PHARMA

Enhancing Pharmaceutical Quality Control with Automated Identification Testing

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Innovation with Integrity

Pharmaceutical quality control necessitates efficient and robust identification testing, yet traditional methods can be labor-intensive, require expert interpretation, and depend on physical reference standards. Nuclear Magnetic Resonance (NMR) offers a universal, highly specific alternative, but its adoption has been limited by the complexity typically associated with data analysis. The Advanced Chemical Profiling (ACP) tool addresses this challenge by fully automating NMR-based identification testing, eliminating manual data interpretation and allowing true end-to-end workflows. In this document we demonstrate how ACP, combined with Bruker's benchtop Fourier 80 NMR spectrometer, enables cost-effective, fully automated workflows for pharmaceutical identification. Starting from compendial monograph examples, the study highlights how ACP can help establish robust and reliable identification processes, making routine, cost-efficient NMR testing even more accessible for quality control laboratories.

Introduction

Identification is the most common test performed in pharmaceutical quality control. While all materials used in drug manufacturing must be assessed for conformity with relevant specifications before release, only identification tests are systematically repeated upon receipt of materials from suppliers and prior to use. Therefore, robust, compliant and easily transferable identification testing procedures are essential to ensure efficient batch control and timely product release.

A recent whitepaper[1] discussed the benefits of using Nuclear Magnetic Resonance (NMR) technology for rapid, cost-effective identification testing. NMR indeed facilitates the development of straightforward identification procedures with exceptional robustness and transferability. As an absolute, universal technique, NMR spectroscopic characteristics of an analyte remain identical under a defined set of analytical conditions, consistently yielding the same fingerprint. While this consistency is a common feature of spectroscopic techniques, NMR offers a wealth of spectroscopic features, such as chemical shifts, resonance shapes, and integration values, that directly correlate to the chemical structure of samples and their environment. As such, NMR can be considered the most specific and informative spectroscopic method.

These unique capabilities of NMR allow the use of reference spectra, without the need for systematic reference material analyses. This approach not only streamlines the testing procedure but also eliminates the recurring need for costly, certified reference standards. The aforementioned whitepaper provides general considerations as well as detailed information on risk assessments and the design of NMR-based identification tests. Additionally, another document presents practical implementations of compendial testing. It uses tobramycin[2] as an example, which identification test in the European Pharmacopoeia (Ph. Eur.) relies on proton (^{1}H) NMR. Importantly, the limitations of visual comparison of spectroscopic results were discussed and strategies for implementing operator-independent criteria in identification procedures were outlined, to leverage all the robustness provided by NMR.

In this supplemental manuscript, we present and discuss how the new Advanced Chemical Profiling (ACP) analysis tool offered by Bruker enables fully automated workflows for identification testing, supporting all stages from analysis to reporting.[3] This innovation helps quality control laboratories to implement true end-to-end automated procedures for identification testing, eliminating the need for expert interpretation to reach conclusions. When the ACP is combined with the Fourier 80 benchtop NMR system, testing laboratories can benefit from a cost-effective, compact NMR solution that supports highly reliable, fully automated, GMP-compliant analytical procedures.

