BIOPHARMA

Automated Analysis of Solutions and Formulations with Zero Sample Preparation on Benchtop NMR

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Innovation with Integrity

Pharmaceutical product development and quality control increasingly require rapid and reliable analytical methods that can analyze solutions and formulations directly, without involving tedious sample preparation. Bruker Fourier 80 benchtop NMR can address these needs by delivering automated, robust and high-quality analytical data from neat solutions through the new Multisupp package. This offers a pre-configured, fully automated setup that effectively overcomes key challenges associated with aqueous sample analysis at benchtop field strengths. It offers efficient water suppression while delivering consistently high spectral quality, reducing operational complexity and cost. Thus, the solution makes advanced NMR analysis accessible to laboratories without specialized NMR expertise through a compact, cryogen-free and easy-to-use spectrometer. This provides facilities with a new tool to accelerate analytical processes and support critical pharmaceutical applications, such as the demanding quality control of injectable formulations.

Introduction

It is becoming increasingly important for pharmaceutical R&D, process development and quality control (QC) to use rapid, reliable analytical methods for solution analysis that do not require extensive sample preparation. Whether monitoring formulation stability, quantifying excipients in high-value drug products, or tracking intermediates during process optimization, analytical tools that can enable direct measurement without dilution, extraction or solvent exchange are in high demand.

Among commonly used contactless spectroscopic methods-such as ultra-violet, infra-red, or Raman spectroscopies-Proton Nuclear Magnetic Resonance (^{1}H NMR) stands out for its ability to provide direct, absolute quantification combined with high chemical specificity. These advantages make NMR particularly well-suited for the analysis of complex mixtures and the performance of non-targeted analyses.

While these benefits are well established for high-field NMR, the emergence of benchtop NMR solutions, such as the Fourier 80, has significantly increased accessibility. By utilizing permanent magnets, benchtop NMR eliminates the need for cryogens, offering a compact, cost-effective and user-friendly method for direct deployment in development or QC laboratories. Additionally, the Fourier 80 does not require deuterated solvents, commonly associated with high-field NMR,[1] thus truly enabling the direct analysis of neat solutions. This capability extends to support direct process monitoring in flow systems.

One persistent challenge in direct NMR analysis of aqueous or partially aqueous solutions is the dominant signal from water. At benchtop field strengths, reduced spectral dispersion increases the likeliness of the water peak to overlap more with analyte resonances compared to high-field NMR. Effective suppression of this water signal, without distorting adjacent peaks, is therefore essential for accurate detection and quantification of solution components and for fully leveraging the analytical benefits of NMR.

Over the years, extensive strategies to address both single- and multiple-resonance suppression in high-field NMR have been developed and thoroughly discussed in existing literature.[2] These have been tailored to sample complexity, concentration ranges, presence of macromolecules and/or the need to detect exchangeable protons.

While benchtop NMR operates on the same fundamental physical principles as high-field instruments, specific factors related to permanent magnet design and probe characteristics require re-evaluation and adaptation to select the most effective water suppression strategies, as conclusions from high-field applications do not necessarily apply. These considerations have been detailed in a recent primer for the Fourier 80, to support the selection of optimal NMR sequences according to the specific analytical context.[3]

Such sequence selection and optimization can however be daunting for less experienced technicians and operators. Furthermore, certain water suppression techniques may require manual adjustments, limiting their suitability for routine applications by non-specialists. This is a critical consideration to maintain a robust analytical workflow in process development and QC.

In this manuscript, we introduce and discuss a key solution to address these challenges: Bruker's latest "Multisupp" package. This is a new, efficient, ultra-robust, and fully automated software tool designed to enable the direct and seamless NMR analysis of aqueous samples using the Fourier 80 benchtop NMR.

The Multisupp package delivers one of the most effective water suppression techniques on the Fourier 80. This feature is complemented by comprehensive automation that enables real-time optimization customized to each new sample. The combination provides consistently high suppression performance while offering a valuable setup to support the implementation of robust routine analytical procedures that take advantage of the benefits of NMR for direct quantification without sample preparation.

Additionally, Multisupp is easy to customize and does not require any expert knowledge. Its capabilities go well beyond basic water suppression, providing additional functionalities, such as carbon decoupling to improve the spectral resolution and T2 filtering to eliminate interference from large macromolecules, like proteins. It can also be used for protonated non-aqueous solutions, including those in organic solvents, and is effective even when multiple resonances need to be suppressed. Altogether, Multisupp offers a powerful yet user-friendly analytical tool for pharmaceutical applications involving solutions, including high-value protein formulations.

[1] For routine use, standard high-field NMR generally requires at least a fraction of deuterated solvent for lock regulation. On the benchtop Fourier 80, the lock is external and does not require any deuterated solvent.

[2] See for example: Giraudeau *et al. Metabolomics* **2015**, *19*,1041; Simpson *et al. Magn Reson Chem.* **2024**, *62*, 463

[3] Simpson *et al. J. Mag. Res. Open* **2024**, *19*, 100150

Optimized sequence for water suppression on the Fourier 80

The Multisupp package relies on a specific NMR sequence referred to as 'noesycpdcgpps1d' in the Bruker library, which has demonstrated exceptional efficiency on the Fourier 80 unit. A detailed discussion of NMR pulse sequences is beyond the scope of this manuscript, and readers can consult reference 3 for detailed information on the topic. However, for clarity it is important to highlight that the Multisupp sequence combines three key elements: a selective pre-saturation (PR), a gradient-assisted NOESY (ge-NOESY), and a composite pulse (CP) blocks, to maximize water suppression and spectral quality with the Fourier 80 (Figure 1). This optimized combination provides high selectivity in suppression, ensuring that signals of interest close to the water resonance remain observable. The sequence also offers an optional Carr-Purcell-Meiboom-Gill (CPMG) block for T2 filtering and ^{13}C decoupling capabilities,[4] which are discussed further in the last section of this document.

