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Structure Verification of molecules in Protonated Solvents

Structure verification and elucidation using NMR is a routine analysis in many synthetic laboratories, where chemists collect and analyze a suite of 1D and 2D NMR experiments to confirm they have indeed synthesized their desired products, or to elucidate the structure of unexpected side products in their reactions. In the recent years, the development and implementation of effective WET solvent suppression in the Spinsolve ULTRA benchtop NMR spectrometers has enabled the analysis of samples in protonated solvents with not only 1D experiments, but also 2D-NMR experiments such as COSY, and HSQC-ME. This new capability allows for the structure verification or elucidation analysis to be carried out directly in protonated organic solvents. This development has opened useful new scenarios like, the analysis of column chromatography fractions to confirm the structure of products, or the direct analysis of reaction aliquots to characterize reaction intermediates *in situ*.

In this application note, we implement these advanced 2D-techniques to verify the chemical structure of CBDV in protonated methanol. Cannabidivarin (CBDV) is an n-propyl analogue of cannabidiol (CBD) that doesn't exhibit the psychoactive effects commonly found in cannabis[1]. Nevertheless, CBDV has shown great potential with applications in pharmaceutical, nutraceutical, and cosmetics[2], which generates the interest in the synthesis of CBDV to produce in higher quantity, and to have access to different intermediates for derivatization.

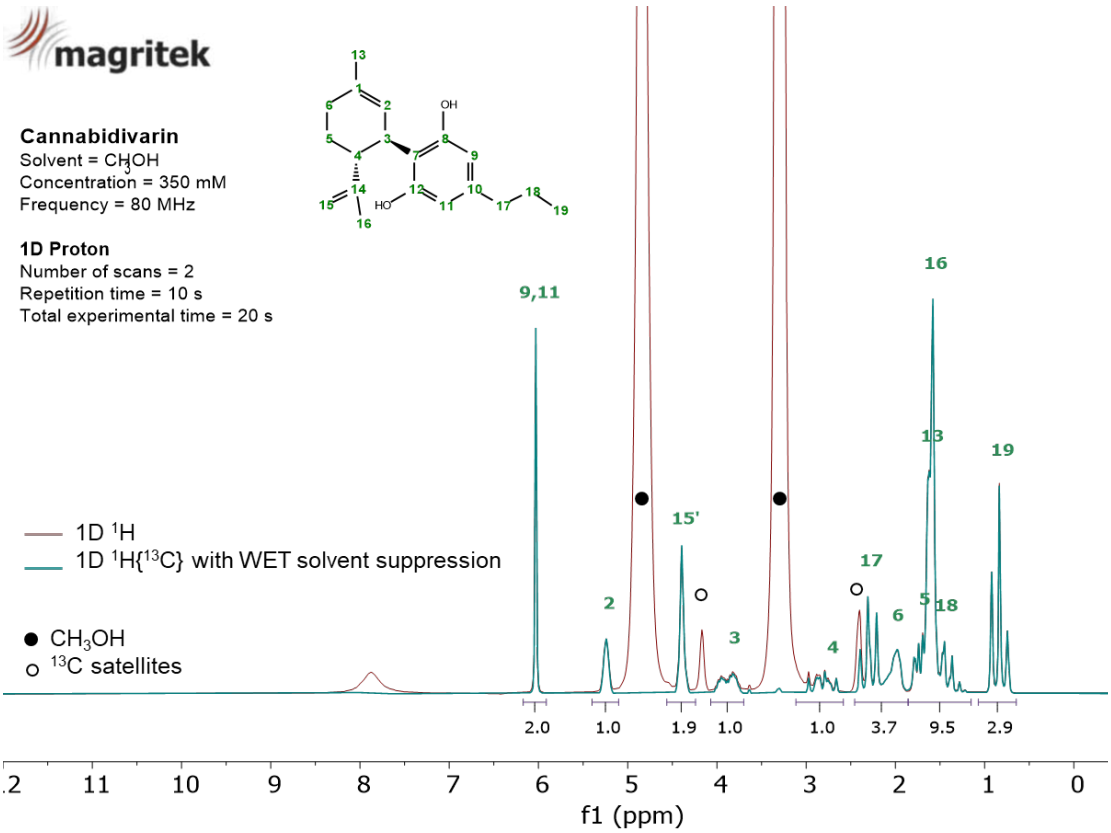

Figure 1. 1D ^{1}H spectrum (maroon trace), and 1D $^{1}H\{^{13}C\}$ spectrum collected with WET solvent suppression (cyan trace) acquired on a Spinsolve 80 MHz ULTRA system.