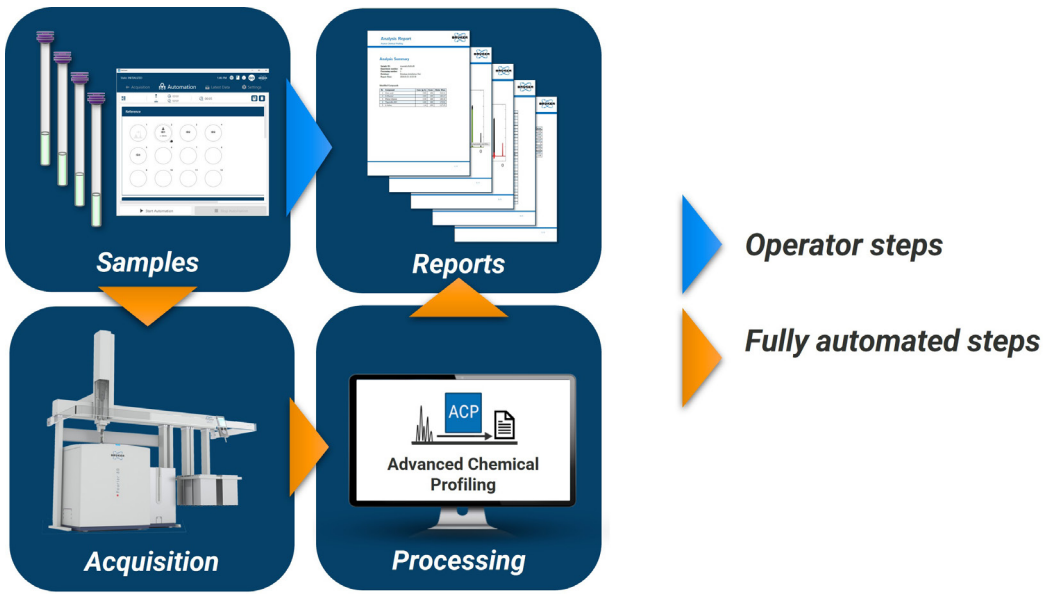


Figure 1: Schematic analytical workflow based on ACP, highlighting the end-to-end workflow.

[1] Bruker, 2024. Streamlining Identification Testing for Quality Control Using Benchtop NMR, July 2024.
[2] Bruker, 2024. Compendial Identification Using Benchtop NMR: Example of Tobramycine According to Ph. Eur. 0645, August 2024.
[3] General information about ACP are available here: Advanced Chemical Profiling | Bruker

ACP for automated identification testing

Aforementioned whitepapers showcase how NMR identification procedures can be developed by identifying the spectroscopic features and numerical criteria that need to be verified for each analyte to confirm its identity. For example, the set of numerical criteria for the identification of tobramycin with the Fourier 80 NMR as reported in Table 1 was suggested as an alternative to visual comparison.

Signal	Shift (ppm)	Integral	Multiplicity[a]	Additional criteria
1	0.95-1.76[c]	2	m	Pseudo quadruplet shape on the right side of the area which two main resonances are 12.3 ± 0.5 Hz apart.
2	1.76[c]-2.40	2	m	/
3	2.45-3.20[c]	6	m	/
4	3.20[c]-4.20	10	m	/
5	5.05	2[b]	d	Coupling constant of 3.7±0.5 Hz
6	5.15		d	Coupling constant of 3.7±0.5 Hz

a) s: singlet, m: multiplet or mix of resonances. b) relative integration reference set to 2. c) no return to the baseline

Table 1: Example of acceptance criteria for the identification of tobramycin by ^{1}H NMR using Bruker Fourier 80 benchtop NMR from ref 2.

Such criteria fulfill modern concepts for robust identification testing. However, understanding and applying them for routine testing require specifically trained analysts and operators, if performed manually. In addition, traditional data processing, such as NMR resonance peak picking and integration, can be cumbersome and time-consuming.

In contrast, ACP offers a new solution to perform the equivalent workload in a fully automated fashion. This approach not only streamlines the process but also enhances robustness by eliminating manual processing in routine analyses. ACP is built around a database system that allows each target to be linked to a customizable definition of the spectroscopic features to be matched. This framework is easy to use thanks to a user-friendly graphical interface. This means that the set of features defined in Table 1 can be quickly transferred into the ACP using a single reference spectrum, as illustrated in Figure 2.

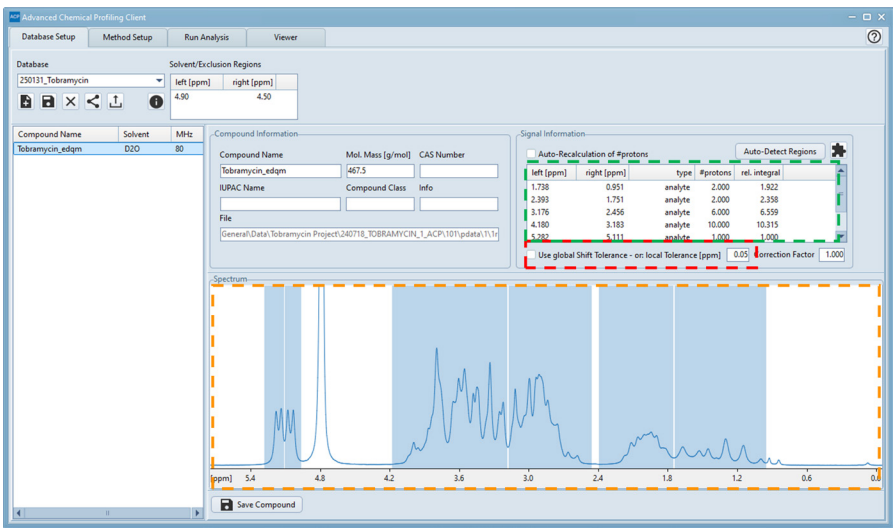


Figure 2: Example of ^{1}H NMR reference definition for tobramycin in D_2O at 80 MHz using ACP. The features can be directly defined graphically on the spectrum (orange box) and/or using numerical values (green box). Tolerance for chemical shifts can also be defined (red box).

