

## BIOPHARMA

# Adapting and Streamlining Compendial Procedures with the Fourier 80 Benchtop NMR Spectrometer: Examples of the USP-NF and Ph. Eur. Betadex Testing

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Innovation with Integrity

Natural cyclodextrins (CDs) and their synthetically modified counterparts, hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD) and sulfobutyl- $\beta$ -cyclodextrin (SB- $\beta$ -CD), are essential pharmaceutical excipients and, in some cases, active ingredients. The chemical modification of CDs significantly impacts their complexation efficiency and safety, requiring precise control. This is achieved in compendial testing using Nuclear Magnetic Resonance (NMR). This whitepaper demonstrates how the USP-NF and Ph. Eur. NMR assay tests for HP- $\beta$ -CD and SB- $\beta$ -CD can be efficiently implemented (or adapted) to the Fourier 80 benchtop NMR spectrometer. This compact, cryogen-free NMR system, perfectly designed for Quality Control laboratories, offers a practical, flexible and compliant solution for determining the grafting ratio of CDs under full automation.

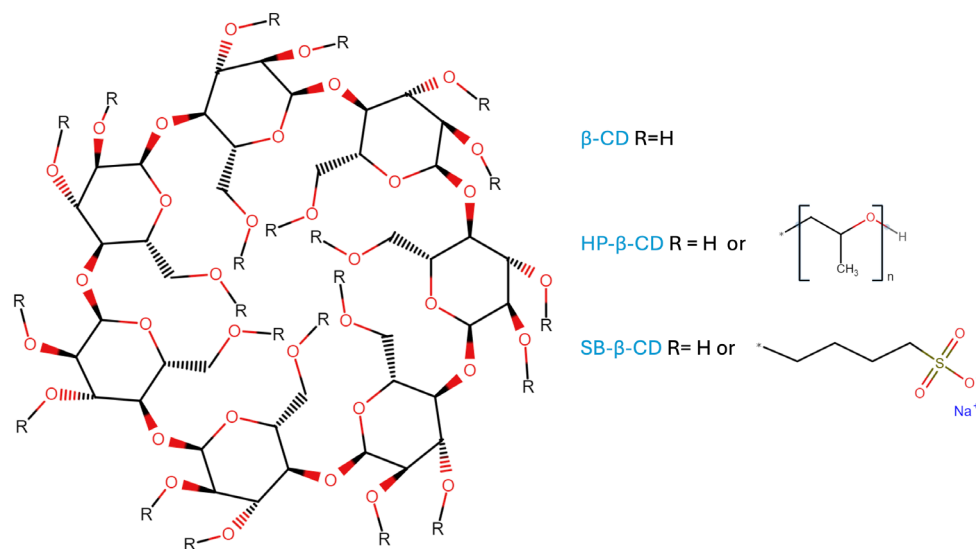
## Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides derived from starch, well-known for their ability to form inclusion complexes with various guest molecules. This property makes them invaluable in pharmaceutical applications where they enhance the solubility, stability, and bioavailability of poorly soluble drugs. Cyclodextrins, particularly  $\alpha$ -,  $\beta$ -, and  $\gamma$ -cyclodextrins (respectively 6, 7, and 8 glucose units), have been extensively utilized to improve drug delivery across various formulations including oral, nasal, pulmonary, and parenteral routes.<sup>1</sup>

To enhance the solubility profiles of cyclodextrins, chemical modifications were attempted. In the early 1980s, both Janssen Pharmaceutica and the National Institutes of Health filed patents for the use of hydroxypropyl- $\beta$ -cyclodextrin (HP- $\beta$ -CD, also named hydroxypropylbetadex) in pharmaceutical formulations. In this chemically modified variant of  $\beta$ -cyclodextrin, (poly)hydroxypropyl groups are grafted onto the hydroxyl groups on the cyclodextrin ring (Figure 1).

<sup>1</sup> See for example E.M.Martin Del Valle, *Process Biochem.*, **2004**, 39(9), 1033-1046

This modification significantly enhances its water solubility and reduces its toxicity, making HP- $\beta$ -CD particularly suitable for use in injectable formulations and other aqueous systems. The increased solubility of HP- $\beta$ -CD allows for the effective incorporation of hydrophobic drugs, thereby improving their bioavailability and therapeutic efficacy. Today, HP- $\beta$ -CD, and another derivative known as sulfobutyl- $\beta$ -cyclodextrin (SB- $\beta$ -CD or sulfobutylbetadex), are well-established excipients, recognized for their ability to improve the solubility and stability of numerous drugs.<sup>2</sup>



**Figure 1:** General structure of  $\beta$ -CD and its chemically modified derivatives HP- $\beta$ -CD and SB- $\beta$ -CD.

	$\alpha$ -CD	$\beta$ -CD	$\gamma$ -CD	HP- $\beta$ -CD	SB- $\beta$ -CD	RM- $\beta$ -CD
<b>Oral</b>		X	X	X	X	
<b>Nasal</b>						X
<b>Rectal</b>		X		X		
<b>Dermal</b>		X	X	X		
<b>Ocular</b>		X		X		X
<b>Parental</b>	X			X	X	

**Table 1:** Use of cyclodextrins in type of pharmaceutical products (RM- $\beta$ -CD: randomly methylated  $\beta$ -CD).<sup>3</sup>

Cyclodextrins and their derivatives not only act as excipients but can also have active properties at high doses. Their use is thus closely monitored and regulated by national agencies. Natural and synthetically modified cyclodextrins have found their way into pharmacopeias such as the United States Pharmacopeia-National Formulary (USP-NF) and the European Pharmacopoeia (Ph. Eur.). Both have specific monographs for the naturally occurring  $\alpha$ -CD,  $\beta$ -CD, and  $\gamma$ -CD. For the chemically engineered cyclodextrins, the predominant HP- $\beta$ -CD is also present in both USP-NF and Ph. Eur. (1804) while SB- $\beta$ -CD is only described in Ph. Eur. (2804).<sup>4</sup>

For these two modified cyclodextrins, the ratio of (poly)hydroxypropyl or sulfobutyl grafts on the  $\beta$ -cyclodextrin ring is a critical factor influencing complexation efficiency and safety. The required substitution ratio will depend on the use and desired properties of the modified cyclodextrin and thus must be tightly controlled as a critical functionality-related characteristic. Insertion of hydroxypropyl or sulfobutyl moieties on the hydroxyl groups of CDs is obviously statistical and depends on the manufacturing process. In order to control the ratio, it is therefore necessary to have an appropriate analytical method which can quantitatively control the product at the molecular level with a high degree of accuracy.