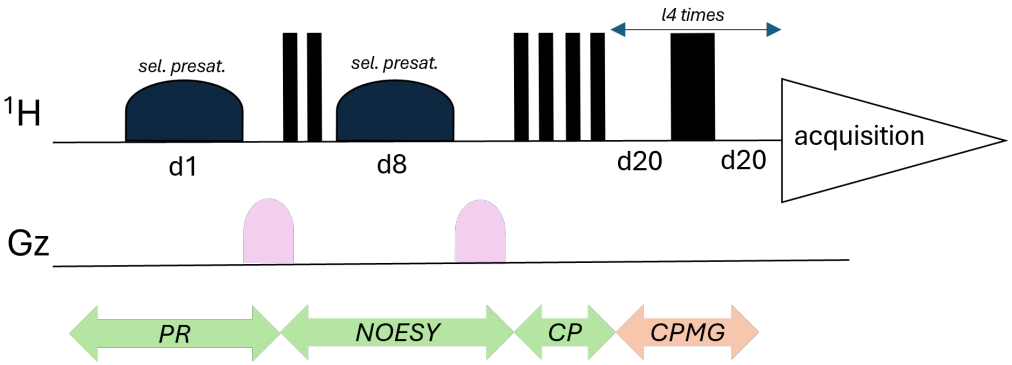

Figure 1: Simplified schematic representation of the noesycpdcgpps1d sequence. The green arrows indicate the three blocks for water suppression. The orange arrow indicates the optional CPMG block for T2 filtering. For clarity purposes, phase information and secondary channel are not reported in this diagram.

Figure 2 illustrates typical NMR spectrum obtained on the Fourier 80 for a mixture of excipients commonly used in monoclonal antibodies (mAb) formulations, made of histidine buffer, sucrose, histidine, methionine, and polysorbate 80 (PS80). The figure clearly demonstrates the effectiveness of the sequence in suppressing the water signal, which is almost completely eliminated. As a result, the spectrum is notably clean, supporting the straightforward detection and quantification[5] of each component in the mixture. In particular, the sequence produces a flat, interference-free baseline between the anomeric signal of sucrose at 5.2 ppm and its aliphatic region, beginning at 4.3 ppm. Without effective water suppression, these areas would typically be difficult, if not impossible, to analyze due to massive overlap.

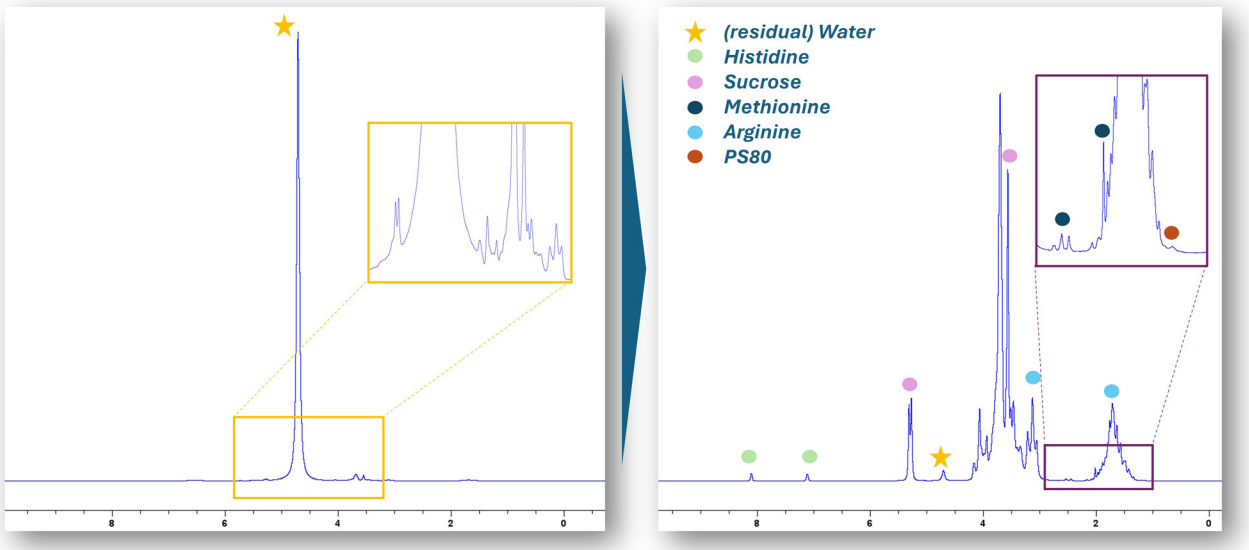

Figure 2: Example of NMR spectra of a typical mixture of excipients used in mAbs formulations without (left) and with (right) water suppression using the noesycpdcgpps1d sequence on the Fourier 80. The main specific signals for each specific species are indicated with colored circles. Inserts provide zoom-in views to highlight signal intensity.

[4] As of May 25, the Multisupp package provided with Topspin (starting with Topspin 4.4.0) does not contain CPMG block and is limited to D1 below ca. 30s. An updated version with CPMG and no D1 limit is available upon request and will be integrated in future releases of Topspin.

[5] Details on automated identification and quantification can be found in supplementary documentation.

The advantages of the noesycpdcgpps1d sequence on the Fourier 80 are further highlighted through comparison with alternative methods (Figure 3). Several sequences can provide acceptable results, such as combinations of PR with NOESY blocks (noesygppr1d, noesypr1d, noesygpps1d) or with CP (zgcppr). Nonetheless, the optimized combination (PR/NOESY/CP) of noesycpdcgpps1d can deliver superior result, offering enhanced water resonance suppression and an improved, flat baseline.

