ASAPHMQC – Quick Multidimensional Spectra

Application Note

Author

Jarrett Farias, Ph.D.
NMR Applications Scientist
Research Products Group
Santa Clara, CA USA

Abstract

VnmrJ 3 software provides easy-to-use, interactive tools for setting up advanced experiments. Simplified set-up procedures allow even novice users to get critical information about their research samples using the most advanced NMR experiments available. This application note is one of a series designed to provide step-by-step guidance for setting up sophisticated experiments to collect exactly the data you need for your analyses.

Introduction

The Acceleration by Sharing Adjacent Polarization Heteronuclear Multiple Quantum Coherence (ASAPHMQC)[1] experiment, similar to the commonly used Heteronuclear Single Quantum Coherence (HSQC)[2] experiment, provides information regarding one-bond (^{1}H-^{13}C) connectivity and heteronuclear chemical shifts for the protonated carbons. ASAPHMQC, however, requires far less time than most two-dimensional (2D) experiments, and, as such, provides an elegant tool for the collection of inspection or survey data.

In this application note, comparative ASAPHMQC and gradient heteronuclear single quantum coherence (adiabatic version) (gHSQCAD) data were acquired with default settings on 5 mg of clindamycin HCl. All 16 ^{1}H-^{13}C correlations are clearly identifiable in the ASAPHMQC experiment in 56 seconds, whereas the gHSQCAD required 8.25 minutes to complete. Additionally, with a few minor parameter changes, a 10 mg sample of clindamycin HCl gives a complete ASAPHMQC spectrum in 19 seconds. These examples provide an illustration of the power of the ASAPHMQC experiment.

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The efficiency gain for ASAPHMQC comes from a much shorter relaxation delay as compared to an HSQC-type experiment (0.06 seconds versus 1 second for the default). The loss of longitudinal magnetization from incomplete relaxation is compensated for by a magnetization transfer from nearby ^{12}C-attached protons (which are kept along z during the pulse sequence) to the observed ^{13}C-attached protons. The default parameters give a total experiment time of 56 seconds, not much longer than a simple proton spectrum.

One way to exploit the efficiency of ASAPHMQC is to use the experiment, along with a one-dimensional (1D) ^{1}H spectrum, as an efficient and elegant strategy for the investigation of a molecular structure at the initial stage. Typically, if the ^{1}H spectrum does not provide sufficient information, the sample is resubmitted with strategic homonuclear or heteronuclear correlation experiments. Adding heteronuclear information in the inspection stage can often solve the structure and, if not, more targeted data can be acquired, for example, selective 1D or band-selective 2D experiments. This approach to structure elucidation will often be much faster and minimize the impact to spectrometer usage.

Another use of the efficiency of ASAPHMQC is to obtain high-resolution (in F1) data when necessary. For 2D ^{1}H-^{13}C correlations, the resolution in F2, determined by the acquisition time, is often limited by the carbon-decoupling power requirements. Resolution in F1, however, requires additional t1 increments (ni), which can be expensive in terms of time. Because of the rapid recycle time of the ASAPHMQC experiment, a high-resolution 2D proton carbon correlation (for example, ni = 2048) can be obtained in approximately 13 minutes for a sufficiently concentrated sample. This can be extremely useful for compounds with spectral overlap issues.

An ASAPHMQC example

A sample of clindamycin (Figure 1) was used to demonstrate the speed and data quality obtained using the ASAPHMQC experiment. All data was collected on an Agilent 400-MR DD2 instrument with a OneNMR probe.

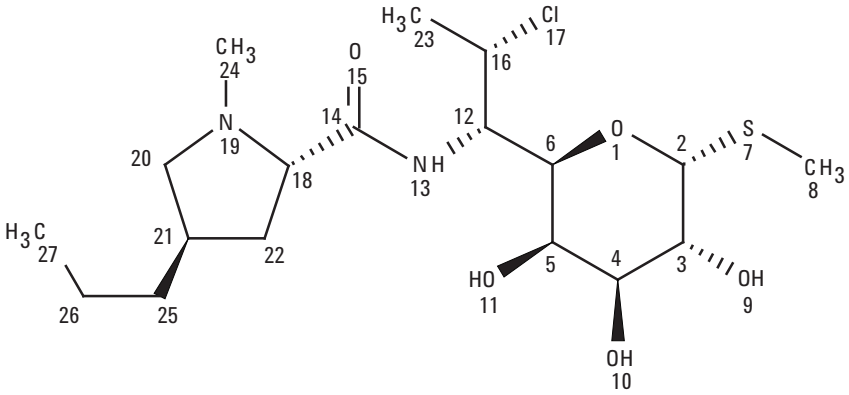

Figure 1. The chemical structure of clindamycin.

