The essential upgrade for modern pharmaceutical NMR

Recent conversations with a leading user of NMR – currently working on structural elucidation in the early development group of a major US-based pharmaceutical company – concentrated on the theme of contemporary developments in NMR.

With more than 25 years' experience in the lab, and a perspective gained from working in applications development and customer training for a major analytical instrument company, he offers a unique view on how modern NMR hardware platforms, probes and software stack up against industry needs for rapid, straightforward analysis of fluorine-containing drug candidates.

Challenges and trends in pharma NMR

Against a background where analysts in the pharmaceutical industry are increasingly looking for accurate, easy-to-use and scalable methods throughout the R&D, QC and drug manufacture pipeline, Nuclear Magnetic Resonance spectroscopy (NMR) has become established as a leading technique. NMR gives an unmatched breadth of information on the sample, including strikingly detailed structural elucidation. When properly implemented, the technique also offers straightforward method development, allows rapid throughput without destroying the samples used and, importantly, is a direct quantitative method with no requirement for response factors and calibration curves.

> **"Five years ago, we could see Fluorine coming; today – with more than 50% of the new compounds that come to me from discovery being fluorinated – it's in your face every day!"**

As regulators expect a company to know much more about their drug compounds – and to have this information early in the development process – NMR is now often used in parallel with Mass Spectrometry so that discovery and development scientists can access structural information and mass data at the same time.

In recent times, Fluorine-containing compounds have become increasingly important. By 2011, more than 200 marketed medicines, and approximately one third of the most successful – so called, 'blockbuster' – drugs contained fluorine atoms in their structure[1].

Moreover, around 40% of small molecules entering advanced (phase III) clinical trials in 2012 and 2013 were fluoro-organic compounds[2].

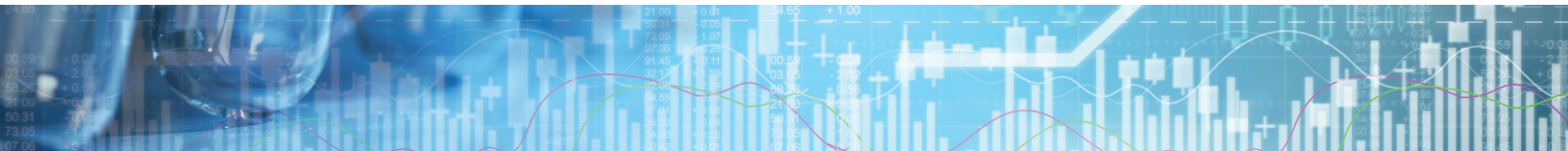
Fluorine chemistry is used in a wide variety of drug applications, including: antibiotics, antacids, antibiotics, anti-depressants, antihistamines, Arthritis/anti-inflammatory agents and psychotropics, for example[3].

> **"We now have a hardware platform that allows us to do investigations that were never available before – and we have a library of well worked out experiments that just work right out of the box – so we are at the stage of exploring the new limits and defining best approaches..."**

Consequently, the spotlight has fallen on the techniques used for the analysis of fluorine-containing pharmaceutical substances – including active pharmaceutical ingredients, branded and generic finished products. Traditionally, NMR analysis is based on ^{13}C and ^{1}H, however, ^{19}F NMR of fluorine-containing compounds can be very useful because of much higher ^{19}F NMR sensitivity. ^{19}F also has less risk of signal overlap due to its broad response range, in comparison to ^{1}H. Moreover, ^{19}F NMR provides a wealth of detail, including the coupling (between fluorine nuclei and other atoms), and chemical shift data that provides the assignment of both the location and nature of fluorine atoms within a molecule. But fluorine (^{19}F) exhibits some specific NMR properties that make it difficult to excite and decouple from other nuclei. Importantly, any new 'multinuclear NMR spectroscopy' approach, centred on ^{19}F NMR, lacked a robust routine HFX probe that could do every experiment well – until recently.

