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 chenomx.com/support to read SOP S-001.

2.0 NMR Data Acquisition

NMR Spectral Acquisition

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:

Pulse sequence:	noesypr1d (Bruker), or an adaptation of metnoesy (Varian). Varian users can download the pulse sequence C code from **https://www.chenomx.com/support/** under the Sample Preparation section of the FAQ.
Solvent:	90% H_2O, 10% D_2O
Temperature:	25°C
Acquisition time:	4 s
NOE Mixing time:	0.1 s
Initial delay:	0.01 s
Pre-saturation delay:	0.99 s
Steady state scans:	4
Spectral width:	12 ppm

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

NMR Suite Standard Operating Procedures

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 (proper shimming, no detector overflow).
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**.
6. 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: (We recommend the Whittaker Spline).
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.
12. Calibrate pH: Initial peak clusters resonance frequencies, as well as their transform windows, are calculated using this setting.

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

4.0 Manual Profiling

1. Select the correct library under the compound sets tab to start.
2. Start with the most intense 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.

4.1 Profiling with the Compound Snapper and Automatic Fitter (V9)

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. Select desired Compound from Compound Table and right-click on suggested cluster on the Spectrum Graph. Choose Snap Selection → Snap Compound on Spectral Line (or Subtraction Line when appropriate). Suggested Cluster will snap to the center of the Spectrum Cluster. Shortcut Key: “,” or “;”
6. Right-Click suggested cluster again. Choose Fit Concentrations → Fit Concentrations with/without GUI. Shortcut key: ‘SPACEBAR’ or ‘SHIFT + SPACEBAR’
7. Inspect the results. Add compounds and/or repeat steps 5 and 6.

4.2 Profiling with Complete Autofit (V10 only)

1. Ensure your Processing Steps were completed carefully according to Chenomx Recommendations listed above.
2. Go to Tools → Batch Tools → Complete Autofit.
3. Select your Processed CNX file you wish to AutoFit as well as an output file for the fit report (TSV file) and click Next.
4. Choose source of compounds to autofit: an existing, profiled CNX file ; a custom library, or compound set you'd like to Autofit to your Spectrum.
5. Linear Fit Setup:

   (a) Set Region Selections for regions to be included/excluded in the Autofit.

   (b) Set additional fitting options for the concentrations (constrained fit vs unconstrained, etc).
6. Select Number of Internal autofitting Iterations and the Number of Export Files for each file. NOTE: the default values are not necessarily recommended for your spectrum. Increasing either value has potential to increase accuracy but will increase processing time.
7. Review the exported CNX files manually for systematic errors or missing compounds that could be added.
8. Repeat the fitting process if additional compounds need to be included.
9. The Autofit Spectrum with the lowest chi-square value will be named FILENAME_BEST. Review the error report in your log (TSV) file.

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5.0 Reviewing/Reporting

1. Go to batch export and select all the profiled spectra. Export.
2. Compare concentrations of each compound across all the samples. Check anomalous data and return to spectrum/profiling where necessary.
3. Review each spectrum and ensure peaks have been fit. Focus on overlapped areas.
4. Check unprofiled peaks (shown with black line), ensure they cannot be profiled.
5. Finish your review. Export again.
6. The data on the excel file is the concentration of the compounds in the NMR tube. Values should be corrected by applying a correction factor due to adding the internal standard and any other dilution factor prior to statistical analysis.