



## PHARMA

# Quantitative Assays with Fourier 80 Benchtop NMR: Benefits and Examples

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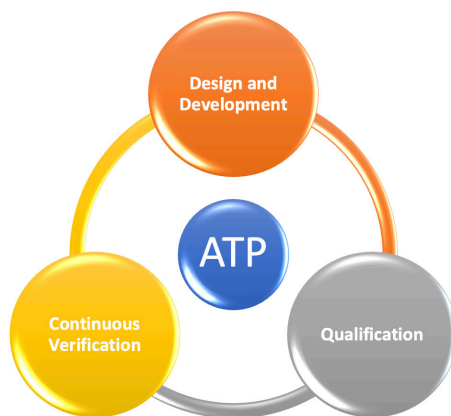
Innovation with Integrity

Robust quantitative assay procedures are paramount for pharmaceutical quality control testing. Cornerstones of the certificates of analysis, they ensure the quality of incoming raw materials, manufactured drug substances and finally release of the drug products. In this whitepaper, development and implementation of such procedures based on the Bruker Fourier 80 benchtop NMR spectrometer are discussed. Examples using model drug products illustrate the combination of simplicity and robustness provided by  $^1\text{H}$  and  $^{19}\text{F}$  qNMR methods. They allow straightforward and streamlined development of analytical procedures, on a compact system tailored for quality control laboratories and in regulatory compliance.

## Introduction

Effective quality control (QC) of pharmaceutical products assures that marketed drugs are safe and efficacious for patients. This assurance is underpinned by the employment of highly robust qualitative and quantitative analytical techniques that are designed to meet stringent regulatory standards, such as current Good Manufacturing Practices (cGMP) and, in certain development stages, Good Laboratory Practices (GLP). These practices are not merely procedural but are integral to safeguarding patient health. A recent shift in the pharmaceutical QC paradigm towards a more holistic approach known as Analytical Quality by Design (AQbD)<sup>1</sup> encourages management of regulatory analytical procedures through life cycle, risk-based considerations. It is centered around the Analytical Target Profile (ATP) which defines both the scope and the performance criteria associated to design and qualify the procedure. AQbD necessitates a profound understanding of the analytical parameters, which must be meticulously monitored throughout the lifecycle of the analytical procedure (Figure 1). While this may initially present as a laborious task, it ultimately affords greater flexibility in lifecycle management and ensures that the procedures are fit for their intended purpose.

<sup>1</sup> As introduced in ICHQ2(R2) / ICHQ14 and USP <1220>



**Figure 1** Schematic and simplified representation of the Analytical Procedure Lifecycle according to the AQBd.

Although Nuclear Magnetic Resonance (NMR) spectroscopy has traditionally been associated with fundamental research and the drug discovery phase, it is emerging as a powerful tool for QC. In the context of AQBd, NMR is recognized for its unique benefits over conventional spectroscopic and chromatographic techniques, as evidenced by ongoing or approved revisions of pharmacopeia and international guidelines that now incorporate and describe in detail NMR for release testing. The unmatched robustness and versatility of NMR indeed makes it an ideal candidate for streamlining the analytical procedure lifecycle and managing the demanding requirements of AQBd. Moreover, NMR addresses common challenges such as the need for reference substances or materials while providing a high degree of confidence during standard qualification because of its inherent quantitative nature and sensitivity to chemical structure. The development of platform NMR procedures, which can be applied to multiple products, reduces the effort required for method validation and monitoring, offering a clear advantage over traditional chromatography-based methods.

The intrinsic benefits of NMR in GxP environments for AQBd are readily apparent and well-documented for high field spectrometers, using super-conducting, cryogenically cooled magnet technology.<sup>2</sup> Interest in utilizing low field benchtop NMR systems, such as the Bruker Fourier 80 (magnetic field strength of 80 MHz), for QC applications is growing because the above benefits of NMR are achieved in a low-cost, compact, cryogen-free system. The reduced footprint of the benchtop system allows for more versatile use in QC labs and manufacturing facilities where available space for a high-field system is impractical. The environmental sustainability and maintenance of benchtop systems are significantly improved compared to high-field counterparts due to the elimination of cryogenic liquids to sustain the magnetic field, which is ideal for QC applications. In this whitepaper, we will demonstrate advantageous applications of quantitative NMR (qNMR) using the Fourier 80 spectrometer in designing assay procedures. We illustrate the streamlined implementation of qNMR for new analytical procedures of two model drug products, using two different strategies.

