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Using Unsymmetrical Indirect Covariance Processing to Calculate GHSQC-COSY Spectra

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Abstract: GHSQC-TOCSY experiments allow sorting of proton–proton connectivity information as a function of ^{13}C chemical shift. GHSQC-TOCSY is a relatively insensitive 2D NMR experiment. Given two coherence transfer experiments, A → B and A → C, it is possible to indirectly determine B ↔ C. Unsymmetrical indirect covariance processing of a ^{1}H–^{13}C GHSQC and a GCOSY spectrum afforded a GHSQC-COSY spectrum, with an information content analogous to a GHSQC-TOCSY experiment. However, GHSQC-TOCSY is of significantly lower sensitivity and the data require considerably more time to acquire than either of the component experiments. Investigators needing access to GHSQC-TOCSY type data can, in principle, access it from more readily acquired 2D NMR data. Strychnine (**1**) was used as a model compound to illustrate this capability.

Two-dimensional NMR methods have unquestionably had a major impact on the elucidation of complex chemical and natural product structures.[1,2] COSY and some form of heteronuclear chemical shift correlation, most commonly HMQC among natural products chemists (although HSQC is a highly preferable choice),[3] are routinely used to characterize structures. As molecular structures increase in complexity, it becomes necessary to resort to more sophisticated 2D NMR methods to disentangle potentially overlapped connectivity information in complex NMR spectra. One particularly useful experiment is GHSQC-TOCSY (or alternately, GHMQC-TOCSY),[3] which provides the ability to extract proton–proton connectivity information from heavily overlapped spectra by sorting proton–proton correlations as a function of the ^{13}C chemical shift of the directly bound carbon.[4] Unfortunately, despite the utility of GHSQC-TOCSY, the experiment suffers from a severe sensitivity penalty that has undoubtedly limited the number of applications of the technique in natural product structure elucidation.[5–12]

Recently, a new approach to handling NMR data, indirect covariance NMR, has been developed.[13] In an effort to suppress processing artifacts in the conversion of IDR-GHSQC-TOCSY data to a ^{13}C–^{13}C correlation spectrum, a further modification of the indirect covariance method was developed.[14] Unsymmetrical indirect covariance processing of IDR-GHSQC-TOCSY data eliminates one type of artifact response and renders a second type diagonally asymmetric, allowing them to be eliminated from the data presentation by the symmetrization algorithm applied to COSY spectra. An alternative and far more interesting application of unsymmetrical indirect covariance processing capabilities arises when one considers using this approach to co-process discretely acquired 2D NMR spectra. Given two coherence transfer experiments,

$$A \rightarrow B$$

and

$$A \rightarrow C$$

where, for example the coherence transfer experiments might be GCOSY and ^{1}H–^{13}C GHSQC, it is possible to use unsymmetrical indirect covariance processing to indirectly determine

$$B \leftrightarrow C.$$

For the present example case, the result would be a GHSQC-COSY spectrum. We have previously shown using a 2 mg sample of the small molecule autumnolide the considerable time/sensitivity savings that can be realized using this approach.[15] GCOSY and a ^{1}H–^{13}C GHSQC spectra acquired in 10 and 60 min, respectively, were co-processed using unsymmetrical indirect covariance processing methods to afford a GHSQC-COSY spectrum with a S/N ratio, based on projection through the F_1 frequency domain, of 77:1. In comparison, directly acquiring an 18 ms IDR-GHSQC-TOCSY spectrum on the same 2 mg sample required 16 h to obtain a spectrum with a S/N ratio, again based on projection through the F_1 frequency domain, of 8:1. This type of gain in apparent sensitivity

