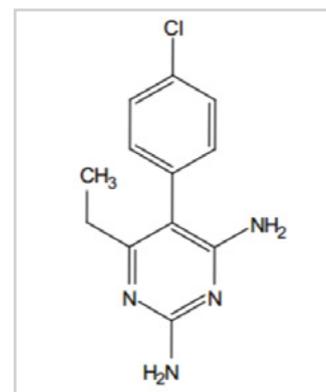


Introduction

In December 2016 it was widely reported[†] that students from Sydney Grammar School reproduced the drug Daraprim®, also known as pyrimethamine. The students followed a reaction pathway to produce it, and the final product was analysed at the University of Sydney, School of Chemistry (200 MHz ¹H NMR spectrum shown in Figure 1 below)[‡]. This substance is used as an example to illustrate 60 MHz NMR spectrometer performance (also Figure 1), and the difference between the two-dimensional (2D) experiments, COSY-90 and COSY-45.



Method

This application note shows a comparison of these two experiments at 60 MHz (Figures 3 and 4), and detailed data treatment with Mestrelab's Mnova software; a full suite of advanced routines for processing and analysing data.

1D ¹H NMR spectra (as in Figure 1) are commonly performed as they provide rich qualitative and quantitative information about the environment of atoms in molecules, which can be used to identify and characterise compounds and reactions. 2D NMR experiments such as COSY provide further information about connectivity between atoms, enabling structural elucidation.

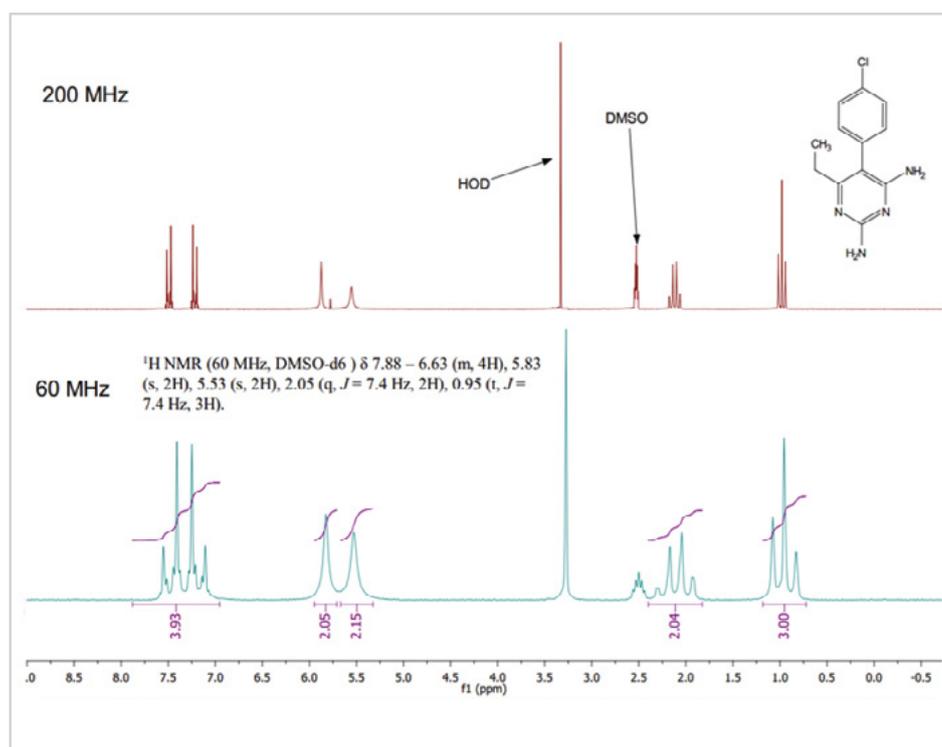


Figure 1. ¹H NMR spectra of pyrimethamine at 200 MHz (top) and 60 MHz (bottom). Sample details at 60 MHz = 16 scans. Approximately four minutes acquisition, 13.5 mg in 0.5 mL DMSO-d₆ = approximately 100 mmol dm⁻³ concentration.

[†] The Guardian / Melissa Davey. 2016. Australian students recreate Martin Shkreli price-hike drug in school lab. [ONLINE] Available at: <https://www.theguardian.com/science/2016/dec/01/australian-students-recreate-martin-shkreli-price-hike-drug-in-school-lab>. [Accessed 9 October 2017].