## QNMR for Assays

qNMR is a well-established primary method for absolute quantification. It eliminates the need for an authentic reference material, instead any compound of known purity can serve as a reference standard by which the target can be quantified, given suitable specificity. This attribute offers a distinct advantage compared to traditional QC methods, notably chromatographic approaches. The other general benefits and considerations for qNMR method have been extensively documented in the growing body of scientific literature.<sup>3</sup> The ongoing revision of USP <761> and <1761> also describes in detail how (q)NMR method should be designed and qualified for pharmaceutical-grade QC. In a very simplified overview, there are two main strategies for qNMR procedures:

1. Internal calibration: this method offers the highest accuracy. It is most commonly used for assays and involves mixing a known amount of internal calibrant (IC) to a sample prior to analysis.
2. External calibration: this method offers simpler sample preparation because the sample and internal calibrant are recorded separately but provides a somewhat lower accuracy. This approach is out of the scope of the present work but can be typically implemented for impurities quantification where the accuracy criteria are less stringent (but the limit of quantification is critical).

<sup>2</sup> See ref. 1 and current draft of UPS <761> and <1761> as of June 2024

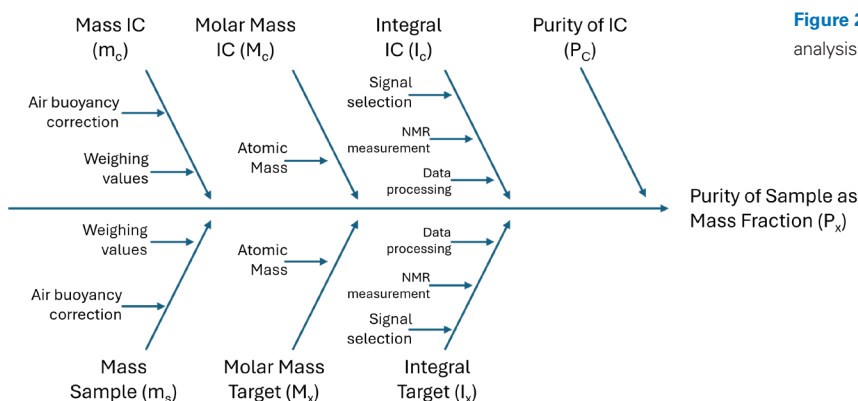
<sup>3</sup> See for example Diehl *et al.* J Pharm Biomed Anal. **2020**, 177, 112847 ; Pauli *et al.* Magn Reson Chem. **2021**, 59, 7

Provided suitable experimental conditions are used (as described in current revision of USP <761> and <1761>), the determination of absolute purity or mass fraction becomes straightforward with qNMR procedures, based on the ubiquitous equation below.

$$P_x[\%w] = \frac{n_c \cdot I_x \cdot M_x \cdot m_c}{n_x \cdot I_c \cdot M_c \cdot m_s} \cdot P_c$$

**Equation 1** With c: internal calibrant, x: target molecule, s: sample, P: absolute purity, I: integral values, M: molar mass, m: mass, n: number of spin yielding the resonance

This universal equation associated with qNMR underscores a key aspect: the majority of variability sources are shared across different qNMR methods. This commonality streamlines the process of risk analysis and facilitates the estimation of the measurement uncertainty, anticipating alignment of the method under development with the ATP. As stated in the ongoing revision of USP <1761> this is usually straightforward as the purity of the IC is usually the major contributor to the method uncertainty. Errors from sample preparation and spectral processing come as the next sources but are comparatively minor. Additional sources of variability are minimal and readily identifiable as exemplified in Figure 2.



**Figure 2** Typical Ishikawa fishbone for qNMR uncertainty analysis adapted from ref 2.

Proton ( $^1\text{H}$ ) is the most utilized nucleus in qNMR due to its high sensitivity and ubiquitous presence in organic and biological compounds. However, the limited spectral width and proton-proton spin couplings can reduce selectivity, which is crucial for qNMR applications. In certain instances, this limitation can be overcome by using alternative nuclei, such as fluorine ( $^{19}\text{F}$ ), which offers a broad spectral range without greatly compromising sensitivity. This is particularly pertinent for pharmaceutical QC considering that approximately 20% of approved drugs contain fluorine, with a significant increase, exceeding 50 new approvals between 2018 and 2023.<sup>4</sup> Generally, fluorine is present only in the active pharmaceutical ingredient (API) and not in the excipients, making it an exceptionally selective marker for detecting and quantifying the API in complex formulations. The Fourier 80 benchtop spectrometer facilitates the analysis of both  $^1\text{H}$  and  $^{19}\text{F}$  nuclei, providing an effective means to develop and implement qNMR methods for pharmaceutical applications. Its design allows for flexible and versatile analytical procedure development that align with the required ATP, as demonstrated in the next section.

