Automated, High-Throughput Data Processing & Quantification:

Illustrated by a series of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

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INTRODUCTION

Technological advancements paired with automated data processing methods (*e.g.,* spectral databases, PCA/PLS methods) have facilitated the widespread use of analytical techniques by both experts and non-experts to answer a variety of practical questions. For example, relaxometers have been tailored towards a single inquiry (*e.g.,* Solid Fat Content) and handheld Raman spectrometers have been coupled with narcotics spectral databases.

Although an extremely powerful technique for qualitative and quantitative analysis, Nuclear Magnetic Resonance (NMR) Spectroscopy has largely lagged behind other characterization tools in this regard. Instead, it has largely remained limited to experts.

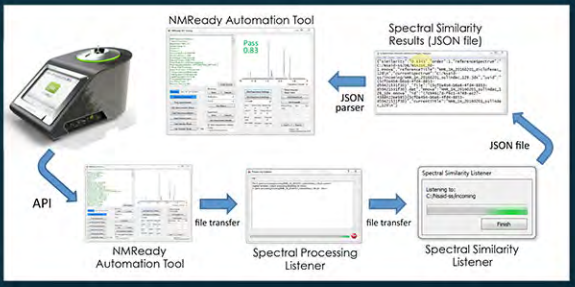
The emergence of the low-field, high-resolution benchtop NMR spectrometer, however, looks to proliferate the use of high-resolution NMR in non-traditional industrial applications. These compact, low maintenance spectrometers, if properly paired with modern data analysis techniques, can increase ease-of-use and make the powerful technique accessible to non-experts.

Automatic Data Processing

The link between spectrum acquisition hardware/controlling software and more sophisticated data processing tools, although not common in NMR Spectroscopy are vital to increase efficiency and address industrial data flow needs, including the:

- generation of NMR data reports by non-experts
- automated downstream data analysis
- automatic ('silent') processing

Mnova offers a number of 'listeners' that can automatically process and analyze NMR data as soon as it is generated. Here we describe the use of a 60 MHz benchtop NMR spectrometer, the NMReady-60, to acquire ^{1}H NMR spectra for a series of nine non-steroidal, anti-inflammatory drugs (NSAIDs) that, paired with three different Mnova listeners (processing, spectral similarity (SS) and simple mixture analysis (SMA)), are automatically processed and/or analyzed.



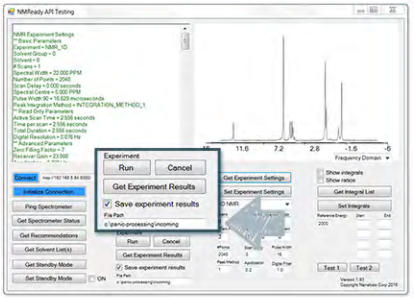
AUTOMATED DATA PROCESSING & REPORTING

To decrease human error, streamline data processing & create institution wide report consistency

To facilitate greater adoption of benchtop NMR in industry, data processing must be more efficient & more accessible to the non-expert. To this end, we describe the use of the modern interface of the NMReady to network directly with the Mnova processing listener plugin. The simple set-up workflow is depicted in 5 simple steps:

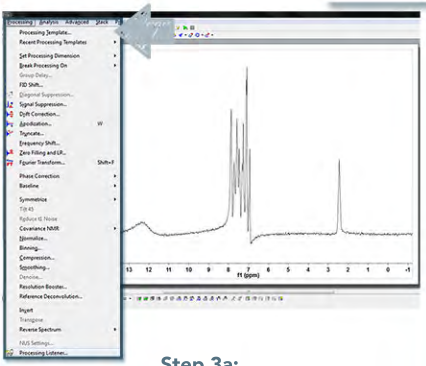
Step 1:

Network the NMReady spectrometer with Ethernet or Wi-Fi connectivity



Step 2:

Connect NMReady Automation tool to spectrometer & configure it to write to MNova listener folder



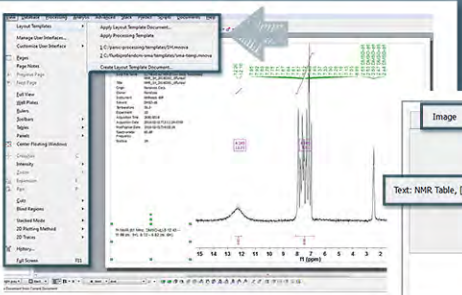
Step 3a:

Launch processing template

Step 3b:

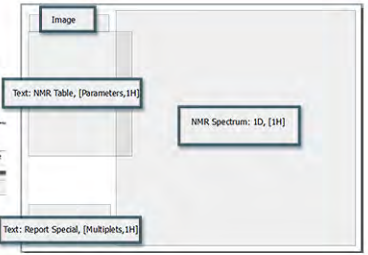
Select desired processing parameters & save

