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Spinsolve 80 ULTRA

The power of solvent suppression and ^{13}C decoupling for synthesis and reaction control

Figure 1. Spinsolve NMR spectrometer used in the chemistry lab for quick analysis 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.

Solvent suppression

Benchtop NMR systems installed in chemistry labs are typically applied to identify or quantify products dissolved in protonated instead of deuterated solvents. The presence of protonated solvents in the sample introduces two main challenges at the time of acquiring an NMR spectrum. The first challenge is due to the large signal from the protonated solvent which can saturate the receiver of the spectrometer. This forces a reduction of the receiver gain which very quickly leads to losses in the signal-to-noise ratio (SNR), or sensitivity in the spectrum. The issue particularly affects high-field NMR systems. A common solution to this problem is to apply solvent suppression methods to attenuate the solvent signal before the measurement is performed. Although the use of these methods is mandatory for high-field systems, they are not required on benchtop spectrometers. At the lower field strengths of these systems, the NMR signals have a smaller amplitude, and the receiver amplifier is designed with a large enough dynamic range to acquire the signals of pure solvents without saturation. This enables the optimum SNR in the spectrum to be maintained. To demonstrate the large dynamic range of the receiver in a Spinsolve spectrometer, the spectrum of histidine dissolved at a concentration of 10 millimolar in neat water was acquired without solvent suppression. The neat water has a concentration of about 55 Molar, which defines an effective concentration of 110 Molar of ^{1}H contributing to the singlet observed for water. The full-scale and zoomed spectra (red spectra in Fig. 2a and 2b) show no distortion caused by saturation of the receiver. Taking into account that the solute has a concentration that is four orders of magnitude smaller than the solvent, a large zoom of the vertical scale is required to see the signals of the solute (see Fig. 2b).

At this point we can identify the second challenge introduced by the presence of a large signal in the spectrum. The small signals from histidine in the standard spectrum shown in Fig. 2b (red) appear mounted on the tails (or sides) of the water peak, which covers a big fraction, if not all, of the spectrum and makes quantification almost impossible. Solvent suppression can be very useful for benchtop systems to remove the solvent peak and its tails, but because these methods are based on selective frequency excitation, they can only work efficiently if the magnetic field of the magnet is highly homogeneous. In a homogeneous magnetic field all nuclei of a particular chemical group have the same resonance frequency and the shape of the NMR peak in the spectrum is described by a Lorentzian function defined only by the relaxation time of the sample. If this criteria is fulfilled it is said that the NMR signal is homogeneously broadened and all nuclei have actually the same resonance frequency.

