MyGEARS

Application Note

Mestrelab Research

chemistry software solutions

MyGEARS

Application Note

Mnova MyGears in action
Automated qNMR, Structure Verification and Spectrum Suitability Testing with ONE Click

NMR has never been so easy!

Camil Joubran 10/2020

Introduction

Quantitative NMR (qNMR) has had great impact on the drug development process. qNMR can replace traditional assay methods and the dependence on multiple techniques to determine potency of an API [1]. A single spectrum can determine the API potency in the Drug Substance or Drug Product, while traditional approaches involve chromatographic assay, moisture analysis, residual solvent analysis and elemental analysis. In addition to potency, structural information can also be ascertained from the same dataset and provide structure ID. Potency is typically determined relative to an internal reference standard, which are readily available through multiple vendors accompanied by certificates of analysis (CoA). Validation of qNMR methods has been established for both pre- and post-GLP studies [2]. qNMR meets the ICH guidelines for specificity, accuracy, precision/repeatability, and robustness [3]. These attributes make qNMR an extremely powerful methodology. However, there can be pitfalls for the unwary: inconsistent data processing, transcription errors in calculation, or the use of spectra of insufficient quality can all cause problems. These issues largely exist because software designed for spectral acquisition rarely includes simple and intuitive workflows for quantification. Users are often forced to transfer information (such as absolute integrals) to alternative tools and the equations needed to determine concentration must be manually defined – the result is multiple sources of transcription and calculation errors. Furthermore, the software does not facilitate the systematic use of identical processing parameters which is required to make a meaningful comparison between replicate samples. Finally, there are few to no tools which quickly and easily assess spectral quality to confirm that the data is suitable for the required analysis, and that inter-sample comparison will be meaningful. Mestrelab Research Inc. has solved these problems by building the qNMR plugin to standardize and automate the process which can add robustness to the technique. Thus, the same process parameters, peak integration, and calculations can be applied to each sample, and each replicate, to reduce error. Furthermore, qNMR automation

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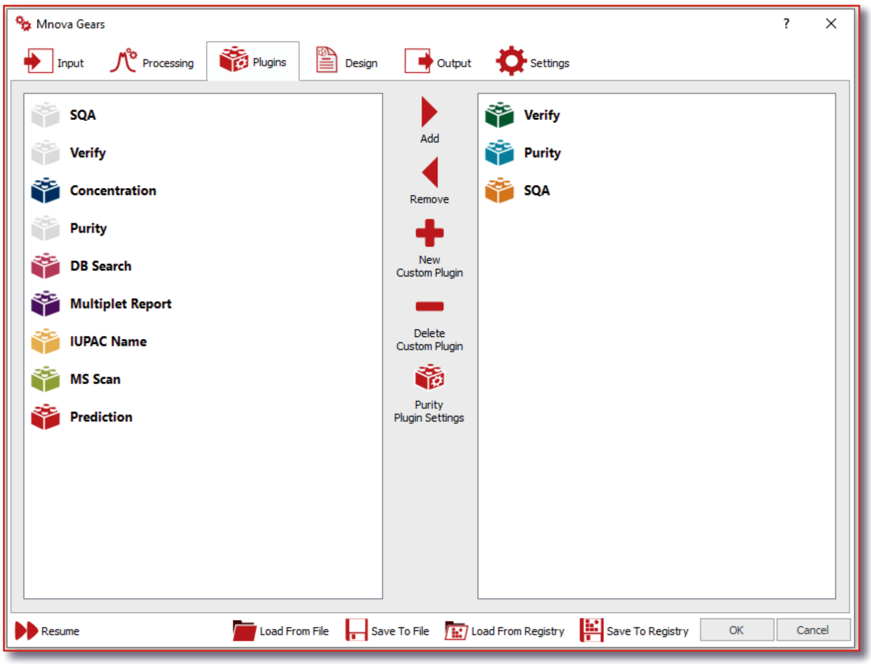
can be easily applied to replicates, using Mestrelab's MyGears automation framework. By developing a 'MyGears' workflow, multiple replicates can be analyzed with one click. In this application note, we will focus on how such a workflow can be built and used, demonstrating not only the ease with which qNMR can be automated, but also providing a template for how other similar tasks can be tackled.

Workflow Sequence

Once a method (processing parameters, integration regions, reference standard selection, solvent) has been developed within the qNMR plugin, the MyGears workflow can automate triplicate analysis and link together various other analyses in the form of 'bricks' (plugins) to generate additional information about each sample. The bricks are executed in a sequential mode to generate quick results. The resultant output can also be automatically exported into a database or other location. At the heart of MyGears are the analysis bricks (e.g. Verify, Purity, SQA, Concentration, etc.) which can be used for processing an open Mnova document (Figure 1).