The spectrum in Figure 2 is of a ^{1}H-^{13}C ASAPHMQC experiment. Figure 3 shows a ^{1}H-^{13}C gHSQCAD for comparison. Using the unmodified default parameters to set up both experiments, ASAPHMQC provided signals for all 16 protonated carbons with a significantly reduced experiment time, 56 seconds as compared to 8 minutes 18 seconds for the gHSQCAD. The intensities of correlations 12 and 20 are smaller in the ASAPHMQC experiment than other correlations due to the limited number of ^{1}H-^{13}C pairs for magnetization transfer, and can be increased with a longer relaxation delay than the default of 60 milliseconds.

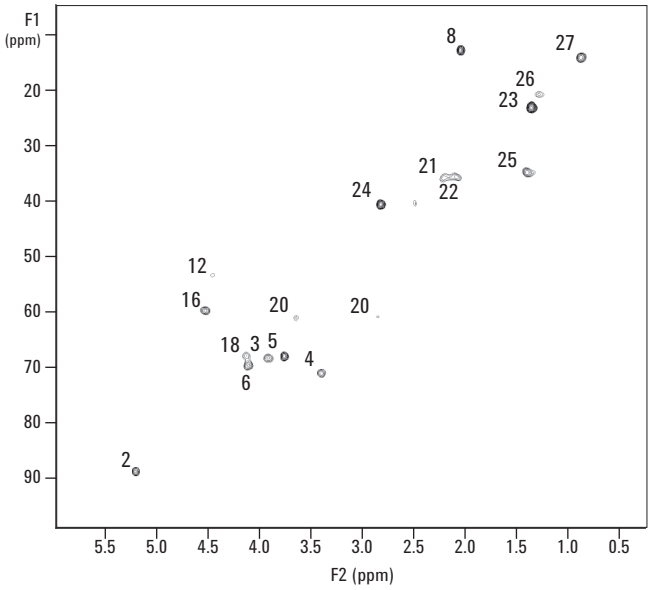

Figure 2. ^{1}H-^{13}C ASAPHMQC on 5 mg clindamycin dissolved in 600 µL DMSO-d_6. The total experiment time was 56 seconds.

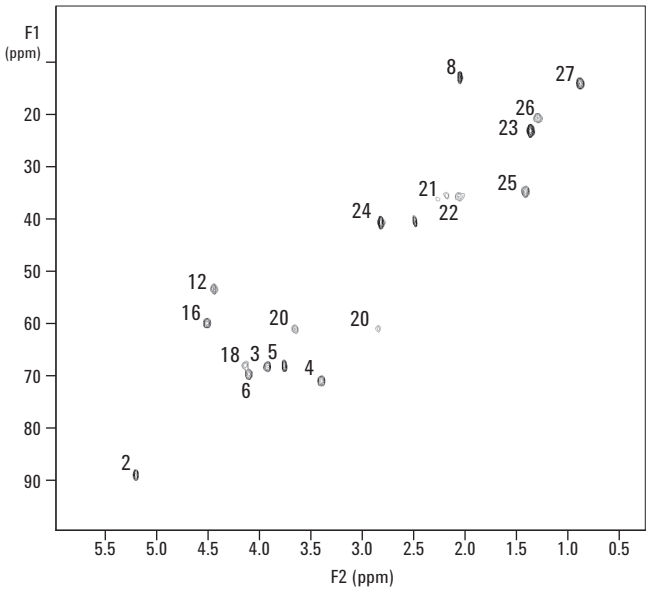

Figure 3. ^{1}H-^{13}C gHSQCAD on 5 mg clindamycin dissolved in 600 µL DMSO-d_6. The total experiment time was 8 minutes 18 seconds.

Depending on the sensitivity of the probe and the amount of sample present, the default number of scans per increment could be decreased to ensure the rapid acquisition of an ASAPHMQC experiment with minimal impact to spectrometer time. In Figure 4, the concentration of clindamycin was doubled, so fewer repetitions are required to achieve an adequate signal-to-noise ratio. The relaxation delay was increased to 70 milliseconds to demonstrate the impact to correlations 12 and 20, and the number of repetitions per increment was decreased to one. All 16 protonated carbons are still clearly identifiable, with correlations 12 and 20 more intense than in Figure 2. The total time for this ASAPHMQC experiment was only 19 seconds, which is similar to the time needed to acquire a short 1D ^{1}H experiment.

