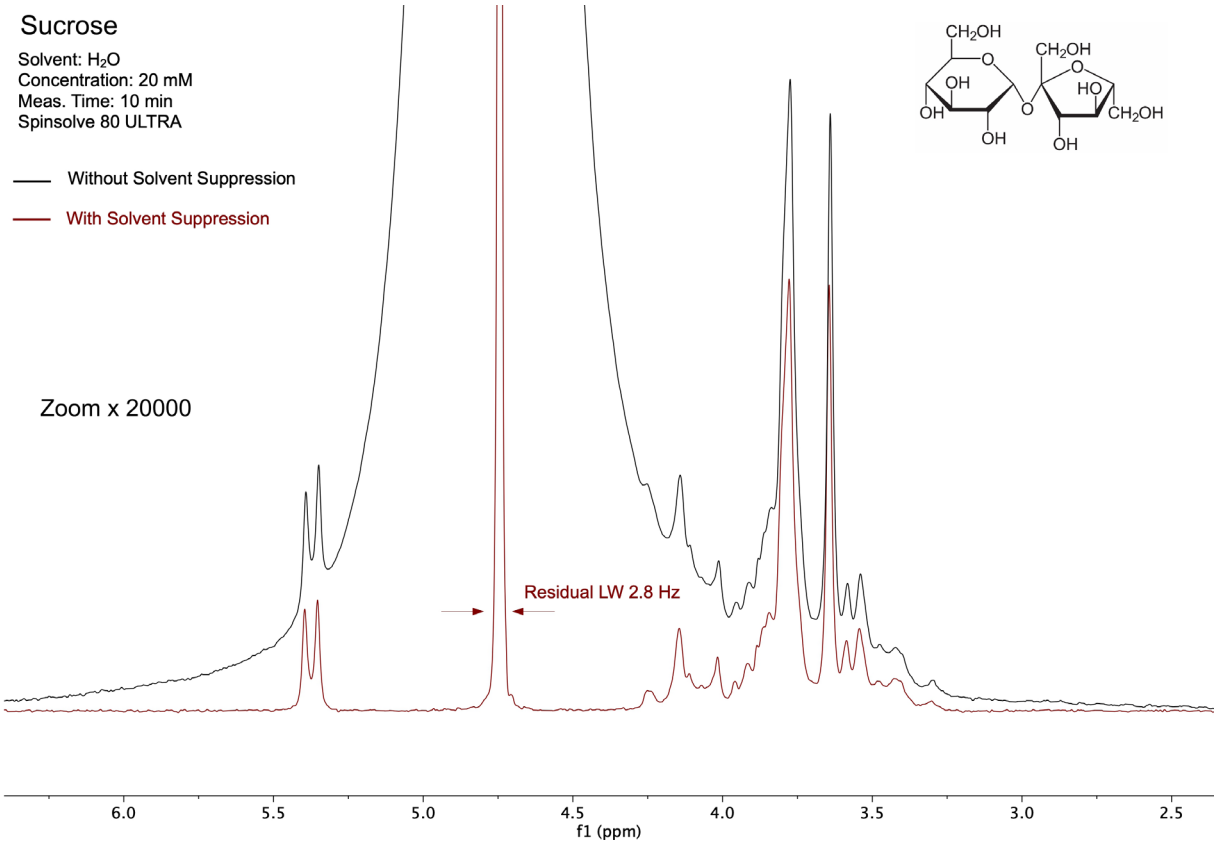

Figure 3. Spectra of sucrose dissolved at a concentration of 20 mM in neat water acquired with a standard pulse sequence (black) and with a solvent suppression method (red). The spectra were acquired using 64 scans and a repetition time of 10 seconds on a Spinsolve 80 MHz Carbon ULTRA.