Conversely, the classical pre-saturation approach (zgpr) exhibited a more substantial residual water signal without a clear return to baseline. Finally, approaches based on the binomial Watergate and WET yielded significant residual signals and considerable suppression of nearby sucrose resonances.

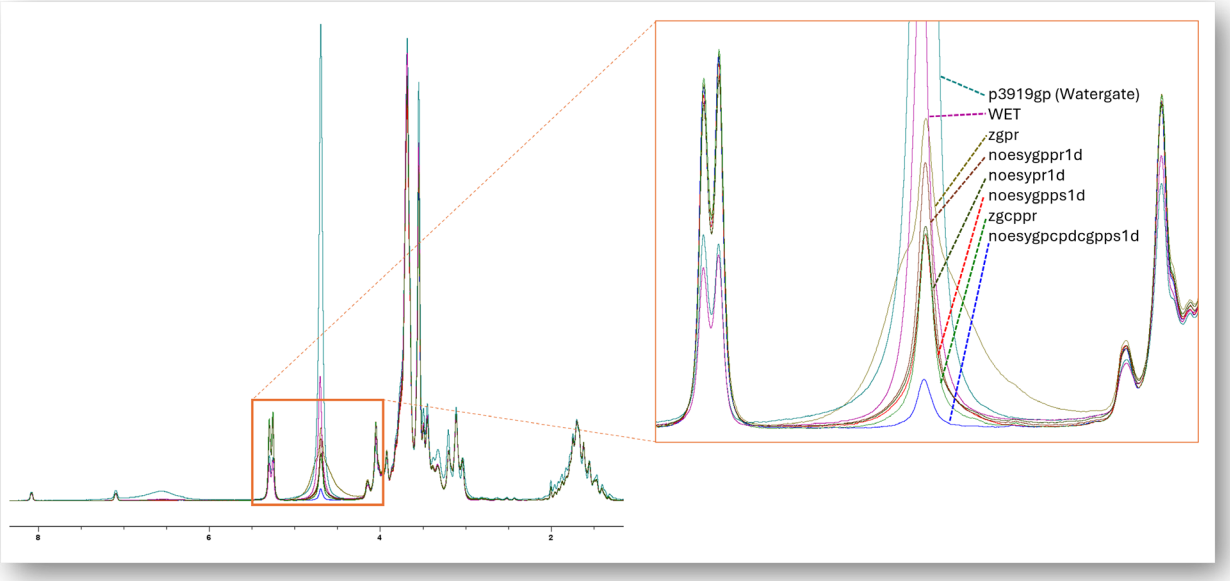

Figure 3: Comparison of several water suppression sequences for ^{1}H NMR recorded on a Fourier 80 on a model mixture of excipients and using identical settings (when applicable).

It is important to note that water suppression efficiency and resulting baseline quality can be significantly affected by sample composition and magnetic field homogeneity. Additionally, careful optimization of a particular sequence for specific sample types can lead to substantial performance improvements. Consequently, the comparisons presented in Figure 3 should not be interpreted as absolute conclusions but as indications. In fact, they showcase the strengths of the noesycpdcgpps1d sequence as one of the most effective methods available on the Fourier 80 NMR, especially for analyzing typical pharmaceutical excipient solutions, including formulations containing proteins (see last section for additional details).

Fully automated, on-the-fly optimized water suppression for robust, routine testing

The Multisupp package is designed to provide an end-to-end solution for optimized, fully automated water suppression on the Fourier 80, significantly reducing the expertise and effort typically required to effectively suppress water signals manually. The package not only uses the carefully designed noesycpdcgpps1d sequence but also includes comprehensive automation scripts ("AU") to manage all necessary settings for each new sample. This automation is crucial to ensure consistent suppression performance and simplify the analytical workflow for less experienced users. In effect, the noesycpdcgpps1d sequence involves shaped pulses that would otherwise require complex recalculation for each sample, particularly when common NMR parameters are changed, such as recycle delay (D1) or frequency offset (O1P). These steps are made entirely automated, transparent to the user, and managed by the script, which performs the following operations (see also Figure 4):

1. Detection of the exact resonance frequency of water in each sample via a scouting run
2. Generation of all necessary shaped pulses for optimal water suppression
3. Recording of the NMR data
4. Processing of the data

As a result, less experienced operators are empowered to conduct even highly sophisticated experiments using standard ICON-NMR or GoScan interfaces for sample submission without compromising suppression quality. Importantly, this automation eliminates potential user errors or subjective assessment in identifying the exact water resonance frequency. This, in turn, helps ensure robust and reproducible results while enhancing scalability, as the analyses can be performed without user intervention beyond sample insertion, thereby maximizing productivity.

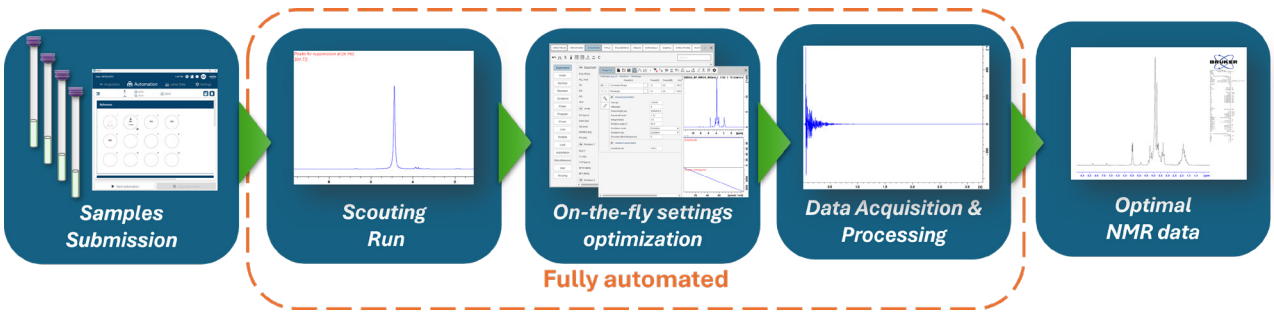

Figure 4: Scheme of the automated workflow followed by the Multisupp package.