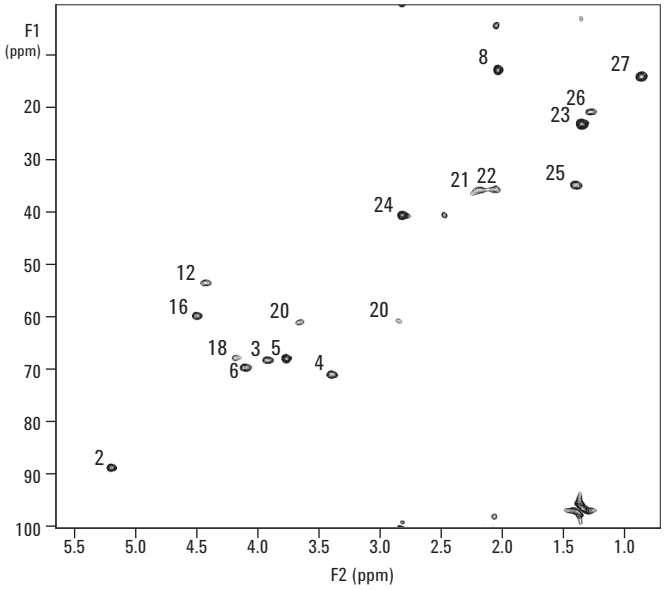

Figure 4. ^{1}H-^{13}C ASAPHMQC on 10 mg clindamycin dissolved in 600 µL DMSO-d_6. The total experiment time was 19 s, using 56 increments, a spectral with of 100 ppm in F1, and a delay of 70 ms.

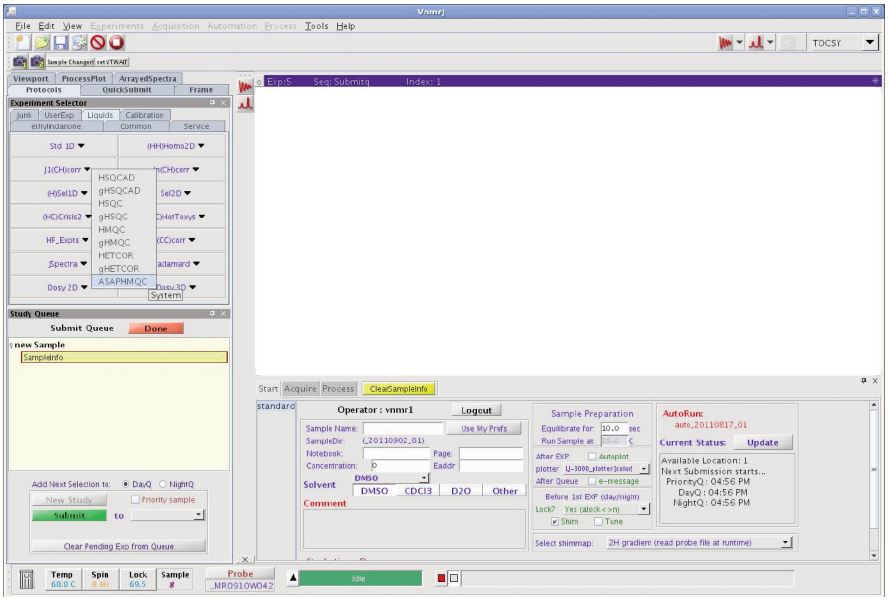

Figure 5. Adding the ASAPHMQC protocol to the Study Queue. The ASAPHMQC protocol is found in the **J1(CH)corr** drop down menu and will automatically add a PROTON protocol to the queue if no PROTON is already present.

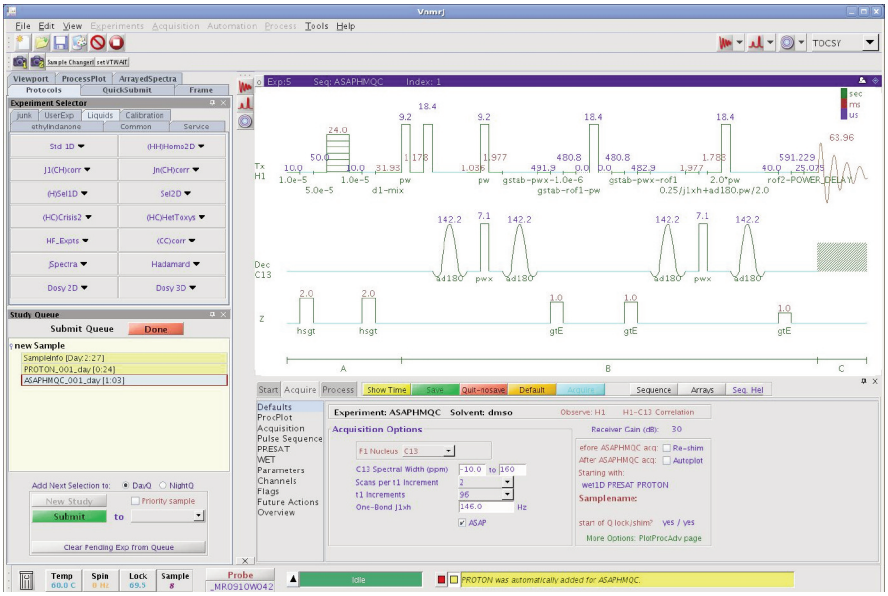

Figure 6. Customizing the ASAPHMQC experiment. The **Defaults** page contains the fields most commonly modified, such as **Scans per t1 Increment**, **t1 Increments**, and **C13 Spectral Width (ppm)**.

