APPLICATION NOTE

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A brief analysis of 2D and ^{13}C-NMR at low field

Limitations, information content, and practical alternatives

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- Benchtop NMR systems are low-field instruments, most suitable for small molecules whose spectra do not contain the ambiguity that heteronuclear and 2-dimensional (2D) experiments were specifically designed to resolve. 2D experiments on benchtop systems often provide little to no new spectral information
- The low isotopic ratio and nuclear susceptibility of ^{13}C requires benchtop spectrometers to use very high concentrations or long acquisition times for these experiments. Spectral acquisitions can take anywhere from 10 minutes (>>5 M) to several hours (100 mM) to complete.
- Introducing a complementary analytical technique can often provide more valuable information over a broader range of samples than a heteronuclear 2D capable benchtop NMRmaximet antio. Lendae.

Abstract

There are a number of benchtop NMR solutions being promoted as alternatives to high-field NMR. While these instruments are capable of performing some of the same experiments as high-field NMR spectrometers, the physics of low-field NMR severely limits the number and types of samples that can be used.

In addition, despite the premium paid for these additional features (i.e. ^{13}C, 2D, heteronuclear, etc.), the added information content is minimal at best. In this application note we look more closely at these features, their information content and limitations, and demonstrate a more robust alternative.

Benchtop NMR spectrometers, all of which have field strengths below 90 MHz, are significantly less sensitive, have broader lines, and have much more spectral overlap than high-field NMR instruments. This places practical limitations on the samples that can be reasonably studied utilizing benchtop NMR spectrometers. While concentrations as low as 1 mM can be measured with very long acquisition times, concentrations of 100 mM are more reasonable for these instruments. Similarly, the size and complexity of the small molecule(s) that can be reasonably studied with benchtop NMR is roughly limited to molecular weights of 500 g/mol, polymers being an exception.

When the above limitations are taken into account, it becomes apparent that very often there is no ambiguity in the spectrum to justify the use of heteronuclear and 2D experiments. In those cases where additional information is necessary, there are alternative methods that can provide the needed structural information much faster than a ^{13}C or 2D NMR capable benchtop NMR spectrometer.

In the following examples, we will examine two molecules whose spectra have been analyzed by ^{1}H, ^{13}C, and 2D NMR experiments and consider what information is gained in each case. Although these are common molecules and the structures are known, we will approach the analysis as though they are unknowns, understanding that in practice knowing nothing about a sample is rather rare.

Case study one: Ibuprofen

Below are examples of 60 MHz 2D spectra of 2 M ibuprofen. While these 2D spectra look impressive, we must ask ourselves what new information they actually contain (Figure 1).

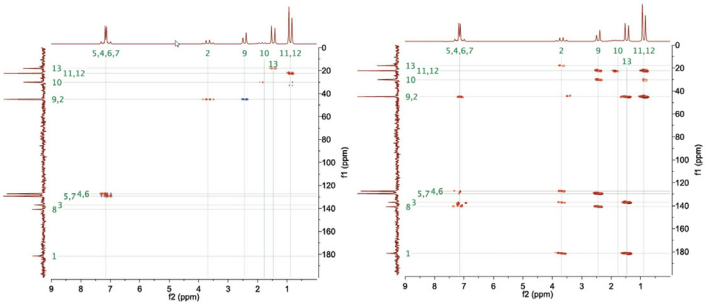

Figure 1: 2M Ibuprofen, HSQC-ME, ~1 hour 2M Ibuprofen, HMBC, ~2 hours[a]

NMR spectrum is virtually always acquired before embarking on any 2D experiment; let us first consider what information is contained within the 1D-^{1}H-NMR spectrum as a foundation for the structural interpretation (Figure 2).