Figure 1 shows the superimposed spectra of regular 1D ^{1}H spectrum (maroon trace), and 1D $^{1}H\{^{13}C\}$ spectrum collected with WET solvent suppression (cyan trace). The signals for methanol are greatly attenuated. The solvent suppression, along with the removal of ^{13}C satellites simplify the 1D ^{1}H spectrum and result in the baseline resolution of signals from H2, H15, H3 and H4. Even though these four signals are very close to the methanol signals, their integral values are not being affected by the solvent suppression, which highlights the effectiveness and selectivity of the WET solvent suppression.

To assign and resolve signals overlapping in the 1D ^{1}H spectrum, 2D-NMR techniques are widely used. However, like for the 1D methods, the presence of protonated solvents in the sample difficult the signal assignment. Figure 2 shows a comparison of a COSY spectrum of CBDV acquired without (left) and with solvent suppression (right). In the regular COSY spectrum, the methanol signals along the diagonal and cross peaks (marked by a yellow box) dominate the spectrum, and introduce T_1 noise, which obscure the identification of cross peaks of the signals of the product. Moreover, ^{13}C satellites signals of the solvent peaks generate further cross peaks (marked by blue arrows in the regular COSY spectrum) that make the interpretation of the spectrum even more complicated. When solvent suppression is applied in WET-COSY, the intensity of the methanol signals (also marked by a yellow box) and T_1 noise is considerably reduced. On the other hand, by applying ^{13}C decoupling during the sequence, the ^{13}C satellites are eliminated together with its cross peaks, leading to the acquisition of a much cleaner 2D spectrum with the WET-COSY sequence.