For expert users, the Multisupp package can streamline configuration and method optimization. For example, the acquisition script allows users to directly set or adjust the pre-saturation field by entering the desired value into the designated configuration field (Figure 5). This eliminates the need for time-consuming manual pulse power calculations, which is typically required in conventional setups. Additional advanced functionalities, such as carbon decoupling (see last section) and multi-resonance suppression (beyond the scope of this document), can also be configured easily, making more complex implementations more accessible and efficient.

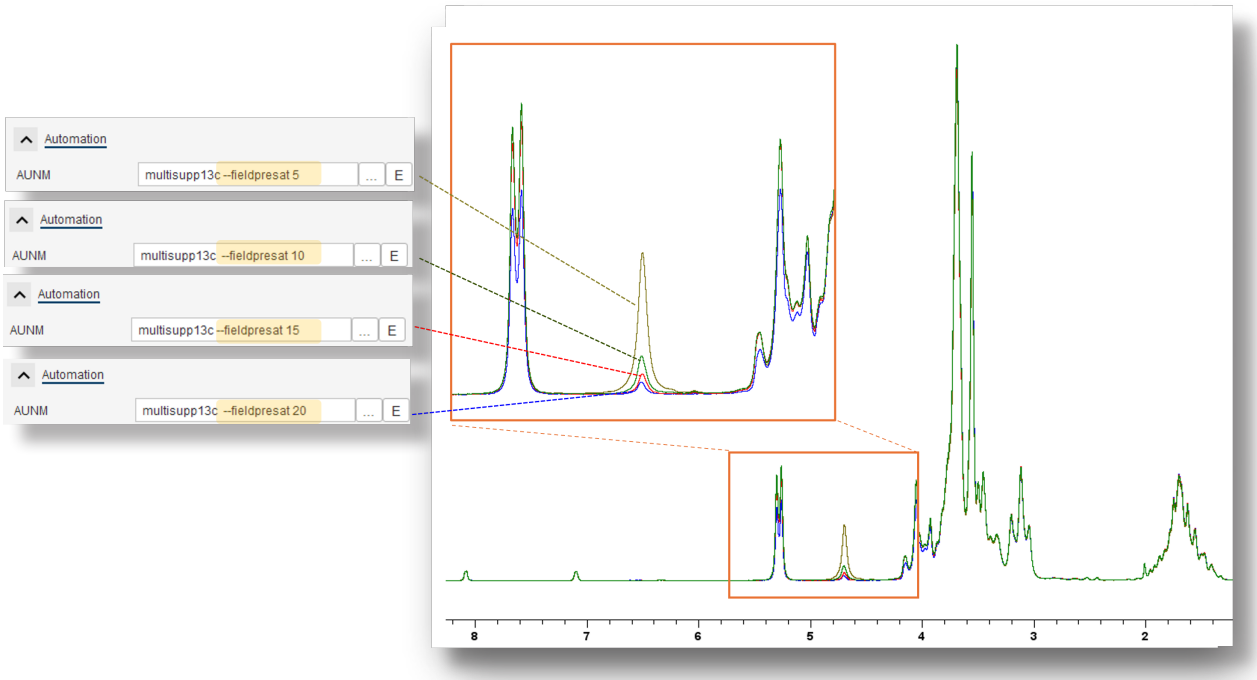

Figure 5: Example of field pre-saturation optimization performed directly, using the acquisition AU command "--filedpresat" with variation from 5 to 20 Hz done on an excipient mixture in water.

As reliability and robustness are critical for automated processes, Figure 6 illustrates how the Multisupp package can meet these requirements. The image shows overlaid spectra obtained from samples of similar composition (excipients in water), recorded and processed automatically, with triplicate measurements conducted on different instruments.

These resulting spectra are virtually identical, independently of the acquisition and spectrometer used, demonstrating how the tool can deliver excellent robustness in the analytical workflow. Thus, the Multisupp package delivers both highly repeatable NMR data acquisition capabilities on the Fourier 80 and consistent performance in water suppression.

