PHARMA

Assays for Fluorinated API Using the Fourier 80 Benchtop NMR

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Innovation with Integrity

Robust and GMP-compliant quantitative assay procedures are essential for quality control in pharmaceutical manufacturing. In this whitepaper, the benefits of using quantitative Nuclear Magnetic Resonance (NMR) with the Fourier 80 benchtop spectrometer on fluorinated active pharmaceutical ingredient are discussed. Due to its multinuclear capabilities, the Fourier 80 allows for easy implementation of ^{19}F NMR quantitative assays, which are applicable to regulatory requirements. Procedure design and implementation are significantly simplified and streamlined, even in complex mixtures such as drug products, leveraging the very high specificity provided by ^{19}F NMR.

NMR as a Modern Technology for Quality Control

Quality control (QC) in pharmaceutical manufacturing is dependent on the robustness of the employed quantitative assay procedures. The challenge in developing such procedures extends beyond meeting stringent technical criteria. Compliance with regulatory requirements such as cGMP or EU-GMP is paramount to ensure patient safety and product quality. In the context of quality control, these comprehensive frameworks are supplemented by the internationally adopted guidelines ICH[1] Q2 and ICH Q14.

A renewed focus on analytical process understanding introduced by the concept of Analytical Quality by Design (AQbD) in ICH Q14 and USP <1220> has created increased demand for state-of-the-art technology. In this context, Nuclear Magnetic Resonance (NMR) spectroscopy stands out as it combines several features highly beneficial for the control of therapeutics. As a primary, universal method, analytical procedure development and management are straightforward with NMR. It is also one of the few techniques where the modern concept of a platform procedure can be leveraged, e.g. a central NMR method can be used to test quality attributes of several products without significant changes.

[1] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

This significantly reduces the efforts needed to develop, qualify,[2] and monitor analytical procedures, especially when dealing with closely related substances. Additionally, NMR allows the use of surrogates instead of reference standards specific to an analyte for quantitative applications, further simplifying procedure development and execution.

These benefits of NMR are now recognized as potent tools for QC, as evidenced by the ongoing or approved revisions of pharmacopeia and international guidelines.[3] Recently, NMR has gained further interest for QC because of the availability of benchtop NMR systems such as the Fourier 80. Benchtop systems can be placed in traditional laboratory or manufacturing environments instead of dedicated, specialized rooms because of the limited footprint and a cryogen-free, permanent magnet configuration. These features alleviate the last barriers to adopting NMR in QC laboratories, without compromising on the intrinsic benefits of NMR technology.

To illustrate these benefits and the overall simplicity provided by benchtop NMR, we will demonstrate how an assay to quantify a fluorine-containing active pharmaceutical ingredients (API) can be easily designed and implemented using the Fourier 80 in few steps.

^{19}F qNMR for Highly Specific Assays on Fluorinated API

Quantification by Nuclear Magnetic Resonance (qNMR) is a well-established primary method for the absolute quantification of organic material.[4] Key features can be summarized as follows:

- As a universal, absolute method, qNMR relies on physical principles with minimal empirical contributions. This makes the method design, risk assessment, and definition of the method operable design region (MODR) straightforward.
- It does not require an authentic reference material but instead, any surrogate of known purity can be used. This is a distinct advantage compared to more traditional QC technologies, such as those based on chromatography.
- Several methodologies are possible, including choice of the nucleus to be used, internal or external calibration, single- or multi-dimensional techniques, etc. This allows for significant flexibility and adaptability, ultimately ensuring a fit-for-purpose procedure.

The most used nucleus in NMR is proton (^{1}H) due to its ubiquitous presence in organic material and high sensitivity. However, this can be detrimental in complex molecules and mixtures such as drug products, where all organic components contribute to the resulting ^{1}H NMR spectrum. This can prove even more challenging on benchtop NMR systems where resolution is intrinsically limited when compared to high magnetic field NMR instruments. The challenges associated with resolving ^{1}H resonances at low magnetic field can be circumvented by instead using a more chemically selective nucleus such as fluorine (^{19}F). With only a minor trade-off in sensitivity,[5] NMR detection of ^{19}F can provide exceptional specificity because its intrinsic spectral width is larger than that of ^{1}H,[6] providing improved resolution, and a limited abundance of fluorine atoms in organic substances. For drug products, this is even more true, since common excipients do not contain fluorine while the number of APIs with at least one fluorinated functional group is significant and continues to grow.[7]

To demonstrate the benefits of such approaches, a commercially available formulation of a fluorinated API (Fipronil) was used as a model. In this veterinary drug product, the content of the drug substance is low, approximately 10%w/w. The resulting ^{1}H spectrum of the drug product after a simple dilution with deuterated dimethyl sulfoxide (DMSO-d_6) was logically dominated by signals from the excipients (Figure 1) Designing a quantification method based on a ^{1}H NMR strategy would thus present a considerable challenge, even using high-field NMR systems.

