Can benchtop NMR detect ^{15}N at natural abundance?

Nuclear magnetic resonance (NMR) relies on powerful multidimensional and multinuclear experiments such as COSY, HSQC, and HMBC, among others, to obtain useful information for molecular structure verification and elucidation. These methods are routinely collected on high-field NMR systems to explore and confirm the couplings between homo and hetero atoms in the molecule. The hetero nuclei that are typically measured are ^{13}C and ^{15}N, which are present in most organic compounds. In the last years these methods have been extended to benchtop NMR spectrometers. While ^{13}C NMR experiments produce high-quality spectra, ^{15}N has remained a challenge for benchtop NMR instruments. ^{15}N has a natural abundance of 0.3%, which is **three times lower** than ^{13}C (1.1%) and, on top, its gyromagnetic ratio is 2.5 times lower than carbon. For these reasons many believed that ^{15}N **at natural abundance** could not be detected at the lower magnetic fields of benchtop instruments. However, with increasing magnetic fields and the development of advanced multinuclear probes, benchtop NMR technology has been progressing to make these experiments possible.

In this application note, we report the first ^{1}H-^{15}N heteronuclear correlation experiments collected on a Spinsolve 90 MultiX benchtop NMR system. The high sensitivity of this spectrometer combined with the ability of the MultiX probe to automatically switch between ^{13}C and ^{15}N has allowed us to detect these nuclei at natural abundance in reasonable measurement times. This capability extends the power of benchtop NMR systems for molecular structure elucidation. It's important in molecular systems where there's a lack of hydrogen, carbon or both types of atoms to have the capability to establish correlations with other heteronuclei. It's precisely here where the two-dimensional ^{1}H-^{15}N will be of help. Since many molecules of interest for the pharmaceutical and chemical industries contain nitrogen atoms, NMR experiments that observe nitrogen can greatly help when performing structural confirmation and elucidation. Another advantage of nitrogen NMR is that the chemical shifts of amine, amide, nitro, and nitroso species have different and distinct chemical shifts. Hence, from the chemical shift position of the nitrogen one is able to determine the oxidation state of the group under investigation and what structure it is part of.

As a first example, we collected ^{13}C and ^{15}N HMBC spectra to verify the structure of Epoxiconazole. This chemical is a fungicide that features a molecular framework comprising a 1,2,4-triazole structure - a five-membered heterocyclic ring with three nitrogen atoms. While for ^{13}C, the single bond correlation experiment (HSQC) is routinely acquired, for ^{15}N, the chemical exchange of the directly coupled hydrogens reduces the potential of this method and makes the HMBC the method of choice. By detecting the multiple bond couplings, the different nitrogen atoms within the structure can be well assigned. Figure 1 shows the ^{1}H-^{13}C HMBC and ^{1}H-^{15}N HMBC spectra, acquired on a Spinsolve 90 MultiX instrument for a sample with a concentration of 250 mM (25 mg dissolved in 300 µL). The time required to acquire the ^{13}C HMBC is just 8.5 minutes, so a total time of 68 minutes would be required to get a similar SNR on a ^{15}N HMBC. The factor 9 in measurement time is required to compensate for the three times lower natural abundance of ^{15}N. The ^{15}N HMBC shown in Fig. 1 was collected in 136 minutes to deliver a high SNR to resolve the *J*-couplings of the different nitrogens. The HMBC was collected with 128 steps along the indirect dimension, 32 scans per step, and a repetition time of 1 second.