AUTOMATED DATA PROCESSING - Setup



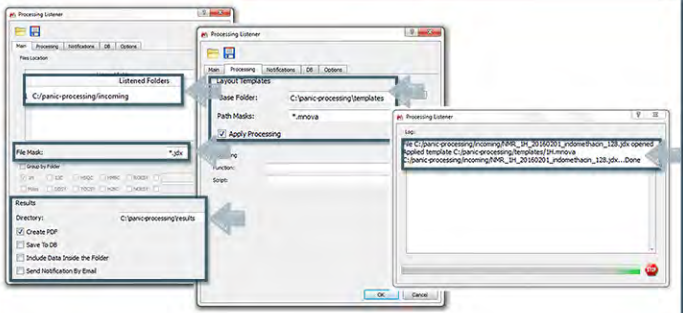
Step 4a:

Setup a single spectrum with the desired layout and information (*e.g.*, spectral parameters, company logo, compiled data, etc.) to create layout template.



Step 4b:

Created template document is saved to be applied to incoming folders.



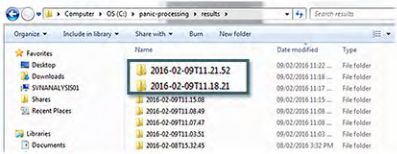
Step 5:

Launch Processing Listener Template & customize processing listener to specify incoming & outgoing data folders, report file formats, and appropriate processing & layout templates.

AUTOMATED DATA PROCESSING - Day-to-day Workflow

Step 1:
Prepare NMR sample

Step 2:
Acquire desired NMR data
(*e.g.*, 1D ^{1}H, ^{19}F, 2D, etc.)

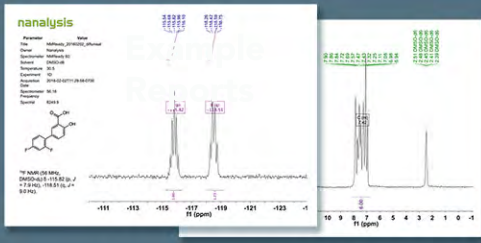


Step 3:
Results automatically opened, processed with predefined template, placed in layout template and saved to results folder with date & time stamp.

Step 4:
Review and/or share processed data files

SUMMARY:

The set-up and daily workflow procedure is presented as an automated analysis for routine processing and reporting of NMR data.



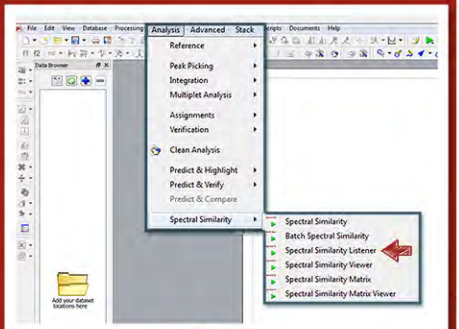
Spectral Similarity Listener

Chemoinformatic method to increase efficiency and optimize derivitization & drug discovery

The idea of a 'distance' between chemical objects is a common. Well-known algorithms such as the 'Tanimoto Score' can be applied to the molecular attributes. Attempts to apply this principle to an objective comparison between NMR spectra are not as common, but a first generation, proprietary algorithm has been developed for Mnova and is presented as the 'Spectral Similarity' (SS) functionality. The functionality considers two spectra, and provides a number (0 to 1) that reflect the similarity.

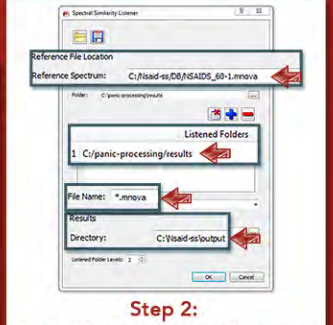
Although there are unlimited applications for this type of spectral assessment, we used the 9 NSAIDs processed with the processing listener. Once seen by the SS listener, a ^{1}H NMR spectrum was opened, compared to a reference spectrum & the calculated similarity score is exported as a text file saved into 'SS results'. For the NSAIDs using Mnova SS, we see high similarity numbers between the compounds, which is reassuringly reasonable, as these pharmaceuticals are expected to have similar mechanisms of action. The test also has potential in a QA/QC scenario where batch samples are analysed & compared against a reference material.

The analyst would have to 'calibrate' the coefficient in terms of a criteria of acceptability. The set-up is shown here.