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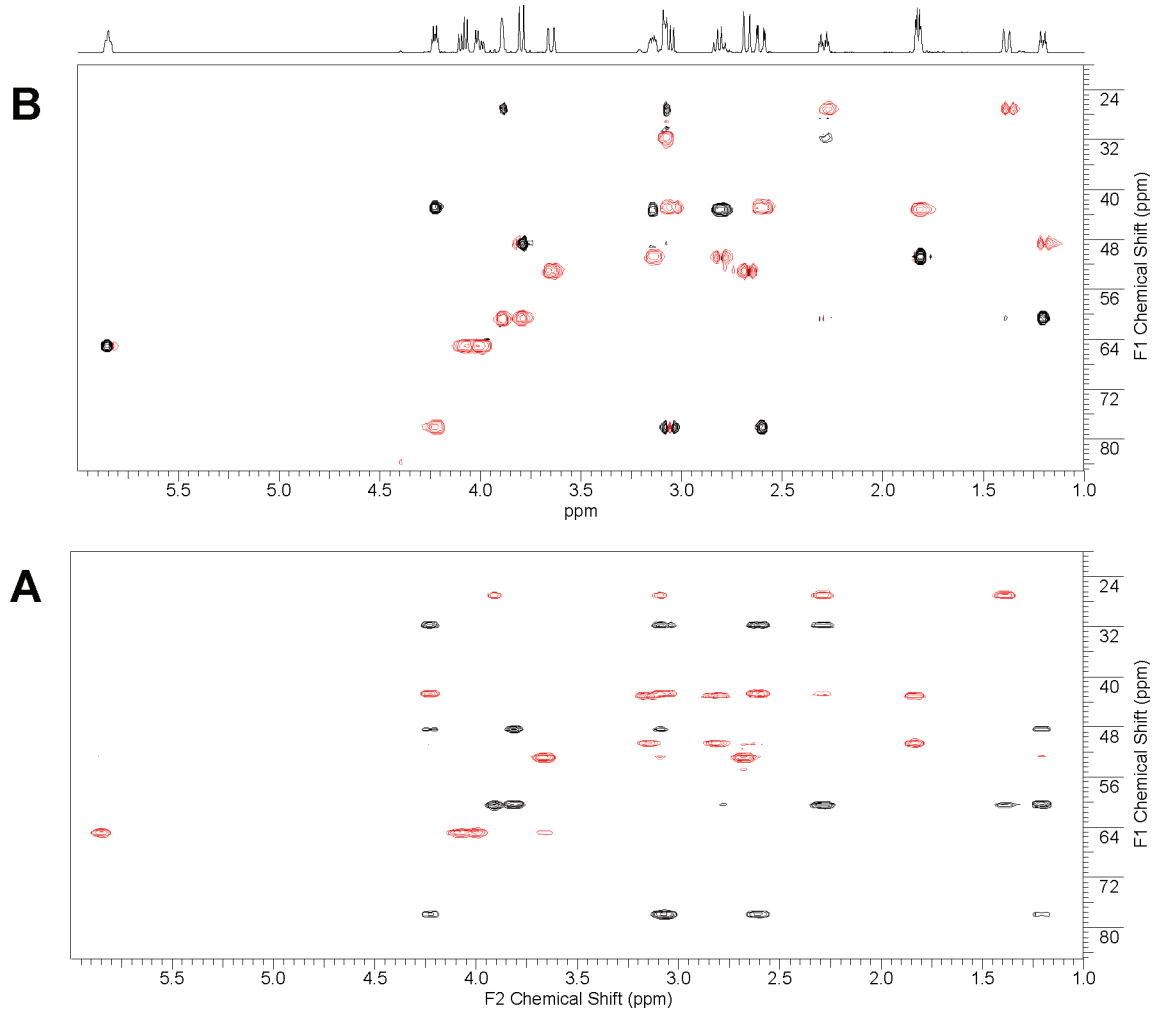
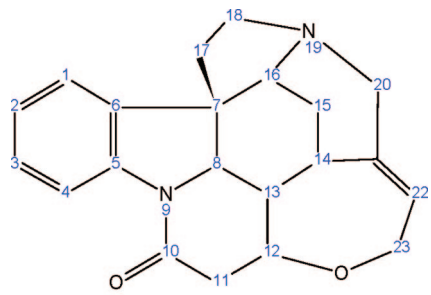


Figure 1. Unsymmetrical indirect covariance processed GHSQC-COSY spectrum calculated from a GCOSY and ^{1}H–^{13}C GHSQC spectrum is shown in panel A. The component COSY and ^{1}H–^{13}C GHSQC spectra used to calculate the GHSQC-COSY spectra were acquired using a Varian 500 MHz NMR spectrometer equipped with a 3 mm gradient inverse detection probe in 5 and 10 min, respectively. In contrast, the 24 ms inverted direct response GHSQC-TOCSY data were acquired in 1.5 h. The two contour plots shown were prepared with identical threshold levels. Comparison projections through the F_1 frequency domain of the spectra above are shown in Figure 2.

