Exploring the use of Generalized Indirect Covariance to reconstruct Pure Shift NMR spectra: *Current Pros and Cons*

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Introduction

The development of novel experimental strategies to significantly enhance signal resolution by broadband <u>homodecoupling is a current topic of high interest in ¹H NMR</u> <u>spectroscopy</u>¹. A number of different building blocks have been implemented into 1D and 2D homo- and heteronuclear experiments in order to provide resolution-enhanced pure chemical shift ¹H NMR spectra, where signals appear collapsed to singlets. On the other hand, Covariance processing methods have been used to generate challenging NMR spectral representations². We present here the first attempts towards a general solution to generate <u>Pure Shift NMR spectra by using Generalized Indirect Covariance</u> (psGIC) co-processing³. The current strategy is based on the calculation of a new 2D psGIC spectrum from the combination of a parent homo- or heteronuclear spectrum and a reference 2D F1-homodecoupled ¹H-¹H correlation spectrum only showing diagonal cross-peaks (DIAG), which share a common ¹H frequency dimension. Using psGIC, the F1 dimension in the DIAG spectrum is transferred to the F2 dimension of the parent spectrum, thus generating a new pure shift 2D spectrum.

Methodology

The key point of this proposal is a highly resolved 2D DIAG spectrum that can be obtained by two methods: i) experimentally, from a modification of the recent 2D PSYCHE-TOCSY pulse sequence with omission of the DIPSI-2 pulse train⁴ (Fig. 1A); ii) a faster method reconstructs it from an experimental 1D broadband homodecoupled ¹H spectrum⁵ followed by the *make2D*^{*} reconstruction (Mnova) (Fig. 1B). As proof of concept, the conventional 2D HSQC and 2D DIAG spectra of strychnine were combined following the GIC formalism (λ =1). This co-processing directly provides a reconstructed pure shift HSQC (psGIC-HSQC) spectrum showing well defined singlet signals for all cross-peaks (Fig. 2).



make2D is Mnova script (available in Mnova 11) that synthesizes 2D-DIAG spectra by automatically deconvolving a ps-1D spectrum and generating 2D diagonal peaks using Lorentzian-type lineshapes

Results

This GIC procedure can be used to reconstruct any pure shift spectrum, even for some experiments that can be **very challenging** or **impossible to acquire** through experimental pulse schemes. Some examples are illustrated by the psGIC-HSQC-TOCSY (Figure 3) and psGIC-HSQMBC (Figure 4) spectra.

Overlapping artifacts

Artifacts generated during covariance processing due to ¹H signal overlap along the F2 dimension is the most critical issue. This problem of signal overlap is clearly observed in psGIC-HSQC spectrum of estradiol that presents a crowded area in the aliphatic 1.2–1.4 ppm region, including several diastereotopic CH₂ protons with different degrees of strong coupling effects (Figure 5). Our current work is aimed at detecting and removing these artifacts characteristics of covariance processing. A new script (psGICClean) has been developed for the automatic removal of these artefacts based on the comparison of the peak list between the original (no artefacts) and psGIC spectra.



Figure 3.

Aliphatic region corresponding to (A) the regular 2D HSQC-TOCSY and B) the psGIC-HSQC-TOCSY spectra of strychnine generated by GIC. A comparison of the internal F2 projections at the top of each 2D spectrum and the selected C14 rows in C) shows the absence of *J* multiplicity and the excellent reproducibility of signal intensities for all cross-peaks.



Figure 4.





References:

 ¹ L. Castañar, T. Parella, Magn. Reson. Chem., 53 (2015) 399.
² M. Jaeger, R.L.E.G. Asper, Ann. Rep. NMR Spectroscopy, 83 (2014) 272.
³ A. Fredi et al., J. Magn. Reson., 266 (2016) 16. DOI: 10.1016/j.jmr.2016.03.003 Aliphatic region corresponding to (A) the regular HSQMBC and (B) the psGIC-HSQMBC spectra of strychnine generated by GIC. A comparison of the internal F2 projections at the top of each 2D spectrum and the selected C14 rows in (C) shows the absence of J coupling pattern and the excellent reproducibility of signal intensities for all crosspeaks.

⁴ M. Foroozandeh, et al., J. Am. Chem. Soc., 136 (2014) 11867.

⁵ M. Foroozandeh, et al., Angew. Chem. Intl. Ed., 53 (2014) 6990.

the Mnova psGlCclean script.

✓ Simple and fast processing tool.

✓ General application to a broad range of experiments.

 ✓ Generation of novel synthetic pure-shift spectra, even for NMR experiments that are "impossible" nowadays.

✓ Complement for conventional dataset analysis

✓ Artifacts and false peaks generated by GIC processing can be detected and removed.

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