This high performance solvent suppression can only be achieved if the magnetic field is highly homogeneous, which is a challenge for benchtop systems. Permanent magnet geometries designed for benchtop spectrometers are required to be as small as possible, generate the strongest magnetic field, and keep the sample volume constrained to use standard 5 mm NMR tubes. In such an optimization process the specification that suffers the most is the homogeneity of the magnetic field. In general, while a reasonable homogeneity can be achieved in the center of the tube, it tends to become inhomogeneous as the volume approaches the wall of the tube, leading to a line shape that is relatively narrow at half height, but that can be very broad in the tails at the bottom of the peak. In this case, the solvent suppression methods do not work properly.

This is because the selective excitation used by the method only saturates the nuclei with a resonance frequency close to the center of the peak. As the tails are simply the signal of nuclei located where the magnetic field has a different strength, such as close to the wall of the tube, they will not be efficiently saturated because they have a different resonance frequency to the one of the nuclei in the homogeneous center of the tube. Depending on the frequency spreading caused by the magnetic field inhomogeneity, the selective excitation part of the sequence will saturate the center of the peak, burning a hole in the center, leaving, in extreme cases, the tails unaffected. So, although the center of the peak can be attenuated, the overlapping of the tails would remain and the solvent suppression would be of no advantage for our measurements.

As the homogeneity of the magnetic field defines the line shape of the peaks in the spectrum, the accepted way to specify the homogeneity of a magnet requires to measure the line width (LW) at different heights of the NMR peak of a standard chloroform sample. While it is common to use the LW at 50% of the peak as main indicator, it is actually the LW at its base which determines the performance that can be expected for the solvent suppression method. For high field systems, the width of the line is typically specified at 50%, 0.55%, and at 0.11% of the peak height. To achieve the highest performance in applications where solvent suppression is required, Magritek introduced in 2017 the Spinsolve ULTRA model. While the classic version of the Spinsolve delivers the highest homogeneity available in the benchtop market today (with a LW <0.4 Hz at 50% and <16 Hz at 0.55% of the peak height), the Spinsolve 80 ULTRA model specifies an even superior linewidth <0.25 Hz at 50%, <10 Hz at 0.55%, and <20 Hz at 0.11% of the peak height, which is comparable to the homogeneity delivered by high field magnets. An example of such a line shape is shown in Fig. 4, where a standard chloroform sample was used for the measurement. At this point it is important to mention that the Spinsolve ULTRA is the only model on the market that specifies the LW at 0.11%. This ULTRA high magnetic field homogeneity makes it possible to resolve the smallest signals close to the solvent signals like no other benchtop system.