<sup>4</sup> Albericio *et al.* *Pharmaceuticals* **2023**, 16(8), 1162 ; Wang *et al.* *Chinese Chemical Letters*, **2024**, 109780

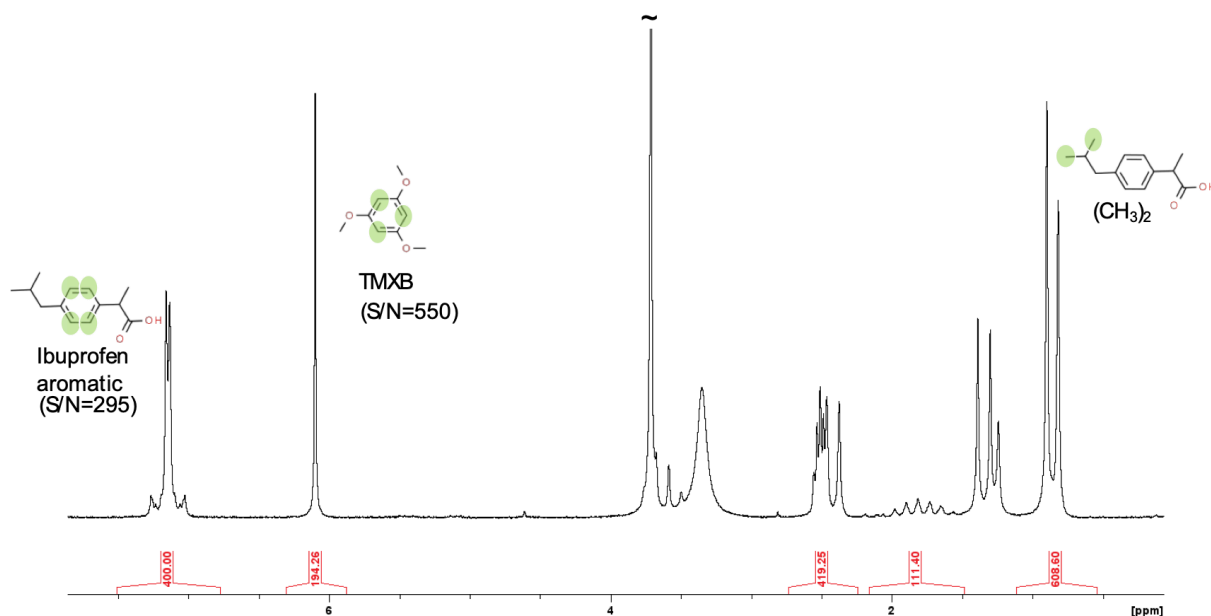
## Example Applications of qNMR for Assays Using the Fourier 80 Spectrometer

To illustrate the simplicity and effectiveness of qNMR analytical procedure design using the Fourier 80 spectrometer, this section will explore two instances. In each scenario, an analytical method was implemented and tested with limited prior knowledge of the mixture being analyzed,<sup>5</sup> serving as representative drug products. Employing strategies based on either <sup>1</sup>H or <sup>19</sup>F nuclei, the assay methods were developed to directly quantify the API.

### Example 1: Ibuprofen Quantification in a Model Finished Drug Product (Tablets) by <sup>1</sup>H qNMR

In this first example, commercially available ibuprofen tablets were examined to establish a qNMR procedure for determining the drug substance content. In this case, the actual content of the API in the formulation is high, about 76% by weight according to the information present on the packaging (200 mg ibuprofen / tablet) and the experimentally determined weight of the tablet (264 mg).

Analytical samples were simply prepared by grinding the tablet into a powder and dissolving it into DMSO-d<sub>6</sub>. A preliminary 1D <sup>1</sup>H NMR spectrum of the sample (not shown) helped to select an appropriate IC that avoids signal overlapping with the analyte, in this case trimethoxy-benzene (TMXB).<sup>6</sup> Figure 3 demonstrates that clear and specific detection of <sup>1</sup>H resonances associated with both the API and the IC is achieved using the Fourier 80. As expected, the resolution is lower than with floor-standing NMR spectrometers (Figure 4), yet it is adequately selective for the method, and a suitable signal-to-noise<sup>7</sup> was reached in a short time (about 25 min in the present example).