[‡] Open Notebook Science Network / Alice Williamson. 2016. Open Source Malaria Daraprim Synthesis. [ONLINE] Available at: http://malaria.ourexperiment.org/daraprim_synthesis/15346/post.html. [Accessed 9 October 2017].

2D NMR experiments at 60 MHz: COSY-90 and COSY-45

Basic 1 and 2D NMR experiments

Benchtop NMR spectrometers have lower sensitivity and resolution than high field superconducting instruments; signals are generally broader and overlap can make interpretation difficult where spectra are crowded.

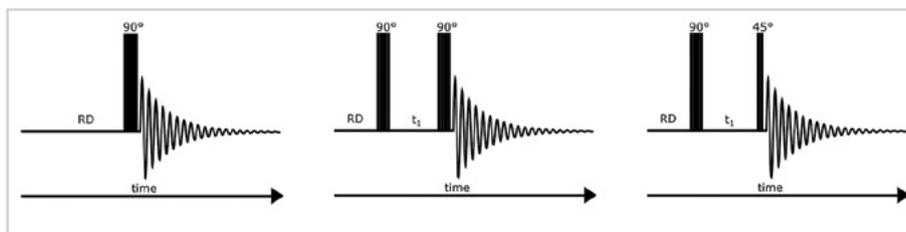


Figure 2. The onepulse, COSY-90 and COSY-45 NMR pulse sequences.

Figure 2 shows three basic pulse sequences which illustrate what happens in a spectrometer during experimental acquisition. Each one begins with an equilibration / relaxation delay (RD) in which spins align with the external magnetic field. In the onepulse experiment, a perpendicular radiofrequency (RF) pulse is applied for a few microseconds, which tilts the bulk magnetisation into the transverse plane. This then relaxes back to equilibrium (the Free Induction Decay, or FID), and can be detected as it induces current in the receiver electronics. Mathematical treatment of the FID with a Fourier Transform (FT) turns the time domain signal into a frequency domain signal, the NMR spectrum, which shows information about the different chemical environments of atoms (usually protons) in the sample.

The standard COSY-90 experiment uses two 90° pulses separated by a delay (t_1). The pulse sequence is repeated with an array of incremented delays. Fourier Transform in both dimensions allows the J-coupling between neighbouring protons to be displayed as off-diagonal crosspeaks.

Results

The COSY-45 sequence is analogous to the COSY-90, with the exception that the second RF pulse has half the duration. After 2D Fourier Transform, the resulting spectrum has a reduced diagonal signal, at a penalty to the signal to noise ratio. However, the reduced diagonal signals enable close resonances to be more easily distinguished as shown in Figure 4.

The aromatic doublet of doublets crosspeaks at around 7 ppm can more clearly be seen in the COSY-45 spectrum after diagonal suppression, notably with the wavelets and convolution approach.