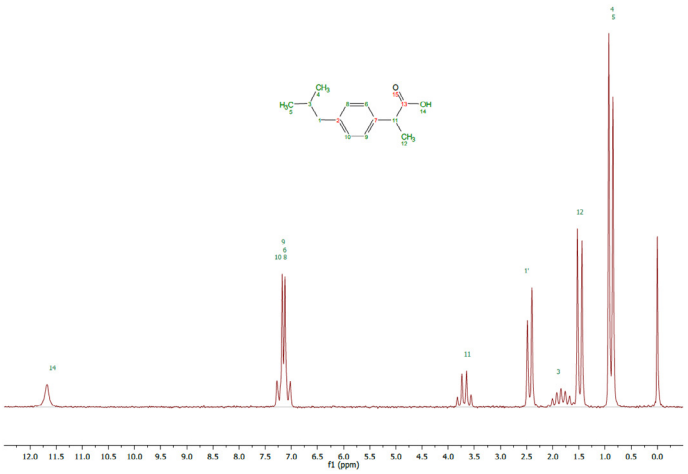

Figure 2: Thermo Scientific picoSpin 80 Series II NMR spectrum of 2 M ibuprofen

A careful analysis of the 1D proton (^{1}H) spectrum results in only a single possible structure despite the low-field spectral overlap obscuring part of the nonet (δ ~1.8 ppm). The ^{1}H-spectrum leaves no ambiguity in the structure of ibuprofen, forcing the question: what new information do these additional 2D and heteronuclear experiments provide? The answer of course, is that once the structure is confirmed, there is no new structural information to be gained from additional experiments. Some would argue the pedagogical value of interpreting these spectra, but with 2 M sample concentrations requiring acquisition times of more than 1 hour, integrating these experiments into a hands-on laboratory session is impractical.

Case study two: Lidocaine

Next, let us consider the value of benchtop 2D and ^{13}C experiments for lidocaine. As we can see from the Thermo Scientific™ picoSpin™ 80 Series II NMR spectrum of lidocaine (Figure 3), the 1D-^{1}H-NMR spectrum contains almost all the information needed to determine the structure, and would be more than enough to confirm it in most real world applications. However, we are approaching this as a true unknown, and therefore some ambiguity remains due to the lack of protons on the carbonyl of the amide. This makes lidocaine a good example of a benchtop NMR sample whose ^{1}H spectrum does not contain enough information to elucidate the structure on its own. Now, let us look at two methods we can utilize to eliminate any doubt in the structure: the use of “advanced” benchtop NMR experiments (i.e. 2D and ^{13}C) and orthogonal spectroscopic technique, infrared spectroscopy.

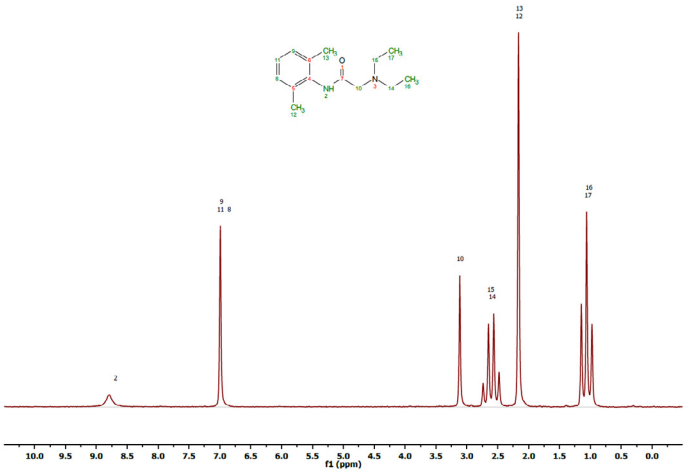

Figure 3: Thermo Scientific picoSpi 80 Series II NMR spectrum of 2 M lidocaine

[a] http://www.magritek.com/products/spinsolve60, accessed on 3/16/2017

Method one: ^{1}H and ^{13}C-NMR

All the peaks in the ^{1}H-NMR spectrum of lidocaine are fully resolved, and all but two signals are singlets, so there is no benefit to running a proton correlation experiment (COSY). A potential method for removing the aforementioned ambiguity in the structure of lidocaine is ^{13}C-NMR, which is the fastest and simplest NMR experiment available to benchtop spectrometers that provides the additional information needed. Lidocaine is very soluble in chloroform, so working from published 1 M data (Figure 4), we can estimate the 1D-^{13}C-NMR acquisition time of a 2 M solution on a 60 MHz benchtop system to be about 10 minutes