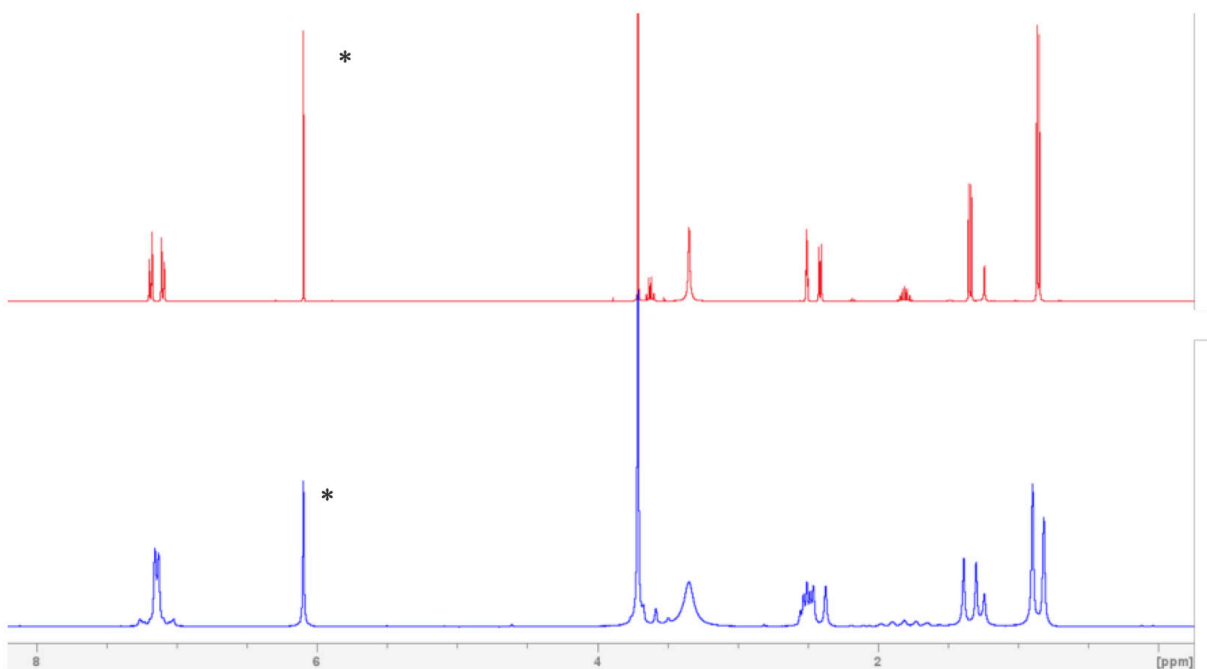


**Figure 3:** Example of <sup>1</sup>H spectrum of the model drug product under investigation mixed with the internal calibrant (TMXB) in DMSO-d<sub>6</sub> using the Bruker Fourier 80 NMR spectrometer (NS 16). Specific resonances of the API and the IC and their attribution are indicated. S/N are the experimental signal to noise reached.

<sup>5</sup> For pharmaceutical analytical design procedure, accurate knowledge of the composition is obvious since all manufacturing steps must be highly controlled.

<sup>6</sup> Selection is straightforward once the spectrum characteristics of the substance under investigation are known, since those of the available NMR-grade standards are well documented.

<sup>7</sup> Schoenberger *et al.* Anal. Bioanal. Chem., **2018**, 28, 7397-400



**Figure 4:** Comparison overlay of the  $^1\text{H}$  spectra of the model drug product under investigation recorded in  $\text{DMSO-d}_6$  at 400 MHz (red, Bruker 400 NEO NMR spectrometer) and at 80 MHz (blue, Bruker Fourier 80 NMR spectrometer). The internal calibrant is already present and indicated by \*.