Step 1:

Select 'Spectral Similarity Listener' from Analysis

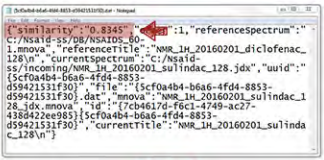


Step 2:

Setup 'SS listener' to watch for mnova files from the processed results folder, it then takes them and compares them to a reference spectrum & saves the results to the defined directory.

Step 3:

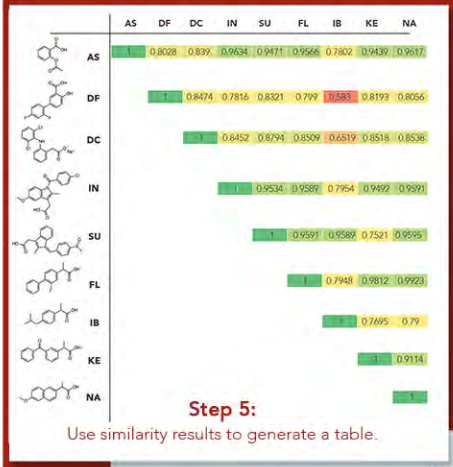
The experiment runs in the background waiting for files to be saved into the processing listener results folder.



Step 4:

Open text file exported to results file with appropriate time stamp and look for similarity value.

NSAIDs DATA FROM SPECTRAL SIMILARITY LISTENER



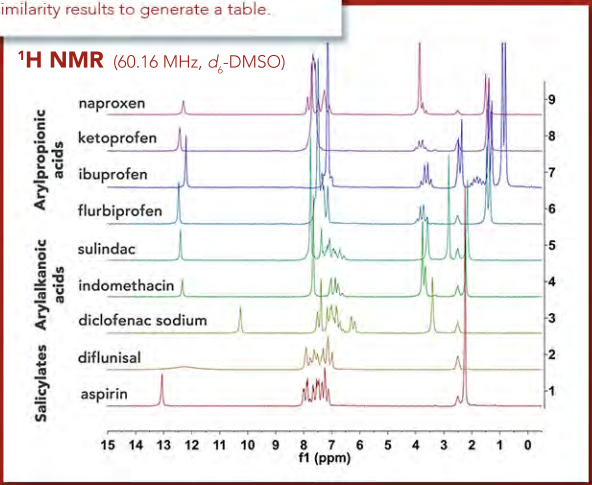
Step 5:

Use similarity results to generate a table.

SAMPLE PREPARATION:

	MW (g/mol)	Added (mg)	Concentration (mol/L)
AS	180.157	18	0.10
DF	250.198	18	0.07
DC	318.13	28	0.09
IN	357.787	19	0.05
SU	356.412	31	0.08
FL	244.261	30	0.12
IB	206.29	30	0.15
KE	254.281	18	0.07
NA	230.259	30	0.13

Each sample was prepared by dissolving NSAID powder in 1 mL of d_6-DMSO and transfering to 5 mm NMR tube.



^{1}H NMR data collected with a spectral width of 16 ppm centered at 7ppm, 4096 points, & scan delay of 5 sec. This is a 10.5 sec/scan acquired at 16 (2.8 min), 32 (5.6 min) and 64 (11.2 min) scans.

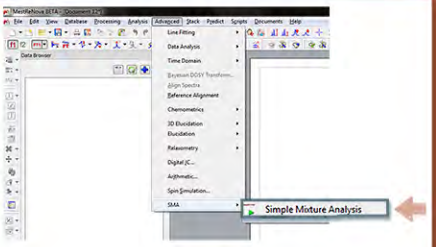
Simple Mixture Analysis Listener

For targeted, repeatable, reliable, and rapid routine quantitative analysis

Many QA/QC inquiries are quantitative (*e.g.*, relative amount of two components, determining amount of residual solvent). The SMA Listener allows a technician to make reliable & repeatable measurements of a validated method using the automated SMA listener.

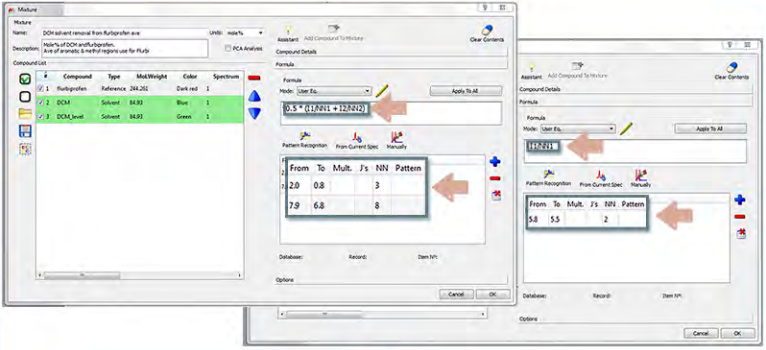
SMA is a plugin that uses global spectrum peak deconvolution (GSD) or conventional running integration to identify various components on the basis of chemical shift ranges in either 1D or 2D spectra. This quantitative information can be generated from the integration information with a flexible, user specified equation.