(S/N) is analogous to the results obtained following a two-dimensional Fourier transformation.

To further illustrate the capabilities of unsymmetrical indirect processing, we have used strychnine (**1**) as a model compound. GCOSY and ^{1}H–^{13}C GHSQC spectra were acquired with identical



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^{1}H (F_2) spectral widths (which is no longer required in the unsymmetrical covariance processing software, ACD/SpecManager v10.02, but is useful in that it avoids interpolation required when the F_2 spectral widths are different). Spectral widths in the second frequency domain (F_1) were set as appropriate. The data were processed to afford data matrices of 2048 × 512 points. The GCOSY data were acquired as 1024 × 256 points and were zero-filled to the final data matrix size. The GHSQC data were acquired as 1024 × 96 data points. The GHSQC data were zero-filled in F_2 and linear predicted to 192 points and then zero-filled to 512 points in F_1 during processing. Weighting functions were independently optimized for the two experiments. For comparison, an IDR-GHSQC-TOCSY spectrum with a 24 ms mixing time was acquired and processed to afford a 2048 × 512 point final data matrix. The aliphatic region of the unsymmetrical indirect covariance processed GHSQC-COSY spectrum of strychnine (**1**) is shown in Figure 1A; the corresponding region of the IDR-GHSQC-TOCSY spectrum is shown in Figure 1B. Direct responses in the latter are inverted (red contours). Relayed responses have positive phase and are shown in black. Responses in the GHSQC-COSY spectrum have phases corresponding to that of the multiplicity-edited GHSQC spectrum used in the unsymmetrical indirect covariance processing calculation. Methine-derived signals have positive phase (black contours); methylene-derived signals have negative phase (red contours). Projections through the F_1

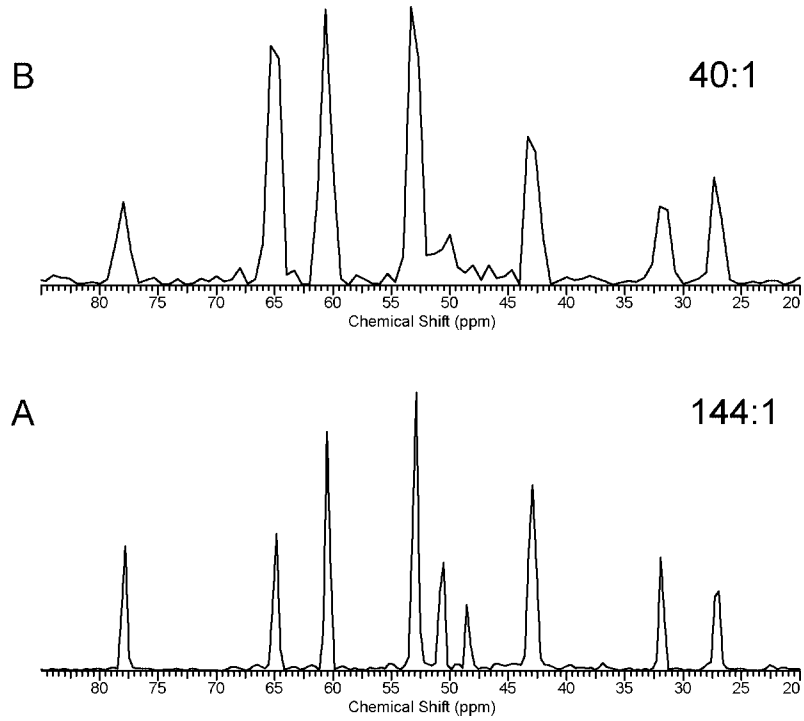

Figure 2. Projections through the F_1 (^{13}C) frequency domain in the GHSQC-COSY spectrum calculated using unsymmetrical indirect covariance processing (A) and the 24 ms GHSQC-TOCSY spectrum after magnitude calculation (B). The S/N ratio of the two projections was 144:1 and 40:1, respectively. Both spectra shown in Figure 1 were magnitude calculated prior to plotting the projections shown above.

frequency domain to compare the S/N performance of the two experiments shown in Figure 1 are shown in Figure 2 and were computed following the magnitude calculation of both 2D NMR spectra.

