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Parallel Acquisition of Two-Dimensional NMR Spectra of Several Nuclear Species

Ēriks Kupče, Ray Freeman,* and Boban K. John

Varian Limited, 6 Mead Road, Yarnton, Oxford OX5 1QU, United Kingdom, Jesus College, Cambridge University, Cambridge CB5 8BL, United Kingdom, and Varian Incorporated, 3120 Hansen Way, Palo Alto, California 94304

Received May 18, 2006; E-mail: rf110@hermes.cam.ac.uk

High-resolution NMR spectroscopists routinely deploy a range of standard pulse sequences, often designed to study the interactions between ^{1}H, ^{13}C, and ^{15}N nuclei. Many of them are two-dimensional, for example COSY,[1] TOCSY,[2] and heteronuclear correlation spectroscopy.[3] Whereas the accepted procedure is to run these experiments separately, it would be more efficient to acquire several multidimensional correlation spectra in a single shot, *parallel acquisition* NMR spectroscopy (PANSY). The basic requirement is a standard "triple resonance" or broadband radio-frequency probe equipped with a separate receiver for each nuclear species under investigation. The detection stage is optimized to accommodate the lower intrinsic sensitivities of the heteronuclei (often ^{13}C and ^{15}N in natural abundance or isotopically enriched). Preferably, cryogenic receiver coils are employed to take advantage of the higher Q factors and lower intrinsic Johnson noise at low temperatures. To date, parallel acquisition techniques appear to be confined to magnetic resonance imaging[4,6] or to high-resolution spectroscopy of separate spectral regions of a single nuclear species.[7]

We demonstrate two simple illustrative examples based on parallel acquisition of two-dimensional ^{1}H and ^{13}C spectra on a Varian 600 MHz spectrometer, although the method is clearly applicable to more sophisticated experiments involving three or more different nuclear species. To demonstrate the principle of the method without distracting complications, we have chosen some of the simplest pulse sequences for illustration. The first test combines the classic H−H COSY experiment with simultaneous heteronuclear C−H correlation, using the pulse sequence set out in Figure 1a. The probe for this experiment is fitted with cryogenic ^{1}H and ^{13}C receiver coils feeding preamplifiers at the temperature of liquid nitrogen and is optimized for ^{13}C detection. The spectrometer hardware and software are modified to control independent receivers tuned to ^{1}H and ^{13}C frequencies. Signals from these two nuclei can be acquired either simultaneously or at different stages of their respective pulse sequences. Further instrumental details are set out in the Supporting Information. Note that the speed advantage of such experiments is inevitably sacrificed in situations of very low sensitivity. At first sight, there is an apparent conflict between the standard phase cycling schemes for H−H and H−C correlation experiments. For simplicity, we adopt a solution that combines real and imaginary ^{1}H signals in the evolution dimension to give an absolute-value COSY spectrum, together with the conventional phase-sensitive ^{13}C display. Alternative remedies are possible.

We present simultaneous two-dimensional H−H and H−C correlation spectra of brucine (about 3% in $CDCl_3$). Because the ^{13}C spectra are recorded directly, wide spectral bands and high resolution are readily accommodated without any significant time penalty, in contrast to the conventional indirect detection schemes involving ^{13}C evolution, such as HSQC or HMQC. Full spectra

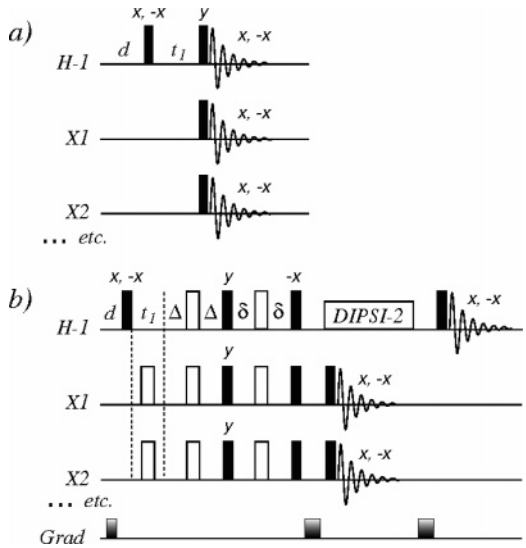

Figure 1. Pulse sequences used in this work. (a) PANSY H−H COSY and H−C correlation spectra. (b) PANSY H−H TOCSY and H−C correlation spectra. Filled rectangles are 90° pulses, while open rectangles represent 180° pulses. In the test experiments the X1 channel was ^{13}C, but additional X channels could be activated in parallel.

are recorded, but for clarity of presentation, only partial spectra are displayed in Figure 2. With just four time-averaged transients, the entire measurement is complete in 40 min. The relatively long evolution times implicit in the H−H COSY sequence correspond to the efficient detection of both long-range and one-bond H−C correlation peaks in the heteronuclear experiment. In Figure 2, both direct and long-range C−H correlation peaks are highlighted in the inset with expanded frequency scales.

PANSY experiments can take many different forms. We show here a combination of H−H TOCSY with heteronuclear H−C correlation, where the ^{13}C acquisition takes place during the mixing period (122 ms) of the TOCSY sequence (Figure 1b). This generates a proton-decoupled ^{13}C spectrum with the anticipated improvement in sensitivity. Following the initial INEPT[3] polarization transfer sequence, both ^{13}C- and ^{12}C-bound proton magnetizations are returned to the *z* axis, where they are purged by a field gradient pulse. The resulting TOCSY H−H and H−C correlation spectra of brucine (Figure 3) are acquired in only 20 min (two transients). Such experiments can be speeded up even further by employing Hadamard encoding[9] of selective proton excitation at frequencies derived from a rapidly acquired one-dimensional spectrum. The Supporting Information shows two-dimensional Hadamard-encoded PANSY spectra of inosine obtained in just 22 s.