[2] In USP <1220> the term "procedure validation" is replaced by "procedure qualification" to highlight that this step is only a part of the life cycle process.

[3] See ICH Q2(R2), applicable as of 14 June 2024 that details the use of NMR and current draft of USP <761> and <1761> as of July 2024.

[4] See for example Diehl *et al.* J Pharm Biomed Anal. **2020**, 177, 112847 ; Pauli *et al.* Magn Reson Chem. **2021**, 59, 7 and current draft of USP-NF <761> and <1761> as of July 2024.

[5] NMR intrinsic receptivity of ^{19}F relative to ^{1}H is 83% but experimental sensitivity will depend on resonances multiplicity and dynamic range which are favorable in the case of ^{19}F NMR.

[6] Spectral width is a physical property associated with a given nuclei.

[7] 20% of approved drugs contain fluorine, with a significant increase, exceeding 50 new approvals between 2018 and 2023, see for example Albericio *et al.* Pharmaceuticals **2023**, 16(8), 1162 ; Wang *et al.* Chinese Chemical Letters, **2024**, 109780

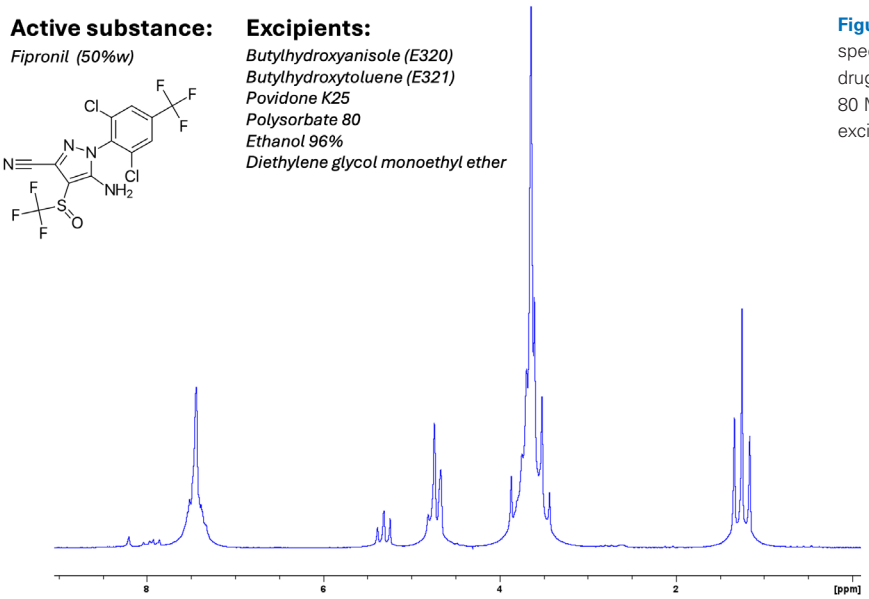

Figure 1: Example of ^{1}H spectrum of the model drug product recorded at 80 MHz, dominated by excipients resonances.

However, the API of the formulated drug product was selectively detected by leveraging ^{19}F NMR, as it is the only molecule containing fluorine in this complex formulation. Representative ^{19}F NMR spectra are shown in Figure 2 and when compared to the ^{1}H spectrum illustrate the gain in resolution and sensitivity for the fluorinated API, which can be further improved by the ability of the Fourier 80 to perform proton decoupling. Consequently, designing a qNMR procedure to quantify the drug substance was straightforward using the Fourier 80 and involved only the following steps:

- A simple sample preparation of mixing the drug product with an internal calibrant (in this case 4,4-difluorobenzophenone) and adding solvent (DMSO-d_6).[8]
- NMR data acquisition, using suitable parameters for quantification,[9] which was completed in less than 10 minutes per sample.
- NMR data processing, which comprises only a few, conventional steps and can be fully automated after the initial method development effort.

This procedure can be further optimized by suppressing fluorine-proton spin coupling using proton decoupling during acquisition (top spectrum in Figure 2), ensuring that all NMR resonances are sharp singlets, even when protons are structurally adjacent to the fluorine atoms.