While the ^{1}H-^{13}C HMBC aids in assigning all present carbons, the ^{15}N HMBC also contributes to completing the assignment for the nitrogen atoms. The ^{15}N trace displayed on the vertical axis of the HMBC shows that the three nitrogen nuclei can be detected with a high signal-to-noise ratio, even for this relatively small sample quantity. Notably, two of these signals (17 and 19) exhibit a coupling to a CH_2 (16) group outside the ring, showcasing different chemical shifts attributable to the inequivalent protons of the CH_2 group. A closer examination of the couplings within the diazole

ring (7.5 - 8 ppm in the ^{1}H spectrum) enables the determination of distinct *J*-coupling patterns and the assignment of peaks within the heterocyclic ring. The traces for the different nitrogen atoms are shown on the bottom-right plot together with a 2D spectrum where the spectral region of H18 and H21 has been zoomed-in. The traces show the good SNR obtained in 136 minutes.

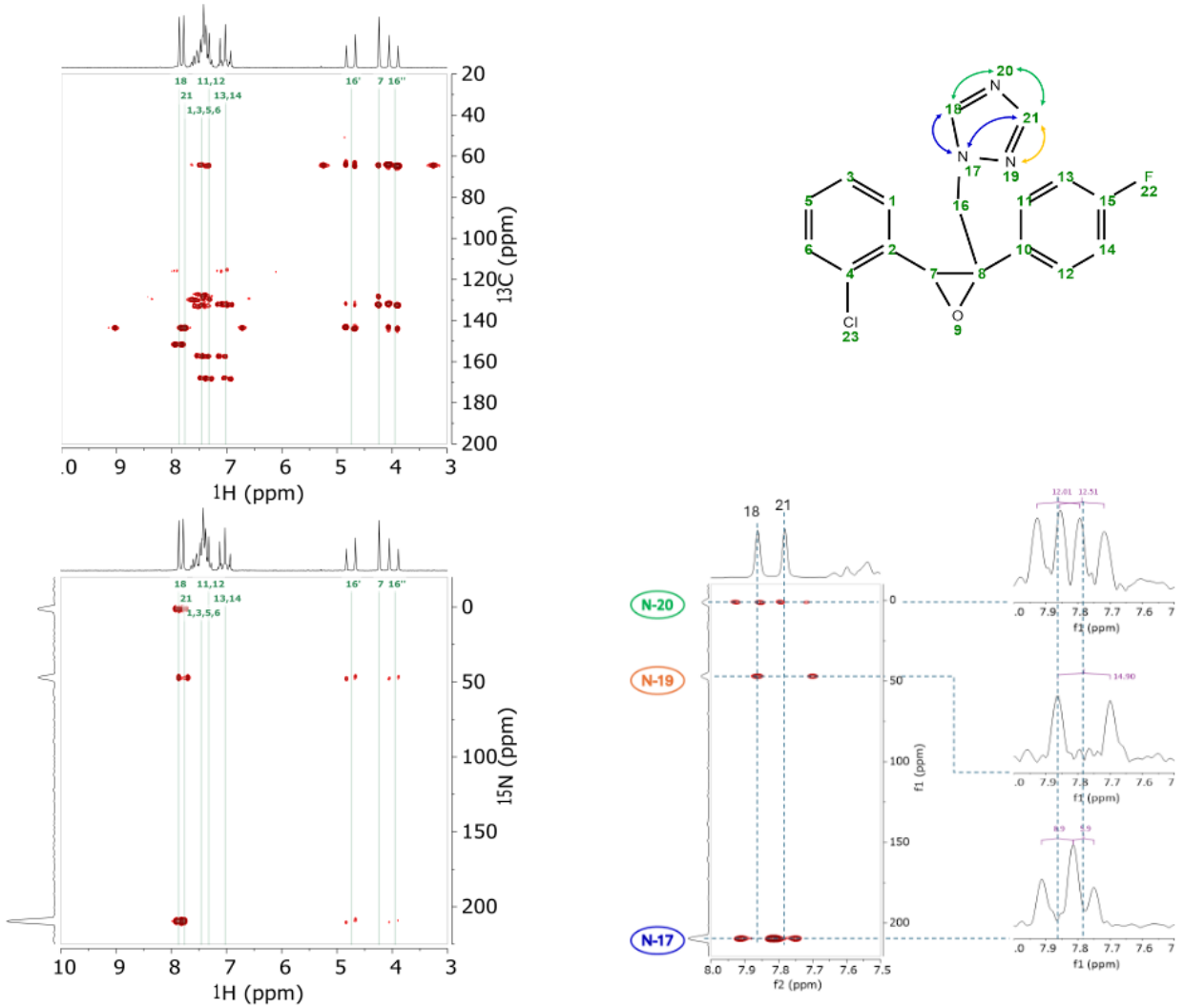