Determination of the drug substance content is then straightforward using Equation 1 and integration values of the  $^1\text{H}$  spectra resonances (identified in Figure 3). Results are summarized in Table 1. The average content of the API after duplicate experiments was measured to be 74.3% using a floor-standing 400 MHz NMR spectrometer and 74.8% using the Fourier 80. The remarkable match between the experimental results and the putative theoretical value (76%) illustrates the unique ability of qNMR to directly yield accurate values without a reference standard or correction factor. Since the actual content in ibuprofen in this specific batch of tablet is unknown and the replication count is limited, a comprehensive statistical analysis is not feasible. Nevertheless, the general precision of the method and consistency of the results are noteworthy (given the standard error associated with the qNMR method). There is thus a perfect agreement between the results obtained on the benchtop Fourier 80 and the 400 MHz high field system (which is regarded as the reference method in this instance).

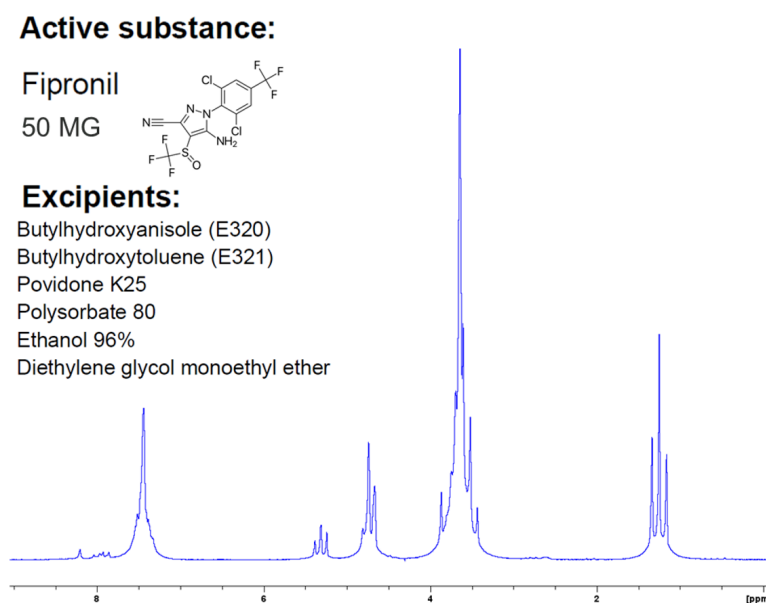
Consequently, further development to refine the procedure and qualify it against the corresponding ATP would require minimal effort. Although the ATP was not explicitly defined for this example, based on these scouting results, qualification against the criteria of USP-NF <761> — specifically, an accuracy within  $\pm 5\%$  and a relative standard deviation below 2% for a drug product — would be clearly feasible and straightforward.

Replicate	Ibuprofen Content (% w/w) in Tablet 400 MHz method	Ibuprofen Content (% w/w) in Tablet 80 MHz method
#1	74.2	75.1
#2	74.3	74.5
<b>Average</b>	<b>74.3</b>	<b>74.8</b>

**Table 1:** Results obtained for the quantification of ibuprofen in the model drug product using either 400 MHz or 80 MHz NMR spectrometer.

## Example 2: Fipronil Quantification in a Finished Drug Product (Unidoses) by $^{19}\text{F}$ qNMR

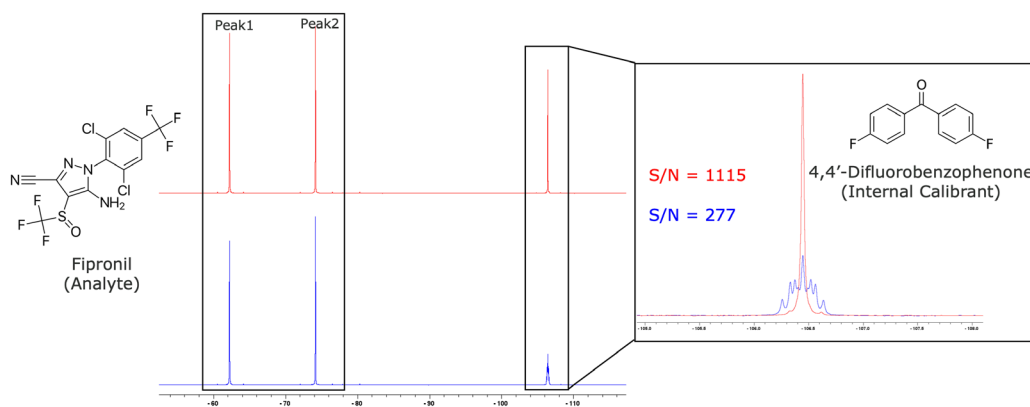
In this second example, a commercial veterinary drug product served as a model to illustrate the development of a  $^{19}\text{F}$  qNMR method for the content determination of the drug substance. In this case and as for most drug products, the active substance represents only a fraction of the formulation (50 mg for 0.5 mL of formulation) and thus high specificity must be reached for its quantification. Owing to the high prevalence of protons in organic molecules, the resulting  $^1\text{H}$  NMR spectrum is dominated by the excipient resonances (Figure 5). Consequently, designing a quantification method based on  $^1\text{H}$  NMR would present a considerable challenge.