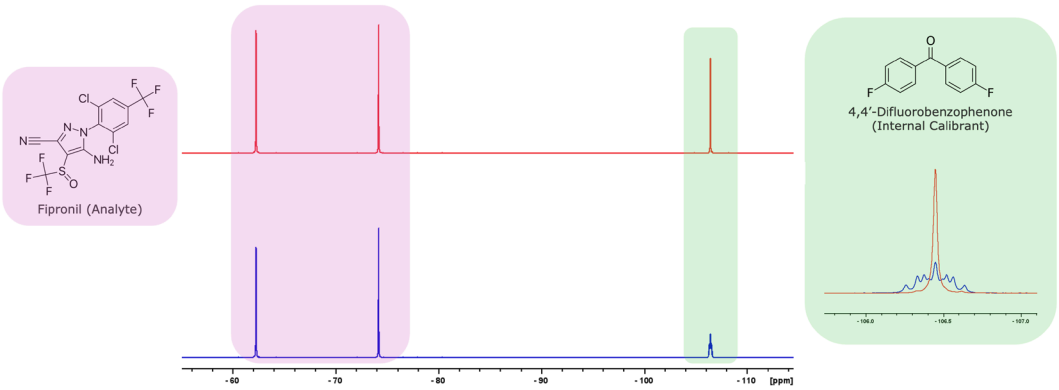

Figure 2: Example of ^{19}F spectra recorded at 80 MHz with (top) or without (bottom) proton decoupling of the model drug product after sample preparation with an internal calibrant.

[8] Use of deuterated solvent is not required. It was use if this case to simplify initial investigations by ^{1}H NMR.

[9] Critical acquisition parameters for quantifications are well know and documented. They can be selected in only few steps, namely a preliminary qualitative spectrum to determine the chemical shift and a T1 measurement to select the appropriate relaxation delay. These steps are well documented in the literature and compendial monographs.

To illustrate the typical results achievable with qNMR, two independent sample preparations were conducted. Each preparation was recorded and processed with five replicates, both with and without proton decoupling. The results are summarized in Table 1 (without decoupling) and Table 2 (with decoupling).

Firpronil content (mg per single dose)		
Acquisition	Sample 1	Sample 2
#1	46.98	47.69
#2	47.09	47.79
#3	47.04	47.51
#4	47.22	47.52
#5	47.26	47.79
Average	**47.12**	**47.66**
RSD	0.26%	0.30%
	Total Average	**47.39**
	Total RSD	**0.66%**

Table 1: Results obtained for the quantification of Fipronil in the model drug product using the Fourier 80 spectrometer without proton decoupling.

Firpronil content (mg per single dose)		
Acquisition	Sample 1	Sample 2
#1	46.84	47.59
#2	46.97	47.55
#3	46.95	47.46
#4	46.87	47.88
#5	47.22	47.63
Average	**46.97**	**47.63**
RSD	0.32%	0.33%
	Total Average	**47.30**
	Total RSD	**0.80%**

Table 2: Results obtained for the quantification of Fipronil in the model drug product using the Fourier 80 spectrometer with proton decoupling.

The average quantity of the API per dose of drug product determined with or without decoupling were very precise, given that the relative standard deviations are well below 1%, and statistically similar with averaged results of 47.30 mg and 47.39 mg, respectively.

These results exemplify the typical simplicity and precision of qNMR methods for determining the API content in drug products, even within complex mixtures. With minimal steps and limited knowledge about the formulation, an analytical procedure could be designed and implemented, yielding high specificity and preliminary precision results well below the standard criterion of 2%.[10] As the exact quantity of the drug substance in the batch under investigation is unknown,

[10] USP-NF <761> criterion for a drug product

it precludes a detailed discussion on accuracy. However, the obtained values were within the anticipated range from the theoretical value, and minimal additional effort would be required to fine-tune the procedure.

Finally, it should be noted that the excellent agreement between the results with and without decoupling is not coincidental. It highlights the universal and absolute nature of NMR: regardless of the specific method design, applying appropriate parameters to ensure quantitative conditions yield consistent and accurate results. In this instance, proton decoupling proved beneficial, enhancing the lineshape and signal-to-noise ratio for the internal calibrant resonance and simplifying data processing. However, it did not alter the absolute response, thereby providing identical outcomes.

