

CHENOMX CONDENSED GUIDE

The Chenomx Compound Signature Library was acquired using the **Sample Preparation** and **NMR Data Acquisition** standard operating procedures summarized below. Use our method for your samples to match the conditions of our library and improve the accuracy of your results.

1.0 Sample Preparation

Sample Filtration through a 3KDa filter tube.

Chenomx is uniquely designed to identify and quantify small molecules in liquid samples. Filtration is recommended for samples containing large molecules. For a step-by-step guide for *filtering your sample*, visit www.chenomx.com/support to read SOPs C-001 and C-002.

Mix/vortex Sample with an Internal Standard

Chenomx Internal Standard (IS2) contains a DSS Chemical Shape Indicator. For a step-by-step guide to *Internal Standard (IS2) and Sample Preparation*, visit chemomx.com/support to read SOP S-001.

2.0 NMR Data Acquisition

NMR Spectral Acquisition using METNOESY Pulse Sequence

Chenomx uses a 1D-1H NMR pulse sequence. Remember to Export an Uncompressed FID file. Automatically processed FIDs can include automatic phasing and baseline correction, which are not best practice for metabolomics research. Use the following parameters for best results:

Temperature:	25°C
Acquisition time:	4s
Mixing time:	0.1s
Initial delay:	0.01s
Pre-saturation delay:	0.99s
Transients/scans:	32
Steady state scans:	4
Spectral width:	12ppm

For more details on NMR Acquisition Parameters, visit www.chenomx.com/support and read SOP N-001.

For information on the Compound Builder, Batching and Binning, please refer to the Video Tutorials and Chenomx User Guide at www.chenomx.com/support/

The following steps reflect best practices for using Chenomx NMR Suite. Please follow these steps *in sequence* for best results.

3.0 Processing

Automatic Processing is available, but it is recommended to check and modify manually prior to profiling.

VALIDATION OF DATA

1. Check quality of acquired NMR Data, ensure upper limits of peaks are within measurable range.
2. Check Pulse Sequences and other parameters to ensure compatibility with Chenomx Library
3. Confirm no broad peaks in spectra (indicating improper filtration of samples)

INTERNAL STANDARD

4. Check Internal Standard peak shape and width to ensure it is symmetrical, sharp, and narrow.
5. Confirm Internal Standard linewidth with 'Calibrate CSI': maximum acceptable linewidth is **2.2 Hz**.
5. Correct minor imperfections with **Shim Correction**. If unable to correct, reacquire data.

PROCESSING STEPS

7. Phase correction: correct asymmetric baselines on either side of peaks and peak clusters in the spectrum.
8. Baseline Correction: remove "smile", "frown" and "wing"-type baseline distortions.
9. Shim Correction: remove lineshape distortions and reconstruct an ideal spectrum using a single, isolated peak.
10. Line Broadening: Double check before Calibrating your CSI. max acceptable linewidth is **2.2 Hz**
11. Calibrate CSI NOTE: If any steps are corrected, you must repeat subsequent steps and Calibrate CSI again.

For more information, watch our **Tutorial Videos** at www.chenomx.com/support.

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4.0 Profiling

1. Select the correct library under the compound sets tab to start.
2. Start with large peaks first, and right click on a peak and search for best fit.
3. If you are doing a targeted analysis (you know what compounds you are looking for in your samples), type the name of the compound in the search box and fit.
4. If you cannot find targeted compounds, enter “0” as the concentration. This indicates the compound has been accounted for.
5. Fit all clusters of a compound where possible. Overlapping or low concentration may make this difficult.
6. Label compounds profiled with low level of confidence by a colour and check them again at the end.
7. If compounds overlap, focus on a different, isolated cluster for that compound. In other words, you should find at least one reliable (isolated and intact) peak for profiling.
8. Be careful profiling compounds with only one singlet peak (such as Acetate, Pyruvate, Acetone, Formate etc.). If they are showing up with low concentration and overlapped with larger peaks, be careful when reporting.
9. When you are done with profiling, go through the spectrum again and ensure un-profiled peaks (black colour) are accounted for.
10. Profile all spectra.

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5.0 Reviewing/Reporting

1. Go to batch export and select all the profiled spectra. Export.
2. Ensure no blank cells in your report. Blank cells have not been profiled, analyze that compound again in your spectrum and enter “0” for concentration if it cannot be located. Export again.
3. Compare concentrations of each compound across all the samples. Check anomalous data and return to spectrum/profiling where necessary.
4. Review each spectrum and ensure peaks have been fit. Focus on overlapped areas.
5. Check unprofiled peaks (shown with black line), ensure they cannot be profiled.
6. Finish your review. Export again.
7. The compound concentration data in the excel file is the concentration measured in the NMR tube. Correct these values to reflect correction/dilution factors such as: the internal standard (usually 10%); *adjustment for low volume samples; buffering agents*.
8. Make a second spreadsheet and name it as FINAL RESULTS and apply the correct dilution factor.
9. Put *project name, client name, name, number and type of samples, dilution factor, magnet frequency, number of scans, and the version of library used to profile* at the top of both sheets.

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