

ACD/LABS [ADVANCED CHEMISTRY DEVELOPMENT, INC.]

# Automated Structure Verification for High-Throughput Quality Control in Pharmaceutical R&D—Part 1



Katharina is one of three NMR scientists on the NMR team that is part of Syngenta Chemistry Research at Syngenta Crop Protection R&D in Stein. The team mainly works to support their synthesis colleagues in structure elucidation and verification among many other duties, even beyond NMR.

NMR Team Responsibilities	Open Access NMR
<ul style="list-style-type: none"> <li>• NMR structure elucidation &amp; verification</li> <li>• Special NMR experiments</li> <li>• High-throughput NMR (HTNMR)</li> <li>• Reaction kinetics by NMR</li> <li>• Byproduct analysis by NMR</li> </ul>	<ul style="list-style-type: none"> <li>• 3x400 MHz instruments</li> <li>• ~50,000 samples/year</li> <li>• &gt;100 users</li> </ul>
	<b>Expert Equipment</b> <ul style="list-style-type: none"> <li>• 2x600 MHz instruments</li> <li>• HTNMR capabilities</li> <li>• <math>^1\text{H}</math>, <math>^{13}\text{C}</math>, <math>^{19}\text{F}</math></li> </ul>

For scientists at Syngenta, quality control (QC) is important—even in a research environment. For example, their chemical store contains materials of different ages and from various sources. Performing periodic quality control allows research scientists to confidently use the materials they obtain from the store in their experiments. Similarly, when something is synthesized, it is important to ensure the quality before it is used in further experiments/decisions. Finally, they also perform quality control for exchanging materials in collaborative projects—ensuring the quality of what they are sending out and receiving.

In all these cases, ensuring quality comes down to correctly verifying the chemical structure. To minimize the time they have to spend verifying known structures, Katharina and her colleagues rely on automated structure verification (ASV) with NMR Workbook Suite™.

## Selecting NMR Experiments for ASV

The first step in implementing a QC NMR workflow with ASV was to identify the dataset that would give them optimal accuracy without compromising efficiency. Estimating that they have approx. 20,000 samples that could be used with ASV in a year, Katharina and her team calculated the time it would take to measure the datasets. (Table 1)

Table 1. Approximate time required to acquire various NMR datasets.

Dataset	Time per Sample (including tune, shim, etc.) [min]	Days of 24/7 Measurement Time per Year	Comments
<sup>1</sup> H	4	56	~1 day/week
<sup>1</sup> H + HSQC on cryoprobe	7 (with decent concentration)	97	Highly dependent on sample availability
<sup>1</sup> H + HSQC on RT probe (400 MHz)	25	347	Not realistic, but may still be useful for individual cases
<sup>1</sup> H + COSY + HSQC + HMBC + <sup>13</sup> C + <sup>19</sup> F on cryoprobe	Maybe 40 (with decent concentration)	556	Impossible

Knowing that it would be impossible to measure the complete dataset for all their samples, they next aimed to identify a smaller dataset that could provide comparable accuracy in ASV. To do this, they measured the complete dataset for two different batches of 96 samples each. They then went back and selectively excluded certain experiments prior to ASV. Based on the two batches, they found that using <sup>1</sup>H + HSQC along with a rigid match factor (MF) threshold provides similar accuracy to the complete dataset in much less time.

## Putting ASV for QC into Practice

Performing quality checks of over 2000 materials in their chemical store was a big undertaking. To cover the wide variety of samples, they used both LC/MS and NMR data to verify the structures. (Figure 1) Working in batches of 96, they simultaneously prepared samples, then performed measurements using both analytical techniques.

The LC/MS data was automatically processed and analyzed using the instrument software. Samples passed when the expected mass was found, unless the approximate purity calculated from the DAD was particularly low.

Meanwhile, NMR data was automatically processed and analyzed using NMR Workbook Suite, with batch ASV settings optimized for their “typical” dataset. They used the database view to quickly see which samples had passed, were ambiguous, or failed based on the MF in a color-coded grid.

They kept the scores from each technique separate, so that failure in one method would not bias the overall result. If the results from both techniques indicated a “pass” for the expected structure, then the structure passed overall. For the remaining samples, they found it quickest to manually revisit the data to determine whether it supported the proposed structure.