Figure 1. Analysis Tab



For this example, we have set up a sequence of verification, purity, and quality assurance. The first step in the workflow is the confirmation of structure utilizing the Verify brick. Verify calculates a predicted spectrum based upon the structure, compares it to the experimental data and provides a confidence score for the match. This first analysis builds support for the structure and, very importantly, determines the number of protons per peak, which are essential for the qNMR calculation. The second step in the sequence calculates the potency of the API based upon an internal reference standard utilizing the Purity brick. The purity calculation utilizes a

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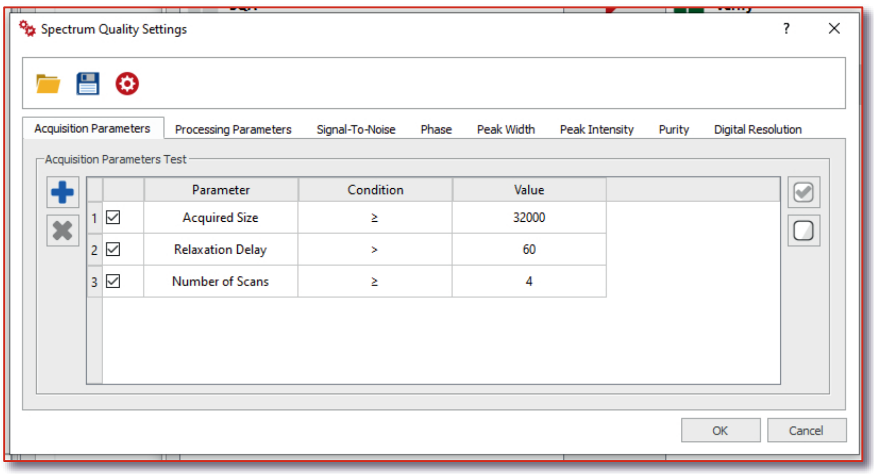
predefined method, held in a library, specific to the APIs being analyzed. The method includes the method directory, reference library, processing parameters, replicate analysis options, predefined regions of integration, and reporting formats. Weights of the sample and reference standard are automatically extracted from the spectral metadata and used in the purity calculation.

The last step in the sequence is the check of the spectral quality utilizing the SQA (spectral quality assurance) brick. SQA is a particularly important step, confirming that the spectra are of sufficient quality to enable a reliable analysis. SQA can be used to check and confirm that the acquisition parameters are adequate for the analysis required – for example, to confirm that the acquisition has been done under quantitative parameters which ensure that there is adequate relaxation delay, etc. SQA evaluates various parameters including dataset size, relaxation delay, number of scans, processing, S/N, peak width, digital resolution, and many more. In this example, minimum thresholds for acquired data size, relaxation delay, and number of scans are defined (Figure 2).

Figure 2. Quality Settings



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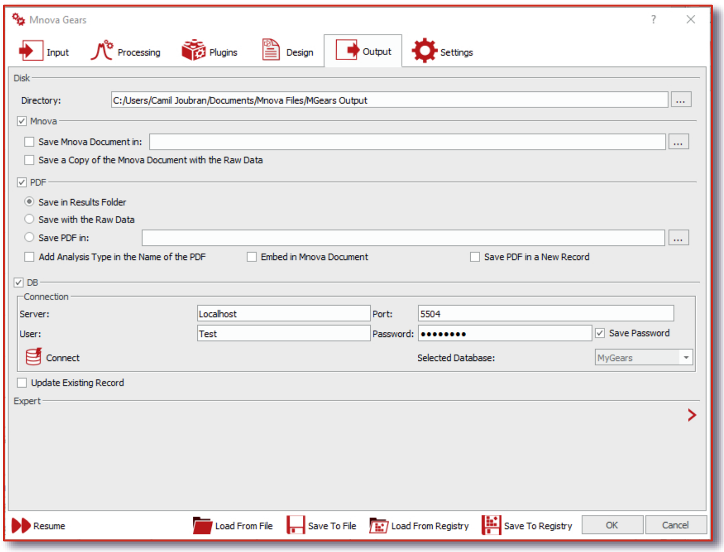
Once the sequence is complete, the results are exported into a database for storage (Figure 3), as well as formatted to produce a PDF, Mnova, or HTML files.

Figure 3. Output Settings



Output

The resultant analyzed spectra contain the purity, RSD, specified integral regions, weights of sample and standard, and acquisition parameters. Assessment of structure is indicated by color highlights in the structure (green = match), (Figure 4). A detailed analysis of structure match can be found in the HTML files. In addition, a summary spreadsheet of all three results is generated that can be imported into excel for further manipulation (Figure 5). The report for the quality assessment of each spectrum (based upon data size, relaxation delay, number of scans, and processing parameters) is also displayed and stored to demonstrate that the specified requirements are met (Figure 6).