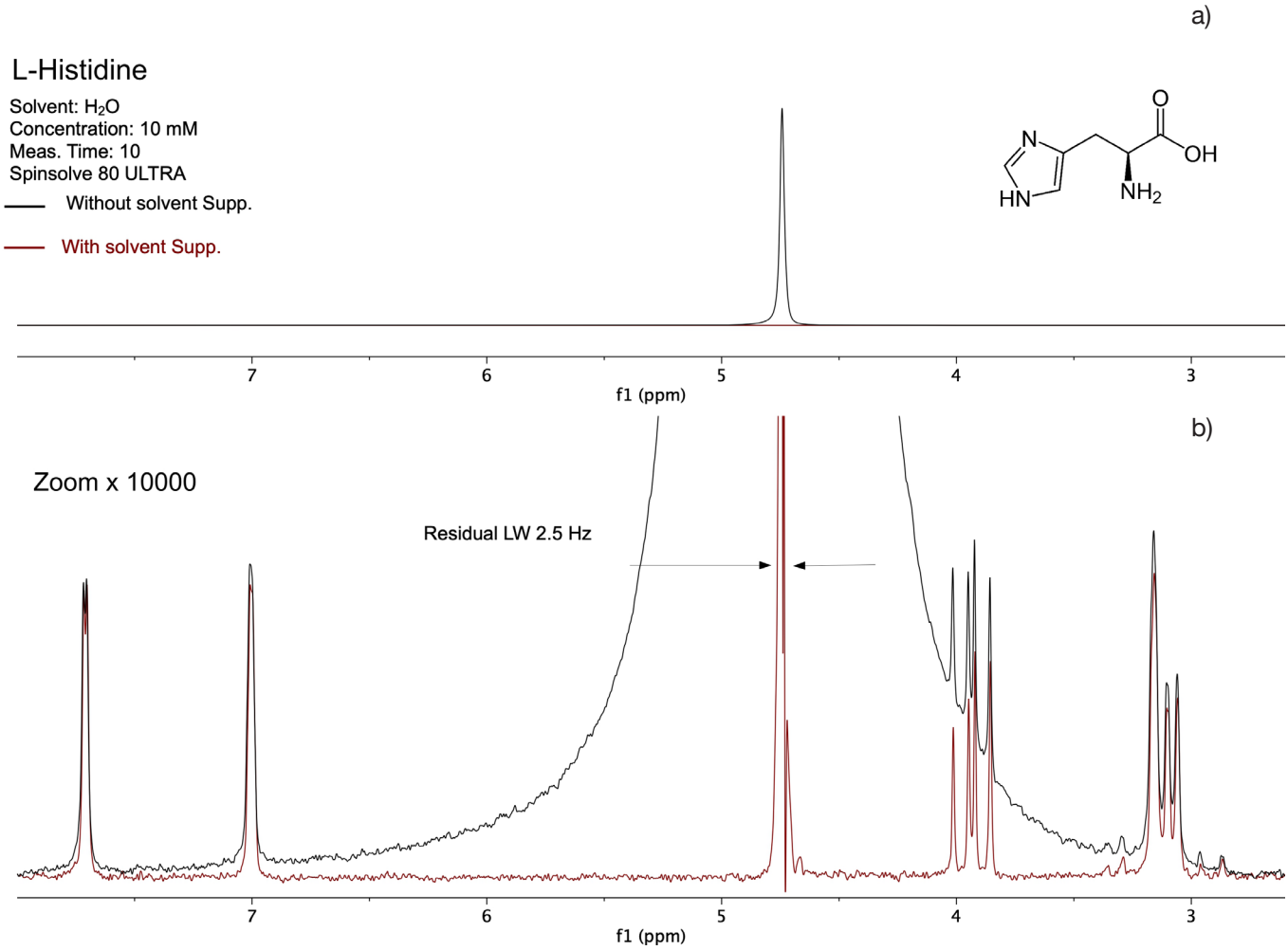

Figure 2. a) Spectra of histidine dissolved at a concentration of 10 mM in water acquired with a standard pulse and acquire sequence (black) and using a solvent suppression sequence (red). Shown in full vertical scale, only the water signal is visible as it has a concentration of 55 Molar (effective 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 peak may extend over a wide region in the spectrum it can be attenuated as a whole by applying continuous excitation at the central frequency of the line. In this situation, solvent suppression can considerably reduce the tails of the solvent peaks and allow the signals of the products to be more accurately quantified. The cyan spectrum shown in Fig. 2a and 2b was obtained when a selective excitation pulse is applied at the resonance frequency of the water peak for a period of two seconds prior to the acquisition of the NMR signal. In this experiment the water peak was attenuated a factor of about 100, while the signals corresponding to histidine remained unaffected.

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. 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. 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).

