

Spinsolve 80

Just a few milligrams available for a ¹³C measurement?

Use advanced NMR methods for quick structure confirmation



Sensitivity of benchtop NMR systems

The yield of substance from reactions in synthesis R&D labs is often only a few milligrams. This is certainly enough material to run ¹H NMR measurements in very short times, however in many cases ¹³C is essential for structure confirmation. As ¹³C is much less sensitive than ¹H, directly detected ¹³C measurements on samples with low concentration can become very time consuming. While in certain cases overnight measurements are acceptable, in general, strategies to obtain the required information faster are mandatory. Although one might initially exclude benchtop NMR for quick structure confirmation, we show in this note that by choosing advanced methods, selecting the right hardware, and optimizing sample preparation one can achieve ¹³C spectra in times below 1 hour for low concentration samples.

The most common way to measure a ¹³C spectrum is by acquiring a proton decouple directly detected ¹³C signal. While the Spinsolve systems provide excellent results with this type of sequence, the measurement times can become long when only a few milligrams of sample are available for the analysis. However, more advanced pulse sequences like HSQC or HMBC enhance the sensitivity of ¹³C nuclei by detecting them indirectly via the signals of the ¹H nuclei in the molecule. As these are two-dimensional methods one might initially expect them to take longer than a standard 1D measurement, but due to the sensitivity enhancement the measurement can become an order of magnitude faster compared to direct 1D Carbon experiment. On top of this large time saving factor, these methods deliver richer information as they provide the correlation between the ¹H and ¹³C nuclei in the sample. While the HSQC sequence correlates directly coupled ¹H and ¹³C nuclei, the HMBC correlates chemical shifts of protons with carbons separated through multiple bonds.

When running ¹³C measurements on samples with small amount of material the NMR system of choice is the one providing the highest sensitivity available. This is currently the Spinsolve 80 Carbon, which has been introduced by Magritek in 2017. An important consideration is to maximize the amount of material inside the rf coil (maximize concentration of the sample) in order to get the largest Signal-to-Noise ratio (SNR) in the spectrum. Users of high field NMR instruments work with sample volumes of about 600 μ L. However, the Spinsolve requires less than 300 μ L, resulting in 2 times higher concentration. As the SNR of an NMR measurement increases with the square root of the number of averages, this factor two increase in concentration results in a 4 times faster measurement time.

In this application note we selected caffeic acid as case study. This compound $(C_9H_8O_4)$ is found naturally in a wide range of plants and has potential anti-oxidant, anti-inflammatory, and anticarcinogenic activities. To prepare the sample we dissolved about 5 mg of caffeic acid in 300 µL deuterated methanol, which corresponds to a sample with a concentration of 100 mmol. This amount of sample is a typical quantity available from synthesis processes carried out in R&D labs.

¹H spectroscopy

The ¹H NMR spectrum of 100 mM caffeic acid in CD₃OD measured in a single 10-second scan is shown in Figure 1. The peaks corresponding to the different chemical groups in the molecule are well resolved and their integral ratios match the number of protons in each group with very high accuracy. At first sight the absence of saturated aliphatic protons, as well as, the strong coupling (16 Hz) of the olefinic doublets at 6.2 and 7.6 ppm can be recognized, while the three aromatic protons are observed as a group between 6.7. and 7.2 ppm. Furthermore it can be noticed that the carboxyl and hydroxyl groups are not separated due to the exchange with the hydroxyl group of methanol.



Figure 1: ¹H NMR spectrum of 100 mM caffeic acid in CD₃OD measured on a Spinsolve 80 MHz in a single scan.

The 2D COSY experiment enables us to identify ¹H nuclei within a spin system as they generate cross peaks in the 2D data set. In this case the olefinic CH groups at positions 2 and 3 (blue) couple to each other. Additionally, position 3 couples to the aromatic protons proving that this group must be the one attached to the aromatic ring.



Figure 2: COSY experiment of a 100 mM caffeic acid sample acquired in 10 minutes on a Spinsolve 80 MHz.

¹³C Spectrum

Figure 3 shows the ¹³C NMR spectrum of 100 mM caffeic acid in CD₃OD acquired employing NOE signal enhancement as well as ¹H decoupling. The 1D carbon experiment is sensitive to all ¹³C nuclei in the sample and clearly resolves the nine expected resonances.