Figure 4. Resultant ^{1}H Spectrum

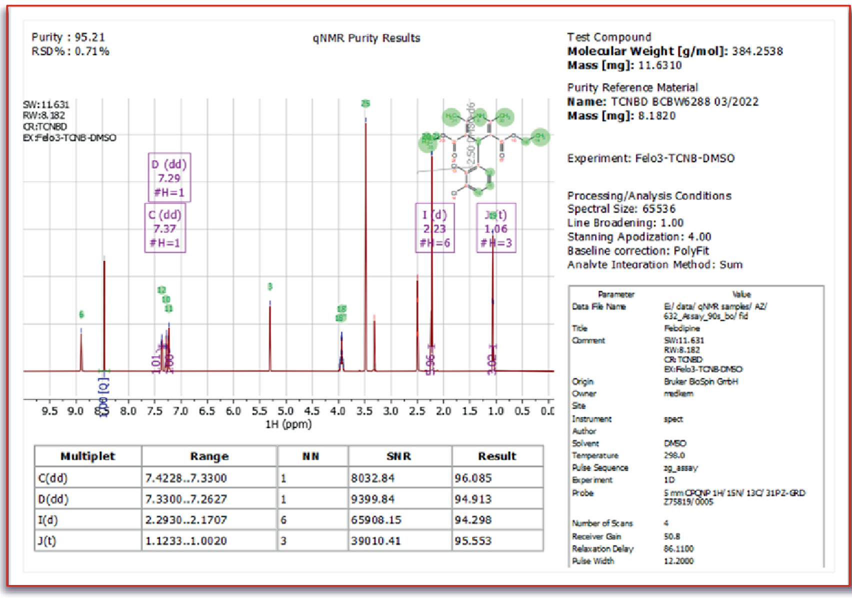


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Figure 5. Purity Summary Report

#	Sample Name	Multiplet 1	Multiplet 2	Multiplet 3	Multiplet 4	Purity	SD	RSD
1	Felodipine	96.08%	94.91%	94.30%	95.55%	**95.21%**	**0.67**	**0.71%**
2	Felodipine	95.93%	94.76%	94.14%	95.40%	**95.06%**	**0.67**	**0.71%**
3	Felodipine	95.98%	94.81%	94.19%	95.45%	**95.11%**	**0.67**	**0.71%**
	Purity	**96.00%**	**94.83%**	**94.21%**	**95.47%**			
	SD	**0.07**	**0.06**	**0.06**	**0.06**			
	RSD	**0.07%**	**0.07%**	**0.07%**	**0.07%**			
Total						**95.13%**	**0.67**	**0.71%**

Figure 6. SQA Results for each Spectrum

Spectrum 1

```
[INFO]: Quality test started on 2020-08-04T10:13:54
[INFO]: Analysis performed using the original spectrum
[INFO]: The specific analysis of SQA has been performed
[INFO]: Quality test spectrum for Felodipine:
[INFO]: All tests have been PASSED
[INFO]: Acquisition parameter test(s): PASSED
        [INFO]: The Acquired Size acquisition parameter meets the condition: 32768≥32000
        [INFO]: The Relaxation Delay acquisition parameter meets the condition: 86.11>60
        [INFO]: The Number of Scans acquisition parameter meets the condition: 4≥4
[INFO]: Processing parameter test(s): PASSED
        [INFO]: The BC[1].PolyOrder processing parameter meets the condition: 3=3
```

Spectrum 2

```
[INFO]: Quality test started on 2020-08-04T10:13:56
[INFO]: Analysis performed using the original spectrum
[INFO]: The specific analysis of SQA has been performed
[INFO]: Quality test spectrum for Felodipine:
[INFO]: All tests have been PASSED
[INFO]: Acquisition parameter test(s): PASSED
        [INFO]: The Acquired Size acquisition parameter meets the condition: 32768≥32000
        [INFO]: The Relaxation Delay acquisition parameter meets the condition: 86.11>60
        [INFO]: The Number of Scans acquisition parameter meets the condition: 4≥4
[INFO]: Processing parameter test(s): PASSED
        [INFO]: The BC[1].PolyOrder processing parameter meets the condition: 3=3
```

Spectrum 3

```
[INFO]: Quality test started on 2020-08-04T10:13:58
[INFO]: Analysis performed using the original spectrum
[INFO]: The specific analysis of SQA has been performed
[INFO]: Quality test spectrum for Felodipine:
[INFO]: All tests have been PASSED
[INFO]: Acquisition parameter test(s): PASSED
        [INFO]: The Acquired Size acquisition parameter meets the condition: 32768≥32000
        [INFO]: The Relaxation Delay acquisition parameter meets the condition: 86.11>60
        [INFO]: The Number of Scans acquisition parameter meets the condition: 4≥4
[INFO]: Processing parameter test(s): PASSED
        [INFO]: The BC[1].PolyOrder processing parameter meets the condition: 3=3
```


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Conclusion

For many years, Mnova has provided powerful, easy to use tools for the analysis of qNMR data, for verifying structures and determining concentration. But never has it been so easy as to bring all this power together into a single easy to use interface common across all your automation needs.

References

[1] "Expanding the Analytical Toolbox: Pharmaceutical Application of Quantitative NMR", Analytical Chemistry, 86, 2014, 11474.

[2] "Validation of Pharmaceutical Potency Determinations by Quantitative NMR", Applied Spectroscopy 64 (5), 2010, 537.

[3] "Guidelines from the International Conference on Harmonization (ICH)", Journal of Pharmaceutical and Biomedical Analysis, 38, 2005, 798.

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