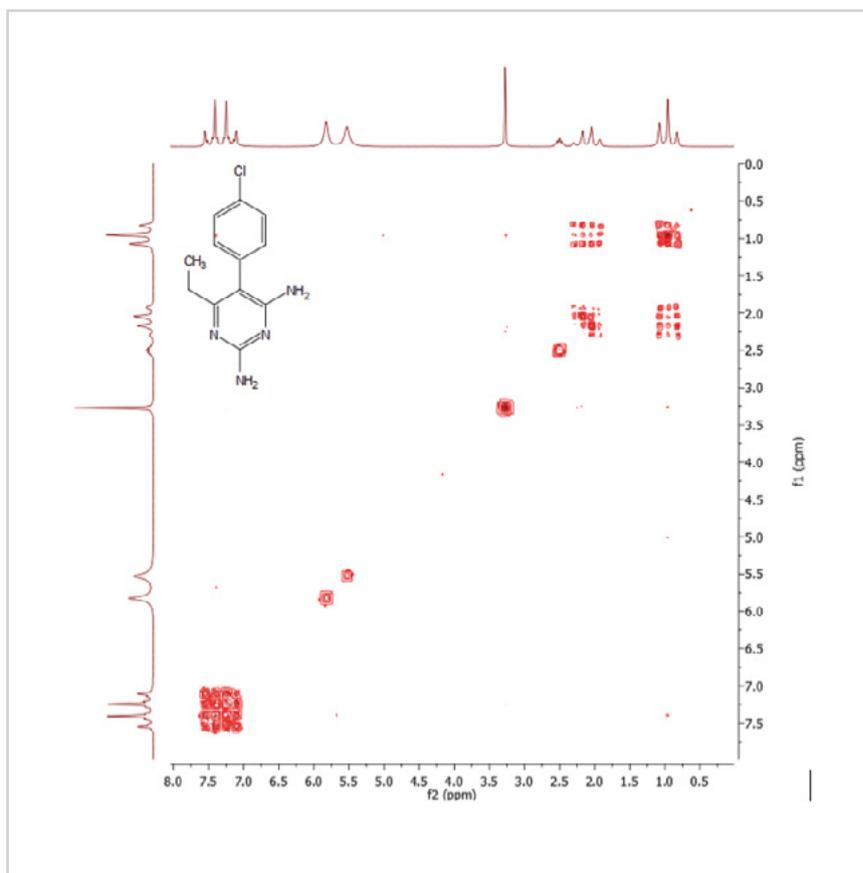


Figure 3. ^1H COSY-90 spectrum of pyrimethamine. Four scans, 128 increments, approximately 17 minutes' acquisition.

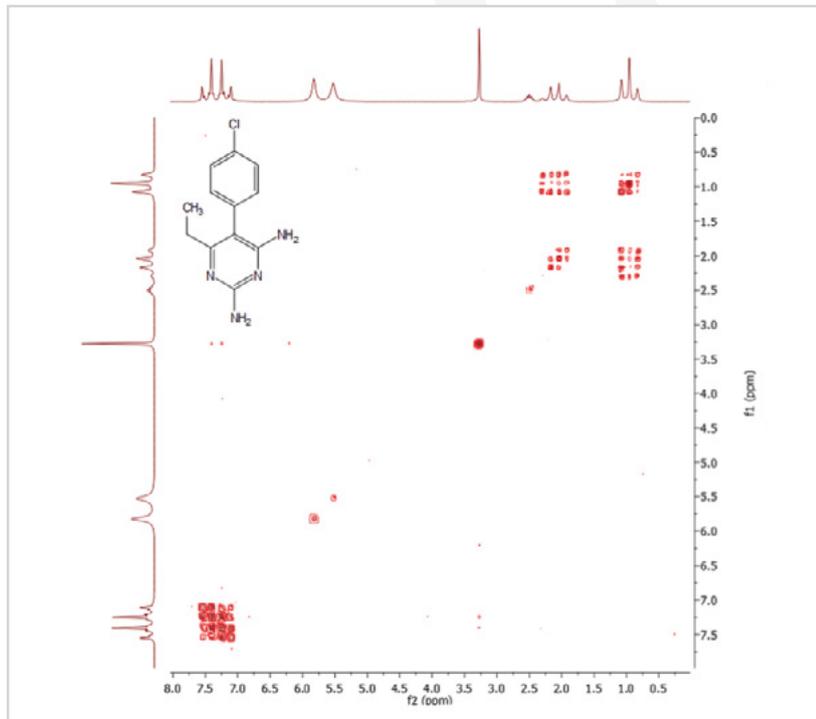


Figure 4. ^1H COSY-45 NMR spectrum of pyrimethamine. Four scans, approximately 17 minutes' acquisition.