Figure 3: Carbon NMR spectrum of 100 mM caffeic acid in CD₃OD measured on a Spinsolve 80 MHz using NOE enhancement.

Superimposed 2D HSQC-ME/HMBC spectra

Figure 4 shows an HSQC-ME and an HMBC spectrum superimposed. Both methods detect the ¹³C nuclei via the ¹H signal, which makes the methods far more sensitive than the directly detected ¹³C spectrum shown in Figure 3. The red signals correspond to the HSQC and the green ones to the HMBC. The HSQC protocol is widely used to correlate ¹H nuclei with directly attached ¹³C nuclei. The Spinsolve is equipped with a multiplicity edited version of this method (HSQC-ME), which additionally includes the editing power of the DEPT-135 sequence to distinguish the signals of CH₂ from the CH and CH₃ groups. The HSQC-ME in this case study was acquired in just 35 minutes, which is more than 10x faster than the 1D ¹³C spectrum shown above. It shows the five directly coupled ¹H-¹³C signals that can as well be seen in the 1D Carbon spectrum. Additionally, it provides information about which Protons are connected to wich Carbons. In the 2D spectra the aromatic signals are separated which helps now to assign the peaks in the ¹H spectrum. As the HSQC does only show directly bond Carbons signals from the quaternary Carbons are not observed. To obtain ¹H-¹³C correlations through two, three or even longer-range bond couplings including quaternary carbons, the heteronuclear multiple bond correlation (HMBC) experiment is used. The orange circles mark the quaternary Carbons and one can assign to which ¹H they are coupled over multiple bonds.

To read the combined HSQC/HMBC spectra one can focus one by one on the ¹H signals shown in the horizontal trace and identify the ¹³C nuclei they are coupled to by looking at the peaks appearing along the vertical direction. As an example, the couplings of the olefinic proton at position 3 are marked in Fig. 4. The ¹H at position 3 is directly coupled to the ¹³C at position 3, as confirmed by the HSQC signal. The circles in blue and red mark the ³J C-H couplings of the olefinic proton (3) to the aromatic carbons at positions 5 (purple circle) and 9 (red circle), while the coupling to the carboxylic carbon at position 1 is indicated by a green circle. Note, that the coupling to the neighbor olefinic proton at position 2 is not observed because the sequence is set to efficiently detect single-bond ²J and ³J couplings. Since the ²J double-bond couplings are larger they are not observable with the present settings and a second experiment would be needed to confirm those. In conclusion, advanced NMR heteronuclear correlation experiments on the Spinsolve provide efficient ¹³C acquisition strategies for structure elucidation with low concentration samples. In the case of caffeic acid presented here, the complementary information of HMBC and HSQC-ME allows measurement of the ¹³C information in under 2 hours instead of the 8 hours required for a conventional 1D ¹³C. Furthermore, the additional information about how the different nuclei are connected to each can be of great help in the confirmation of the molecule structure.



Figure 4: Superimposed HSQC-ME and HMBC spectra of 100 mM caffeic acid in CD₃OD showing the direct and long-range correlations between the ¹H (horizontal) and ¹³C (vertical) signals on a Spinsolve 80 MHz.

Spinsolve 80

Specifications

- Nuclei: ¹H, ¹⁹F, ¹³C (Other Nuclei Available)
- Operating frequency: 80 MHz (¹H)
- 1 H 50% Linewidth: < 0.5 Hz
- ¹H 0.55% Linewidth: < 20 Hz
- ¹H Sensitivity (dual channel): >200:1 for 1% Ethyl Benzene
- Operating Temperature Range: 18° C to 28° C (64.5° F to 82° F)
- Dimensions: 58 x 43 x 40 cm (23" x 17" x16")
- Weight: 72.5 kg (160 lb)
- Stray Field: < 2 G all around system
- Power requirement: 110-240V AC



Pulse sequences available on the Spinsolve 80 Carbon spectrometer

Proton	Fluorine	Carbon
1D with carbon decoupling	1D	1D with proton decoupling
Paramagnetic	Paramagnetic	DEPT
2D COSY	2D F - COSY	APT
2D TOCSY	2D F - JRES	HETCOR
2D JRES	2D FH - COSY	HMBC
T ₁ , T ₂	Reaction Monitoring	HMQC
Solvent Suppression		HSQC
Reaction Monitoring		HSQC-ME

Other sequences may be available, contact Magritek for details.

Contact us now for a quote, to request a demo or to measure your samples

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