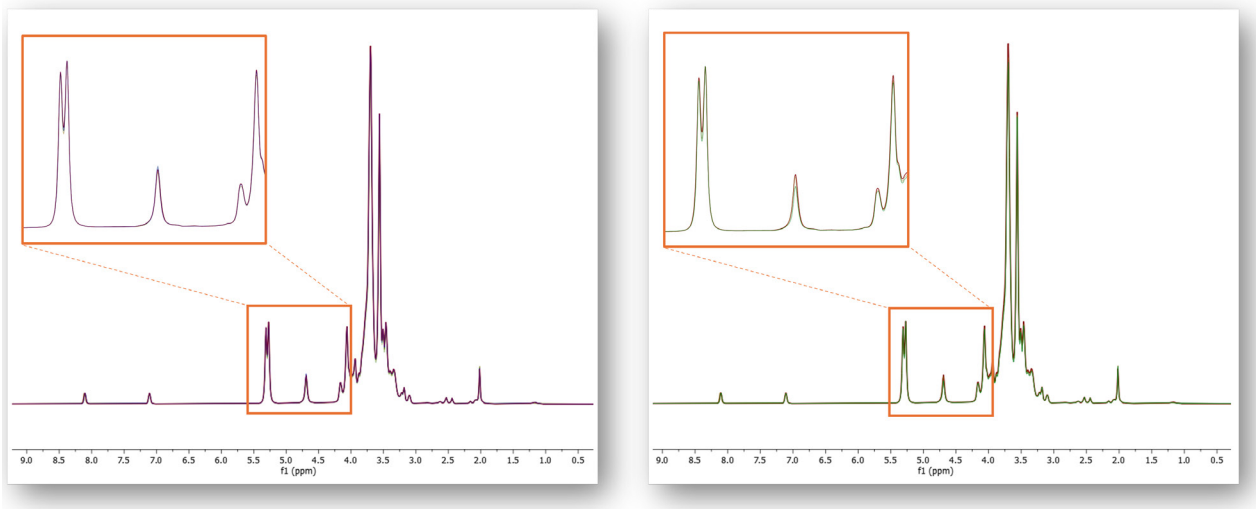

Figure 6: Overlays of ^{1}H NMR spectra from three replicate samples recorded in triplicate on a Fourier 80 (nine spectra in total, left) and from triplicate measurement of the same sample recorded on two different Fourier 80 (six spectra in total, right). All spectra were acquired and processed in a fully automated manner using the Multisupp package. Samples consist of a mixture of excipients in water (histidine buffer, sucrose, methionine, PS80), and correspond to sample "6" in Figure 7.

To support these qualitative conclusions with quantitative data, the ratio between water resonance areas before and after suppression was systematically evaluated across a series of similar samples on two Fourier 80 instruments. The results, shown in Figure 7, highlight the efficiency of the water suppression, with residual signals averaging 0.035% (350 ppm) relative to the unsuppressed spectra. The results also highlight the exceptional robustness, with negligible variability observed across samples, replicates and systems. Slightly lower residual water ratios observed on "Instrument 2" compared to "Instrument 1" are likely attributable to minor differences in magnetic field homogeneity, and do not impact the overall quality of the resulting spectra in Figure 6.

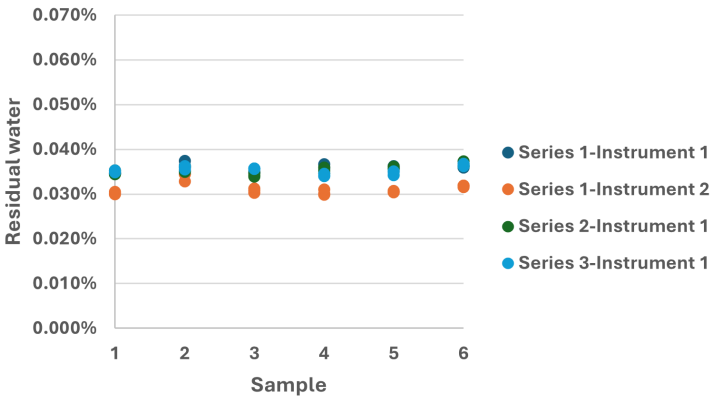

Figure 7: Plot of the residual water expressed as the % ratio of the absolute areas of the water resonance after and before water suppression (normalized by the number of scan and the flip angle). Three series of six samples were analyzed. Series 1 was recorded on two different Fourier 80 benchtop NMR units. Acquisition and processing were fully automated through the Multisupp package, in systematic triplicates for each sample. Series 1 to 3 are of similar composition (mixture of histidine buffer, sucrose, methionine, PS80 in water) with increasing PS80 concentrations from sample 1 to 6.

As previously mentioned, the efficiency of water suppression is influenced by sample composition and concentration, meaning the absolute residual water ratio is most useful for comparing performance within samples with similar compositions. To illustrate this, Figure 8 shows the residual water ratios across a dilution series of an excipient mixture, where the two most concentrated compounds, arginine and sucrose, range from 300 mM (sample 1) to 30 mM (sample 6).

At higher concentrations, water suppression efficiency decreases slightly (e.g. residual water ratio increase). This is expected, as both concentrated species contain exchangeable protons that rapidly exchange with water, affecting the suppression scheme. Despite this, overall suppression remains excellent, and the automated process demonstrates high robustness, with consistent results obtained across replicates and days. Furthermore, the slight differences in residual water signals between dilution levels are nearly imperceptible when inspecting the corresponding ^{1}H NMR spectra (see Figure 9). This is particularly valid when comparing the efficiency of the noesycpdcgpps1d sequence to alternative sequences (see Figure 3), as these differences have no impact on the baseline quality or the resolution of proximal resonances.

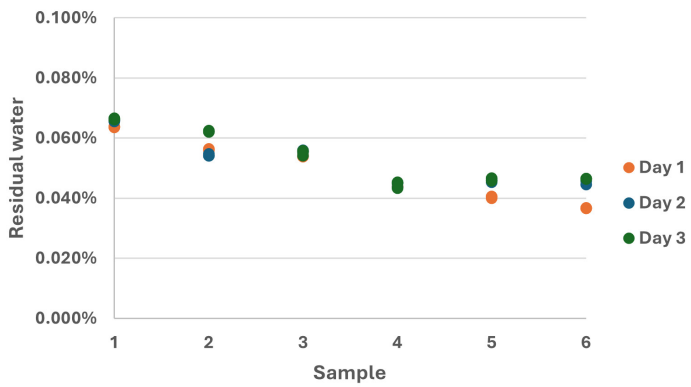

Figure 8: Plot of the residual water expressed as the % ratio of the absolute areas water resonance after and before water suppression (normalized by the number of scan and the flip angle) recorded on a dilution series of six samples across three days. Acquisition and processing were fully automated using the Multisupp package. Each sample was analyzed in systematic triplicate. The composition of the samples consisted of histidine buffer, sucrose, methionine, arginine and PS80 in water, with different dilutions from sample 1 to 6.