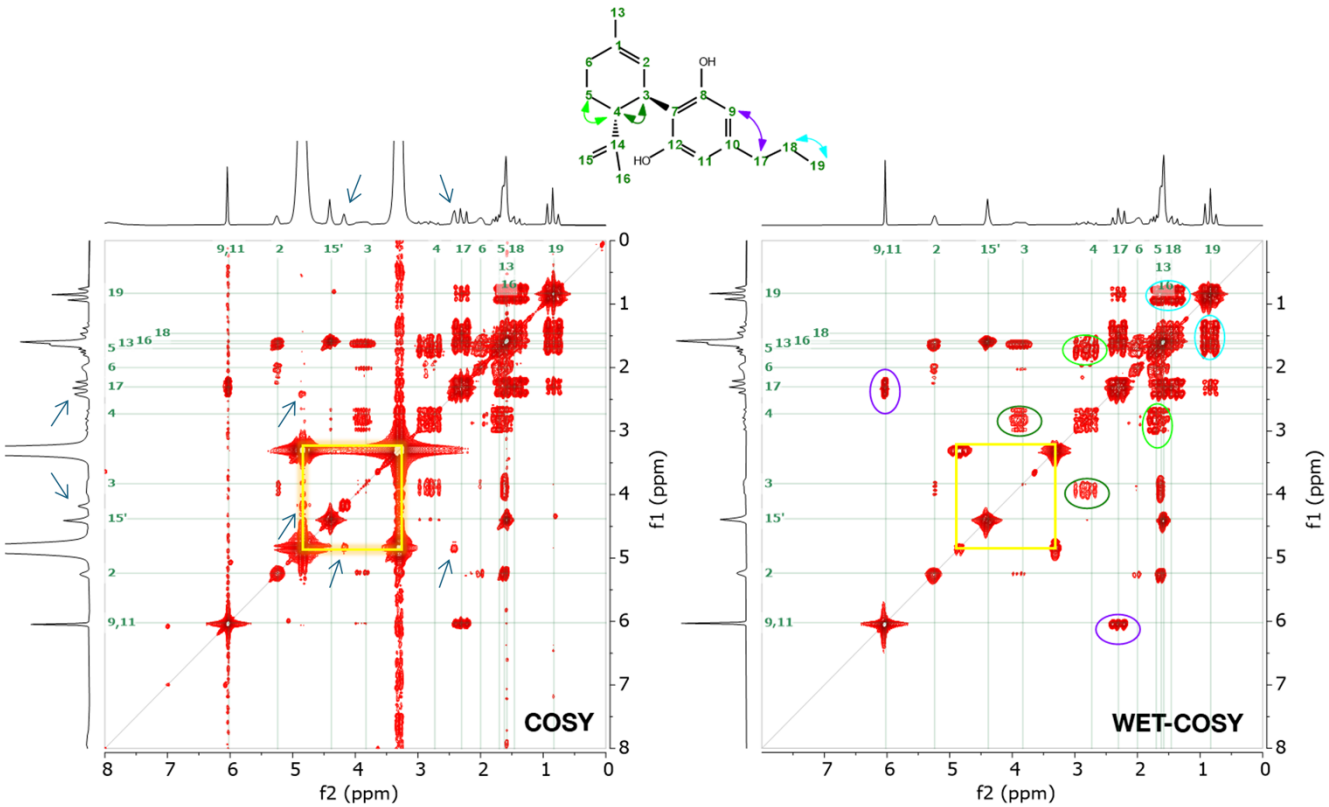

Figure 2. ^{1}H 2D COSY (left) and 2D WET-COSY (right) experiments of CBDV in MeOH acquired in 34 minutes on a Spinsolve 80 MHz ULTRA system. The signals of protonated methanol dominate the spectrum in the regular COSY, but are strongly attenuated by the WET module applied in the WET-COSY sequence. Moreover, the WET-COSY includes ^{13}C decoupling during the sequence to eliminate the ^{13}C satellites (marked with blue arrows on the regular COSY spectrum).

The measurement of ^{13}C 1D spectra is typically of great assistance to confirm the structure of a molecule. In general, this measurement can be done in protonated solvents without suffering from the signal overlapping described for the ^{1}H counterpart. The 1D ^{13}C spectrum, shown in Fig. 3, shows all the expected signals for CBDV, with a prominent singlet of protonated methanol at 49 ppm. When available, a ^{13}C

spectrum can be conclusive to confirm the structure of the molecule under investigation, however, depending on the concentration of the sample, this measurement can be prohibitive long. As a powerful alternative, multinuclear experiments like the HSQC and HMBC combine the high resolution of ^{13}C with the higher sensitivity of ^{1}H to obtain very powerful information in much shorter measurement times.