Once the reference features are defined, the routine, automatic identification of samples can be set up. In this process, ACP matches the reference data with the sample results to conclude on the identification result. It does so by detecting all the pre-defined features within specified tolerances. This process effectively ensures that the chemical shift, shape and integration of all defined resonances are correctly identified in a fully automated manner. Results can be accessed as individual reports or via an intuitive user interface, helping users to quickly review a full sequence of results. These visualization options are exemplified in Figure 3, where ACP was used to analyze two batches of tobramycin (positive control) and a related substance with a very similar structure (kanamycin), as a negative control. Results are thus identical, in essence, to the manual steps described in the whitepaper mentioned above. However, because these are obtained in a fully automated manner, it significantly streamlines the analytical process.

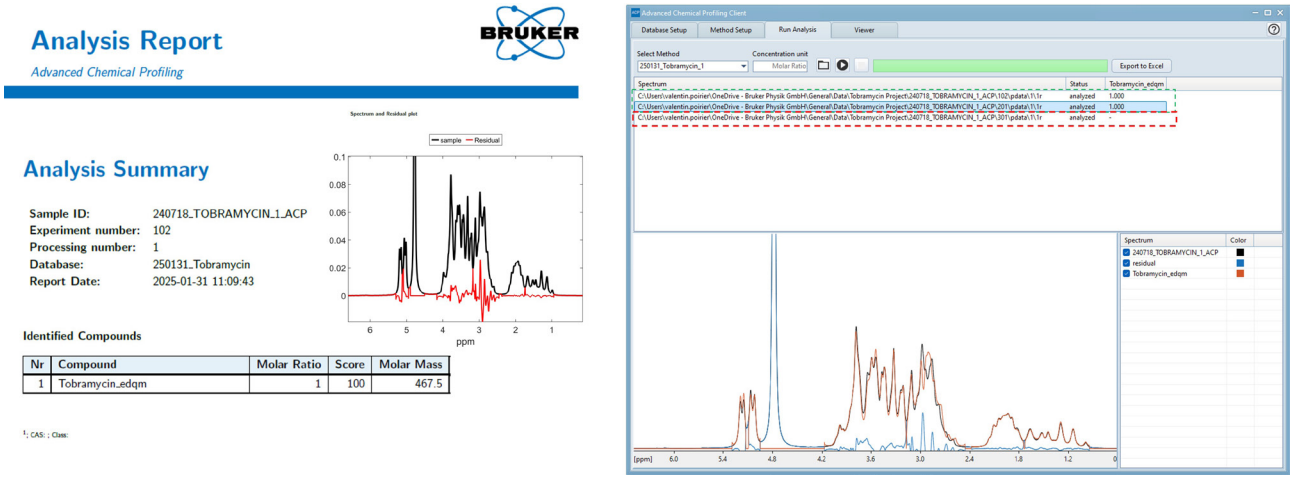


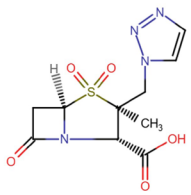
Figure 3: Left: example of a report from ACP indicating the positive identification of a sample as tobramycin. It includes the experimental spectrum (black) and the residual data after matching (red). Right: screenshot of the dedicated user interface for intuitive visualization. Positive (green box) and negative results (red box) can be easily identified. Dynamic spectral data are also available, displaying the sample raw spectrum, the reference data and the quality of the matching via the residual plot.

It is important to highlight that ACP is fully integrated with Bruker's spectrometer control software, TopSpin. Once methods are implemented, automated data analysis is performed immediately after data acquisition, enabling a truly integrated end-to-end workflow. As such, the operator only needs to submit the sample for analysis using standard interfaces for routine NMR analysis (IconNMR, GoScan, or Mdrive). The analytical report is generated automatically, without any additional manual intervention. While the ACP user interface can be accessed for the visualization and review of results as well as for re-analysis, operators do not necessarily have to interact with it for standard workflows (see Figure 1).