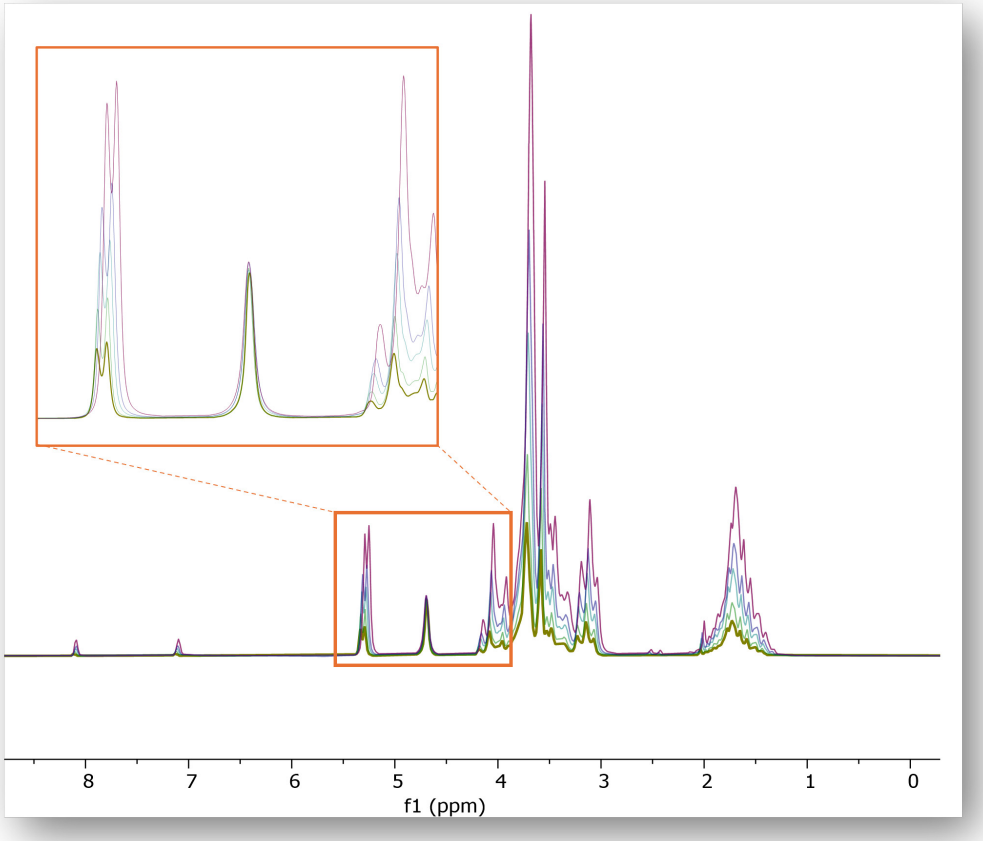

Figure 9: Overlays of ^{1}H NMR spectra from samples with six dilution levels recorded on a Fourier 80 benchtop NMR. All spectra were acquired and processed in a fully automated manner using the Multisupp package and correspond to the first replicate of day 1 shown in Figure 8. Samples are a mixture of excipients in water (histidine buffer, sucrose, methionine, arginine, PS80).

Overall, these examples showcase the capabilities of the Multisupp package as a tool that can offer highly efficient and robust automated water suppression for aqueous solutions. Through these capabilities, the technology can facilitate the implementation of reliable, straightforward and standardized analytical workflows for detecting and quantifying organic solutes via ^{1}H NMR, elements that are typically required in pharmaceutical development and QC applications.

Versatile solution for high-end analysis

For direct, quantitative analysis of aqueous solutions and formulations, additional NMR tools may be necessary to further enhance the quality of ^{1}H NMR spectra:

- Suppression of background signals generated by large macromolecules, such as proteins and mAbs. This can be achieved using a so-called T2 filter (via a CPMG block) in the NMR sequence, which exploits the significant relaxation time differences between large molecules and smaller molecules (e.g. excipients) to effectively eliminate protein-related interferences.
- Suppression of the coupling between protons and the NMR-active ^{13}C isotope, which constitutes approximately 1.1% of the total carbon present. This decoupling acquisition method merges satellite resonances with main resonances, resulting in a cleaner baseline and eliminating potential interference. This is particularly important when compounds with closely spaced ^{1}H NMR resonances have significantly different concentrations, as the satellite resonances from more concentrated compounds can otherwise interfere significantly with the signals from less concentrated compounds.

Both improvements are integrated into the Multisupp package and can be activated when needed. ^{13}C decoupling can be directly enabled through a simple script parameter, while the duration of the T2 filter can be set using the standard loop number (L4) and echo time (D20) parameters. For less experienced users, standard settings can be saved directly within a dedicated parameter set, eliminating the need for expert knowledge during routine use.

Figure 10 presents an example of baseline distortion due to protein background, which can be completely eliminated by activating the T2 filter in the noesycpdcgpps1d sequence. This produces a completely flat baseline, simplifying and facilitating the identification and quantification of excipients within the formulation.