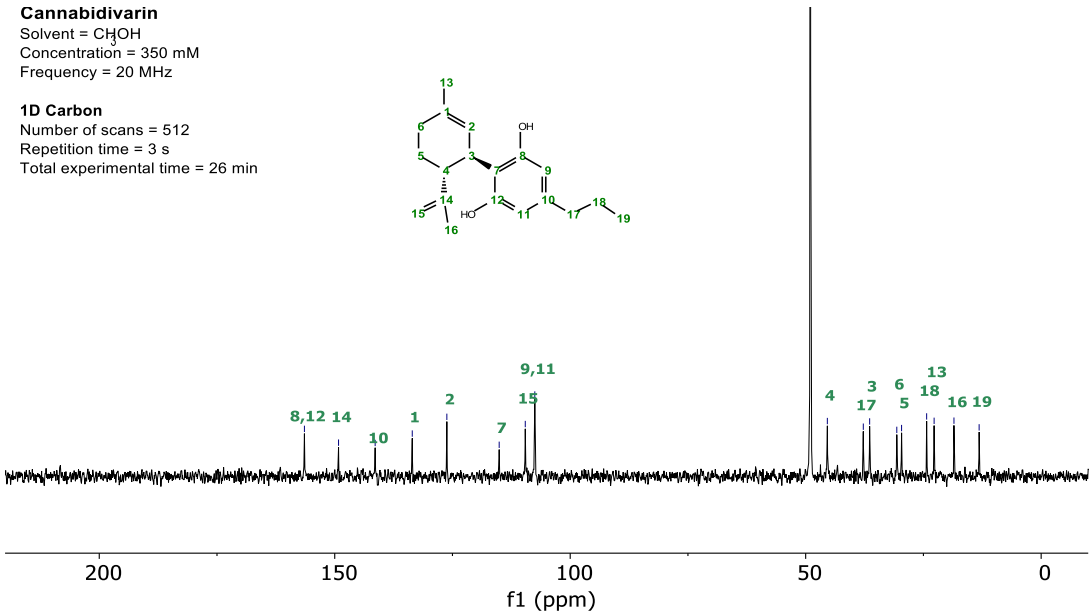

Figure 3. 1D $^{13}C\{^{1}H\}$ spectrum of CBDV in MeOH acquired on a Spinsolve 80 MHz ULTRA system in 26 minutes.

Like the COSY, the data collected with the regular HSQC-ME pulse sequence (see Fig. 4) shows high T_1 noise intensity of the methanol signals, which makes the C3-H3 and C4-H4 correlations hard to observe and interpret. The implementation of solvent suppression in WET-HSQC-ME removs the T_1 noise to reveal these correlations for unambiguous analysis.

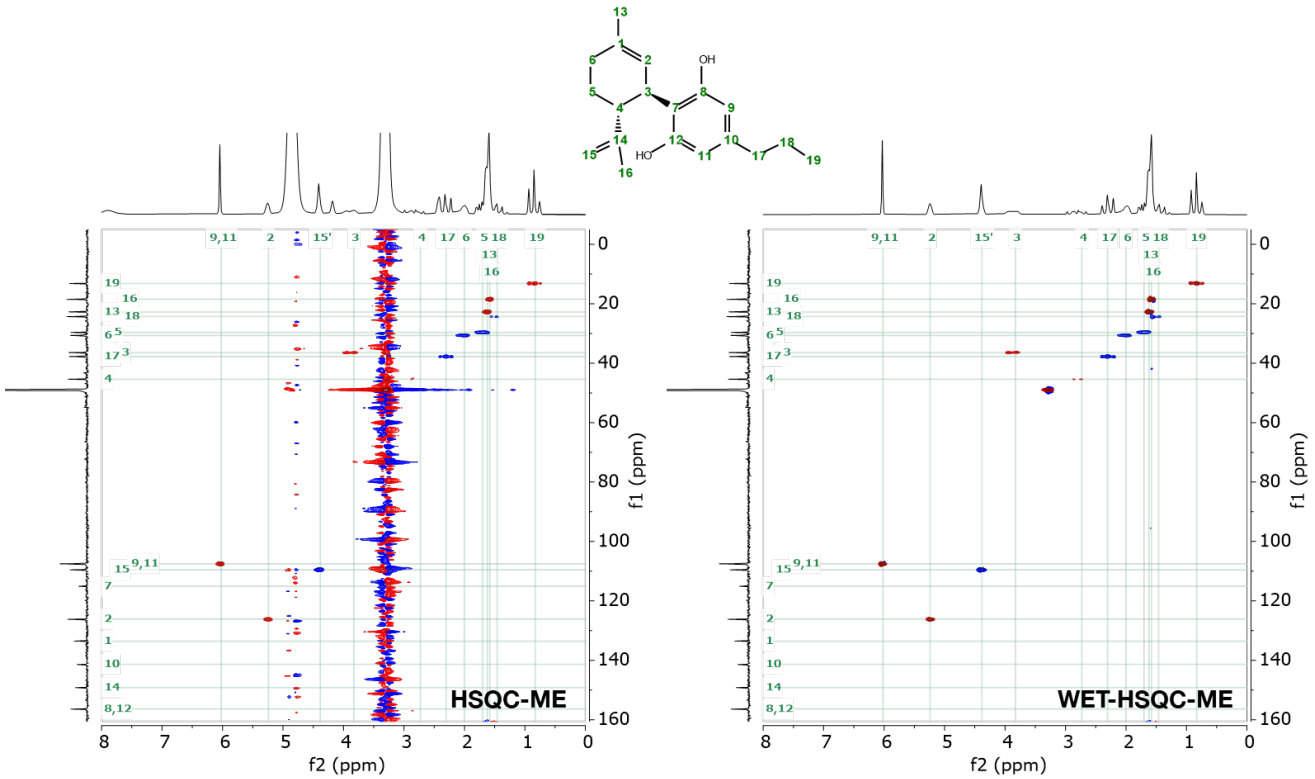

Figure 4. HSQC-ME (left) and WET-HSQC-ME (right) spectra of CBDV in MeOH showing the correlation between the ^{1}H and ^{13}C signals, acquired in 17 minutes on a Spinsolve 80 MHz ULTRA system.