Assessing performances – Identification according to the Japanese Pharmacopeia

To demonstrate the potential and effectiveness of ACP for identification testing, several examples from the Japanese Pharmacopoeia (JP) were tested. The JP includes approximately 50 compendial identification tests using NMR, most of which are for antibiotic active pharmaceutical ingredients (APIs). Notably, the NMR tests described in the JP leverage the key advantage of NMR being a universal method. As such, the monographs list the spectroscopic features to be controlled and provide the reference data. Selected examples of JP criteria are illustrated in Figure 4 and Figure 5, showing the NMR identity testing criteria of tazobactam and piperacillin, along with the corresponding experimental spectra recorded on the Fourier 80.

This approach contrasts sharply with the United States Pharmacopeia–National Formulary (USP–NF) and the Ph. Eur., which systematically require the simultaneous analysis of a physical reference standard, thereby undermining some of the inherent benefits of NMR. Nonetheless, while the JP is a pioneer when it comes to NMR reference data, this paradigm is also evolving in the USP–NF, with the recent introduction of the "digital reference" concept in the current revision draft of General Chapter <11>.[4] This development could potentially reduce the need for systematic physical reference standards in compendial testing when the analytical technique is deemed appropriate.



Signal	Chemical shift (ppm)	Shape	Relative integration
A	1.3	Singlet	3
B	7.8	Doublet	1
C	8.1		1

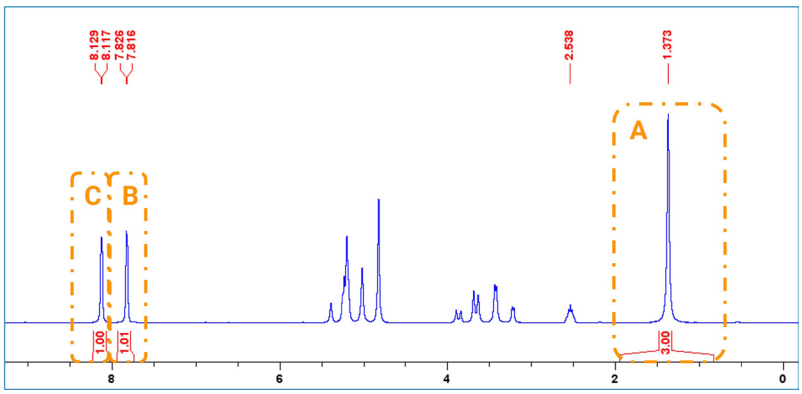
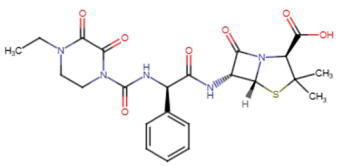


Figure 4: Left: Structure and reference data for ^{1}H NMR identification testing in DMSO-d_6 of tazobactam according to JP. Right: example of corresponding ^{1}H NMR recorded on a Fourier 80. Orange boxes indicate the resonances to be controlled for positive identification.



Signal	Chemical shift (ppm)	Shape	Relative integration
A	1.1	Triplet	3
B	4.2	Singlet	1
C	7.4	Multiplet	5

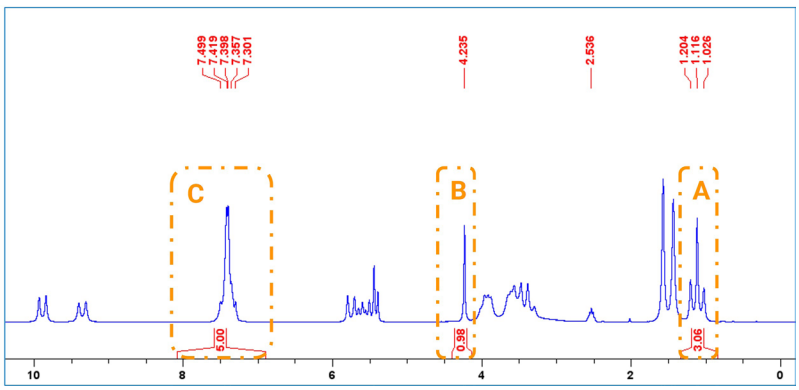


Figure 5: Left: Structure and reference data for ^{1}H NMR identification testing in DMSO-d_6 of piperacillin hydrate according to JP.
Right: example of corresponding ^{1}H NMR recorded on a Fourier 80. Orange boxes indicate the resonances to be controlled for positive identification.

[4] As of February 2025.