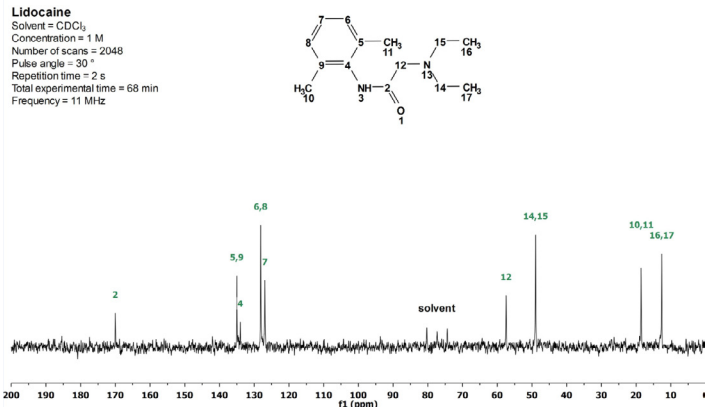

Figure 4: Carbon ^{13}C-NMR spectrum of 1 M lidocaine, published acquisition time 68 min.[b]

The nine resonances displayed in the spectrum allow us to conclude the presence of an additional carbon nucleus that is not bonded to any hydrogen nuclei. Combining this with the ^{1}H-NMR information, we can say conclusively that there is a carbon atom separating the methylene and amine protons and we can finalize the lidocaine carbon backbone. There remains some ambiguity as to what is bonded to the carbon in question, oxygen or sulfur? While either nuclei are possible, the chemical shift of ~170 ppm leads us to the conclusion that it is a carbonyl, confirming the structure of lidocaine.

Method two: Orthogonal techniques

The idea of combining multiple analytical techniques to solve problems is well established and has proven to offer a number of advantages over single technique solutions. When run in parallel, these orthogonal spectroscopic methods have proven to be fast and effective at characterizing a broad range of samples. In this case, combining ^{1}H-NMR and IR spectroscopy offers a rapid and effective solution to fully characterize the structure of lidocaine in less than 10 minutes depending on concentration and application.

While the ^{1}H-NMR spectrum leaves us with some ambiguity as to the structure, and no information about the presence of a amide, an IR spectrum immediately reveals the presence of a carbonyl from the strong absorption at ~1660 cm^{-1}. The amide is further confirmed by the broad, weak N-H absorption at 3243 cm^{-1}. While the IR spectrum contains a great deal more information, at this point the structure of lidocaine can be elucidated and there is no need to further analyze the IR spectrum (Figure 5).

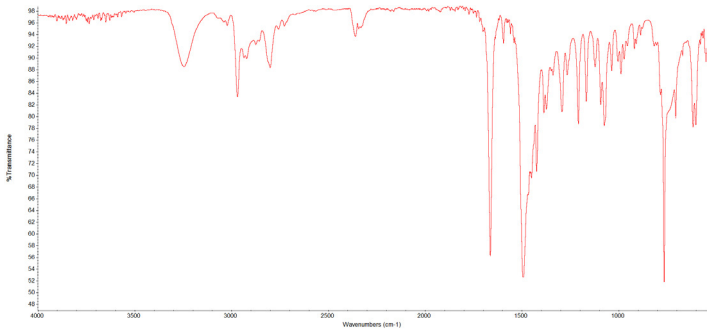

Figure 5: Spectrum of 100 mM lidocaine after 16 scans, (<30 seconds acquisition time) acquired using an Thermo Scientific™ Nicolet™ iS ™ 5 FT-IR spectrometer with iD5 ATR accessory.

We have two equally effective methods for determining the structure of a concentrated (2 M) sample of lidocaine, and both the NMR-IR and ^{13}C methods have reasonable acquisition times at 1 and 10-18 minutes, respectively. The NMR-IR method displays a significant throughput advantage over ^{13}C-NMR with total acquisition times of less than 1 minute at this concentration.