<sup>2</sup> S. Gould *et al.* Food Chem. Tox., **2005**, 43(10), 1451-1459; M. Malanga *et al.* J. Pharm. Sci., **2016**, 105, 2921-2931; Z. Li *et al.* J. Mol. Liq., **2022**, 365, 120105

<sup>3</sup> EMA/CHMP/333892/2013

<sup>4</sup> As of January 2026

Nuclear Magnetic Resonance (NMR) is the only available analytical technology able to directly provide such information, at least without tedious destructive and empirical techniques. NMR spectroscopy is a very well-established method for structural characterization. In the case of polymeric structures, NMR is the analytical technique of choice, able to provide detailed information of their composition and examine their microstructure with the significant advantages of being inherently quantitative and non-destructive. In the case of HP- $\beta$ -CD and SB- $\beta$ -CD, NMR was thus selected from the start as the compendial method for controlling the conformity of this critical value.

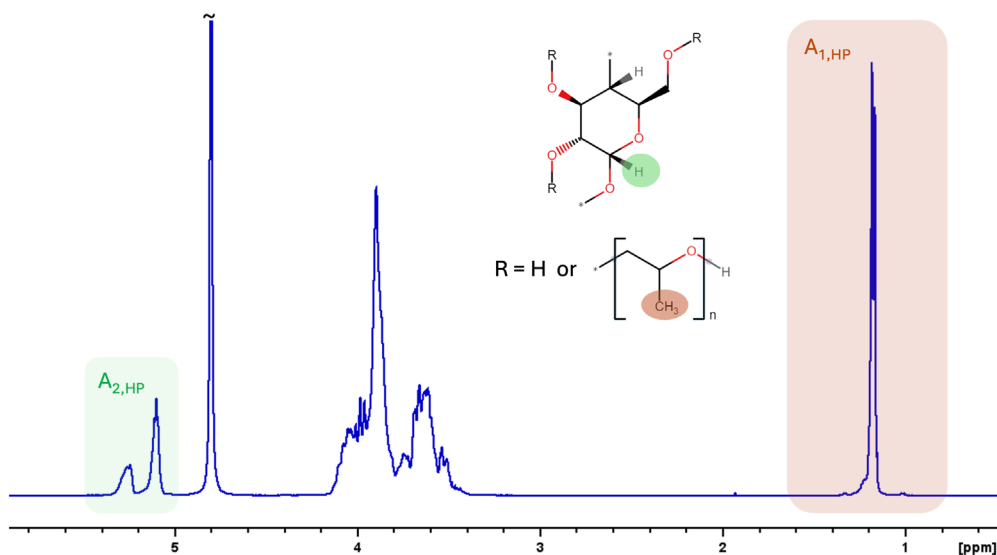
It is worth mentioning that this is quite an exception as very few compendial tests rely on NMR technology. NMR has indeed been rapidly established as one of the fundamental analytical techniques in academia. In the case of CD, it proved a valuable tool to study molecular interactions between cyclodextrins and guest molecules, including the stoichiometry of complexation, binding constants, and the orientation of guest molecules within the cyclodextrin cavity. Nonetheless, the availability of NMR in the private sector has been much more limited due to the cost and the level of expertise required. GMP-compliant systems were even rarer. Thus, for a long time, NMR was perceived as a technique to avoid for compendial procedures. This paradigm is now rapidly changing. Spectrometers are simpler to use and can be operated on a routine basis by non-experts because of significant improvements in user interfaces, fully automated procedures, and simplified maintenance operations. The recent progress in GMP compliance of NMR spectrometers and the introduction of benchtop systems have also further accelerated the process, as evidenced by the recent introduction of NMR in the ICH Q2(R2) and the revision of USP-NF NMR general chapters <761> and <1761> effective as of Dec, 1<sup>st</sup> 2025.

In this respect, the Fourier 80 is an ideal tool for QC laboratories as it brings the benefits of NMR in a compact system with virtually no maintenance and supports full compliance with pharmaceutical regulations. In this whitepaper, we will discuss how the current USP-NF and Ph. Eur. monographs for the molar substitution determination of HP- $\beta$ -CD and SB- $\beta$ -CD can be easily implemented or adapted to this modern system to alleviate some of the challenges associated with high-field NMR systems while ensuring a fit-for-purpose procedure.

## Control of Hydroxypropylbetadex and Sulfobutylbetadex by NMR

The grafting ratio determination of HP- $\beta$ -CD and SB- $\beta$ -CD by NMR is straightforward as proton ( $^1\text{H}$ ) NMR resonances specific to the cyclodextrin ring and to the grafted moiety are directly accessible. As NMR is inherently quantitative (provided suitable acquisition conditions), integration values of these resonances can be directly leveraged for molar ratio calculation.

In the case of HP- $\beta$ -CD, the  $A_{2,HP}$  area is specific of the glycosidic protons (1 per glucose unit) and the  $A_{1,HP}$  are specific to the three protons of the methyl group of the (poly)hydroxypropyl moieties as illustrated in Figure 2.



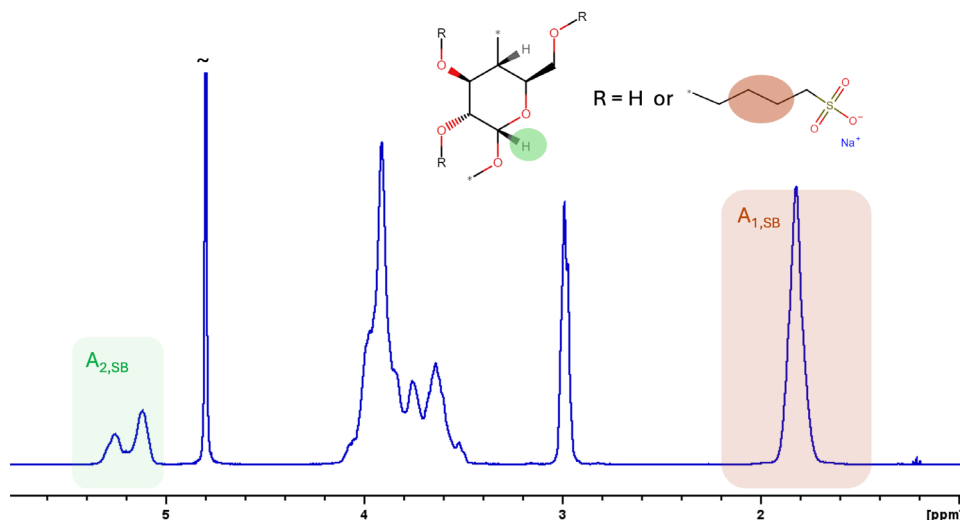
**Figure 2:** Example of  $^1\text{H}$  NMR spectrum of HP- $\beta$ -CD in  $\text{D}_2\text{O}$  as recorded on a 400 MHz AVIIIHD NMR Bruker spectrometer. Areas defined in USP-NF and Ph. Eur. 1804 for HP- $\beta$ -CD are indicated as colored boxes with the corresponding structural attribution. Only one anhydroglucose unit of the CD ring is represented.