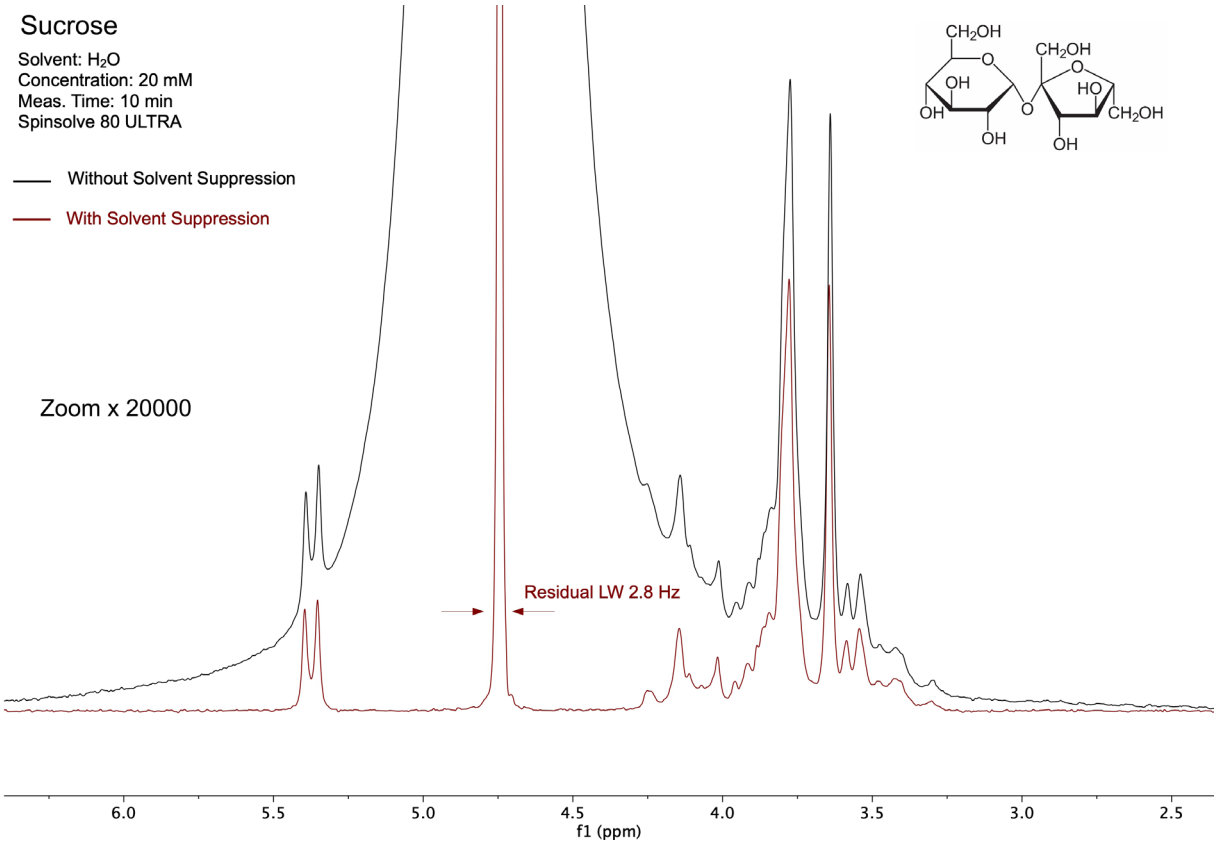

Figure 3. Spectra of sucrose dissolved at a concentration of 20 mM in neat water acquired with a standard pulse sequence (black) and with a solvent suppression method (red). The spectra were acquired using 64 scans and a repetition time of 10 seconds on a Spinsolve 80 MHz Carbon ULTRA.

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. When the tails are defined by the magnetic field inhomogeneity, 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 is by providing the line width (LW) at different heights of the NMR peak of a chloroform standard 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 homogeneity makes it possible to resolve the smallest signals close to the solvent signals like no other benchtop system.

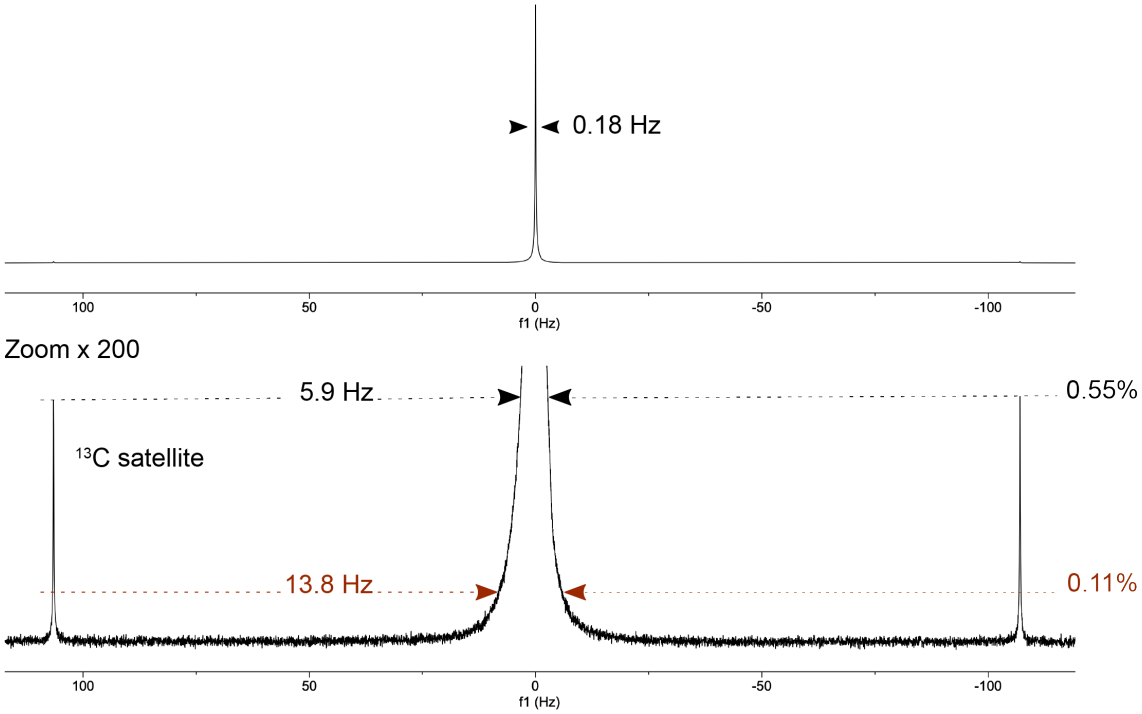

Figure 4: Spectrum of a reference chloroform sample dissolved in deuterated acetone at a concentration of 20%. The inset shows the full line shape where the linewidth at 50% of the peak amplitude is specified. The zoomed spectrum shows the carbon satellites of chloroform as a reference to identify the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

^{13}C decoupling

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 pulse applied before the acquisition of the spectrum achieves a saturation of about a hundred fold and the solvent peaks are now comparable in amplitude to the paracetamol signals.