However, 2 M is a concentration often not achievable with every compound, so let us consider a more widely achievable concentration of 100 mM, or 25 mg/mL. Obviously, at 100 mM the acquisition times increase relative to the earlier 2 M example and are outlined in the Table 1 below.

Technique	2 M	0.1 M
^{1}H-NMR	<0.25 minutes	1-9 minutes
^{13}C-NMR	10-18 mininutes	≥120 minutes
IR	0.3 minutes	1 minute

Table 1: To determine the structure, the use of IR spectroscopy to supplement the ^{1}H-NMR spectrum provides an approximately 120 fold advantage in acquisition time over ^{13}C-NMR with 100 mM solution concentrations.

[b] http://www.magritek.com/wp-content/uploads/2016/12/007-Lidocaine-1D-13C-43MHz.jpeg, accessed 3-16-2017

At 100 mM the NMR-IR method shows a significant throughput advantage over the use of a ^{13}C capable benchtop NMR spectrometer. This should be expected as NMR is inherently less sensitive than many other spectroscopic techniques, and the ^{13}C nucleus is less abundant (1.1%) and even less sensitive than hydrogen (0.0176%). A benchtop ^{13}C-NMR acquisition time of 2 hours may seem reasonable, until you consider the alternatives. The higher sensitivity of IR spectroscopy and the fact that the NMR-IR method can be run in parallel, rather than in series as demanded by the 13C method, offers a 14- to 121- fold decrease in acquisition time without information loss (Figure 6). This time saved can dramatically increase the productivity of your lab or classroom.

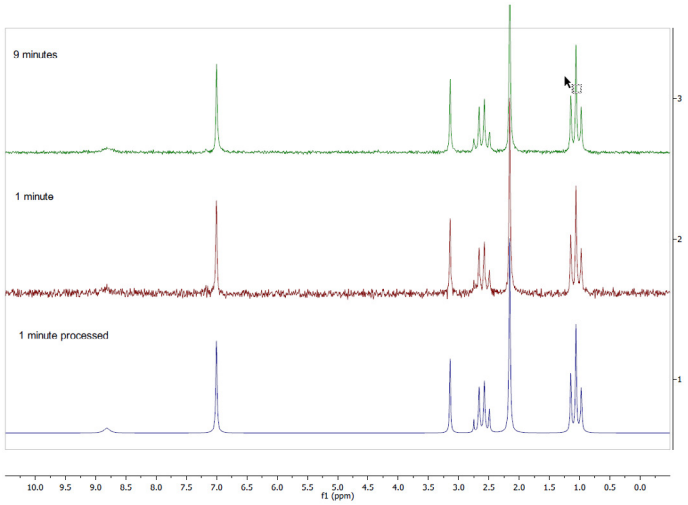

Figure 6: Thermo Scientific picoSpin 80 Series II NMR spectrum of 100 mM lidocaine in $CDCl_3$ after 1 minute (red trace) and 9 minutes (green trace) of data acquisition. While the spectrum at 9 minutes clearly has better signal to noise, the bottom spectrum (blue trace), generated from the 1 minute data, shows that all the spectral information is present after only 1 minute of data acquisition.

Summary

While several benchtop NMR spectrometers are capable of conducting certain 2D and heteronuclear experiments, the fundamental limitations on molecular size, complexity, and solubility severely limit their application and practical utility, sometimes taking more than 100 times longer than that of alternative methods. In this application note, we have discussed how many benchtop NMR samples do not require anything more than the ^{1}H-NMR spectrum to determine the structure. In cases where more data is necessary, the addition of orthogonal spectroscopic techniques such as infrared spectroscopy, offer a viable alternative to ^{13}C-NMR over a broader range of sample types and concentrations than benchtop NMR systems. Lastly, we have shown that combining the picoSpin 80 series II ^{1}H-NMR and iS5 IR spectra is many times faster at a variety of concentrations than ^{13}C-NMR experiments conducted on benchtop instruments.

Find out more at **www.thermofisher.com/nmr**

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