The grafting ratio, in the Ph. Eur. and USP-NF monographs, is expressed as the average number of hydroxypropyl groups per anhydroglucose unit and is referred to as the molar substitution (MS). It is simply accessible via the equation:

$$MS = \frac{A_{1,HP}}{3 \times A_{2,HP}}$$

**Equation 1:** MS calculation for HP-β-CD according to Ph. Eur. 1804 and USP-NF.

In the case of SB-β-CD, the grafting ratio is expressed as the average number of sulfobutyl groups per cyclodextrin ring. In this instance, it is so called average degree of substitution (DS) by the Ph. Eur. monograph. In a comparable manner to HP-β-CD, the  $A_{1,SB}$  area is specific to 4 protons (so-called "inner CH2") of the sulfobutyl moiety of SB-β-CD while the  $A_{2,SB}$  accounts for the glycoside proton (1 per glucose unit, Figure 3). As the whole ring is constituted of 7 anhydroglucose units, the DS is calculated according to Equation 2.



$$DS = \frac{7 \times A_{1,SB}}{4 \times A_{2,SB}}$$

**Figure 3:** Example of  $^1\text{H}$  NMR spectrum of SB-β-CD in  $\text{D}_2\text{O}$  as recorded on a 400 MHz AVIITHD NMR Bruker spectrometer. Areas in Ph. Eur. 2804 for SB-β-CD are indicated as colored boxes with the corresponding structural attribution. Only one anhydroglucose unit of the CD ring is represented.

**Equation 2:** DS calculation for SB-β-CD according to Ph. Eur. 2804.

Prescriptions for the HP-β-CD NMR testing in the USP-NF and Ph. Eur. are virtually identical and the procedure workflow straightforward. It consists of the simple dissolution of the sample in deuterated water ( $\text{D}_2\text{O}$ ) and then recording of the  $^1\text{H}$  NMR of the resulting solutions and data processing to yield the area values described above, allowing calculation of the MS or DS. Few technical prescriptions are specifically provided, and one will have to refer to the general chapters of the pharmacopeia to implement the complete NMR method and notably ensure the quantitative aspect of the procedure. The 1804 monograph of the Ph. Eur. is a little more detailed regarding the instrumental part of the procedure compared to the USP-NF. Both contain two important requirements:

- Originally, a minimal NMR  $^1\text{H}$  frequency of 250 MHz *e.g.* a high field NMR system. USP-NF monograph was however revised in August 2025 to lower this minimal field to 60 MHz, officially recognizing the applicability of benchtop NMR for such testing as discussed hereafter. As of January 2026, Ph. Eur. 1804 (and 2804) was not revised.
- Sample must be dried before preparation – this implies an additional, tedious step as it must be performed in conformance with the corresponding general chapter.

These combine with additional, more subtle "hints" in the procedure that the resonance of residual water may have been identified as a potential issue during the initial development of this assay:

- The Ph. Eur. requires a very high degree of deuteration of the  $\text{D}_2\text{O}$  to be used (99.95% minimum, while USP-NF requires at least 99.8%).
- Both monographs indicate that very little line broadening should be used during data processing (LB factor of not more than 0.2 Hz).

The same global consideration applies to the Ph. Eur. 2804 monograph for the DS determination of SB- $\beta$ -CD. The monograph is more precise and prescriptive in the description of the NMR acquisition and processing parameter settings, but the above requirements are virtually identical (except the LB factor accepted up to a factor of 0.3 Hz).

As it can be seen on the example spectra of Figure 2 and Figure 3, the signal of the so-called residual water is indeed in close proximity to those of the A<sub>1</sub> areas, potentially interfering with them. One can suppose that at the time of the original procedure design, ensuring a minimal NMR field and trying to minimize the presence of residual water were chosen as a control strategy to mitigate the risk of this potential interference. This could, however, be challenged since water uptake may be difficult to control during sample preparation and, more importantly, the “residual water” resonance is not only due to water. Exchangeable protons from the hydroxyl group will also contribute to this signal – as they are numerous on the HP- $\beta$ -CD and SB- $\beta$ -CD structure, this resonance is inherently significantly present (area of at least 3 times that of A<sub>1</sub> resonances in HP- $\beta$ -CD for example).

In the next section, we will demonstrate that this test can actually be advantageously performed on the Fourier 80 benchtop NMR spectrometer (operating at 80 MHz or 1.9 T) even in the significant presence of water, alleviating both the need for a high-field NMR system and the strict control of the source of water during sample preparation. Such modifications of the compendial procedures obviously implicate the necessity of a full revalidation of the procedure instead of a somewhat shorter verification. Nonetheless, the benefits of cost and simplicity of performing this test on the Fourier 80 will probably compensate for the initial effort of revalidation.

## Performing the Compendial Testing with the Fourier 80

Performing the MS testing on HP- $\beta$ -CD and DS on SB- $\beta$ -CD on the Fourier 80 can be seen as an alternative method to the compendial ones, at least with respect to the Ph. Eur. monographs, given that using a magnetic field significantly lower than the minimal one indicated in the monographs can have a significant impact. As such, it is required to demonstrate that the new method can produce comparable results in the framework of a formal validation process. One of the challenges of such approach is that the validation criteria of the compendial method are not known and thus the acceptance criteria for the new method must be carefully selected to justify the acceptability of the alternative method.

In this section, we will describe how to achieve such a demonstration. Given the overall design of the procedure and its purpose, a target deviation compared to the reference method of not more than 3% was chosen, while the precision criterion was defined at better than 1% when expressed as the relative standard deviation. These stringent criteria were selected for the sake of illustration, but the steps described herein do not constitute a formal validation process. They are provided to give some key examples and possible justifications, to help laboratories seeking to implement such testing using the Fourier 80.

Given the strong similarities of the NMR procedure for the HP- $\beta$ -CD and the SB- $\beta$ -CD testing, we will focus on the former as a general example with detailed results. SB- $\beta$ -CD will be illustrated as a concluding example of the present work.

## MS Testing of HP- $\beta$ -CD Using the Fourier 80

To demonstrate the applicability of the Fourier 80 to perform the MS testing of HP- $\beta$ -CD, three different sources of HP- $\beta$ -CD were investigated, and the MS determined using a high field NMR system (400 MHz) are reported in Table 2. It is worth mentioning that only the commercial grade reports an MS value on its certificate of analysis, in agreement with the experimentally determined value.