In comparison, the GHSQC-COSY spectrum replicates the information content of the 24 ms IDR-GHSQC-TOCSY spectrum. All of the responses in the IDR-GHSQC-TOCSY are contained in the calculated GHSQC-COSY spectrum; a number of the responses were observed with considerably better peak intensity. There are also legitimate responses contained in the GHSQC-COSY spectrum that are not observed in the IDR-GHSQC-TOCSY spectrum either because of the response being weak or because the mixing time used was inappropriate for the correlation pathway in question. For example, the H-12 resonance observed at 4.22/77.9 ppm in the IDR-GHSQC-TOSCY spectrum exhibited TOCSY correlations only to the H-11a and H-11b protons at ~3.08 and ~2.61 ppm, respectively. On examination of the F_2 slice at 77.9 ppm, a very weak correlation response can be seen to the H-13 resonance at ~1.18 ppm, although this response is well below the threshold of the contour plot shown in Figure 1B. In contrast, the H-12 resonance in the GHSQC-COSY spectrum calculated using unsymmetrical indirect covariance processing methods exhibited responses to the H-11a and H-11b resonances, as well as readily observed correlations to H-13 resonating at ~1.18 ppm and a long-range correlation across the oxepin ether linkage to the H-23b proton resonating at ~4.01 ppm.

It should also be noted that in cases of proton resonance overlap, artifact responses can be generated during unsymmetrical indirect covariance processing. Care must be exercised by an investigator using these processing methods not to mistake an artifact response due to resonance overlap as a legitimate correlation response.

The other comparison that should be noted is the relative S/N ratio of the two experiments. The GCOSY and GHSQC spectra were both acquired in 15 min in total in comparison to the 90 min required to record the 24 ms IDR-GHSQC-TOCSY spectrum. Projections through the F_1 (^{13}C) frequency domain of both spectra are shown in Figure 2. The S/N ratio of the F_1 projection for the IDR-GHSQC-TOCSY spectrum was 40:1. In comparison the S/N ratio of the F_1 projection of the calculated GHSQC-COSY spectrum was 144:1, a factor of 3.6 higher. When the difference in the S/N ratio of the two experiments is considered in light of the acquisition time for the data, a nearly 22-fold improvement is realized via the unsymmetrical indirect covariance calculated GHSQC-COSY spectrum versus the acquisition of the 24 ms IDR-GHSQC-TOCSY spectrum. This is not as high as was observed in our initial effort, but it should be noted that the proton spectrum of strychnine (**1**) is characterized by sharp multiplets without extensive coupling. A proton spectrum with more extensively coupled multiplets, e.g., that of autumnolide used as a model compound in our previous study,[15] would require a correspondingly much longer data acquisition time to reach an acceptable S/N ratio in the GHSQC-TOCSY spectrum.

We have compared the unsymmetrical indirect covariance calculated GHSQC-COSY and IDR-GHSQC-TOCSY of several complex alkaloids. In each case, the calculated GHSQC-COSY spectrum reproduced the information content of the hyphenated 2D-NMR spectrum. Unsymmetrical indirect covariance processing methods can also be used to mathematically combine other pairs of coherence transfer 2D NMR experiments, including ^{1}H–^{13}C GHSQC and GHMBC,[16] ^{1}H–^{13}C GHSQC and ^{1}H–^{1}H NOESY,[17] and ^{1}H–^{13}C GHSQC and ^{1}H–^{15}N GHMBC or ^{1}H–^{15}N IMPEACH-MBC spectra to afford ^{13}C–^{15}N GHSQC-GHMBC[18] and ^{13}C–^{15}N GHSQC-IMEACH[19] heteronuclear shift correlation plots, respectively. ^{13}C–^{15}N correlation has no experimental equivalent at natural abundance but has also recently been demonstrated by Kupče and Freeman using projection reconstruction methods.[20] We are currently exploring the use of unsymmetrical indirect covariance processing to solve chemical structure problems, which will be the subject of forthcoming reports.

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