Rather than relying on visual, and thus subjective, comparison of NMR spectra, the JP approach relies on more objective comparisons by specifying the verification of selected NMR features. Noteworthily, it does not require a full ^{1}H NMR fingerprint match. Instead, it focuses on certain resonances that need to be detected, whose chemical shifts, shapes and relative integrations must meet the defined values to confirm a positive identification. For both the tazobactam and piperacillin examples, this approach defines seven unique criteria. Given the high sensitivity of ^{1}H NMR to even subtle changes in chemical composition, these tests thus offer exceptional specificity, even without full-spectrum matching.[5]

It is important to note that manual analysis and interpretation of experimental spectra in accordance with the JP monographs require trained NMR analysts that are skilled in correlating compendial spectral descriptions with experimental data.

This is greatly simplified through ACP, empowering even less experienced operators to successfully understand the results. By using this tool, the interpretation steps can be fully automated, as criteria for chemical shifts, resonance shapes and relative areas can be defined once in the software using a reference experimental dataset. These can then be automatically applied to routine samples for identity testing. Figure 6 illustrates the ^{1}H reference definitions for tazobactam and piperacillin in ACP, based on reference data recorded on a Fourier 80. Since the analytical workflow is automated, the minimal set of resonances required by JP monographs can be easily extended without additional effort, unlike manual interpretation. In the present examples, five resonances were used for tazobactam and six for piperacillin.[6]

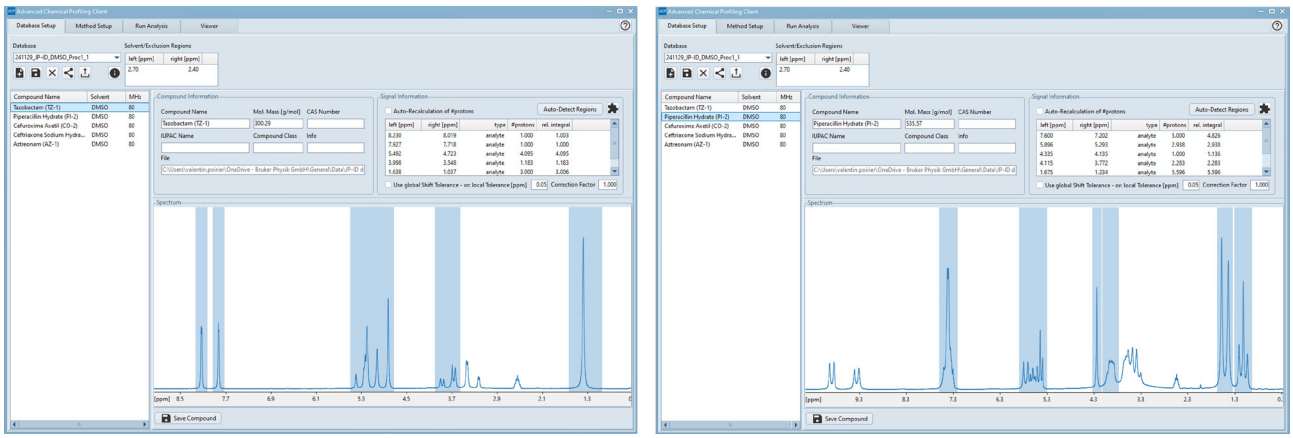


Figure 6: Example of ^{1}H reference definition for tazobactam (left) and piperacillin (right) in DMSO-d_6 at 80 MHz using ACP.

[5] For more detailed discussion about criteria selection see ref 1.

[6] See ref 1 for a discussion about appropriate resonance selection.

Examples of the automated analysis result that can be obtained with ACP are shown in Figure 7. These illustrate how the data are presented within the automatically generated report for each sample as well as on the ACP interface.