Material	Supplier	Reference	Batch	Grade	MS from CoA	Reference MS <sup>a</sup>
1	EDQM	Y0000186	002E1J	EP Reference Standard	/	0.64
2	Supelco	PHR1440	LRAD3826	Pharmaceutical Secondary Standard	/	0.74
3	Merck	1.42020.0050	K55638020 428	Raw Material (comply with Ph. Eur.)	0.62	0.62

a: as determined using a 400 MHz AVIIIHD spectrometer using the compendial procedure CoA: Certificate of Analysis.

**Table 2:** HP- $\beta$ -CD batches used in this study.

After initial testing, the procedure could be adapted in a simple fashion to the Fourier 80. As for any quantitative NMR procedure, it was first critical to ensure full relaxation of the spins between each scan. This was assessed during the initial setup, running the typical inversion-recovery experiment on a representative sample of HP- $\beta$ -CD, yielding a minimal time of 2.1 s.<sup>5</sup>

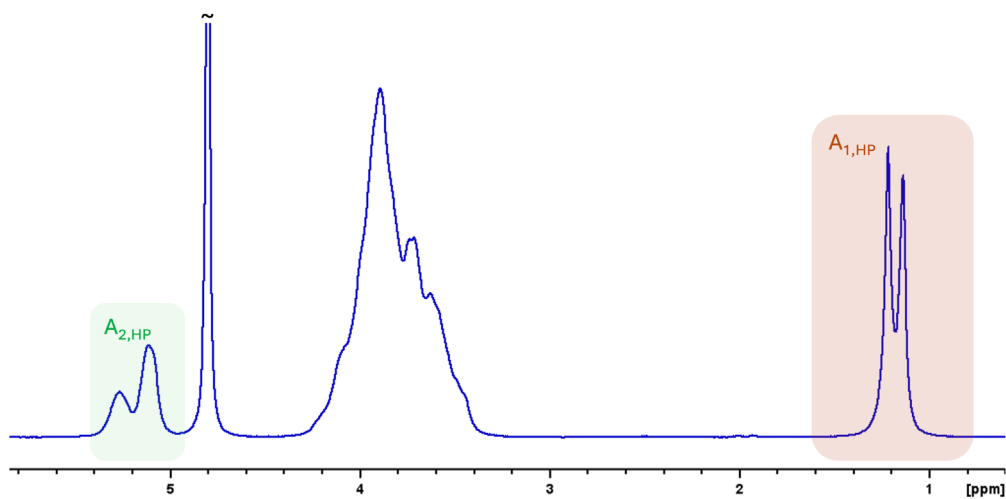
Based on these results, the typical conditions used for <sup>1</sup>H quantitative acquisition on the Fourier 80 and the original monographs, the default parameters used during this study were selected as reported in Table 3. In this instance the total recycle delay, which is the sum of D1 and AQ was 3.5 s, which is well above the minimal one of 2.1 s.

Importantly, after this initial implementation, all data were acquired and processed in full automation, removing any potential bias from operator actions, as it would be required for a routine testing in a QC laboratory.

<b>Sample concentration</b>	50 mg/mL
<b>Temperature</b>	25 °C
<b>Sequence</b>	ZG
<b>Number of scans (NS)</b>	32
<b>Inter-pulse delay (D1)</b>	1 s
<b>Spectral Width (SW)</b>	20 ppm
<b>Transmitter Offset (O1P)</b>	6.2 ppm
<b>Acquisition Duration (AQ)</b>	2.5 s
<b>Time Domain Point (TD)</b>	8K
<b>Frequency Domain Point (SI)</b>	8K
<b>Apodization function</b>	Exponential
<b>Line Broadening Factor (LB)</b>	0.2 Hz

**Table 3:** Default parameters of the method for MS determination HP- $\beta$ -CD using the Fourier 80. Abbreviation of the acquisition and processing parameters as used in Bruker software are indicated in italic.

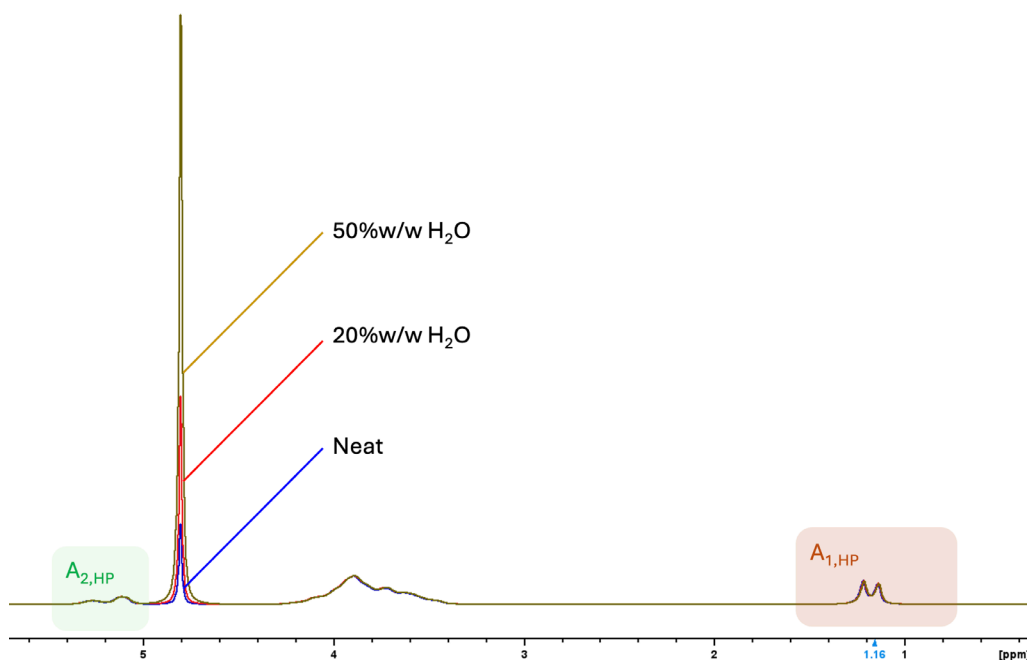
As mentioned in the previous section, the main point of concern when implementing this procedure was the proximity of the exchangeable proton resonance with the A2 area, possibly resulting in interference and thus a lack of specificity (Figure 4).



**Figure 4:** Example of <sup>1</sup>H NMR spectrum of HP- $\beta$ -CD in D<sub>2</sub>O recorded on a Fourier 80 benchtop spectrometer using parameters of Table 3. Areas defined in USP-NF and Ph. Eur. 1804 for HP- $\beta$ -CD are indicated as colored boxes.