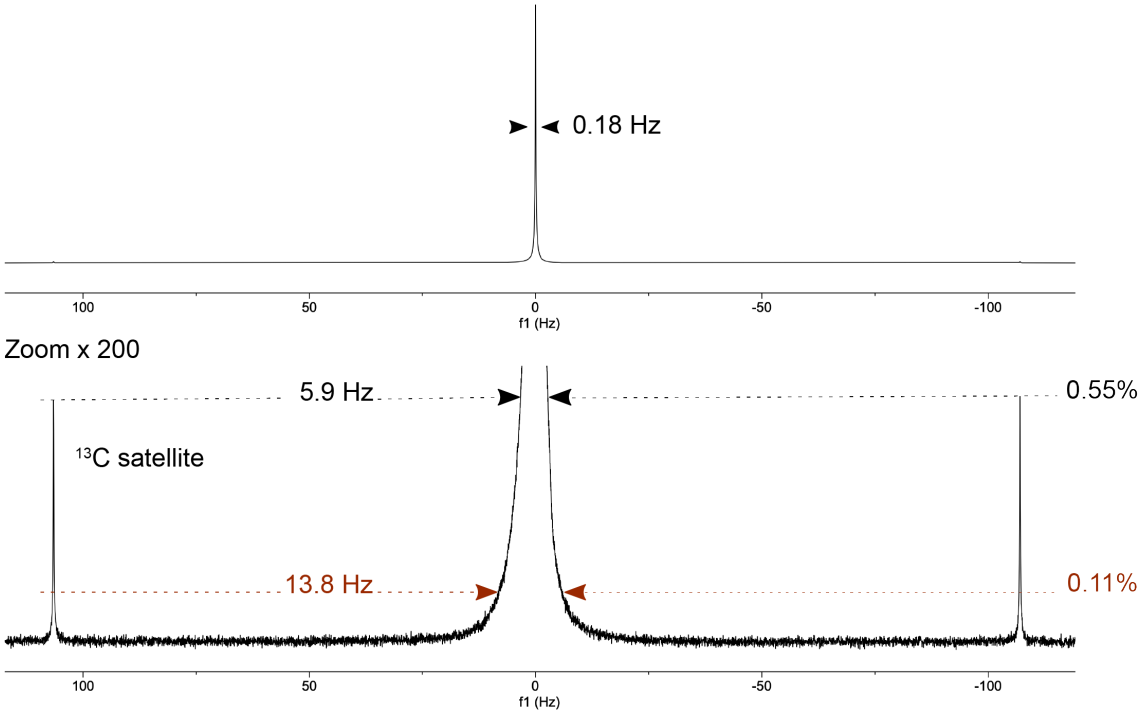

Figure 4: Spectrum of a reference chloroform sample dissolved in deuterated acetone at a concentration of 20%. The upper inset shows the full line shape, where the linewidth can be measured at 50% of the peak amplitude. The zoomed spectrum shows the carbon satellites of chloroform, which can be used as a reference to identify the 0.55% of the peak height. The line width at the base of the peak is shown also at 0.11%.

Suppression of multiple lines and ^{13}C satellites in organic solvents

In the previous examples water was used as solvent. However, organic solvents with multiple lines in the spectrum are frequently used depending on the solubility of the sample, or the reaction under investigation. Figure 5a shows the full scale spectrum of a paracetamol sample dissolved in ethanol at a concentration of 170 mM. As ethanol has a concentration of about 17 molar only the signals of the solvent are visible at full scale. A zoom x100 allows us to identify the signals of paracetamol in the base of the large solvent peaks (Fig. 5b). This example confirms again that the Spinsolve receiver can acquire the signal of a neat solvent without showing effects of saturation or compromising the sensitivity. The result after applying solvent suppression at the center of each of the three solvent signals can be observed in Fig. 5c. The selective excitation pulses applied during the WET module before the acquisition of the spectrum achieves a saturation of about a 300 fold and the solvent peaks are now comparable in amplitude to the paracetamol signals.