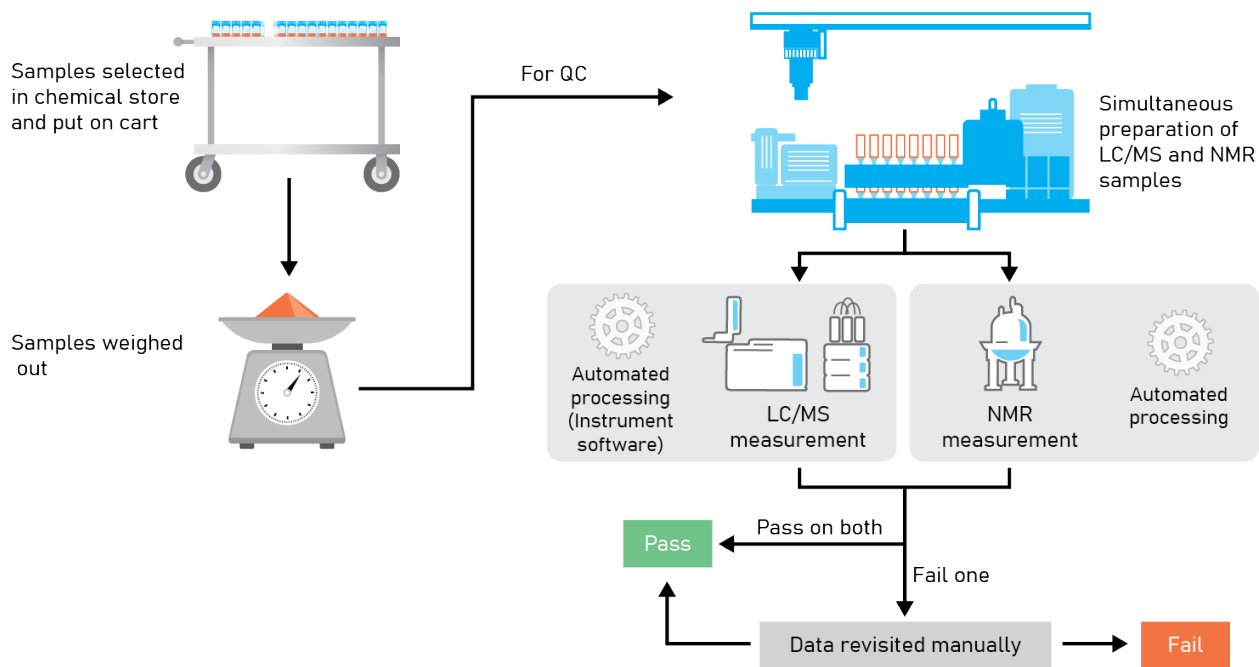


Figure 1. High-throughput quality control workflow for chemical store samples.

## How much time is saved with ASV?

For the NMR component of this project, Katharina and her team looked at the distribution of results to assess the impact of ASV. Of the more than 2000 samples analyzed:

- ~45% passed with no human analysis required
- ~ 45% required 5–10 minutes of manual reprocessing
- ~10% required 10–60 minutes of manual reprocessing, assignment, or structure elucidation

They also compared the time it took to manually analyze a batch of 96 samples with the time it took using ASV. They found that, even with approximately 10 minutes of manual data marshalling, using ASV cut down the amount of expert time required by almost a half.

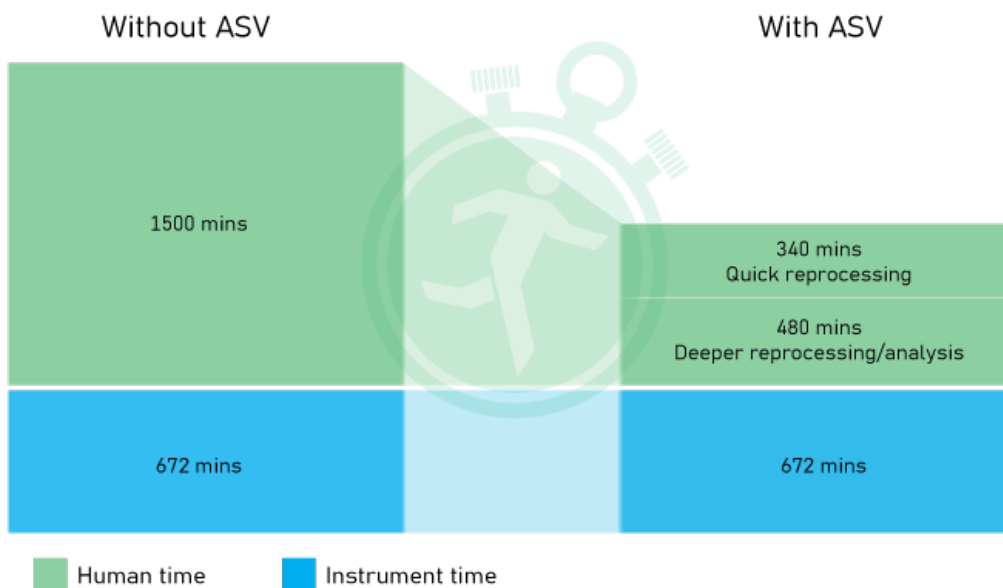


Figure 2. Time required to analyze a batch of 96 samples with and without ASV.

They also noted that most of the samples in the 10% that required significant expert attention had the incorrect structure proposed. For these they chose to elucidate and verify the correct structure. However, they note that they could have saved even more time by simply disposing of those samples, as they were clearly not what they intended to have in their chemical store.

## Future Potential of ASV at Syngenta

With this in mind, Katharina and her colleagues at Syngenta are currently considering other workflows that could benefit from the implementation of ASV. Combining the average time savings of 7 minutes/sample and the estimation of 20,000 samples they produce per year that could be analyzed with ASV, they potentially save 98 days/year on structure verification. As ASV continues to improve in the future, particularly as it evolves to handle more complicated verification tasks (e.g., samples containing rotameric and diastereomeric mixtures or NMR-invisible functional groups), the time savings will only grow.