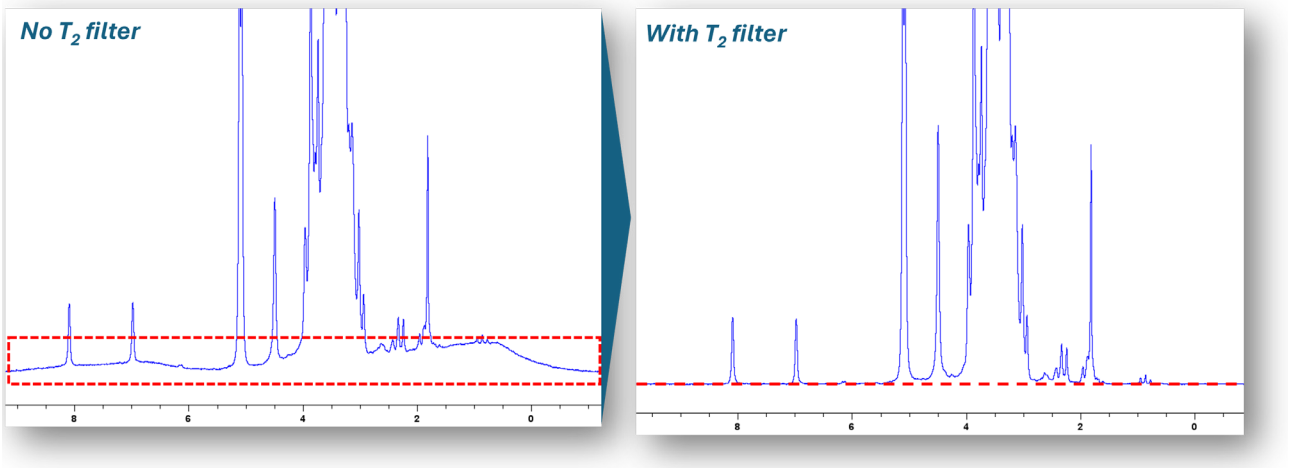

Figure 10: Zoom-in of ^{1}H NMR spectra of a mixture of histidine buffer, sucrose, methionine and bovine serum albumin (BSA) in water as recorded on the Fourier 80 benchtop NMR with the Multisupp package in a fully automated manner without (left) and with the T2 filter activated (right). The BSA background present in the spectrum without T2 filter causes a significant baseline distortion (red box) while T2 filtering completely remove this interference, resulting in a clean, flat baseline.

The benefits of ^{13}C decoupling are illustrated in Figure 11, where low-concentration excipients, such as methionine and PS80, experience interferences from ^{1}H-^{13}C satellite resonances originating from higher-concentration components (sucrose and arginine) in the formulation under examination. When ^{13}C decoupling is used, these interferences are effectively removed, significantly simplifying data analysis and quantification of lower-concentration species.

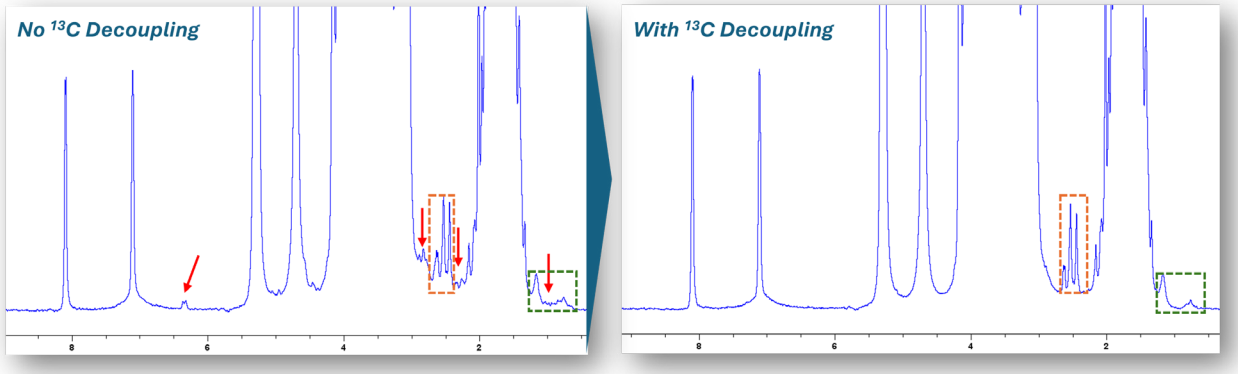

Figure 11: Zoom in of ^{1}H NMR spectra of a mixture of histidine buffer, sucrose, methionine, arginine and PS80 in water as recorded on the Fourier 80 benchtop NMR with the Multisupp package in a fully automated manner without (left) and with ^{13}C decoupling (right). Red arrows point to examples of ^{1}H-^{13}C satellite resonances, some interfering with resonances from the less concentrated methionine (orange box) and PS80 (green box). ^{13}C decoupling enables complete suppression of these interferences, improving resolution and simplifying any quantitative analysis of the data.

Like all previous results, these examples were obtained through fully automated procedures, without requiring any sample-specific setting or user input. All optimizations were performed on the fly by the Multisupp package, further demonstrating the tool's capabilities for implementation and routine use by less experienced professionals, while ensuring consistent and reliable spectral quality across all samples. Even more, T_2 filtering and ^{13}C decoupling are not mutually exclusive and can be combined if required, e.g., for complex biological formulations.

To further illustrate this capability, Figure 12 shows repeated automated acquisitions and processing of the same sample, repeated over three days on a Fourier 80 benchtop NMR instrument. The nine spectra obtained are virtually identical, except for minor changes reflecting the slow degradation of PS80. This similarity confirms the high reproducibility and robustness offered by both the Fourier 80 benchtop NMR and the Multisupp package, even when using complex combinations of water suppression, T_2 filtering, and ^{13}C decoupling. As a result, reliable and consistent day-to-day analytical results can be obtained.