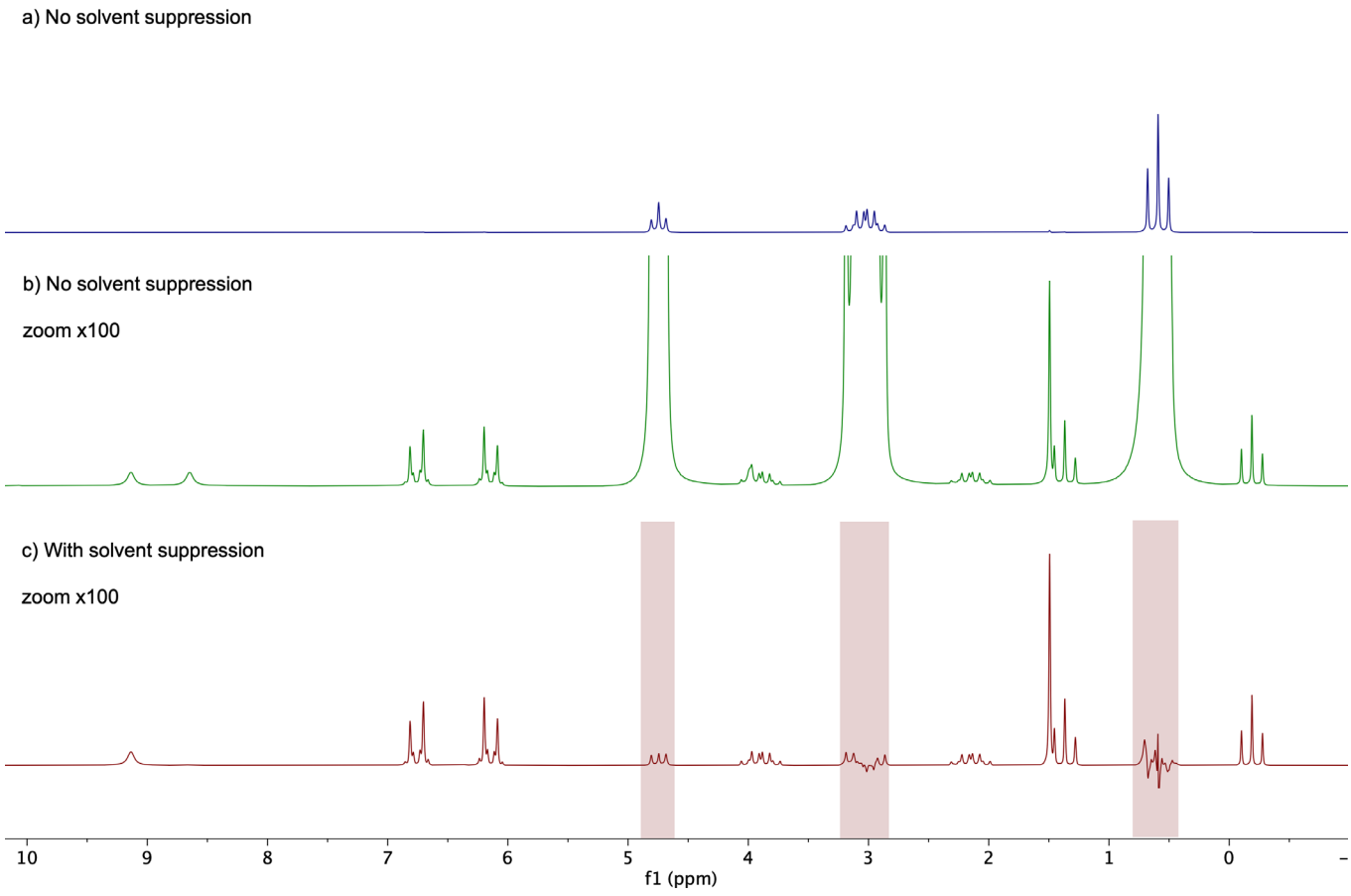

Figure 5: a) Spectrum of paracetamol dissolved in Ethanol at a concentration of 170 millimolar. b) Zoom x100 of the spectrum in a). The signals of paracetamol get visible and appear with a similar intensity as the signals of the carbon satellites of the ethanol peaks. c) Spectrum of the paracetamol sample acquired using a multi peak WET solvent suppression method that saturates the three main signals of ethanol.

While the solvent suppression sequence removes the tails of the solvent peaks, we can quickly identify a new problem introduced by the organic solvent. As the solvent has different C-H groups, the carbon satellites of these groups are visible in the spectrum. The natural abundance of ^{13}C is about 1%, so with a neat concentration of 17 Molar, there are 170 mM of ethanol molecules with a ^{13}C nuclei at each of the two carbon positions. In this example, it is the same concentration as the paracetamol in the sample. The concentrations used here are similar to the concentrations you can expect in a typical reaction, confirming that carbon satellites are a common problem to expect when using organic solvents. A powerful NMR method to remove the satellites from the spectrum is known as carbon decoupling. It requires continuous excitation of the ^{13}C nuclei either during the solvent suppression period or during signal acquisition. Figure 6 shows the results obtained with the second option implemented on a Spinsolve Carbon ULTRA system, which allows simultaneous acquisition of the ^{1}H signals and excitation on the carbon channel. With this we can conclude that an instrument with a carbon channel can also be of advantage for Reaction Monitoring applications, although ^{13}C is not a nucleus that one would typically try to detect in flow applications (this is because of the longer measurement times required for ^{13}C measurements).