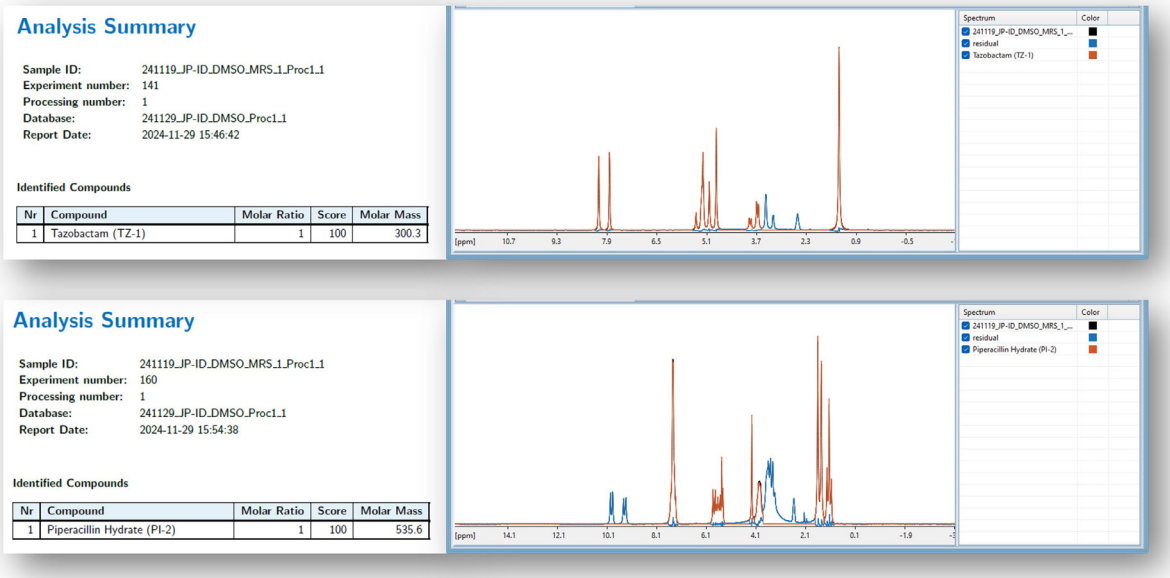


Figure 7: Example of automated reports (selection) and result display in the ACP interface for tazobactam (top) and piperacillin (bottom) in DMSO-d_6 at 80 MHz using ACP and the database shown in Figure 6.

The capabilities and robustness of this new solution were further demonstrated through the systematic analysis of nine different APIs, each with an NMR identification test specified in the JP. In this extensive series of tests, 26 individual preparations were performed, varying the following parameters:

- Batches
- Concentrations
- Number of accumulated scans
- Deliberate deviations, such as the use of incorrect solvents or referencing errors

All preparations were acquired and automatically processed with ACP, with each condition tested in at least triplicate, resulting in a total of 100 spectra. All reference data for a given solvent (D_2O or DMSO-d_6) were consolidated within the same ACP database to evaluate the potential for false positives. Representative results are summarized in Table 2.

Entry	Target	Source[a]	Solvent	Concentration (mg/mL)	NS	ACP identification result
1	Aztreonam (AZ)	A	DMSO-d_6	54	16	**AZ**
2	Ceftriaxone Sodium Hydrate (CR)	A	DMSO-d_6	53	16	**CR**
3	Tazobactam (TZ)	A	DMSO-d_6	26	16	**TZ**
4	Piperacillin Hydrate (PI)	A	DMSO-d_6	36	16	**PI**
5		B		52	16	**PI**
6	Cefuroxime Axetil (CO)	A	DMSO-d_6	53	16	**CO**
7		B	DMSO-d_6	6	16	**CO**
8			DMSO-d_6	53	16	**CO**
9			DMSO-d_6	128	16	**CO**
10			DMSO-d_6	20	2	**CO**
11			DMSO-d_6	20	64	**CO**
12			DMSO-d_6	20	128	**CO**
13	Cefalexin (CL)	A	D_2O w/ TMSP	4	16	**CL**
14	Cefepime Dihydrochloride Hydrate (CP)	A	D_2O w/ TMSP	48	16	**CP**
15	Cefazolin Sodium (CZ)	A	D_2O w/ TMSP	51	16	**CZ**
16		B		51	16	**CZ**
17	Cefotaxime Sodium (CT)	A	D_2O w/ TMSP	5	16	**CT**
18				15	16	**CT**
19				50	16	**CT**
20				137	16	**CT**
21			D_2O w/ TMSP wrong referencing[b]	15	16	**None**
22			MeOD[b]	14	16	**None**
23			DMSO-d_6[b]	16	16	**None**

NS: number of scans. TMSP: 3-trimethylsilylpropionate-d_4 (internal chemical shift reference)
a: A and B refer to two different commercial sources of a given API; b: negative test

Table 2: Selected results from the robustness study for the identification of 9 APIs from the JP by NMR using the Fourier 80 and ACP in full automation. D_2O database contained 4 targets, DMSO-d_6 database contained 5 compounds.

This extensive series of tests resulted in the consistent positive identification of all the intended targets, regardless of the batch. Importantly, no false positives were observed, demonstrating the high specificity of the workflow.