As with any new methodology, limitations on the applicability may eventually emerge. For example, when several different pulse sequences are combined, some compromises may need to be made

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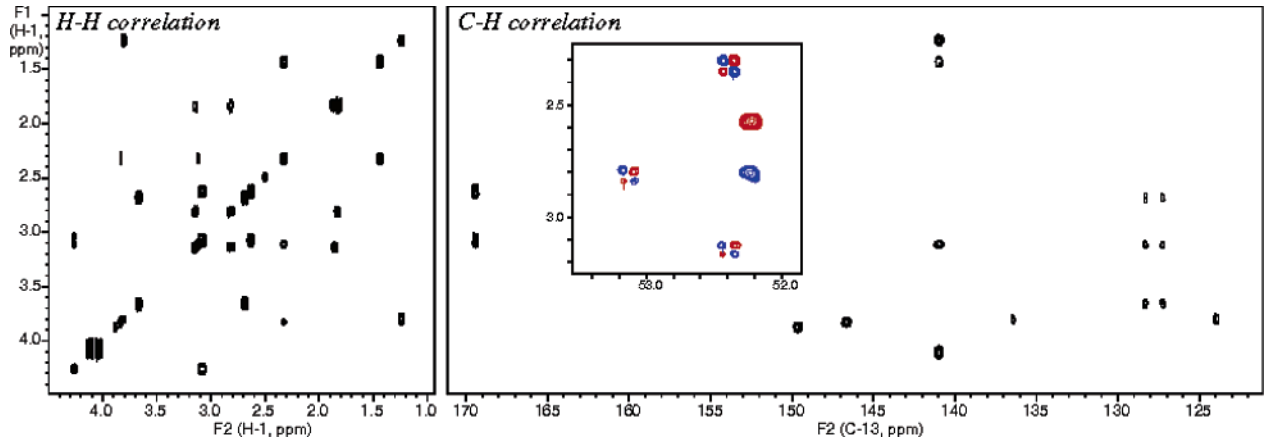

Figure 2. Parallel acquisition of H−H COSY and H−C correlation spectra of brucine using the pulse sequence of Figure 1a. Full spectra were recorded, but for clarity of display, only the crowded regions are shown. Note that both direct and long-range C−H correlation peaks are detected, as illustrated in the color inset representing selected high-field ^{13}C sites.

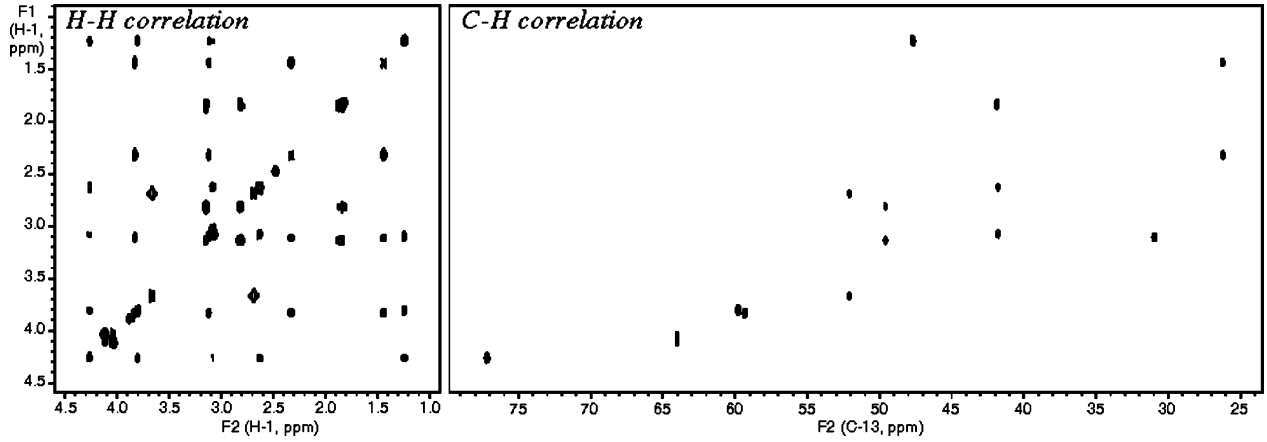

Figure 3. Parallel acquisition of H−H TOCSY and H−C correlation spectra of brucine using the pulse sequence of Figure 1b. For reasons of clarity, only the crowded spectral regions are displayed. The ^{13}C signals are decoupled from protons in both dimensions.

to accommodate the appropriate decoupling protocols, phase cycles, pulsed field gradients, or experimental durations. However, the potential advantages of the general concept look very promising, both in liquid-phase and solid-state NMR. As an initial "proof of principle" of parallel acquisition, these experiments were confined to the case of ^{1}H and ^{13}C spectra. However, there would be considerable interest in simultaneous parallel acquisition of ^{1}H, ^{13}C and ^{15}N spectra, using three radio-frequency receivers, and experiments are currently underway to demonstrate this. More sophisticated experiments are readily envisaged. There is no practical reason why additional nuclear species should not be accommodated, and three-dimensional experiments can certainly be imagined. PANSY offers useful economies of instrument time and can complement other fast multidimensional techniques, such as projection reconstruction.[10]

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Supporting Information Available: Two-dimensional H−H and H−C correlation spectra of inosine recorded by Hadamard encoding and parallel acquisition; experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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