



# Optimizing Spectra for Metabolomics

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*Chenomx Processor allows you to work with NMR spectra from a variety of sources, helping you apply phasing and baseline correction through manual and semi-automated tools, and offering advanced lineshape correction through reference deconvolution [1]. Processor also allows you to define chemical shape indicator (CSI) parameters and determine sample pH via indicator compounds. These are necessary prerequisites to analyzing your spectra with Chenomx Profiler[2] [3] [4].*

## Introduction

Targeted profiling in Chenomx NMR Suite involves comparing signals in an experimental spectrum to a library of spectral signatures in a reference database [2]. This approach works best when you analyze spectra that are well phased, have flat, well-defined baselines, and do not contain systematic lineshape distortions.

To get the most out of your data, maintain a consistent "order of operations" when processing a spectrum. At Chenomx, we have found that the following order offers consistent, high-quality results from most spectra: First, adjust the phasing, then apply baseline correction, use reference deconvolution to correct lineshape distortions, and finally, apply line broadening (if necessary) and water deletion (if desired).

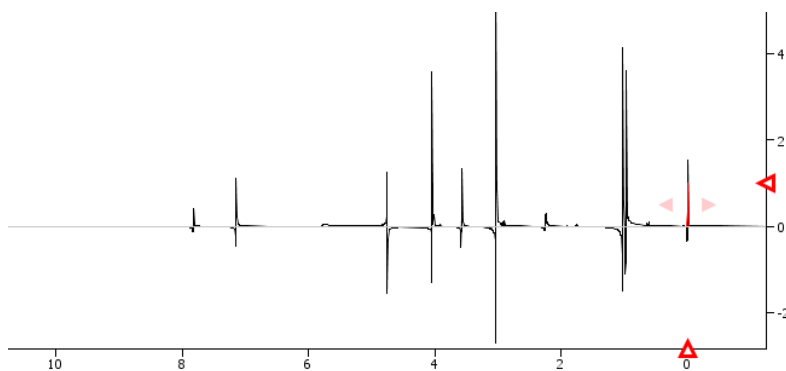


Figure 1. A normal unprocessed spectrum.

## Basic Processing

Upon opening the sample FID, you see a fairly normal unprocessed spectrum (see Figure 1). To begin processing the spectrum, adjust the phasing. Since you are processing the spectrum anyway, do not import the existing VNMR phase angles when importing the spectrum. The automatic phasing algorithm comes close to the desired phase angles, and with a little tweaking, the appearance of the spectrum improves (see Figure 2).

In general, the goal when adjusting phasing in a spectrum is to make the baseline even (symmetrical) on either side of the signals in the spectrum. For signals affected by "tails" of large signals nearby (like water or urea), try to get a smooth curve of the baseline on either side of each signal. While adjusting phasing, ignore the phasing of the water peak in favour of better phasing for signals from other compounds. Many pulse sequences, including the NOESY-derived sequence recommended for use with Chenomx NMR Suite, actively suppress the water signal, with the common result that the water peak itself cannot be accurately phased.

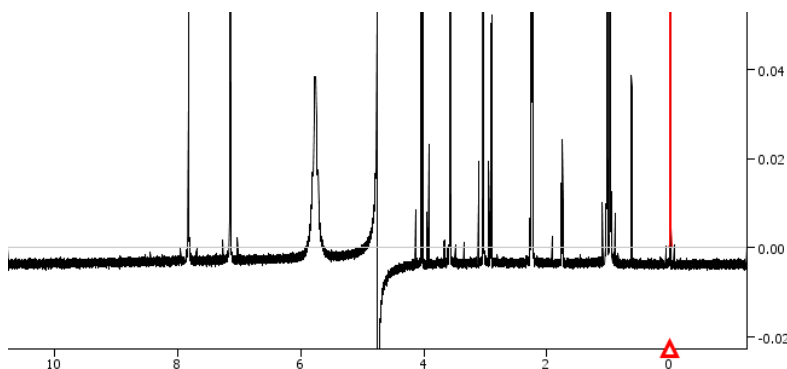


Figure 2. After the phasing is properly adjusted, the baseline describes a smooth curve on either side of each signal in the spectrum.

In preparation for baseline correction, you may find it helpful to zoom in on the baseline. The automatic spline baseline correction suggests fairly reasonable breakpoints, but for the best results, adjust these points manually (see Figure 3). The resulting spectrum looks very good, with a flat, well-defined baseline (see Figure 4).

