Electronic Supplementary Information (ESI) for:

Improved 3D DOSY-TOCSY experiment for mixture analysis

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This section lists all relevant experimental parameters and describes the setup that was used to obtain the results described in the article. The focus is only on the 3D Hadamard-encoded DOSY-TOCSY experiment, since the parameters of all other experiments (conventional and Hadamard-encoded 2D TOCSY experiments) have been described elsewhere (see in the article references 26 and 22, respectively).

1. Samples

The first mixture was an equimolar mixture of methanol, ethanol, propanol, and valine at 0.1 M in D₂O, whereas the second was an equimolar mixture of propanol and 2-butanol at 0.1 M in D₂O.

2. Acquisition parameters

General. First Mixture. 8192 complex data points were acquired with a spectral width of 2125.85 Hz, giving a total acquisition time of 1.92 s. A relaxation delay of 0.5 ms was chosen. The 90° pulse was 9 µs. The diffusion gradient pulse duration was 2.0 ms and the diffusion delay Δ was 200 ms. The diffusion gradients were sine shaped. All other gradients were rectangular. The duration of the first two homospoil gradient pulses was 1.0 ms whereas the third was 3.0 ms, and their amplitudes were -32.0, -17.4, and 28.7 G/cm, respectively. The gradient recovery delay was set to 0.15 ms. The LED was 5 ms and the delay between the last homospoil gradient and the last RF pulse was 2 ms. For the ZQC filters, the swept-frequency pulses were adiabatic 180° CHIRP pulse (90 kHz sweep rate and 10% truncation). \( \tau_f \) and \( \tau_f^x \) were equal to 30 and 18 ms, resp., and their RF power were 2.9 and 3.8 kHz, resp. These pulses were applied in the presence of a rectangular gradient (2.2 and 2.5 G/cm, resp.). Homonuclear mixing was achieved with frequency-swept adiabatic 180° WURST-2 pulses (16 kHz sweep rate and 10% truncation), exhibiting a duration of 0.25 ms and a RF power of 7.3 kHz. These pulses were embedded in a MLEV-16 supercycle, and this cycle was repeated to give a total mixing time of 80 ms. Second Mixture. 4096 complex data points were acquired with a spectral width of 2062.71 Hz, giving a total acquisition time of 0.99 s. A relaxation delay of 2.0 ms was chosen. The 90° pulse was 8.45 µs. All other parameters were as above.

Diffusion encoding. Diffusion encoding was performed using monopolar gradients, as opposed to bipolar gradients usually employed in DOSY experiments to minimize spectral distortions due to eddy currents. This solution was chosen in order to reduce the number of scans and maximize the experimental speed, since the use of bipolar gradients implies extra RF pulses which, in addition to reducing the sensitivity, impose additional phase cycling to prevent the acquisition of spurious signals. With the refinement of active gradient shielding in modern NMR probes and the generalisation of gradient-pulse shaping, eddy current suppression is not necessarily of major concern, especially when it comes to the analysis of mixtures of small molecules for which relatively low intensity gradient pulses are required. An additional advantage of bipolar gradients relies on the inversion RF pulses placed in the middle of the diffusion coding and decoding periods, which allow chemical shift evolution to be refocused, hence preventing the formation of zero quantum coherences (ZQC). These pulses also compensate for background gradients, an issue that may jeopardize the accuracy of the measured D values, but which is less relevant here because the primary objective is to separate the mixture components rather than strive for optimal accuracy.

Phase cycling. A simple two-step phase cycle was employed, alternating the phase \( (x, -x) \) of the first pulse only, in order to minimize the effects due to relaxation during the pulse sequence. Most probably, substantially cleaner results could have been obtained through the use of adequate, more extensive phase cycling. However, to maximize experimental speed, the smallest number of scans required to achieve satisfactory results was chosen. Moreover, to avoid accidental magnetization refocusing, all homospoil gradients, including those used for the ZQC filters, should ideally be orthogonal.

Hadamard. As described in the article, for the first mixture, 10 irradiation frequencies were selected from the peak picking routine. In Hadamard NMR spectroscopy, this implies to use a Hadamard matrix of dimension \( N = 2^m \), where \( m \) is an integer such that \( 2^m \) is equal to or higher than 10. The corresponding \( m \) value is 4 (\( N = 16 \)). The matrix dimension gives the number of polychromatic selective pulses that must be used, and hence 16 Gaussian 90-ms phase-encoded selective pulses were used here. These pulses were automatically created by a macro provided by BRUKER. For the second mixture, the same procedure applied but 6 irradiation frequencies were found, requiring \( N = 8 \).
3. Experimental setup

**Acquisition.** The 3D cube showed $^1$H chemical shifts in the F1 and F3 dimensions (F3 being the directly observed dimension) and gradient amplitudes in F2. To obtain this cube, sixteen 2D experiments were sequentially recorded (one for each Hadamard-encoded Gaussian pulse). For each 2D experiment, the amplitude of the diffusion gradient $g$ was varied from 2.1 to 33.9 G/cm. This procedure was chosen because it was the simplest to implement with respect to the acquisition software in use (XwinNMR 3.5). Each FID of these 2D experiments was composed of 2 transients, and 4 dummy scans were run before the first 2D experiment only. Therefore, the total experimental time for the first mixture was:

$$\left( N \times N_{\text{grad}} \times 2 + 4 \right) \times (1.92 + 0.5) = 20 \text{ min}.$$ 

Similarly, for the second mixture, the total experimental time was:

$$\left( N \times N_{\text{grad}} \times 2 + 4 \right) \times (0.99 + 2.0) = 13 \text{ min}.$$ 

**Processing.** Data processing sequentially required the use of XwinNMR 3.5 and Topspin 2.0 from BRUKER.

[XwinNMR 3.5] A series of macros (AU programs) were written to process the so-obtained 2D files and decode the Hadamard-encoded data. This preliminary processing yielded 16 diffusion-encoded two-dimensional planes (with 1024x1024 data points), one plane for each of the 16 experimentally used gradient amplitudes. The cross-peaks shown in these 2D spectra were obtained through a symmetrisation procedure similar to that described in reference 22.

[Topsin 2.0] All the above mentioned diffusion-encoded planes (except the first one) were written as F3-F1 planes in a 3D file exhibiting 1024, 128, and 1024 points in F1, F2, and F3, resp. In other words, along the F2 dimension, all planes were blank except the first 15. Finally, the diffusion dimension was processed by using the implemented dosy3d command. The F3-F1 planes shown in Fig. 2 and Fig. 3 were then extracted from this cube by scanning the F2 dimension.

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**Notes**

1 Strictly speaking, a Hadamard matrix of dimension $N = 12$ could have been used as well, but our experimental setup required 16 (due to programming reasons only).

1 Importantly, when processing the data of both mixtures and looking at the exponential signal decays, the first point was systematically an outlier. It was thus removed before processing the whole 3D cube. This explains why, in the text, only 15 gradient amplitudes are specified, although 16 were used in total. However, to properly compare our results, the total experimental time mentioned in the text was calculated by accounting for all 16 gradient amplitudes.

**Additional references**