Opportunities ahead

Modern NMR probes often allow easy, straightforward use of ^{19}F NMR or ^{1}H and ^{13}C NMR, but not all three at the same time. JEOL's response was the introduction of the ROYAL HFX NMR Probe (see box overleaf) and a dedicated matrix of complementary 1D and 2D NMR experiments (Figure 1). The ROYAL HFX NMR Probe offers the ability to manipulate the ^{1}H, ^{19}F, and ^{13}C spins simultaneously without the typical loss in performance associated with traditional NMR probes designed for proton-fluorine NMR spectroscopy.



These developments follow historical precedent – where NMR, having been first described in the mid-1940s – has seen many technology developments over the years, each one providing scientists with the ability to extend the range of NMR applications:

- 1960s – FT-NMR
- 1980s/90s – 2D/3D techniques
- 2000s – cool probes
- 2010s – automation and data processing

- 1D
  - $^{1}H\{^{19}F\}$
  - $^{19}F\{^{1}H\}$
  - $^{13}C\{^{1}H,^{19}F\}$
  - $X\{^{1}H,^{19}F\}$, X= ^{15}N to ^{31}P
- 2D with ^{1}H,^{19}F, or ^{1}H & ^{19}F Direct or Indirect Decoupling
  - HSQCAD - ^{13}C & ^{15}N
  - gHMBCAD - ^{13}C & ^{15}N
  - HOESY - ^{1}H-^{19}F

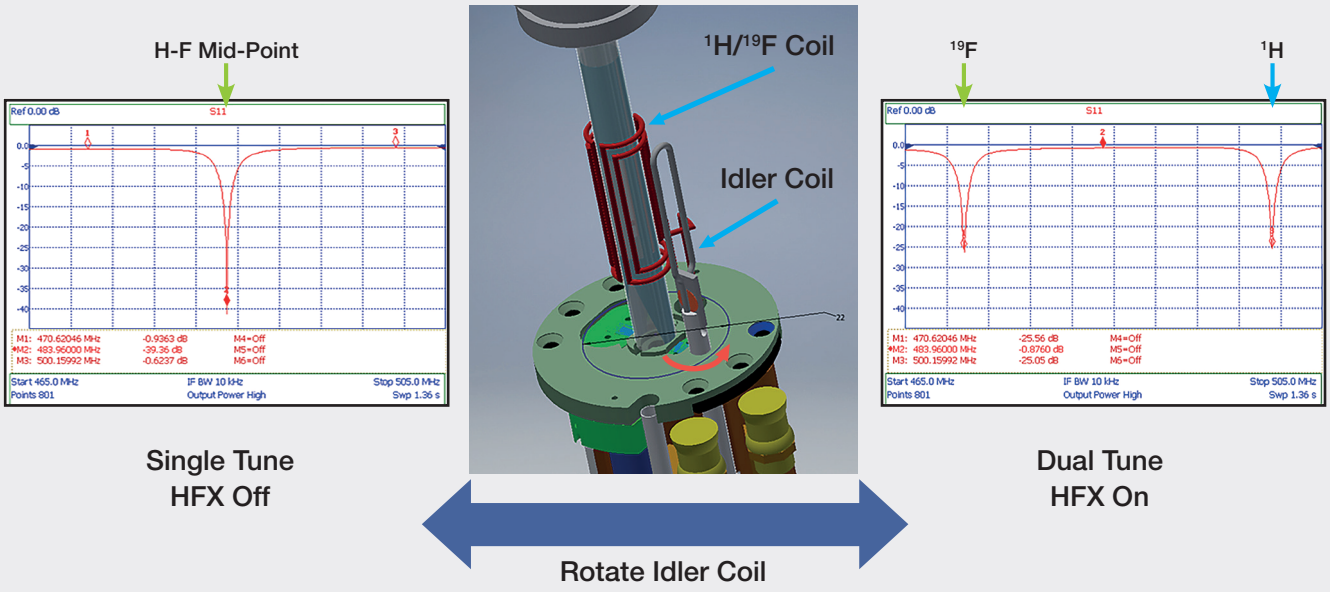
Figure 1: *Matrix of common 1D and 2D NMR experiments*

“ Moving to my current lab around three years ago, I wanted to add a 3rd channel to one of the existing spectrometers. In my opinion it’s an absolute requirement for modern pharma R&D… ”

What’s new in the JEOL ROYAL HFX NMR Probe?