<sup>5</sup> The longest measured T1 was 300 ms and a factor of 7 was selected to ensure full relaxation. See for example USP-NF <761> and <1761>

This risk was certainly present and to avoid the possible contribution of the exchangeable proton resonance to those of the  $A_2$  area, a baseline correction routine was carefully designed. It ensures that integration of the  $A_2$  area is specific to the HP- $\beta$ -CD resonances. This routine combines both global then local baseline corrections to eliminate any potential interference and it was implemented during the fully automatic processing. This strategy was then challenged to ensure a fit-for-purpose procedure. This was accomplished by 1) ensuring that the results matched the reference ones from Table 2 (bias assessment) and 2) deliberate addition of water to the sample prior to performing the NMR testing (robustness assessment). This was done up to a content in water of 50%w/w vs the neat HP- $\beta$ -CD. Figure 5 helps understand visually the impact of such extreme content of water on the resulting  $^1\text{H}$  spectrum.



**Figure 5:** Overlays of  $^1\text{H}$  NMR spectra in  $\text{D}_2\text{O}$  of HP- $\beta$ -CD with increasing content of added water recorded on a Fourier 80 benchtop spectrometer using parameters of Table 3. Areas defined in USP-NF and Ph. Eur 1804 for HP- $\beta$ -CD are indicated as colored boxes.

The results presented in Table 4 demonstrate that the implemented processing strategy was perfectly suited to yield results without bias compared to the reference ones (deviation within the example 3% criteria), while remaining perfectly robust toward significant presence of water.

Material	Experimental MS with Fourier 80	Deviation (%)
1 (neat)	0.65	1.3
2 (neat)	0.76	2.3
3 (neat)	0.63	1.8
3 / water (20%w/w)	0.63	1.0
3 / water (50%w/w)	0.62	0.0

**Table 4:** Bias testing using the Fourier 80 for the MS determination of HP- $\beta$ -CD. Values are rounded for comparison to specifications. Deviation is expressed by comparison to the value obtained at 400 MHz for a given material (see Table 2). For material 3, result of the precisions study is reported.

Such tests not only allow to demonstrate that the procedure based on the Fourier 80 fits the intended purpose but also alleviate the concern about the presence of water in the sample. A content of 50%w/w is indeed unlikely (at least from simple water uptake), yet it still does not impact the results. This first step thus demonstrates the specificity of the method and allows for a very large design space when referring to the content of water. Drying and using very high-quality deuterated water as prescribed in the original monograph is thus most likely not required and more flexible prescriptions can be made. This could be for example a maximal acceptable area for the exchangeable proton resonance relative to those of HP- $\beta$ -CD, based on data collected during a formal validation process, demonstrating the absence of effect below this limit.

Besides a potential bias, the precision of this alternative procedure also needed to be verified. This was assessed through the systematic measurement of 6 independent preparations, each being recorded and processed 6 times (repeatability). Then the first 3 preparations were subsequently remeasured 3 times after 1 and 2 days of storage at room temperature. Even if the same preparations as day 1 were used and neither the operator nor the instrument varied, this can be considered as an intermediate fidelity test as:

- The operator effect in here is not relevant as the procedure is fully automatic.
- The instrument effect is supposed to be minimal, NMR being a universal and absolute technology (e.g. two systems of the same field while yield the same spectrum), as explicitly specified in ICH Q2(R2).
- The sample preparation itself cannot induce any variability since attributes of the same molecule are assessed (relative quantitation). Using identical sample preparations over several day is beneficial as it allows to directly account for the stability of the preparation in the results.

Results are reported in Table 5. Both the precision and the intermediate fidelity are well below 1.0%, validating these criteria. Even more remarkably, the whole set of data (54 values) yield a very narrow confidence interval of 0.05% at the 95% confidence level.

	Day	Number of Preparations	Number of Measurement by preparation	Average MS	RSD (%)	Total Average MS	Total RSD (%)
<b>Repeatability</b>	1	6	6	<b>0.63</b>	<b>0.3</b>	/	/
	1	3	3	0.63	0.3		
<b>Intermediate Fidelity</b>	2	3	3	0.63	0.3	<b>0.63</b>	<b>0.3</b>
	3	3	3	0.63	0.2		

**Table 5:** Results for repeatability and intermediate fidelity for the MS determination of HP- $\beta$ -CD using the Fourier 80 and the default procedure parameters. Values are rounded for comparison to specifications.

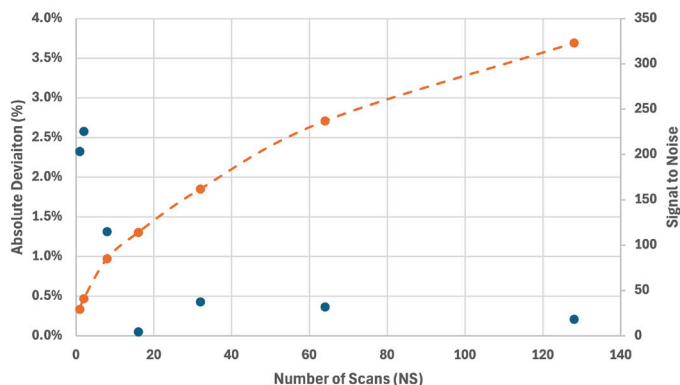
These results definitively confirm that the adapted procedure for determining the MS of HP- $\beta$ -CD using the Fourier 80 is fit for purpose,<sup>6</sup> in agreement with the recent revision of the USP-NF monograph. It also suggests that the initial compendial procedure is probably too rigid, with prescriptions and specific requirements that may involve noncritical variables.

To allow flexibility and large design space, it is indeed useful to study the various variables in the procedure to establish their impact. This will not only assess the robustness of the procedure but also help to manage the risks as recommended by the new Analytical Quality by Design (AQbD) framework. NMR brings the significant advantages of allowing technology inherent justification where many of the variables are known and can be calculated or predicted, simplifying the risk-assessment while providing significant flexibility. In a recent whitepaper, we provided a detailed discussion about managing NMR procedure and their design space.<sup>7</sup> In the present discussion, we are demonstrating these principles with selected examples for the MS determination of HP- $\beta$ -CD with the Fourier 80, to design a controlled yet flexible procedure by understanding and managing the impact of possible variables.

<sup>6</sup> According to the new ICH Q2(R2) and the current draft of USP-NF <761> and <1761> linearity does not need to be validated on a case-by-case basis as it is inherent to the NMR technology. A general verification during the operational qualification is sufficient.

