# Correcting Lineshapes in NMR Spectra

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*In this note we present a method for removing lineshape distortions from nuclear magnetic resonance (NMR) spectra prior to more detailed analysis. Reference deconvolution is a method of reconstructing an ideal spectrum by removing lineshape distortions caused by field inhomogeneity (see [2], [3]). Applying reference deconvolution has been found to improve the quality of fit of a spectrum using targeted profiling in Chenomx NMR Suite. However, successful application of reference deconvolution to a spectrum requires some preprocessing and the presence of an appropriate reference peak.*

## *Introduction*

All experiments performed with NMR spectrometers are subject to a variety of distortions imposed by the instruments. The majority of these affect all signals in the same way. Reference deconvolution attempts to correct these distortions by using an experimental signal for a known resonance to compensate for the distortions imposed on the spectrum by the spectrometer.

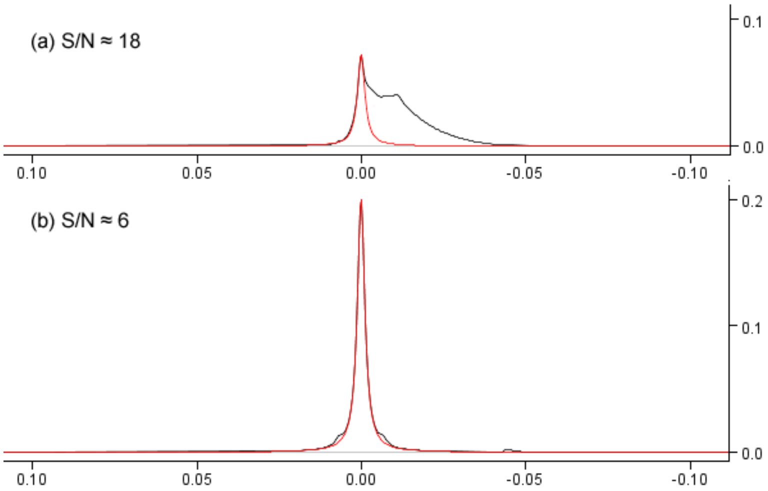

**Figure 1. The main DSS peak of a poorly-shimmed urine spectrum (a) before reference deconvolution and (b) after reference deconvolution**

The general method involves deconvoluting the whole experimental spectrum with the experimental lineshape of the reference signal, and reconvoluting it with the ideal lineshape of the reference signal. Reference deconvolution is most efficiently applied to the time domain, by extracting the component of the experimental free induction decay (fid) arising from the reference signal, and multiplying the original fid by the complex ratio of the ideal and experimental fids for the reference signal [2].

Applying reference deconvolution to a spectrum assumes that all lineshapes in the spectrum are equally affected by field inhomogeneity. Thus, it is only effective in removing lineshape distortions that affect all signals in the spectrum equally. This includes the majority of distortions imposed on spectra by NMR spectrometers, such as distortions due to imperfect static field homogeneity, changes in signal phase and amplitude due to pulse phase and flip angle errors, and frequency shifts due to changes in the static magnetic field [2].

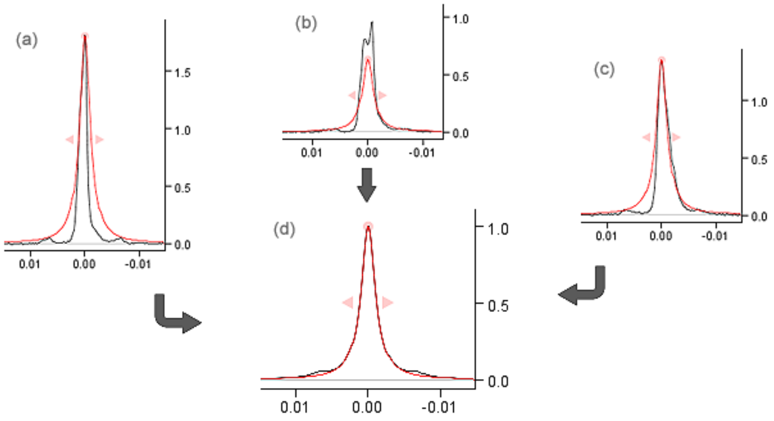

**Figure 2. Based on the chemical shape indicator (CSI), spectra with varying degrees of distortion (a, b and c) can be corrected and standardized (d) for more consistent analysis results**

## *Theoretical Background*

The effectiveness of reference deconvolution in reconstructing a spectrum relies on the selection of an appropriate reference signal. Several factors contribute to the suitability of a signal for use as a reference. To avoid errors resulting from division by very small numbers, the decay envelope for the chosen signal should not drop to zero anywhere in the fid that is being processed. Thus, simple multiplets like symmetric doublets or triplets, or experimental lineshapes with significant doublet character due to "split fields" or poor shimming may not be good candidates for reference signals. The signal should also be well-separated from any other signals, to ensure that neither the absorption nor the dispersion components of the signal experience any overlap. Finally, the ideal form of the signal should be accurately defined throughout the relevant region of the spectrum, including any closely-associated satellite signals [2].