**Figure 5:** Example of  $^1\text{H}$  spectrum of the model drug product recorded at 80 MHz, dominated by excipients resonances.

Fortunately, the presence of fluorine in the API can be leveraged through the use of  $^{19}\text{F}$  NMR experiment, circumventing the specificity issue with  $^1\text{H}$ . As illustrated in Figure 6, this nucleus is selective to the drug substance, with two fluorine moieties, yielding highly resolved and thus specific resonances, without any interferences from the other components of the drug product. Furthermore, the proton decoupling capability of the Fourier 80 spectrometer can further improve the sensitivity and resonance lineshape when proton-fluorine coupling occurs as in the case of the IC (4,4'-difluorobenzophenone) used in this example.

Designing a qNMR method based on  $^{19}\text{F}$  follows the same principles as qNMR using  $^1\text{H}$ . Similar to the previous example, the sample preparation step involved a simple mixture of the drug product with an internal calibrant and addition of solvent ( $\text{DMSO}-d_6$ ). Data acquisition was completed in less than 10 minutes per sample. Lastly, data processing comprised only a few steps, which can be automated after the initial method development effort to directly yield absolute quantification values using Equation 1.



**Figure 6:** Example of  $^{19}\text{F}$  spectra recorded at 80 MHz with (red) or without (blue) proton decoupling of the model drug product after sample preparation with an internal calibrant. Signal to noise (S/N) values are reported to indicate the gain provided by proton decoupling.

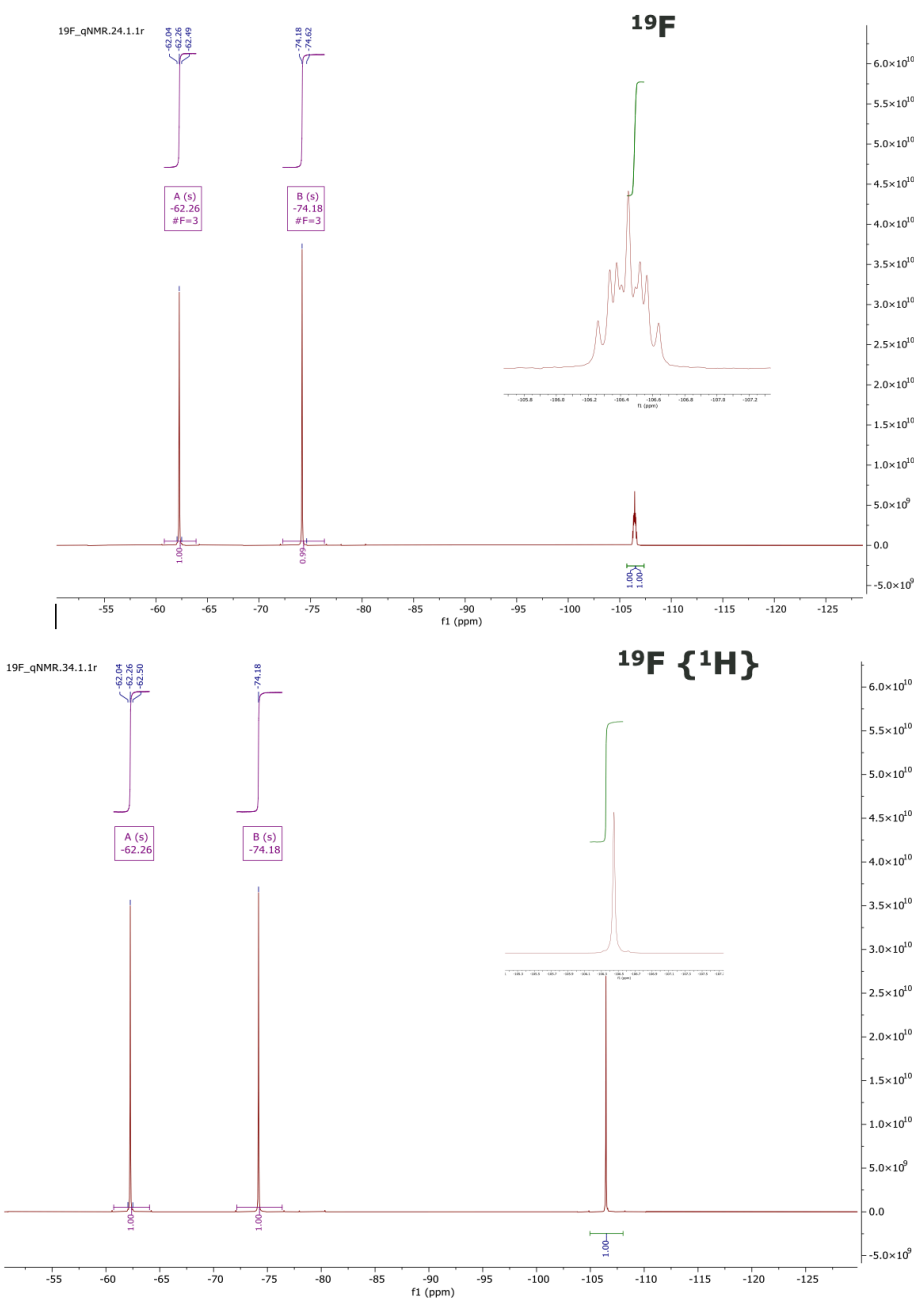
To illustrate the typical results one can obtain using qNMR, two independent sample preparations were performed, and each one was recorded and processed with 5 replicates, with and without proton decoupling. Results are summarized in Table 2 (without decoupling) and Table 3 (with decoupling) and representative spectra after processing with the Mnova software from Mestrelab are illustrated in Figure 7.