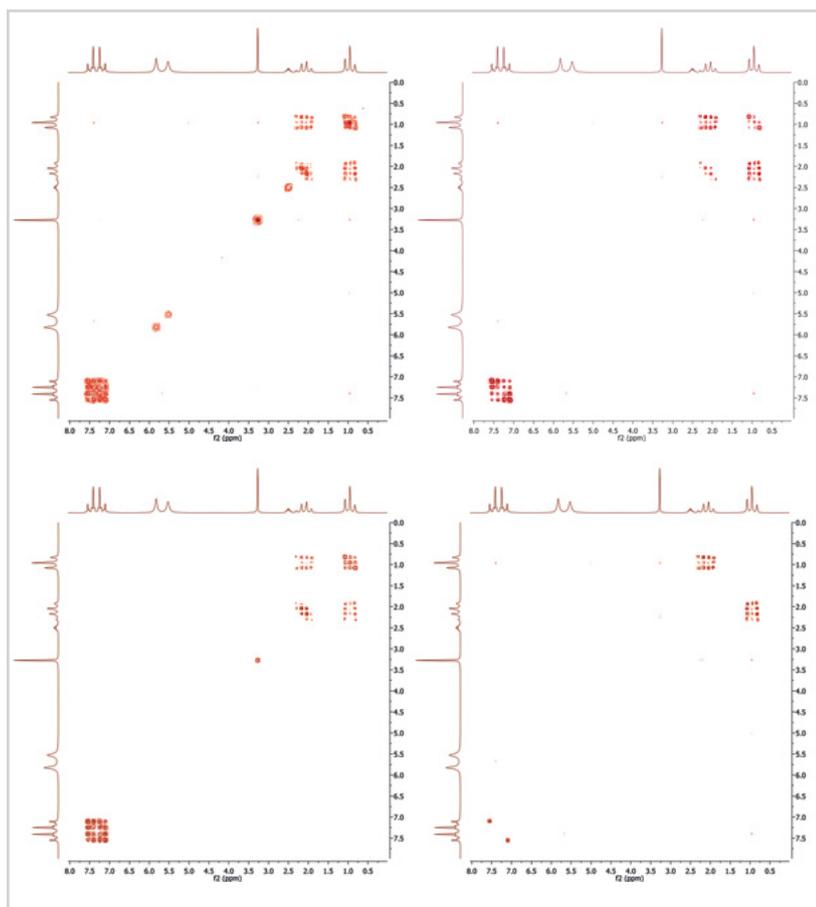


Figure 5. COSY-90 (top left), with convolution diagonal suppression (top right), shifted convolution (bottom left) and wavelets (bottom right).

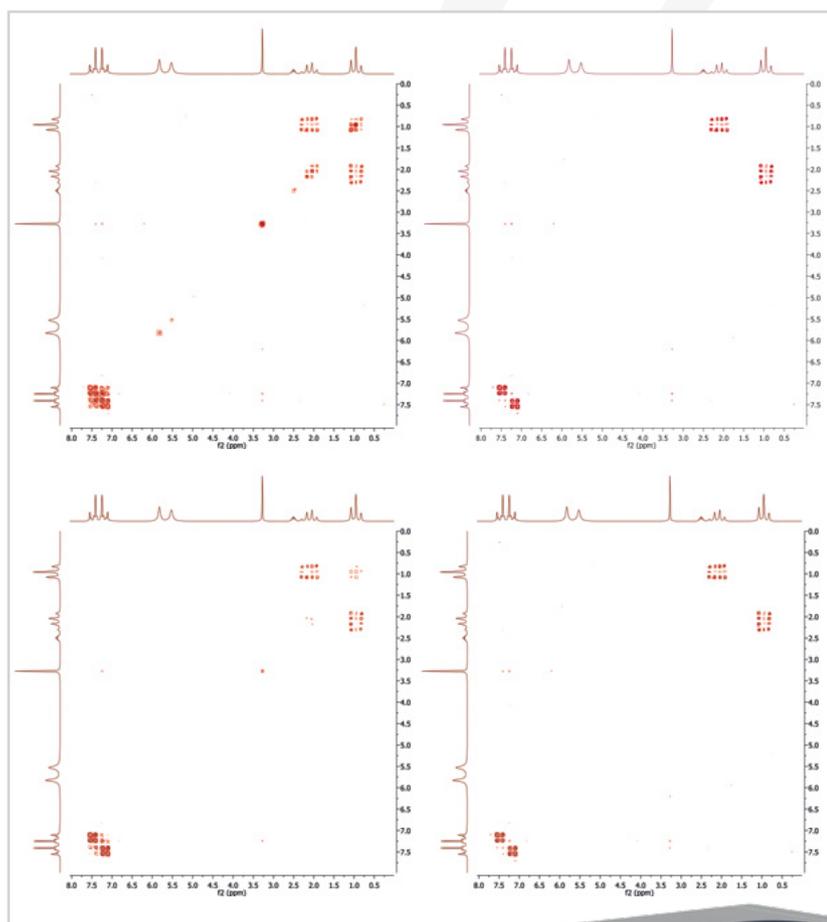


Figure 6. COSY-45 (top left), with convolution diagonal suppression (top right), shifted convolution (bottom left) and wavelets (bottom right).

Conclusion

Various NMR pulse sequences produce different results which can enormously help with interpretation / structural assignment. COSY-90 and COSY-45 are among the most straightforward 2D NMR experiments, and yield rich information which can be exploited further with Mnova's advanced data processing features.



visit nmr.oxinst.com/x-pulse for more information or email magres@oxinst.com

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