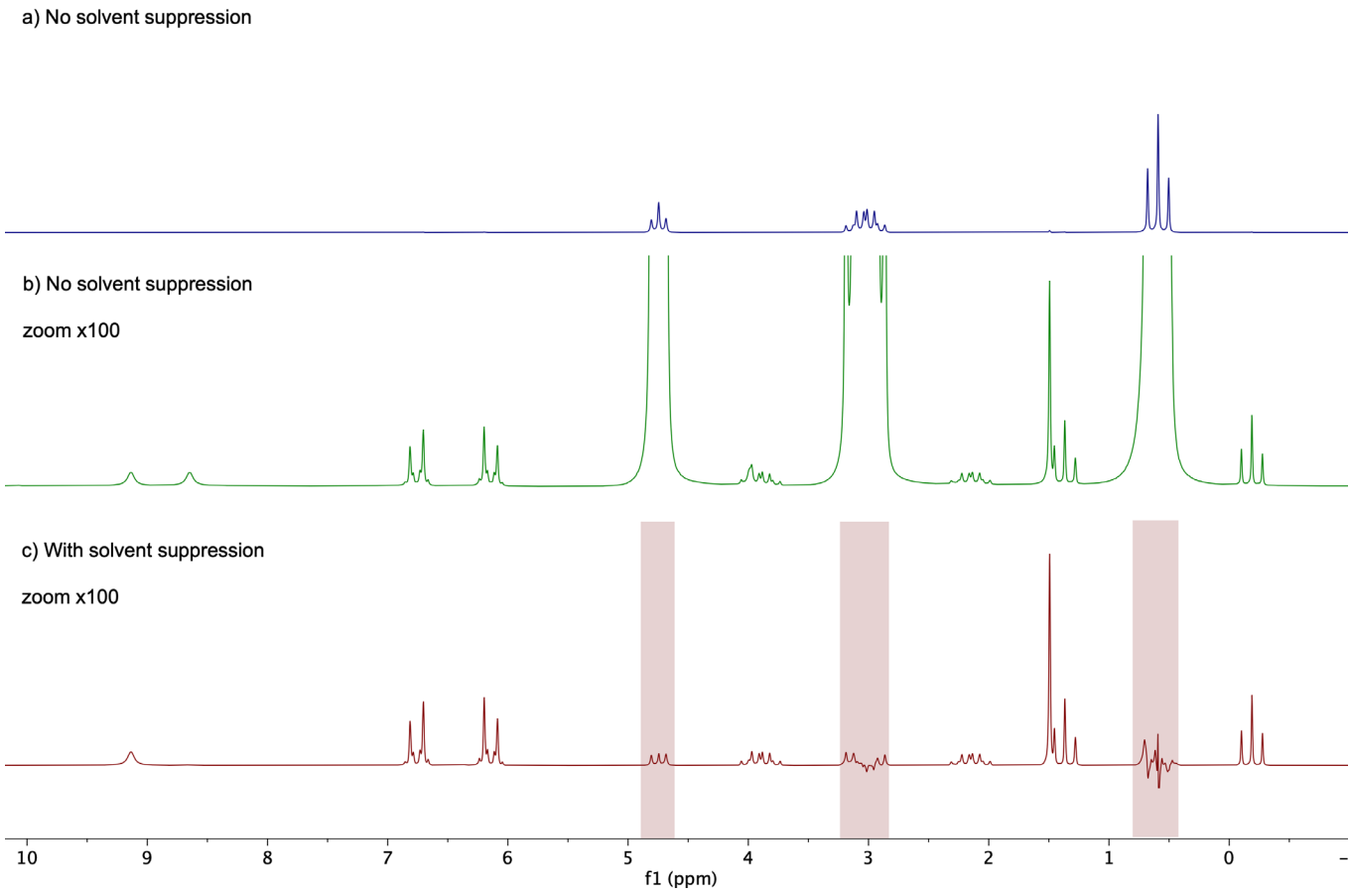

Figure 5: a) Spectrum of paracetamol dissolved in Ethanol at a concentration of 170 millimolar. b) Zoom x100 of the spectrum in a). The signal of paracetamol gets visible and appears with a similar intensity as the signal of the carbon satellites of the ethanol peaks. c) Spectrum of the paracetamol sample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

While the solvent suppression sequence does the job of removing the tails of the solvent peaks, we can quickly identify a new problem introduced by the organic solvent. Because the solvent has 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. 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).