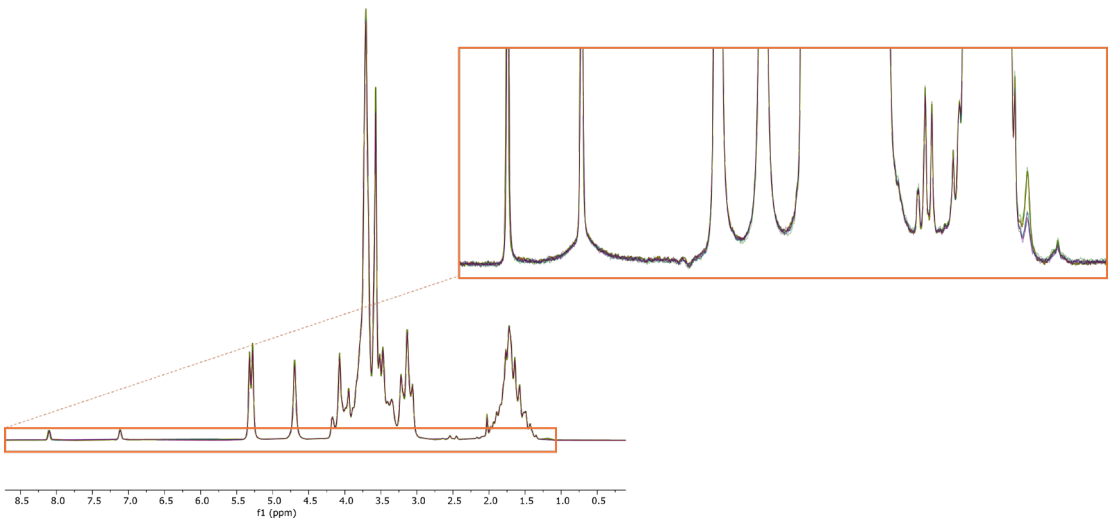

Figure 12: Overlay of nine ^{1}H NMR spectra of a model formulation consisting of histidine buffer, sucrose, methionine, arginine and PS80 in water. These spectra were acquired using the Fourier 80 benchtop NMR and the Multisupp package with both T2 filter and ^{13}C decoupling in a fully automated manner. The analysis was carried out on the same sample in triplicate on three separate days. The image also shows a zoom-in on the baseline for clarity. The only detectable change is attributed to PS80 degradation over time.

Finally, the capabilities of the Multisupp package extend beyond aqueous solutions. As its name implies, Multisupp can suppress multiple solvent resonances simultaneously, a critical advantage when analyzing samples containing organic solvents. Similarly to the process described for water, the tool automatically detects and optimizes the suppression of resonances, which are defined through a simple threshold. Users can therefore benefit from intuitive, robust multi-solvent suppression, a capability that is extremely beneficial for direct reaction monitoring under static or flow conditions.

Consequently, the Multisupp package offers a versatile and powerful tool for direct solution analysis by NMR using the Fourier 80, as it enables the generation of high-quality NMR spectra in a fully automated manner. When combined with automatic data analysis technologies, such as Bruker's latest Advanced Chemical Profiling (ACP), it supports a broad range of applications and analytical end-to-end workflows in pharmaceutical laboratories, from simple solution monitoring to complex and rigorous QC procedure of injectable formulations (Figure 13).

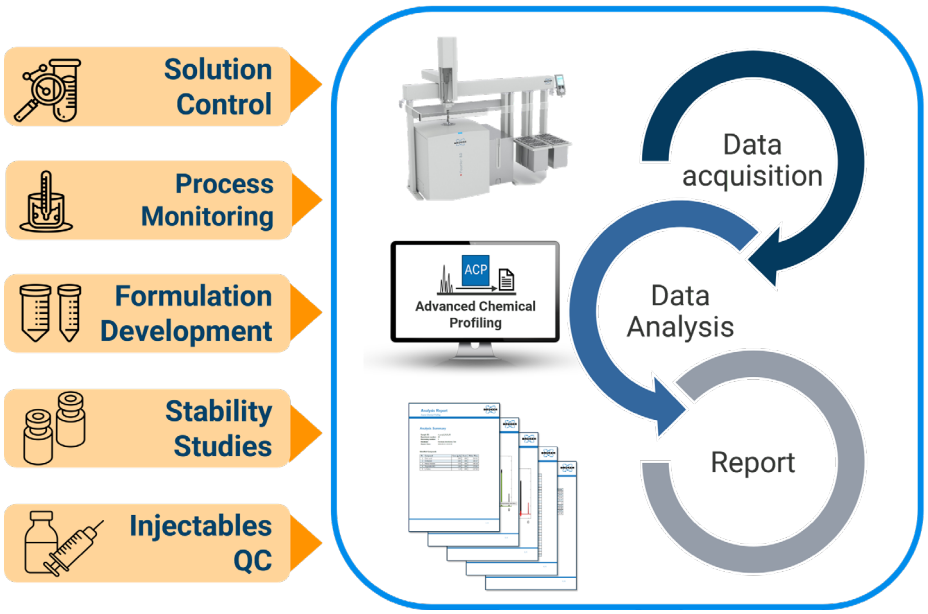

Figure 13: Example of possible use cases enabled by the Multisupp package combined with ACP on the Fourier 80 benchtop NMR for end-to-end analytical workflows without sample preparation.

Conclusions

Compact, cryogen- and maintenance-free benchtop NMR spectroscopy based on Bruker's Fourier 80 technology significantly improves pharmaceutical development and QC by providing a cost-effective setup for fast, reliable and automated analysis. With the optimized Multisupp package, it offers a unique solution for reliable, robust and direct analysis of solutions and formulations that eliminates time- and resource-intensive sample preparation, shortening time-to-result. The fully automated setup helps ensure the generation of consistent, reliable, high-quality data, enabling effective routine use even by less experienced operators.

The innovative combination of the Multisupp package and the Fourier 80 benchtop NMR extends the powerful capabilities of NMR spectroscopy beyond specialized labs, making advanced analysis accessible in standard laboratory settings. In effect, it turns NMR into a scalable, everyday solution for pharmaceutical and analytical workflows.

When this setup is used in combination with the new Advanced Chemical Profiling software, it provides a comprehensive platform for end-to-end automated analytical workflows, from data acquisition to advanced quantitative data analysis. It accommodates a variety of applications in the pharmaceutical industry, including highly regulated environments, as it includes built-in GMP compliance.

Looking ahead, the combination of benchtop NMR and advanced data analysis tools will continue to enhance analytical possibilities, particularly in the rigorous and demanding area of injectable formulation QC, demonstrating the technology's pivotal role in pharmaceutical development and quality assurance. Detailed examples of such critical applications will be provided in supplemental manuscripts, showcasing the Fourier 80 as a comprehensive and innovative solution for direct multi-excipient assays in injectable drug products, including the challenging detection and quantification of surfactant.

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