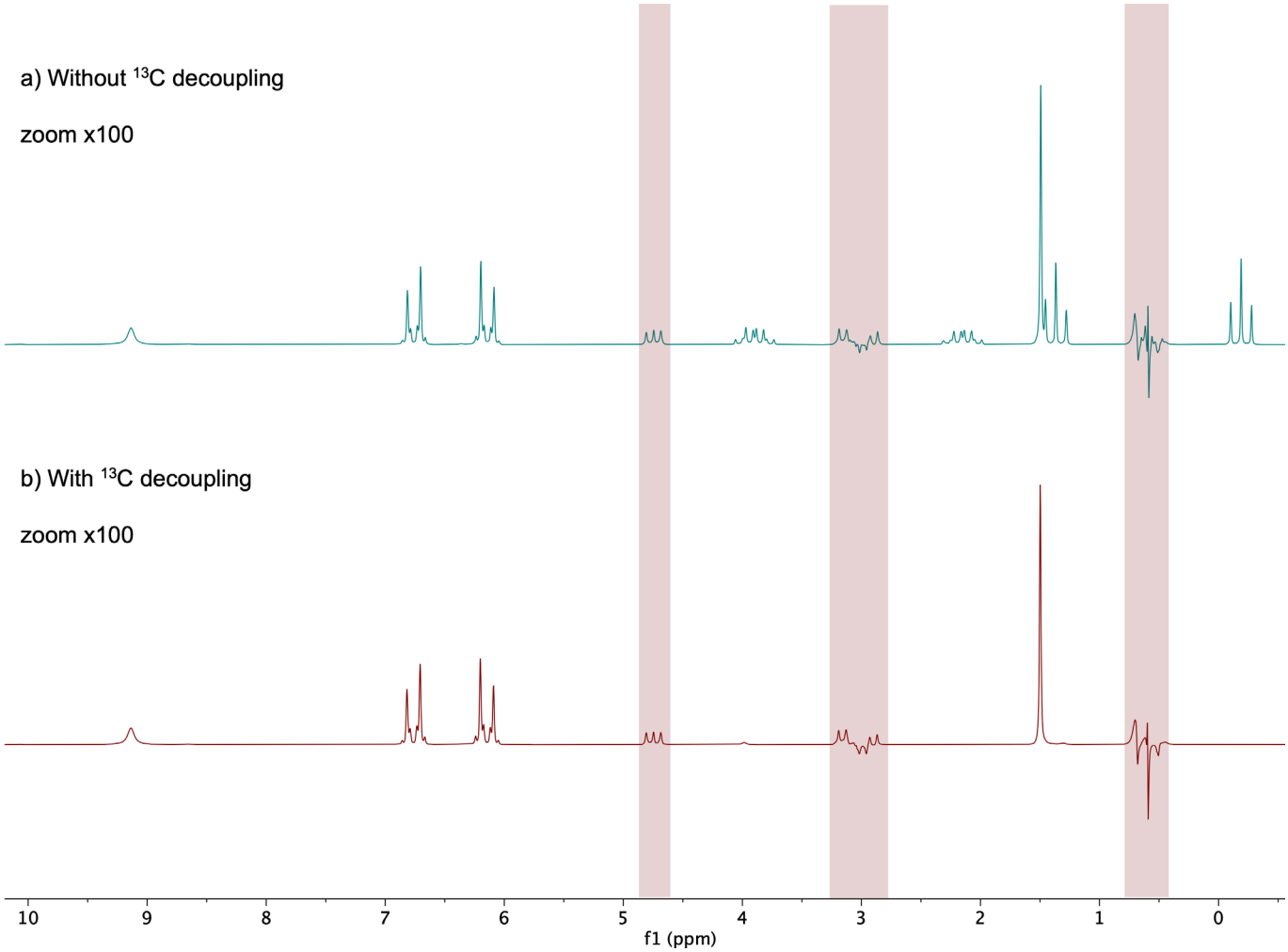

Figure 6: Comparison of the spectra acquired with (a) and without (b) carbon decoupling. By acquiring the signal in the presence of carbon decoupling the ^{13}C satellites are completely eliminated, removing the overlap with the signals of the product that need to be integrated.

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