For this example we look at the isolation & drying of flurbiprofen by monitoring the relative amount of residual dichloromethane (DCM, δ = 5.76 ppm in d_6-DMSO) present.



Step 1:

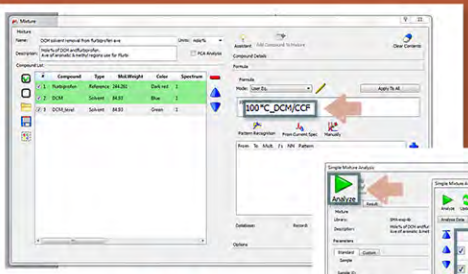
Select "Simple Mixture Analysis" from Advanced



Step 2:

Define experiment for NSAID (flurbiprofen) & solvent (DCM) using ratio of integral region (I) in ppm to number of nuclei (NN) where their signals uniquely occur.

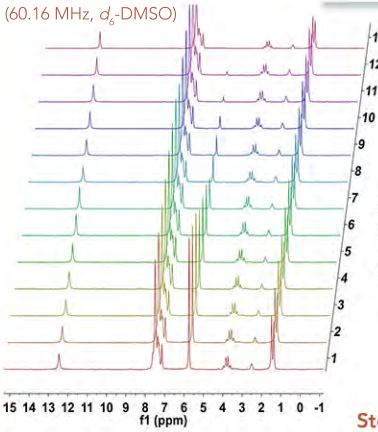
SIMPLE MIXTURE ANALYSIS LISTENER - Setup & Validation

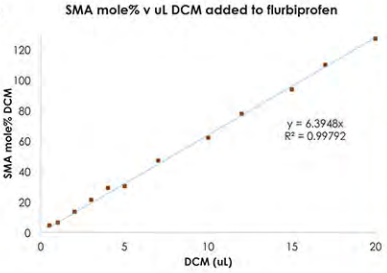


Step 3:
Determine mol% of DCM

Step 4:
Analyze

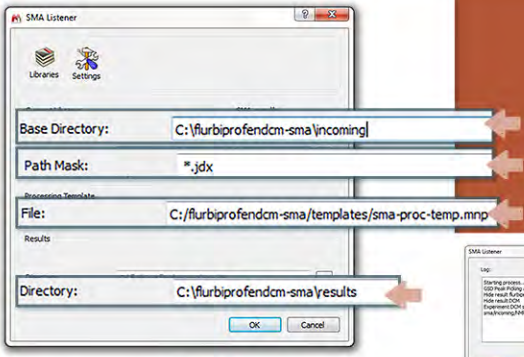
^{1}H NMR
(60.16 MHz, d_6-DMSO)





Step 5:
Validate experiment over a known concentration range of dichloromethane in flurbiprofen to ensure linearity, repeatability and/or accuracy.

SIMPLE MIXTURE ANALYSIS LISTENER - Automation

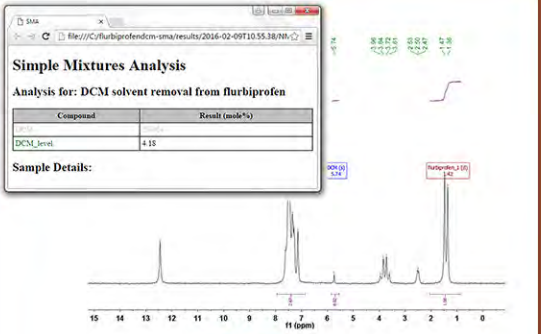


Step 1:

Customize SMA Listener by defining:
(a) Base directory where listener will 'watch' for incoming raw files to be processed
(b) file extension of incoming file .jdx for NMReady
(c) pre-defined processing parameters *(see above)*
(d) file in which results will be saved

Step 2:

Listener watches 'incoming' folder and immediately applies defined SMA experiment saving results in 'results' folder



Step 3:

View spectral results in Mnova or tabulated summary

CONCLUSIONS

The NMReady-60 benchtop NMR system can be easily paired with Mnova for automated data processing via:

(1) Configure the NMReady Application Programming Interface (API) to export all acquired spectra to a specified folder.

(2) Set the appropriate Mnova listener to 'watch' for generated spectral files and process the under pre-set conditions.

This has been shown for:

(1) Spectral Processing & templating
- to streamline data processing & generate consistency

(2) Spectral Similarity -
chemoinformatic information for drug discovery

(3) Simple Mixture Analysis
- for targeted, reliable quantitative analysis

For more information, please visit
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