Figure 1: ^{13}C HMBC (top left) and ^{15}N HMBC (bottom left) measured on a Spinsolve 90 MultiX for a sample of Epoxiconazole at a concentration of 250 mM in DMSO-d_6. Both spectra were collected with 128 t_1 increments along the indirect dimension with a recycling delay of 1 second. The ^{13}C HMBC was acquired setting a *J* constant of 8 Hz and averaging 4 scans per step, while the ^{15}N HMBC was acquired with a *J* constant of 10 Hz and 32 scans.

As the *J* couplings between ^{1}H and ^{15}N vary over a larger range compared to ^{13}C, it is necessary to acquire at least two ^{15}N HMBC for two different *J* values to detect all nitrogen nuclei present in the molecule. The coupling between the H18 and H21 and the nitrogen atoms varies from 5.9 to 14.9 Hz. Such couplings can be directly measured from the splitting of the proton signals. Depending on the coupling chosen to acquire the HMBC, some of the signals may simply disappear from the spectrum as the corresponding evolution time may lead to a zero-crossing for particular coupling constants that are different to the ones set in the experiment. Figure 2 shows the modulation for the three different nitrogen nuclei coupled to hydrogen H18 and H21, respectively. The traces in Fig. 2 were extracted from a series of HMBC spectra collected for *J* couplings ranging from 3 to 25 Hz.