Experimental Method

The ASAPHMQC experiment can be run within or outside the Study Queue mode without any modification. To acquire an ASAPHMQC in the Study Queue:

1. Select **New Study** at the bottom of the Study Queue.
2. In the Experiment Selector, select the **Liquids** tab, then select **J1(CH)corr** and choose **ASAPHMQC**. (Figure 5). A PROTON will automatically add to the Study Queue before the ASAPHMQC. The default settings are 170 ppm (160 to –10) for the indirect dimension spectral width, a relaxation delay of 60 milliseconds, a mixing time of 25 milliseconds, 96 increments, two repetitions per increment, an acquisition time of 64 milliseconds, and a ^{1}H-^{13}C coupling of 146 Hz.
3. To customize the ASAPHMQC experiment, right-click on the **ASAPHMQC** protocol in the Study Queue and select **Open Experiment**. The experiment is retrieved, the pulse sequence displayed, and the Defaults panel is displayed (Figure 6). The typical fields modified are **Scans per t1 Increment**, **t1 Increments**, and **C13 Spectral Width (ppm)**.

4. To lengthen the relaxation delay, select the **Acquisition** page and enter a longer time in the **Relaxation delay** field (Figure 7). Lone ^{1}H-^{13}C pairs without neighboring protons may need a longer relaxation delay than the default of 60 milliseconds.

5. The experiment is now ready to acquire ASAPHMQC data. Use the **Submit** button in the Study Queue to initiate data collection.

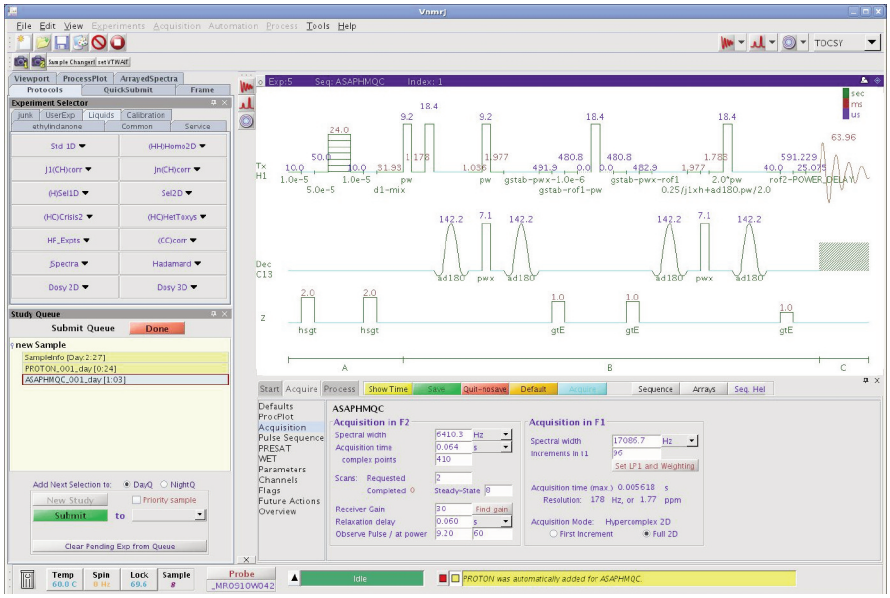

Figure 7. Customizing the ASAPHMQC experiment. The **Acquisition** page contains additional parameters, such as the **Relaxation delay**, and provides information such as resolution in the indirect dimension.

Conclusions

The ASAPHMQC experiment is a superb choice for obtaining quick protonated carbon (or other heteronuclear) information. The efficiency gained from the cross-polarization period and shortening of the relaxation delay, means information can be obtained in the ASAPHMQC experiment eight times faster than in a gHSQCAD experiment. As such, the experiment is an excellent choice complimentary experiment to be collected along with a 1D ^{1}H spectrum to quickly provide additional structural information with no special setup requirements.

References

1. Kupce, E., and Freeman, R. Fast multidimensional NMR by polarization sharing. *Magn. Reson. Chem.*, **2007**, 45:2-4.

2. Bodenhausen, G., and Ruben, D. J. Natural abundance nitrogen-15 NMR by enhanced heteronuclear spectroscopy. *Chem. Phys. Lett.*, **1980**, 69:185-189.

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