Finally, the HMBC spectrum of CBDV shown in Fig. 5 helps assign the quaternary carbons (C1, C7, C10, and C12). Since HMBC focuses on the long-range correlations, it doesn't experience the intense T_1 noise observed in the COSY and HSQC-ME experiments.

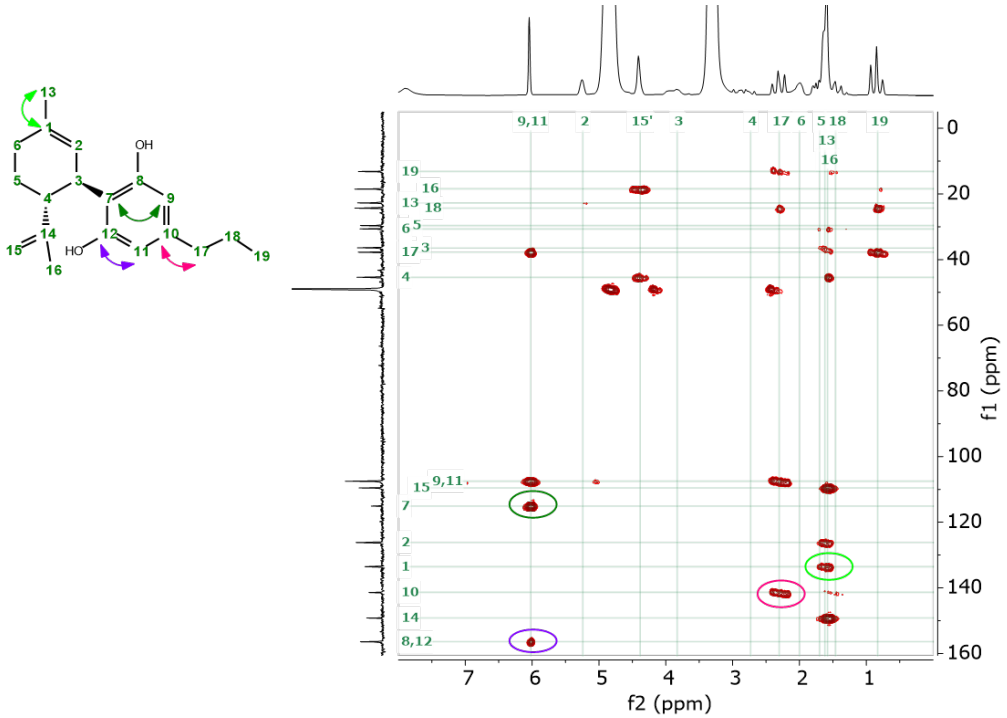

Figure 5. HMBC spectrum of CBDV in MeOH showing the long-range couplings between ^{1}H and ^{13}C nuclei, acquired in 34 minutes on a Spinsolve 80 MHz ULTRA system.

This application note shows the advantages of solvent suppression in 2D techniques. The structural analysis of CBDV in protonated methanol demonstrates the effective implementation of the solvent suppression to allow the structural analysis of molecules in regular organic solvents either during the reaction process, or at the end of the purification.

Acknowledgement

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References

1. Campos DA, Mendivil EJ, Romano M, García M, Martínez ME. A Systematic Review of Medical Cannabinoids Dosing in Human. Clin Ther 2022;44(12):e39–58.
2. Chiurchiù E, Sampaolesi S, Allegrini P, Ciceri D, Ballini R, Palmieri A. A Novel and Practical Continuous Flow Chemical Synthesis of Cannabidiol (CBD) and its CBDV and CBDB Analogues. European J Org Chem 2021;2021(8):1286–9.