While JP monographs typically specify a concentration for ^{1}H NMR identification testing, this parameter is not critical for the effectiveness of an identification test.[7] This holds true for the present automated solution, as demonstrated by the comparison of entries 7 to 10 and entries 17 to 20, where the variation in the concentration by a factor of 20 (DMSO-d_6) and nearly 30 (D_2O) had no impact on the results. Similarly, the number of accumulated scans, which affects only the signal-to-noise ratio, had no effect on the automated outcomes, as evidenced by the comparison between entries 10 and 12.

An identification test should yield a positive result only when the defined set of experimental conditions are met. The series of negative controls in entries 13 to 15 highlights ACP reliability, as it correctly produced negative results (no identification) when deliberate deviations were introduced, such as incorrect chemical referencing or the use of the wrong solvent.

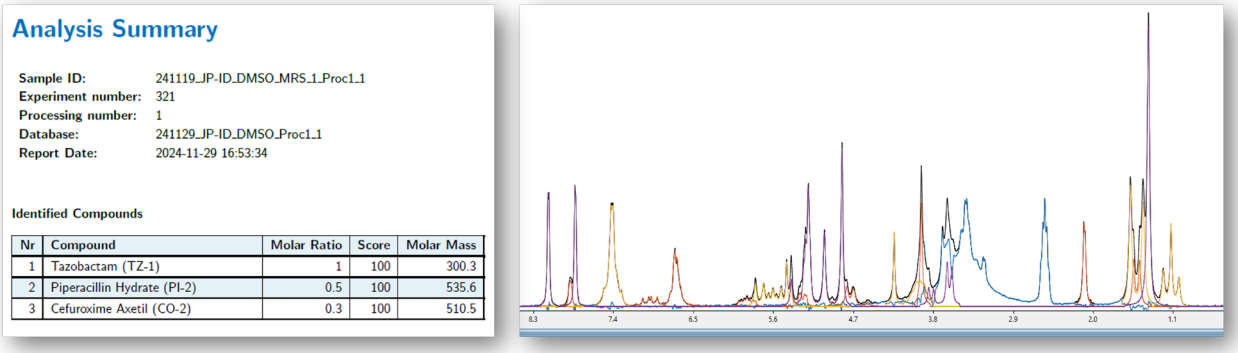
Overall, this study demonstrates the excellent robustness of the automated workflow using ^{1}H NMR data recorded on the Fourier 80, with data analysis performed via ACP. The system consistently delivered results equivalent to manual expert analysis but in a fully automated fashion, significantly streamlining the testing process while ensuring high-quality results. This approach enables less experienced users to successfully perform NMR-based identification tests independently, with minimal system interaction while maintaining a high level of confidence in the results.

[7] See ref 1 for details about concentration risk-assessment.

Beyond identification testing

ACP offers capabilities that extend well beyond "simple" identification testing. It is a powerful toolbox designed to support fully automated qualitative and quantitative NMR data analysis for both pure substances and complex mixtures. It is important to stress that it can handle a variety of workflows, so that adaptation to the specific requirements of each procedure can be implemented. For instance, ACP provides the necessary tools for all three main quantitative NMR (qNMR) approaches—relative quantitation, internal calibration and external calibration[8] —within a single, unified solution.[9]

To briefly showcase the tool's capabilities, various mixtures of the APIs studied in the previous section were analyzed using ACP to model simplified cases of batch contamination or adulteration of organic materials and to evaluate ACP's ability to accurately detect, identify and quantify such adulterations. An example of ACP output for one of such mixtures is shown in Figure 8, with a summary of the results provided in Table 3.



Analysis Summary

Sample ID: 241119_JP-ID_DMSO_MRS_1_Proc1_1
Experiment number: 321
Processing number: 1
Database: 241129_JP-ID_DMSO_Proc1_1
Report Date: 2024-11-29 16:53:34

Identified Compounds

Nr	Compound	Molar Ratio	Score	Molar Mass
1	Tazobactam (TZ-1)	1	100	300.3
2	Piperacillin Hydrate (PI-2)	0.5	100	535.6
3	Cefuroxime Axetil (CO-2)	0.3	100	510.5

Figure 8: Example of automated reports (selection, right) and result display in the ACP interface (left) for a mimic batch of tazobactam contaminated with two other APIs using ACP (DMSO-d_6, 80 MHz).