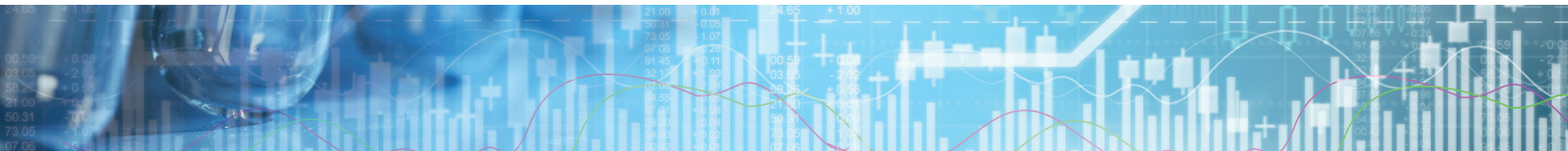
To create the ROYAL HFX NMR Probe, JEOL has merged two of its NMR probes – the ROYAL Probe offers high sensitivity ^{1}H or ^{19}F experiments, and the TFH Probe brings the required ^{1}H-^{19}F dual tune ability. Patented magnetic coupling technology delivers high-resolution NMR in HFX or HX mode with no performance loss in HX mode relative to a dedicated HX NMR Probe. Auto-Tune compatibility ensures easy switching between modes.

The JEOL ROYAL HFX NMR Probe also has the important added benefit of being able to observe other nuclei while pulsing or decoupling ^{1}H and ^{19}F. This results in no loss in performance or sensitivity for observing ^{13}C or other hetero-nuclei. Improved sensitivity and spectral simplification for ^{13}C when decoupling both ^{1}H and ^{19}F is a large advantage when collecting and analyzing NMR data from unknown compounds containing one or more fluorine sites.



“ …and, after looking carefully at all the options, I chose to work with JEOL to upgrade a console, refurbish our magnet and install their ROYAL HFX NMR Probe; all running under Delta software. ”

“ …I expect to see significant new data, and new publications, coming out from my team in the coming months and years. ”



In practice

Installing new NMR hardware should be a straightforward process – as noted above, it's a mature technique and the physical platform is solid and secure. Even adding automation to a set-up is now routine. But implementing new methodology can be more challenging, and the heart of the system – the control and data processing software – has become the vital component for a successful installation in practice.

"On the day we took delivery of our JEOL system with the ROYAL HFX NMR Probe, a colleague came to us with a problem – and it is testament to the robust pre-programmed methodology available from JEOL that I was able to make a measurement with little or no optimization and no reference material or standard and get an answer immediately. It was a great start, and something I don't think would have happened with any other vendor!"

Different manufacturers take dramatically different approaches to software architecture. Many users report that multi-instrument labs – or partners within a company but working at different sites – want to exchange results, share optimized methods with minimum input at each individual system and do simple housekeeping tasks such as using descriptive rather than numerical file names. All things that JEOL's operating system makes available.

"We are spoilt a little bit by how far software has developed on the devices we use every day. Software systems are so intuitive, so easy to use. NMR instrument software – from all vendors – has a way to go to approach this modern state but, in my experience, the underlying architecture available from JEOL gives us some significant advantages in terms of networkability and the ability to share method files in a multi-instrument set-up. In my experience, this is not a trivial matter."

In conclusion

With fluorine established as a major elemental component in many new chemical entities coming through pharma R&D, scientists are again relying on NMR as a frontline analytical technique. Now, the introduction of the unique ROYAL HFX NMR Probe and its matrix of common experiments, establishes an essential upgrade path to the rapid straightforward analysis of these important new drug candidates.

References

1. Wang et al, Chem. Rev. 2014, 114, 2432-2506
2. Zhou et al, Chem. Rev. 2016, 116, 422-518
3. Fluoride Toxicity Research Collaborative (FTRC), Index of Fluorinated Pharmaceuticals www.slweb.org/ftrcfluorinatedpharm.html