Final Considerations

qNMR is beneficial due to its unique ability to perform quantification, even in complex mixtures, without the need for identical reference materials. With limited prior knowledge, assay procedures can be designed and implemented in a straightforward manner using the Fourier 80. The example discussed in this whitepaper presents a complex scenario: a drug product with a low content of drug substance and a formulation with other detectable excipients. Similarly, qNMR procedures can be implemented for simpler assays, such as those on neat API or excipients (with or without fluorine), during batch release or incoming material control.

Fourier 80 benchtop NMR spectrometer is advantageous for QC laboratories as it brings these unique capabilities of qNMR into a compact, cryogen-free form factor. The implementation of platform procedures to streamline method management is now possible without the constraints of a floor-standing system. Lastly, as a prerequisite for all regulatory testing, Bruker GxP kits actively support implementation of NMR analytical procedure with the Fourier 80 in full compliance with applicable regulatory requirements. These kits include all the necessary tools for the instrument and computerized system qualification to enable full electronic data integrity. They enable automated, compliant analytical workflows, illustrated in Figure 3, specifically tailored for QC laboratories.

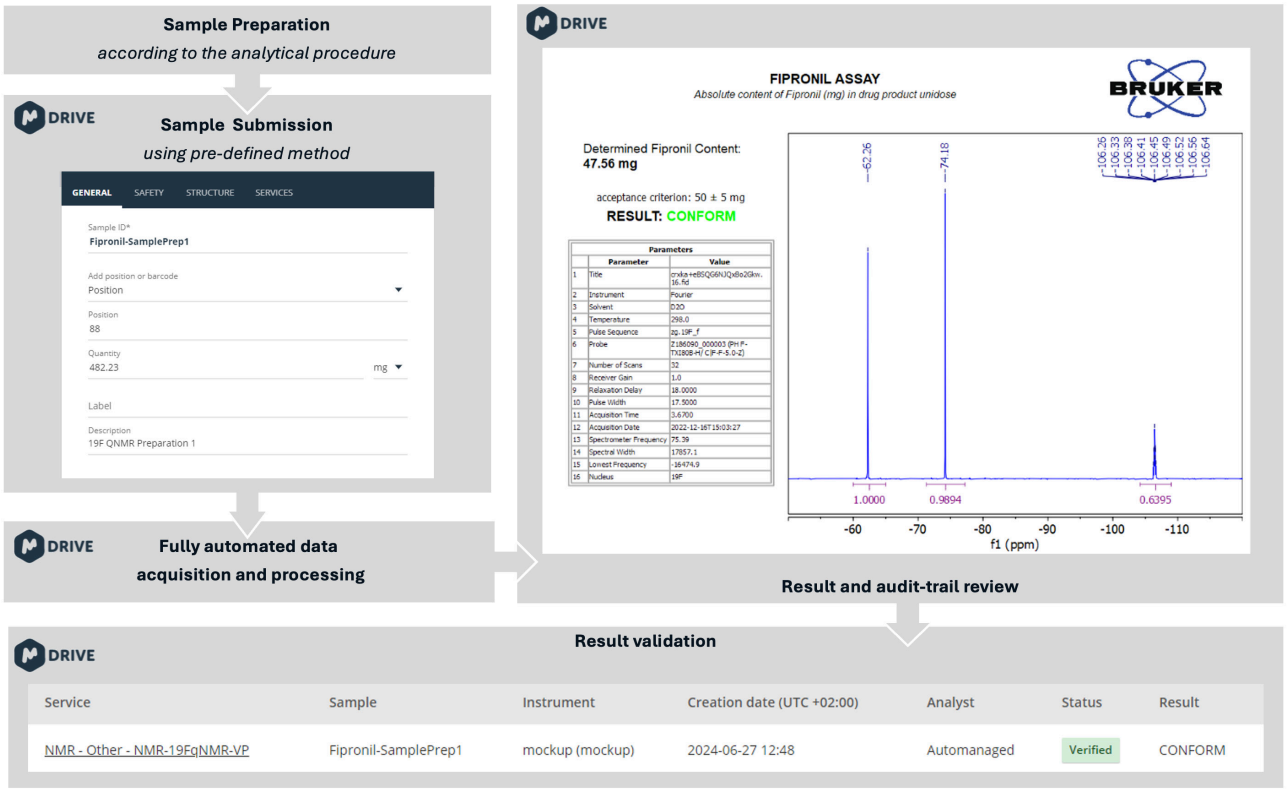

Figure 3: Workflow for automated ^{19}F NMR-based assay on the model drug product using the Fourier 80 and the Bruker GxP Readiness Kit Enterprise. The whole analytical cycle is performed using a web interface (Mdrive). Data and audit-trails are secured on a server database, ensuring data integrity.

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