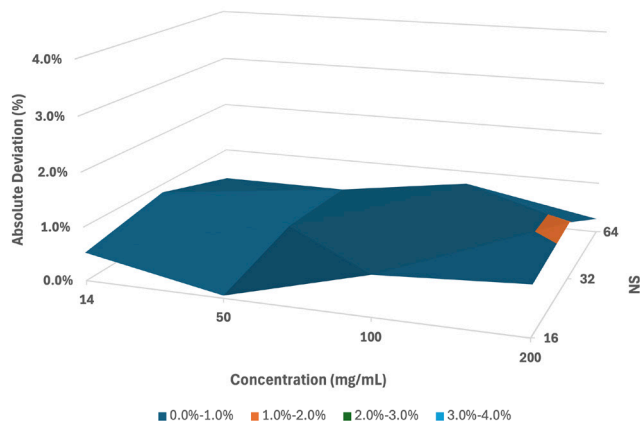
<sup>7</sup> Bruker, [Combining Robustness and Flexibility - Benefits and examples of using Benchtop NMR for Simplified Design and Risk-Assessment of Analytical Quality Control Procedures](#), August 2024

In a first example, Figure 6 illustrates how varying the number of scans impacts the results. Logically, with low number of scans, somewhat more significant deviation occurs, due to lower signal to noise. But as soon as sufficient sensitivity is achieved, the results stabilize and become independent of the number of scans. This is expected due to the underlying principles of NMR, in which the signal to noise is directly proportional to the square root of the number of scans.



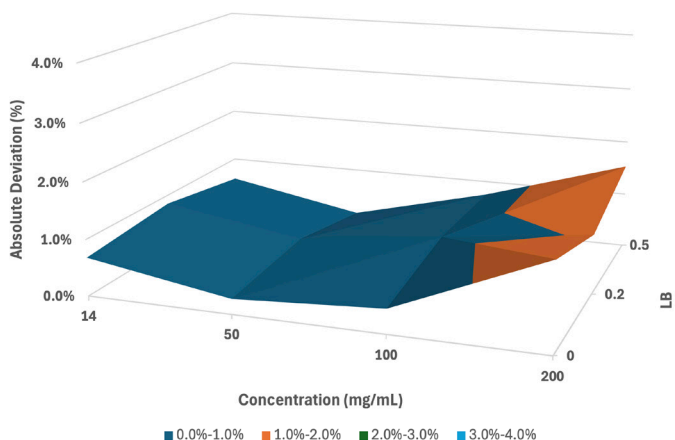
**Figure 6:** Absolute deviation of the measured MS on material 3 using the Fourier 80 (with respect to the average result of the precision study) varying the NS. The dashed orange line is the experimental signal to noise of the smallest resonance in the integrated area, using a 2 ppm noise range. All other parameters are those of Table 3.

In NMR, the signal to noise and thus the accuracy, not only depend on the number of scans but also on the sample concentration. These two variables were therefore studied with a multivariate approach, reported in Figure 7. It confirmed that all possible combinations proved suitable when concentration was within the 13 to 100 mg/mL and with NS from 16 to 64. A start of possible tendance was however visible at 200 mg/mL with slightly higher errors.



**Figure 7:** Surface plot of the absolute deviation of the measured MS on material 3 using the Fourier 80 (with respect to the average result of the precision study) when varying the concentration and NS. All other parameters are those of Table 3.

Sample concentration can in turn affect the broadening of the NMR resonances, which is also dependent of on the broadening factor LB used during the processing. In another example of multivariate study, both were systematically investigated, yielding the surface plot of Figure 8. This showed that for concentration at or below 100 mg/mL, the LB parameters can be varied from 0 to 0.5 Hz without significant impact. However, at 200 mg/mL, it can further increase the tendance observed with the default parameters, with deviation ranging from 1.1 to 1.5%. Although these values remain acceptable, they evidence a limit of the design space and concentration should be limited to a lower value.



**Figure 8:** Surface plot of the absolute deviation of the measured MS on material 3 using the Fourier 80 (with respect to the average result of the precision study) when varying the sample concentration and the LB parameter during processing. All other parameters are those of Table 3.

Based on these correlated results, it appears clearly that instead of prescribing the number of scans in the procedure, it can be more advantageous to control the signal to noise and define a minimal value to be reached. Using the results from Figure 6 this limit can be safely set to 150:1 (for the smallest resonance of interest of the A<sub>2</sub> area). This does not completely alleviate the need to define an acceptable concentration range, as very high concentration can impact the reportable value. Finally, the LB factor has clearly no impact within the defined tested range. Thus, the set of parameters described in Table 3 can be revised as follows:

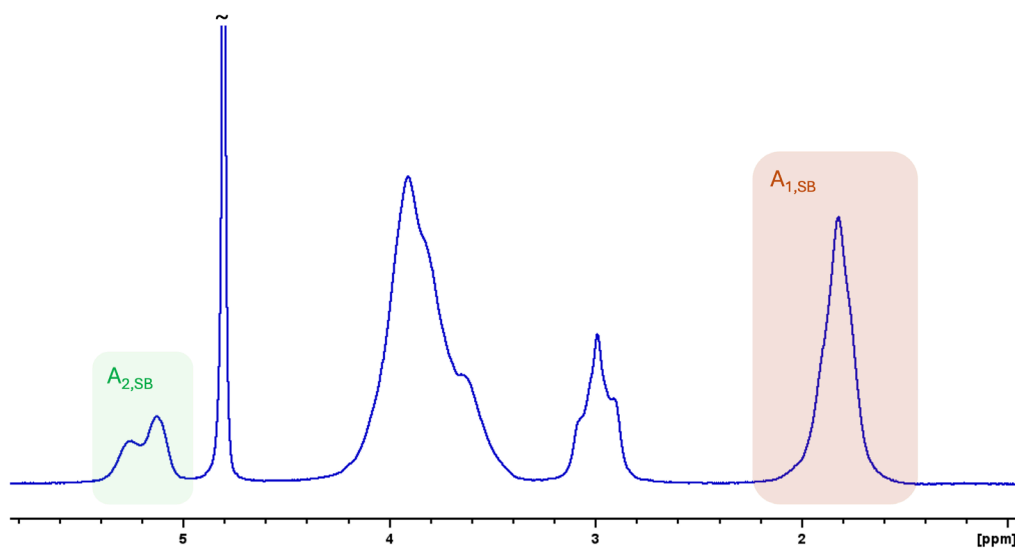
<b>Sample concentration</b>	50 to 100 mg/mL
<b>Temperature</b>	25 °C
<b>Sequence</b>	ZG
<b>Inter-pulse delay (<i>D1</i>)</b>	As needed
<b>Number of scans (<i>NS</i>)</b>	As needed
<b>Spectral Width (<i>SW</i>)</b>	
<b>Transmitter Offset (<i>O1P</i>)</b>	Set so the 6 to 0 ppm range is recorded and (D1+AQ) ≥ 3.5 s
<b>Acquisition Duration (<i>AQ</i>)</b>	
<b>Time Domain Point (<i>TD</i>)</b>	≥8 K
<b>Frequency Domain Point (<i>SI</i>)</b>	≥TD
<b>Apodization function</b>	Exponential
<b>Line Broadening Factor (<i>LB</i>)</b>	0 to 0.5 Hz
<b>Acceptance criteria</b>	The left resonance of the A <sub>2</sub> area has a signal to noise of at least 150:1 using a 2 ppm noise range.