In general, many of the characteristics that make a good peak for use as a chemical shape indicator (CSI) also make a good reference signal for reference deconvolution. Most spectra of samples containing common CSIs like 2,2-dimethylsilapentane-4-sulfonic acid (DSS), trimethylsilylpropionic acid (TSP) or tetramethylsilane (TMS) can benefit from this technique.

Reference deconvolution is a linear process, based on direct and inverse Fourier transforms. Applying the transformation to an experimental spectrum will preserve quantitative relationships in the spectrum. However, the best results from reference deconvolution are possible only with properly phased, baseline corrected and zero-filled spectra [3]. You should zero-fill spectra to at least one full power of two beyond the number of acquired points. For example, if 32k points ($2^{15}$) are acquired, zero-fill to 64k points ($2^{16}$), but if 33k points are acquired, zero-fill to 128k ($2^{17}$).

## *Applications*

Reference deconvolution is an effective method of improving spectrum quality. You can use it to correct experimental spectra by adjusting the acquired data to remove the influence of external, systematic distortions, such as shimming errors or static field inhomogeneities. You can also standardize spectra across a dataset to establish a consistent basis for comparison of the individual spectra or analysis of the dataset as a whole.

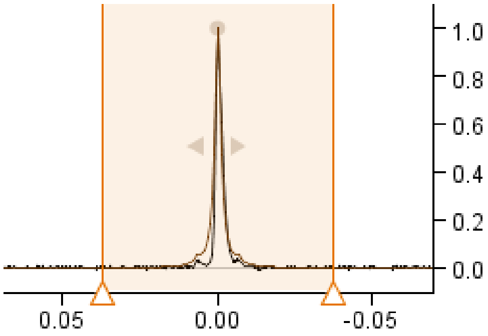

**Figure 3. Use the triangles on the horizontal axis to set a region size that includes the full CSI peak**

To apply reference deconvolution to a spectrum using Chenomx NMR Suite, you must open the spectrum in the Processor module. For maximum effectiveness, the spectrum must be properly phased, baseline-corrected and zero-filled. Use CSI Edit mode to locate and approximate the shape of the CSI in the sample (typically DSS or TSP), then switch to reference deconvolution mode. Set the region size using the triangles along the horizontal axis, making sure to include the full CSI peak, but no other peaks (Figure 3). $^{13}$C satellites should be excluded, but if $^{29}$Si satellites are present, include them and check the Use DSS/TSP Satellites box. Finally, select a target linewidth and click the Accept button to apply the transformation.

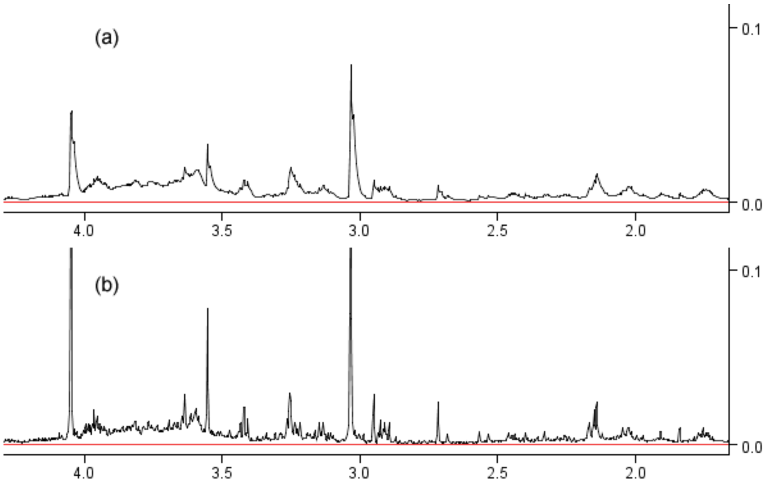

**Figure 4. The aliphatic region of the urine spectrum appearing previously (a) before and (b) after reference deconvolution**

Target linewidths in reference deconvolution can serve two purposes. Specifying a target linewidth value x Hz larger than the native linewidth will have the same effect as applying line broadening of x Hz. Thus, there is no need to apply line broadening if you intend to use reference deconvolution. A target linewidth smaller than the native linewidth will allow a degree of resolution enhancement, but such enhancement will be at the expense of the signal-to-noise ratio. If the target linewidth is equal to the native linewidth, only the lineshape correction effects mentioned previously will apply.