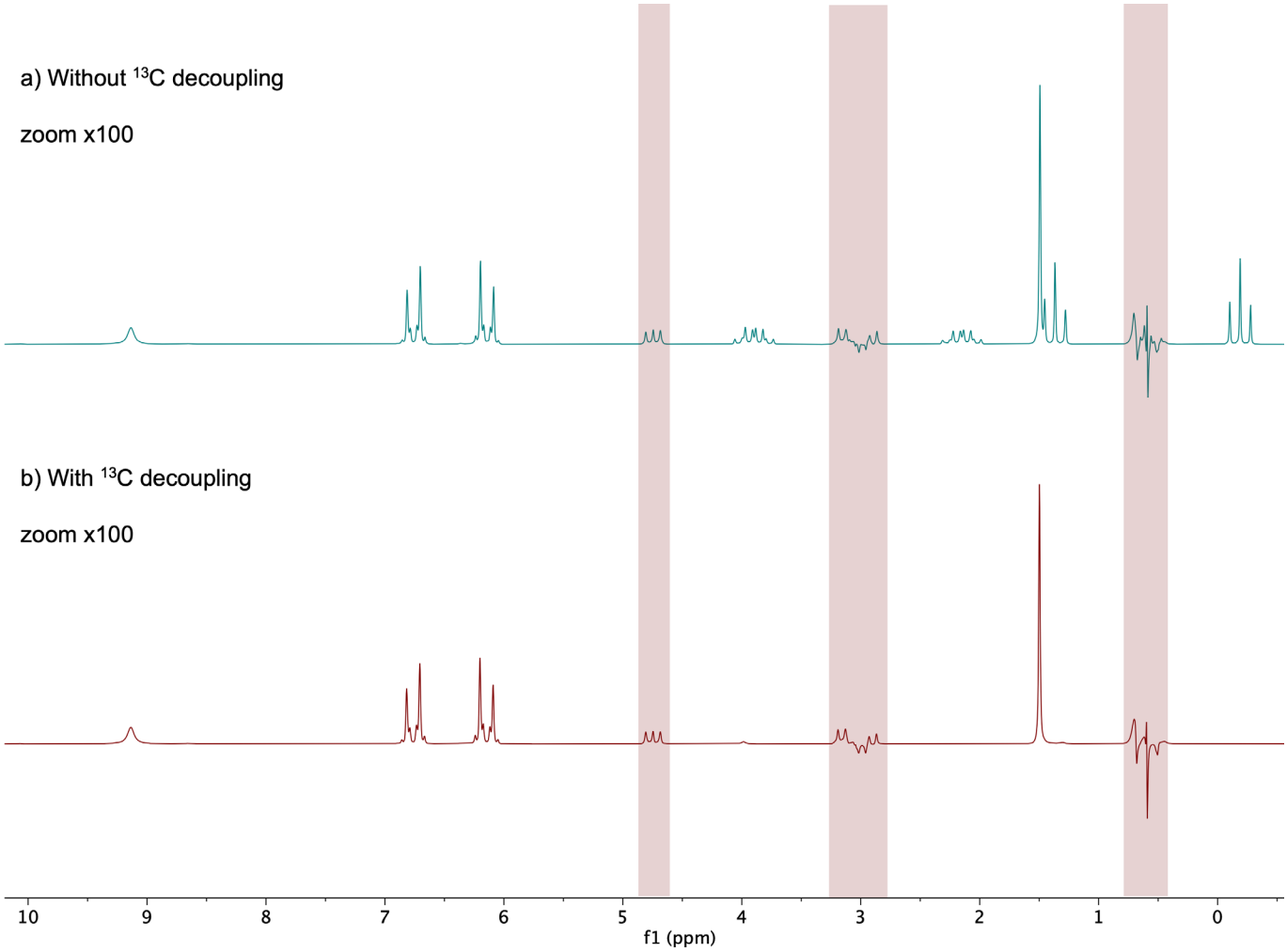

Figure 6: Comparison of the spectra acquired with (a) and without (b) carbon decoupling. By acquiring the signal in the presence of carbon decoupling the ^{13}C satellites are completely eliminated, removing the overlap with the signals of the product that need to be integrated.

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