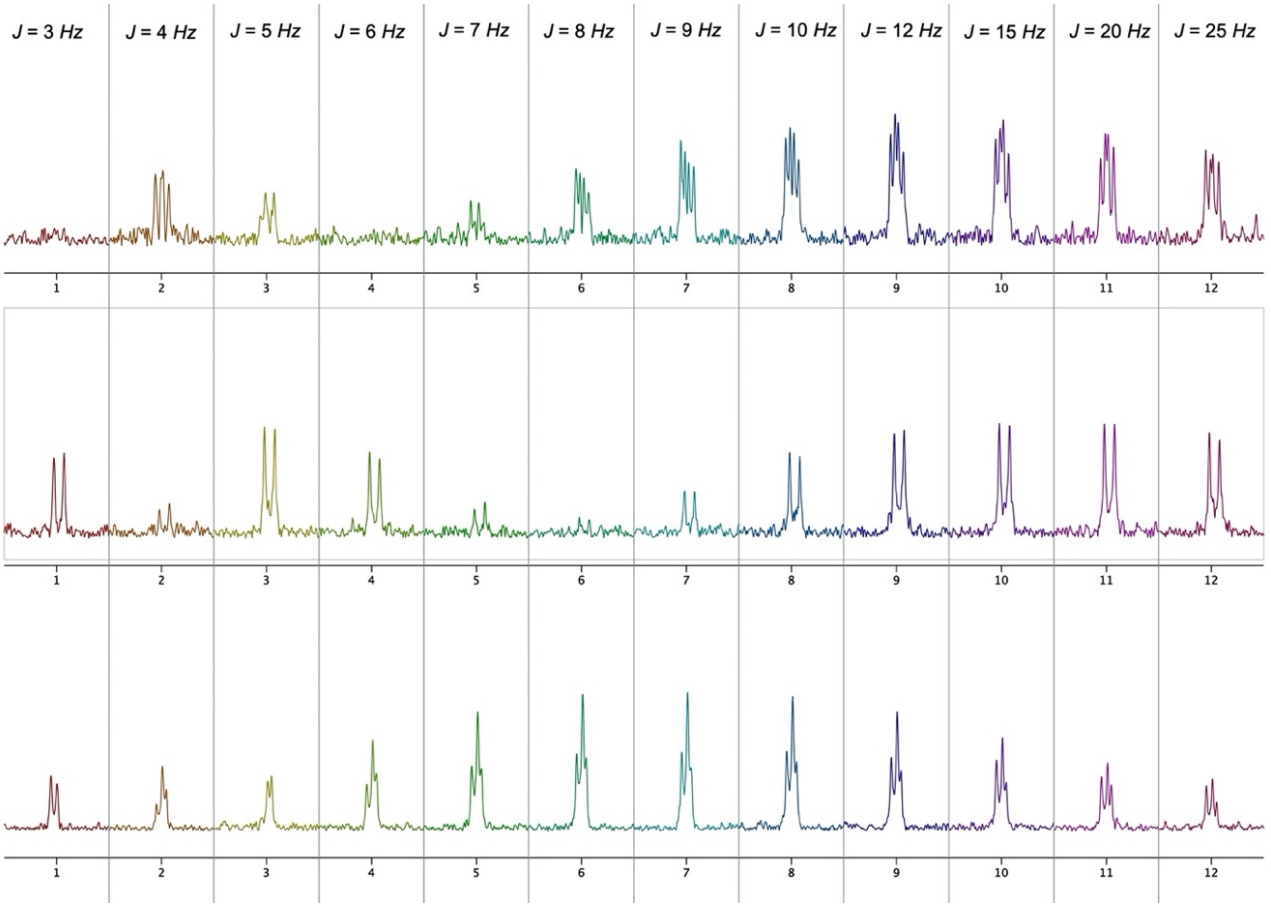

Figure 2: Traces extracted from the ^{15}N HMBC for the three different nitrogen nuclei in Epoxiconazole. The traces are similar to the ones shown at the bottom-right of Fig. 1. The proton ppm range set for these traces goes from 7 to 8.7 ppm, to observe the signals of protons H18 and H21. The different *J* couplings lead to different modulation patterns as a function of the *J* constant set in the HMBC.

As a second example we selected Levofloxacin, which is a known antibiotic drug active against a range of bacterial infections. Structurally, Levofloxacin belongs to the group of fluoroquinolones. It is a chiral compound in the (-)-(*S*) enantiomeric form. Figure 3 shows the ^{13}C HMBC (top) and ^{15}N HMBC (bottom) spectra of a 250 mM Levofloxacin sample in DMSO-d_6 measured on a Spinsolve 90 MultiX. The two HMBC experiments provide rich information to confirm the structure of Levofloxacin. In particular, the ^{15}N HMBC shows the signal of the three nitrogen atoms in the molecule. The assignment of the nitrogen nuclei is straightforward when the couplings between ^{15}N and ^{1}H are analysed. N11 and N16 from the piperazine ring can be easily identified as they both couple to the CH_2 groups in the ring, but only N16 couples with the methyl group. On the other hand, N3 from the morpholine ring couples beautifully with the proton 5 in the ring and the CH_3 26.