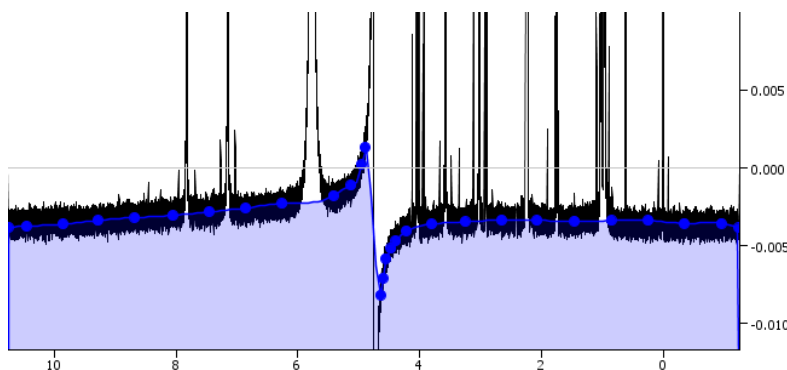


Figure 3. Manual adjustment of the automatically-placed breakpoints will give the best results.

During manual adjustment of breakpoints for baseline correction, place breakpoints along the middle of the baseline region. To avoid introducing "rolling" artifacts, adjust adjacent breakpoints to lie along a smooth curve. If necessary, add more breakpoints; this will often be necessary in regions with strong curvature of the baseline.

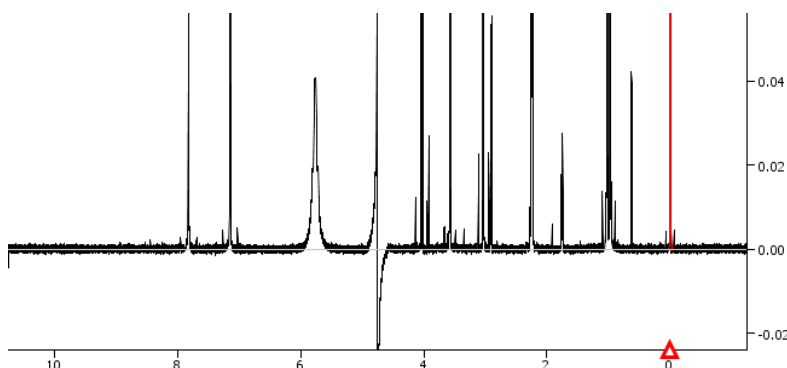


Figure 4. Based on the previously-defined breakpoints, the corrected spectrum baseline is flat and well-defined.

### ***Lineshape Correction***

Before you apply reference deconvolution to correct the lineshapes in the spectrum, establish an initial fit of the CSI peak, which acts as the reference. This also defines starting parameters for the reference deconvolution step. After zooming in on the CSI peak (see Figure 5), adjust the reference peak (brown) to fit the CSI peak (black).

Once you have accurately fit the CSI peak, applying reference deconvolution provides an excellent lineshape (see Figure 6).

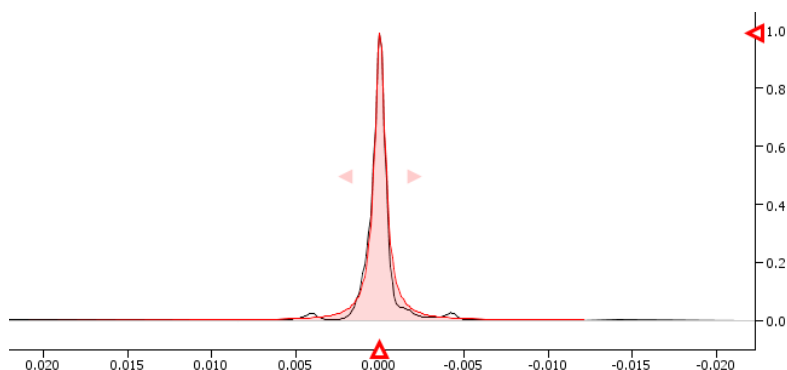


Figure 5. The lineshape of the CSI peak is somewhat distorted.

Since the linewidth of the CSI peak is quite low (around 0.6 Hz), add line broadening to bring the linewidth within a range of 1.0 to 1.5 Hz to get the best analysis results from the Chenomx Metabolite Libraries. For analysis with custom libraries, this step may be unnecessary. After applying line broadening, readjust the CSI fit to account for the change in lineshape.

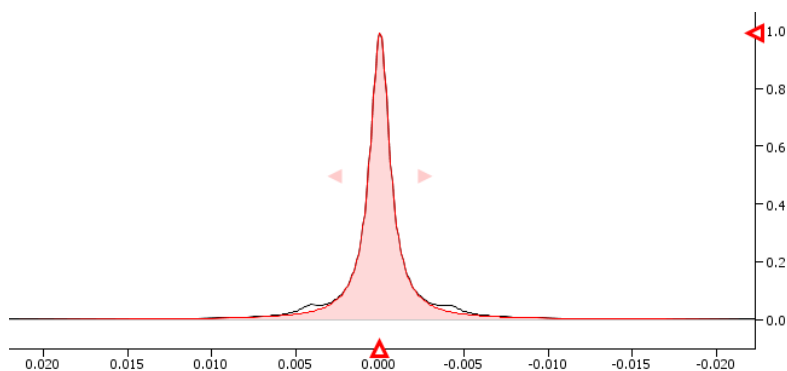


Figure 6. Following reference deconvolution, the CSI lineshape is symmetrical and easily fit.