[8] See current draft of USP-NF <761> and USP-NF <1761> (as of February 2025) for compendial use of the qNMR. General considerations about qNMR with the Fourier 80 for regulated work are provided in Bruker, Combining robustness and flexibility - Benefits and examples of using Benchtop NMR for simplified design and risk-assessment of analytical quality control procedures, July 2024. Examples of procedures are presented in Bruker, 2024. Quantitative Assays with Fourier 80 Benchtop NMR: benefits and examples, June 2024.

[9] A detailed discussion of ACP applications for qNMR is beyond the scope of this document.

Target (solvent)	Contaminant	Molar Ratio[b]	ACP results		
			Main ID[a]	Additional ID[a]	Molar Ratio[b]
Tazobactam (TZ) ($DMSO\text{-}d_6$)	/	/	**TZ**	**/**	**/**
	CO	0.9	**TZ**	**CO**	**0.9**
	PI	0.7	**TZ**	**PI**	**0.7**
	CO PI	1.0 0.3	**TZ**	**CO** **PI**	**1.0** **0.3**
	PI CO	0.5 0.3	**TZ**	**PI** **CO**	**0.5** **0.3**
Cefotaxime Sodium (CT) (D_2O)	/	/	**CT**	**/**	**/**
	CP	0.1	**CT**	**CP**	**0.1**
	CP	0.4	**CT**	**CP**	**0.4**
	CZ	0.7	**CT**	**CZ**	**0.8**
	CZ CP	0.7 0.5	**CT**	**CZ** **CP**	**0.7** **0.5**

a: Main ID is that reported with a molar ratio of 1.0. Additional ID are those reported and with a molar ratio < 1.
b: accurate purity of commercial products unknown - preparations not optimized for quantitative purposes.

Table 3: Summary of selected results on model adulteration detection of APIs from the JP using the Fourier 80 and ACP in full automation using the same database as in Table 2. All preparations were recorded and analyzed in triplicates, average results are given.

These results demonstrate ACP's capabilities to analyze not only pure chemicals but also complex products such as ternary mixtures, as shown in Figure 8 with complex ^1H NMR spectra. In effect, these can be accurately analyzed by ACP for both identification and quantification. The results summarized in Table 3 highlight how ACP can consistently deliver accurate conclusions in a fully automated manner.

From a qualitative perspective, ACP not only reliably identified the correct main species but also accurately detected the model contaminant(s). Even more, the quantitative results—also generated through full automation—provided correct molar ratios. This demonstrates that, in a single, fully automated analysis, ACP can deliver reliable qualitative and quantitative outcomes, even for complex mixtures.

It is important to emphasize that such analyses would be significantly more challenging if performed manually, even when involving expert NMR scientists. Manual workflows would involve tedious tasks associated with the identification and assignment of each resonance to potential species, manual integration of specific signals for each target (if distinct signals are even available), and subsequent manual calculation of quantitative results. ACP-based workflows overcome these challenges, offering a robust, end-to-end solution that enables any analytical laboratory to perform routine NMR analyses without manual processing or extensive operator expertise.

Conclusions

ACP provides a new, unique tool that supports quality control laboratories interested in leveraging the benefits of NMR for routine testing. In this document, we demonstrated how ACP enables automated identification, eliminating the need for manual interpretation of analytical data while maintaining robustness and reliability.

NMR is arguably one of the most powerful analytical technologies for efficient identification, as it offers exceptional specificity and robustness while being universal—thus removing the necessity for systematic physical reference materials. While the intrinsic advantages of NMR are well established, potential challenges associated with NMR data interpretation can discourage testing laboratories from adopting this technique. ACP addresses these challenges, providing a solution that allows laboratories to implement NMR testing without requiring manual data analysis for routine testing. In addition, it streamlines the process, delivering a complete end-to-end workflow, from sample submission to final report generation.

When combined with Bruker NMR spectrometers, ACP can accommodate a wide range of analytical needs, from simple qualitative procedures to advanced quantitative assays in complex matrices. In particular, ACP can handle all field strengths, including those of the benchtop Fourier 80 NMR spectrometer, whose compact footprint, low maintenance requirements and cost-efficiency make it particularly well-suited for quality control laboratories. The combination of ACP with the Fourier 80, supported by Bruker GxP kits, offers a unique, GMP-compliant4 solution for automated testing—from raw materials to finished products. As such, it is ideal to address diverse analytical needs while delivering the unparalleled flexibility and adaptability of NMR technology.

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Benchtop NMR for Quality Control
Solution Page

GxP NMR
Solution Page