Unsymmetrical Indirect Covariance NMR: Creating a $^1$H/$^{13}$C HSQC-COSY and a $^{13}$C/$^{13}$C COSY Experiment from an HSQC and a COSY Dataset

ACD/2D NMR Processor
Version 9.06

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Introduction

Acquiring an HSQC-TOCSY (or HMQC-TOCSY) experiment with reasonable S/N can take several hours to days, even when working with a cryogenic NMR probe. Considering normal lab time constraints, acquiring an HSQC and COSY in a few minutes, and then combining the data into a single experiment, is more practical [1]. In addition, the NMR Spectroscopist is presented with a single experiment for analysis rather than the current method of analyzing two experiments.

Stepping from an HSQC-COSY to a $^{13}$C/$^{13}$C COSY [2,3], an NMR Spectroscopist can directly extract information about the carbon-carbon framework (the $^{13}$C/$^{13}$C COSY dataset represents contiguous protonated carbons free of exchangeable protons). Moreover, the data can be automatically assigned to produce a match factor if a structure is available, or passed to ACD/Structure Elucidator [4] if the structure is unknown.

This technical note will show how to combine an HSQC with a COSY dataset [5,6] to obtain a $^{13}$C/$^{13}$C COSY for strychnine (IUPAC: strychnidin-10-one) (1). This procedure can be extended to incorporate any related TOCSY and HMQC experiments as well.

![Chemical Structure of Strychnine]
Technical Note

Note Further details about ACD/Labs' recent work in the area of Indirect Covariance NMR processing including a detailed description of the matrix manipulations underlying the processing algorithms, will appear in Mag. Res. Chem. shortly [1,7].

Combining Two 2D NMR Data Sets

After processing the COSY and HSQC datasets with ACD/2D NMR Processor [8] as shown in Figure 1, the HSQC is the active experiment and the COSY experiment is selected using the SHIFT key and left clicking on the title bar.

Note Information on how to process 2D NMR data is shown in the user manuals available with the software.

A structure can be attached to the experiment at this stage or later on. The structure would be needed if a verification result is desired.

![Figure 1: A COSY and HSQC NMR dataset shown in Tile mode. Both datasets are selected as shown by the blue color of the title bars, the active experiment being the HSQC. Although the 1D NMR experiments have been attached to the axes of the 2D NMR spectra, this is not necessary for Indirect Covariance NMR.](image)

From the menu toolbar, select Process, then **Indirect Covariance NMR**. The square root of the real part of the spectrum is accomplished as part of Indirect Covariance NMR.
Figure 2: Applying Indirect Covariance NMR as selected from the Process menu.

A dialog box (Figure 3) will ask the user if they would like to combine the two datasets (selecting Yes) or to apply Indirect Covariance on the active experiment only (selecting No). In this case, select Yes.

Figure 3: Select Yes in the dialog box prompting the user on what to do when multiple datasets are selected.
Figure 4: Combined HSQC-COSY NMR spectrum. Contour color selection can be chosen to the user’s preference or to match the convention of the user’s spectrometer.

Producing a $^{13}\text{C}/^{13}\text{C}$ COSY Spectrum

Once again select Process > Indirect Covariance from the menu commands and click No in the dialog box that appears (Figure 3). The result is a $^{13}\text{C}/^{13}\text{C}$ COSY from an HSQC-COSY spectrum.

The direct domain is converted from a $^1\text{H}$ to a $^{13}\text{C}$ and the attached $^{13}\text{C}$ NMR is automatically transferred to the direct domain. The diagonal correlations indicate the protonated carbons and the off-diagonal peaks show connections between the diagonal peaks.

This process produces some asymmetrical artifacts. These artifacts can easily be removed by applying symmetrization. Select Process > Symmetrize > Apply. In the dialog box, select the Symmetry as Diagonal and Filter as the Lower value, as shown in Figure 5. The final product of Indirect Covariance NMR is shown in Figure 6.
Figure 5: Symmetrize dialog box set to Filter Lower value.

Figure 6: A $^{13}$C/$^{13}$C COSY spectrum. The product of Indirect Covariance NMR processing of an HSQC-TOCSY spectrum or from the unsymmetrical covariance processing of HSQC and COSY followed by indirect covariance processing of the resulting HSQC-COSY spectrum. The $^{13}$C NMR spectrum is automatically transposed along F2.
Performing a Structure Verification

The structure can be attached to the experiment at this stage or previously. Automatic peak picking can then be performed followed by automatic peak assignment and automatic structure-spectrum verification for both the direct ($1J$) and long-range correlations.

Figure 7: Auto-Peak Picked $^{13}C/^{13}C$ COSY spectrum with attached structure.

Once the $^{13}C/^{13}C$ COSY has been calculated, auto-assignment is performed with the help of ACD/2D NMR Predictor. In Figure 10, the peaks are assigned and the assigned atoms are displayed in green. The data can then be reported in Microsoft® Word or PowerPoint, or stored in a lab notebook for future reference.
Figure 8: As part of the Verification and Auto-Assignment routine, a $^{13}$C/$^{13}$C COSY is predicted and utilized for assigning the experimental correlations.

Figure 9: The result of Verification of the $^{13}$C/$^{13}$C COSY for 1 provides Match Factor criteria to judge the quality of the structure-spectrum match. A "good" result is defined by a Spectrum Match Factor over 0.7.
Figure 10: Assigned $^{13}$C/$^{13}$C COSY spectrum. The assignments for the protonated carbons are indicated in parentheses on the peak labels. In addition, the assigned atoms on the structure are shown in green so a user can quickly spot an unassigned atom. Hovering over the assigned atom highlights the corresponding correlation on the spectrum.
Conclusion

Following the above steps for Indirect Covariance NMR, an HSQC and COSY can be visually transformed and interpreted as a $^{13}C/^{13}C$ COSY.

For more information on Indirect Covariance, consult the technical note “Indirect Covariance NMR: Creating a $^{13}C/^{13}C$ TOCSY Experiment from an HSQC-TOCSY Dataset” at http://www.acdlabs.com/appnotes/technotes/nmr.html, as well as the references listed below.

For more information on ACD/2D NMR Processor, consult the ACD/2D NMR Processor Tutorial and Reference Manual.

References