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

**Table 2:** Results obtained for the quantification of Fipronil in the model drug product using the Fourier 80 spectrometer without proton decoupling.

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

**Table 3:** Results obtained for the quantification of Fipronil in the model drug product using the Fourier 80 spectrometer with proton decoupling.



**Figure 7:** Example  $^{19}\text{F}$  spectra recorded on the Fourier 80 after processing using the Mnova software without (top) or with (bottom) proton decoupling.

The average amount of API in the drug product was determined to be 47.39 mg (RSD = 0.66%) without proton decoupling and 47.30 mg (RSD = 0.80%) with decoupling. Once again, these data illustrate the simplicity and precision of these qNMR methods for determining API content in drug products, even in a complex mixture. The exact quantity of the drug product in the batch under investigation is unknown and thus precludes a detailed discussion on accuracy. Yet, with minimal knowledge of the product, and in a few steps, the obtained values were within the anticipated range from the theoretical value. Additionally, the method achieved high precision, with a relative standard deviation significantly lower than the 2% criterion outlined in USP <761> for drug products. This was consistent regardless of proton decoupling, underscoring the universal and absolute nature of NMR. Irrespective of the specific method design, applying appropriate parameters to ensure quantitative conditions will indeed yield consistent and accurate results. In this instance, proton decoupling was advantageous, enhancing the lineshape and signal-to-noise ratio for the internal calibrant resonance and simplifying data processing. Nevertheless, it did not alter the absolute response, thereby providing identical outcomes.



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## Conclusion

The examples presented here demonstrate the unique capability of NMR to perform quantification even in complex mixtures, without prior knowledge or identical reference materials. These cases underscore the simplicity of the initial procedure development, and the utility of qNMR in streamlining method life cycle management. While these advantages are recognized for high field NMR systems, this whitepaper confirms their applicability to the benchtop Bruker Fourier 80 NMR spectrometer. It offers the full range of NMR benefits to pharmaceutical QC laboratories and eliminates the complexities associated with traditional floor-standing systems. With a compact design and maintenance-free operation, it enables the development and routine implementation of NMR methods for qualitative applications (such as identification), quantitative assays as demonstrated here, and compendial methods.<sup>8</sup> Automated workflows for data acquisition and processing can be implemented and facilitates user-friendly operation, allowing non-expert to deliver results with minimal input. Finally, as a pre-requisite for all batch release testing, Bruker GxP kits actively support achieving full compliance with applicable regulatory requirements. They include all the necessary tools for instrument and computerized system qualification and enable full electronic data integrity.

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<sup>8</sup> See for example "[Quantification of Oxyethylene in Poloxamers in Full Automation on the Fourier 80](#)"

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