**Table 6:** Possible parameters of the method for MS determination HP-β-CD using the Fourier 80 based on the robustness study. Abbreviation of the acquisition and processing parameters as used in Bruker software are indicated in italic.

Such type of prescription allows more flexibility in the studied design space while ensuring that the critical factors are under control. Compared to Table 3, other correlated parameters were directly adapted based on the intrinsic features of NMR (*SW*, *O1P*, *AQ*, *TD*, *SI*). On the other hand, the more empirical temperature effect was not studied, and thus a fixed-point parameter was kept. These prescriptions are given for example – in a formal validation process, a full risk-assessment to ensure that all variables are identified and controlled would be required. Nonetheless, the example in Table 6 gives a good starting point to implement and validate an analytical procedure for the MS determination HP-β-CD using the Fourier 80, in a flexible framework, alleviating some of the binding prescription of the original monographs while ensuring the control of critical variables.

## DS Testing of SB-β-CD Using the Fourier 80

The procedure described in the previous section can be directly applied to the determination of the DS of SB-β-CD with the Fourier 80 as both the underlying principle and the resonances to be integrated present strong similarities with HP-β-CD. As illustrated in Figure 9, the A<sub>1</sub> area is shifted between HP-β-CD and SB-β-CD due to different chemical nature of the associated proton. Yet the area remains free of any interference. On the other hand, the resonances of the A<sub>2</sub> area are strictly similar between HP-β-CD and SB-β-CD since they correspond to the glycosidic proton of the CD ring, common to both products. Finally, an additional set of resonances is present at about 3.0 ppm (due to another CH<sub>2</sub> group of the SB graft) but this is out of the exploited areas.



**Figure 9:** Example of  $^1\text{H}$  NMR spectrum of SB- $\beta$ -CD in  $\text{D}_2\text{O}$  recorded on a Fourier 80 benchtop spectrometer using parameters of Table 3. Areas defined in Ph. Eur. 2804 for SB- $\beta$ -CD are indicated as colored boxes.

Thus, the procedure was directly assessed for bias using different materials and using the DS value determined on a 400 MHz system, in a similar fashion as for HP- $\beta$ -CD. Results are presented in Table 7.

Material	Supplier	Reference	Batch	Grade	Reference DS <sup>a</sup>	Experimental DS with Fourier 80	Deviation (%)
1	EDQM	Y0001998	00Uu1V	EP Reference Standard	6.1	6.1	0.5
2	Supelco	PHR2923	LRAD6279	Pharmaceutical Secondary Standard	6.3	6.2	-2.2

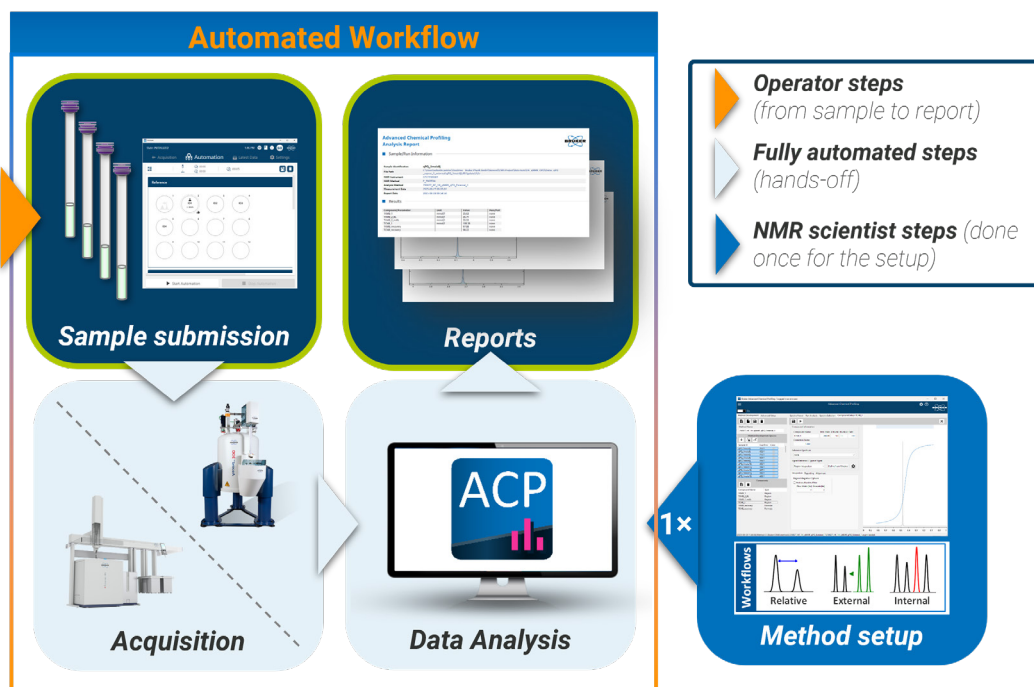
a: as determined using a 400 MHz AVIIIHD spectrometer using the compendial procedure.

**Table 7:** SB- $\beta$ -CD batches investigated and results. Values are rounded for comparison to specifications. Deviation is expressed by comparison to the value obtained at 400 MHz.

As for HP- $\beta$ -CD, the results were comparable to that of the compendial method, with deviation within the 3% example limit range. Given the similarities, an extended precision study was not performed, but it would most probably yield results comparable to those presented in the previous section for HP- $\beta$ -CD, and a global platform procedure could be implemented with the Fourier 80 for the testing of modified cyclodextrins and validated as such in a laboratory, further simplifying the management of the procedure portfolio and their life cycle.