## *Benefits*

Targeted profiling involves comparing an experimental spectrum to NMR spectral signatures of individual metabolites from a reference database [1]. Such comparison is most useful when the observed lineshapes in the experimental spectrum are as close to ideal as possible. Variations in lineshape introduced by different instruments, or different shimming techniques, may adversely affect the results of an analysis. Applying reference deconvolution to experimental spectra prior to analyzing with targeted profiling can increase the consistency of the analysis from spectrum to spectrum by removing the effect of these systematic lineshape distortions. In some cases, spectra that would otherwise be unusable can be restored to a useful condition.

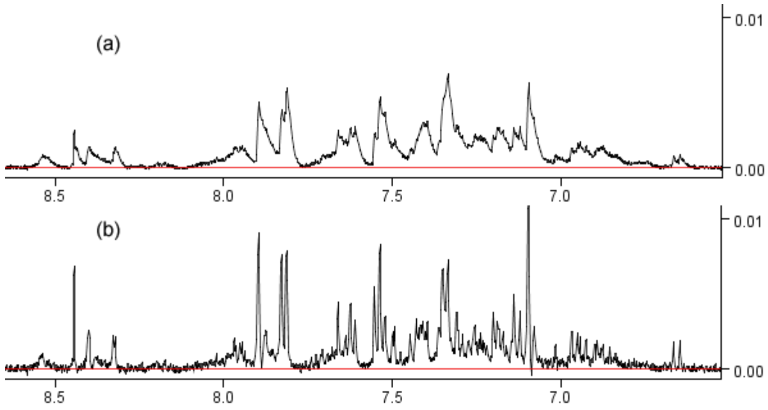

**Figure 5. The aromatic region of the same urine spectrum (a) before and (b) after reference deconvolution**

The lineshape of an internal reference can allow assessing the shimming quality of a spectrum. In the absence of reference deconvolution, a poorly-shimmed urine spectrum (Figure 1) would be unusable, and you would need to reacquire the spectrum. If the original sample was no longer available, the information contained in the spectrum would be lost. Applying reference deconvolution, however, corrects the shimming errors and allows analyzing the spectrum (see Figure 4 and Figure 5).

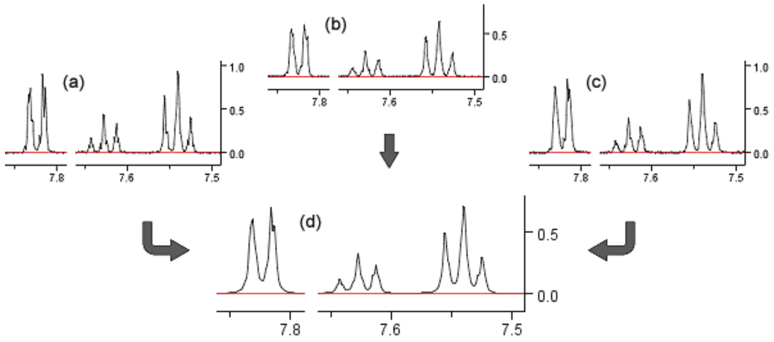

**Figure 6. Spectra that would otherwise yield differing models of hippurate (a, b and c) will yield more accurate models after reference deconvolution (d)**

Creating a compound signature involves building a mathematical model of the compound based on an experimental spectrum. If an experimental spectrum is distorted by external effects, then models based on the spectrum will be inaccurate. Reference deconvolution can remove many of these distortions, bringing the experimental spectrum closer to its ideal shape (Figure 6).

Statistical analysis of NMR spectra using spectral binning (also known as "spectral bucketing") can also be affected by systematic lineshape distortions. Such distortions change the apparent frequency distribution throughout the spectrum, changing the integrated area in each bin. In some cases, this could result in artificial separations in principal component analysis (PCA), based on variables like shimming technique instead of true variation in the samples. Even if the distortions do not necessarily render the PCA results incorrect, subtle variations in frequency distributions can act as a confounding factor when interpreting them. Reference deconvolution can help ensure that the observed variation among samples is inherent to the samples themselves.

## Conclusion

Reference deconvolution is a powerful technique for removing systematic lineshape distortions from NMR spectra, based on reference signals contained in the spectra themselves. It allows standardizing spectra within a dataset, more consistent analysis results during targeted profiling, generating more accurate custom compound signatures, and improved statistical analysis of binned spectra. Although there is still no substitute for proper shimming and good sample preparation, reference deconvolution can help you to ensure high spectrum quality before performing further analyses.

## References

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[2] GA Morris, H Barjat, and TJ Horne. **1997.** *Reference deconvolution methods*. *Progress in Nuclear Magnetic Resonance Spectroscopy.* 31(2):197-257

[3] KR Metz, MM Lam, and AG Webb. **2000.** *Reference Deconvolution: A Simple and Effective Method for Resolution Enhancement in Nuclear Magnetic Resonance Spectroscopy*. *Concepts in Magnetic Resonance.* 12(1):21-42