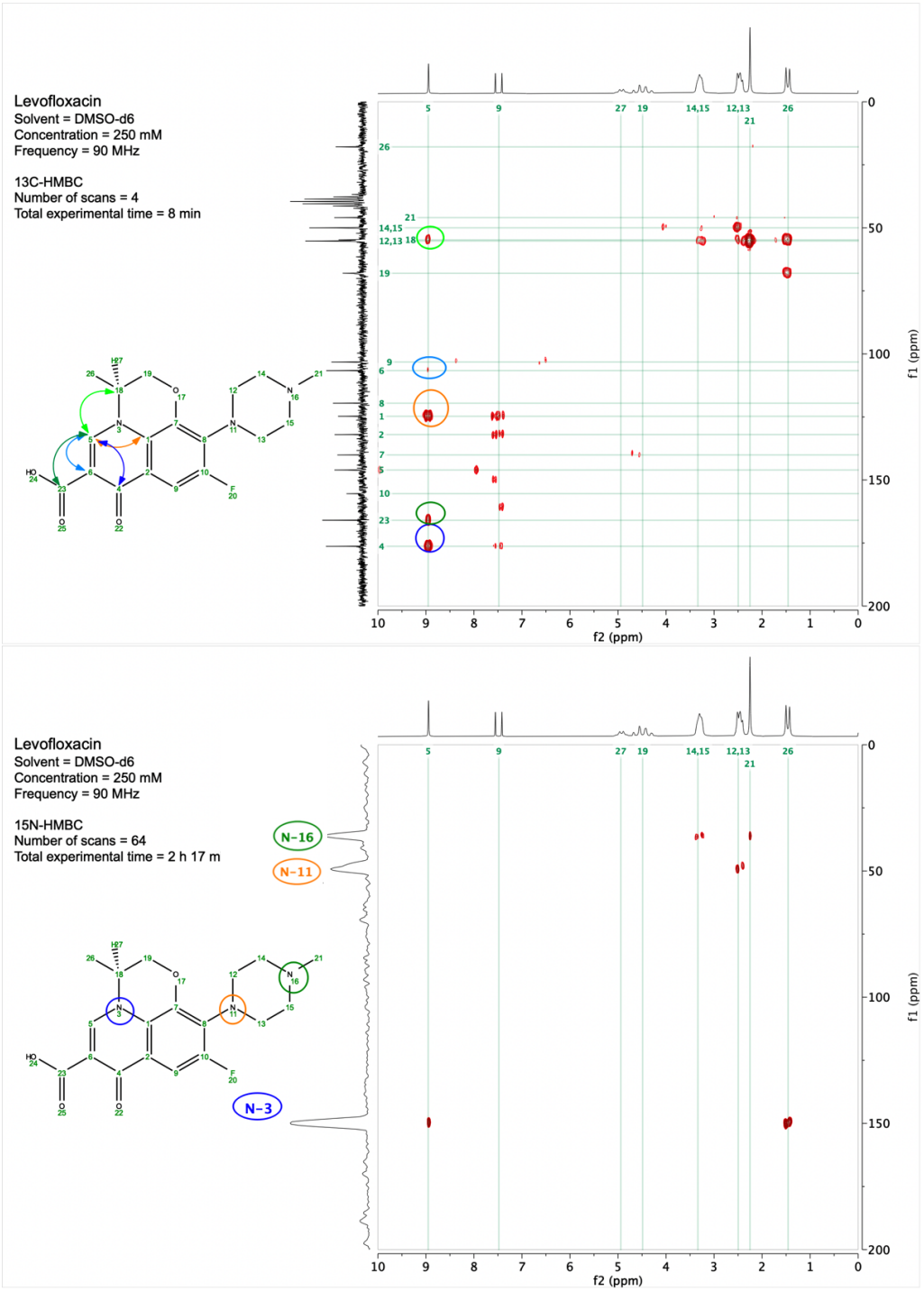

Figure 3: ^{13}C HMBC (top) and ^{15}N HMBC (bottom) collected for a sample of 250 mM of Levofloxacin dissolved in DMSO-d_6. The three different nitrogen nuclei present in the molecule can be clearly detected and the couplings to the different protons make direct assignments possible.

The results shown in this application note demonstrate that the sensitivity of the Spinsolve spectrometers is high enough to detect the signal of ^{15}N at **natural abundance** in relatively short measurement times while using a small amount of compound (~25 mgs). Given the high sensitivity and the automatic switching between heteronuclei to obtain ^{1}H-^{13}C followed by ^{1}H-^{15}N 2D experiments, we have an NMR instrument ready to perform high-quality assignments and structural verification and elucidation.