### Sample-to-report automated workflow implementation

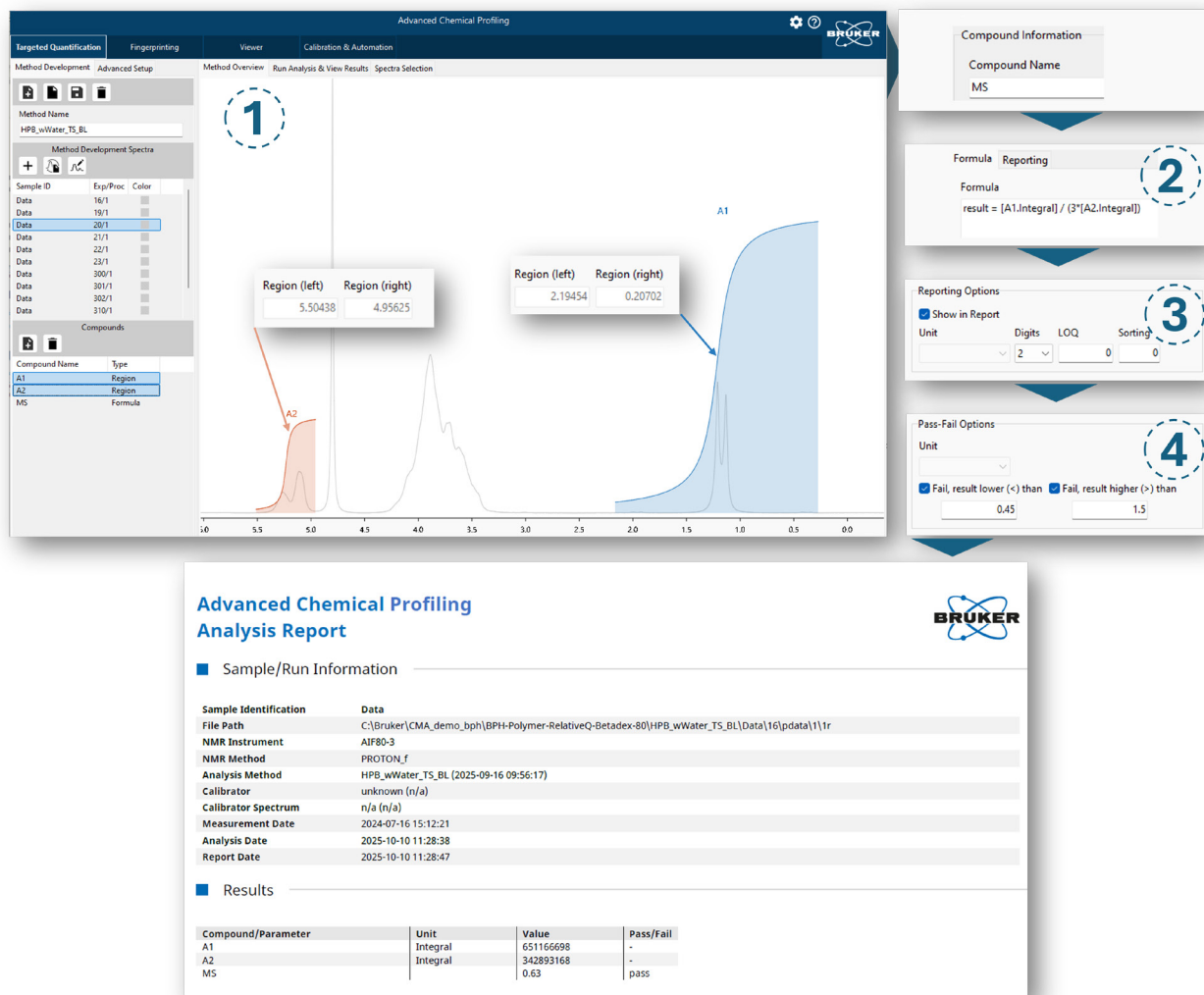
Finally, robustness in routine testing also requires automation to minimize operator induced variability. In this context, the Advanced Chemical Profiling 2.0 (ACP) software now enables fully automated, sample to report workflows. ACP integrates directly with the Fourier 80 main software (TopSpin, IconNMR and GoScan), allowing non expert users to execute virtually any NMR based procedure while ensuring that the method remains fit for purpose. For routine analyses, it operates in the background, with no user intervention beyond selecting the appropriate analytical method during sample submission. All subsequent steps are then carried out under full automation, culminating in a report in which quantitative results are expressed according to the relevant monographs and/or standard operating procedures, without requiring additional interpretation or calculation (Figure 10).



**Figure 10:** Schematic workflow enabled by ACP. Once set up, an NMR method can be performed under full automation by routine users. Following sample submission, all acquisition, processing and analysis steps are performed in the background and end up with the generation of a report containing the reportable values.

A detailed discussion of ACP capabilities, workflows and examples for polymeric raw material testing is available in a separate document.<sup>8</sup> It includes a step by step walkthrough of the fully automated implementation of the HP- $\beta$ -CD test and shows how monograph or alternative procedures can be configured in just a few steps for routine use. Data acquisition, processing and analysis are subsequently performed automatically, directly providing a human and/or machine readable report containing the MS value as defined in the compendia. When desired, an optional pass/fail statement can also be generated to support immediate interpretation (Figure 11). The same manuscript further details how ACP simplifies the implementation of advanced baseline correction strategies, such as those required in the presence of significant amounts of water (see Figure 5), thereby enabling reliable and robust fully automated testing even in challenging cases.

<sup>8</sup> Bruker, [Enabling Scalable and Reproducible NMR Analysis for Polymeric Raw Materials in Pharma QC](#), November 2025



**Figure 11:** Example of the setup of an automated analysis of HP-β-CD according to USP-NF using the Fourier 80 and ACP. Setup is done entirely graphically and in just few steps: 1) definition of resonances to be integrated, 2) reportable value formula, 3) result formatting and 4) optional acceptance criteria. After setup, the method can be used for fully automated analytical process, up to formatted report, including the delivery of pass/fail conclusions according to the monograph.

## Conclusion

The results presented in this whitepaper demonstrate that the procedures for the control of HP- $\beta$ -CD and SB- $\beta$ -CD, as described in the USP–NF and Ph. Eur., can be readily implemented on the Fourier 80 benchtop NMR spectrometer. This aligns with the recent revision of the USP–NF monograph for HP- $\beta$ -CD, confirming that these procedures remain fit for purpose when performed on benchtop systems such as the Fourier 80. This substantially lowers operational cost compared to floor-standing NMR instruments while simplifying day-to-day operation. Furthermore, by leveraging the inherent strengths of NMR, flexible yet robust procedures can be designed, easing some of the restrictive prescriptions of older monographs while maintaining control of critical variables.

This use-case illustrates the value of NMR as a flexible platform analytical technology, which is particularly relevant in the context of AQbD. The Fourier 80 brings the intrinsic benefits of NMR into a compact, cryogen-free system with automated sample-to-report capabilities and built-in support for GMP-compliant workflows. In combination with ACP, it offers an ideal solution for QC laboratories seeking to implement and benefit from NMR-based testing.

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