In general, following the application of any processing layer, ensure that the fit of the CSI definition (red) to the spectrum line (black) is as accurate as possible (see Figure 6). The best method for this will depend on the CSI used in the spectrum. For well-phased spectra with good baseline correction, the automatic CSI algorithm will usually provide the best results for DSS and TSP; if you use formate as a CSI, you will need to use manual adjustments.

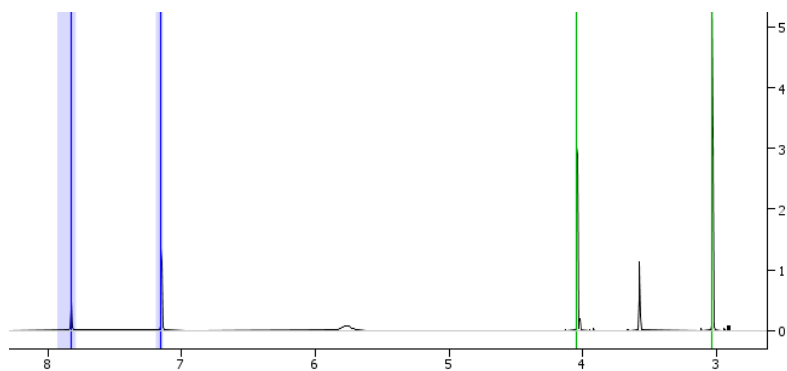


Figure 7. Accurate pH determination is important in performing high-quality analyses of biofluids and other aqueous samples.

## Wrapping Up

To finish processing this spectrum, you need to determine the spectrum pH, or enter a pH that you have measured yourself. Since this sample contains both creatinine (upfield, green) and imidazole (downfield, blue), you can use these pH indicators to determine the spectrum pH by aligning the indicator guides with the indicator peaks in the spectrum (see Figure 7). If there were no pH indicators present, you could simply enter a measured pH instead of using the indicator guides.

Since the water peak in this spectrum has a strong negative intensity, consider applying water deletion. This will reduce the amount that you need to zoom in to the spectrum during fitting, and will provide a generally better-looking spectrum (see Figure 8). However, if there are signals of interest appearing close to the water peak, verify that these are not obscured by water deletion before proceeding, and remove water deletion if necessary.

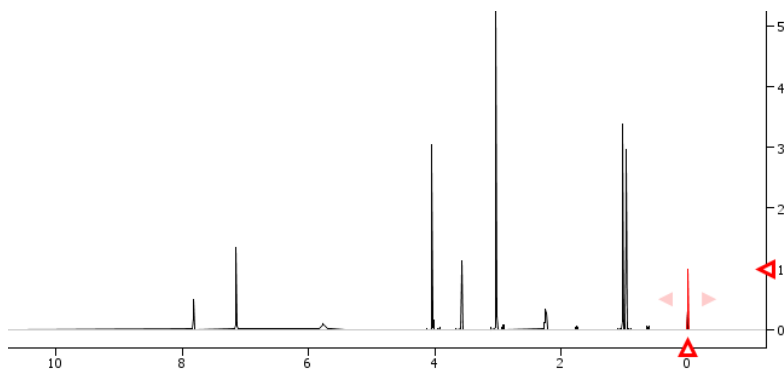


Figure 8. Optional water deletion can yield a better-looking spectrum.

## Analysis

The spectrum is now ready for analysis in the Profiler module. You can remove or reapply any of the processing layers as needed by returning to Processor, as the underlying FID data is not altered during processing.

If you choose to use third-party software to process your spectra for targeted profiling in Chenomx NMR Suite, many of the same steps will still apply. Specifically, ensure good phasing and baseline correction, and add line broadening to yield a DSS linewidth of 1.0 to 1.5 Hz. Do not apply any other weighting functions, such as sinebell or gaussian. These nonlinear weighting functions will adversely affect quantification in Chenomx NMR Suite, which relies on linear combinations of compound signatures.

## References

- [1] P Mercier and C Vitols. **June 2006.** *Correcting Lineshapes in NMR Spectra.* Chenomx Inc.
- [2] R Rosewell and C Vitols. **March 2006.** *Identifying Metabolites in Biofluids.* Chenomx Inc.
- [3] H Fu and C Vitols. **April 2006.** *Targeted Profiling of Common Metabolites in Urine.* Chenomx Inc.
- [4] A Weljie and C Vitols. **June 2006.** *Identifying and Quantifying Metabolites in Blood Serum and Plasma.* Chenomx Inc.