

Bridging the Gap between Theoretical and Experimental Mass Spectrum

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Overview

- Accurate mass measurements alone, even to 1ppm, generally do not produce unambiguous elemental composition results (ECD or Formula ID).
- Line-shape calibration of the mass spec profile data allows extremely accurate matches to be made against theoretical spectra (Spectral Accuracy) which can provide unambiguous formula ID.
- Different types of mass analyzers, detectors, and post processing methods can affect the Spectral Accuracy and must be carefully considered.

Introduction

Mass spectrometry is one of the very few measurement techniques where the exact theoretical response for a given ion can be accurately calculated, all based on first principles and known elemental abundances. Such a theoretically calculated mass spectrum, however, corresponds to a virtual mass spectrometer with infinite resolving power or FWHM = 0. Mass spectral response measured from any physical instrument, from single quadrupole MS to FT ICR MS, however, corresponds to a finite resolving power appropriate for a particular instrument. It is therefore of great importance to bring the theoretical and measured mass spectrum together for accurate spectral comparison, significantly improving elemental composition determination¹⁻⁴ and enabling reliable deconvolution of overlapping ion signals.

Experimental

Previously acquired and archived data was drawn on to provide a large sampling of different mass analyzers, sampling and ionization techniques, concentrations, conditions and compounds. All data were acquired as continuum (profile) data and transferred to MassWorks software⁵ for calibration, analysis, and report generation.

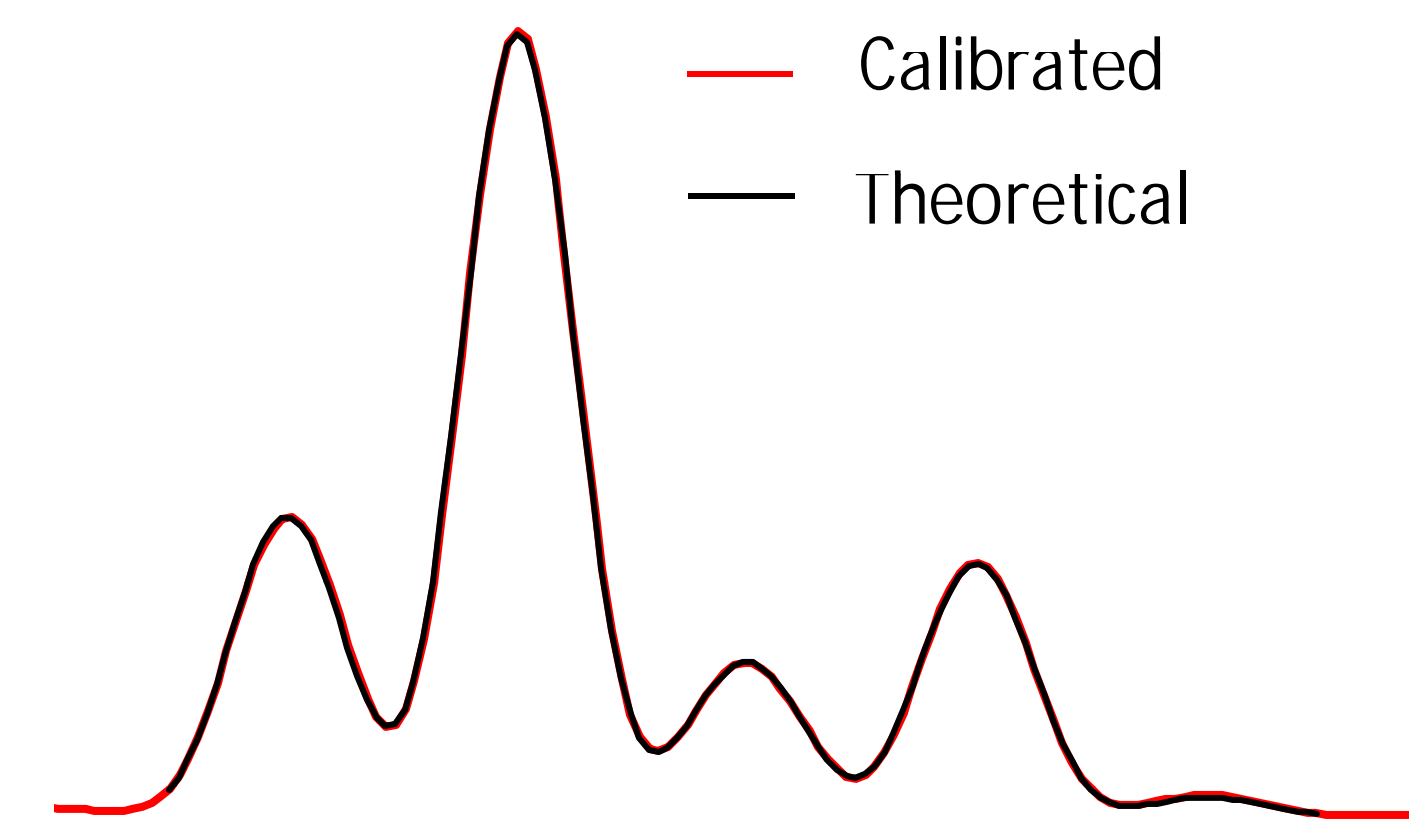
Methods

Due to the mutually overlapping isotopes involved on a low resolution mass spectrometer system, e.g., single quad LC/MS or GC/MS, the peak shape information is not readily extractable from raw profile mode mass spectral data, where the peak shape information has to be indirectly derived from an elaborate numerical process involving calibration ions of known elemental compositions. On higher resolution systems such as qTOF or FT ICR MS, on the other hand, the monoisotopic peak of an unknown ion is well separated from the rest of the isotopes, providing a direct measurement of the peak shape function, without the use of either internal or external standards. Both methods of line-shape calibration are illustrated in Figure 1.

Once data are line-shape calibrated, formula candidates, generated based on the accurate mass capabilities of the instrument, are used to compute theoretical spectra utilizing the exact same line-shape derived from the calibration. A least squares best fit is then performed for the theoretical spectrum against the calibrated spectrum which provides the Spectral Accuracy metric use to identify a unique formula (Figure 2).

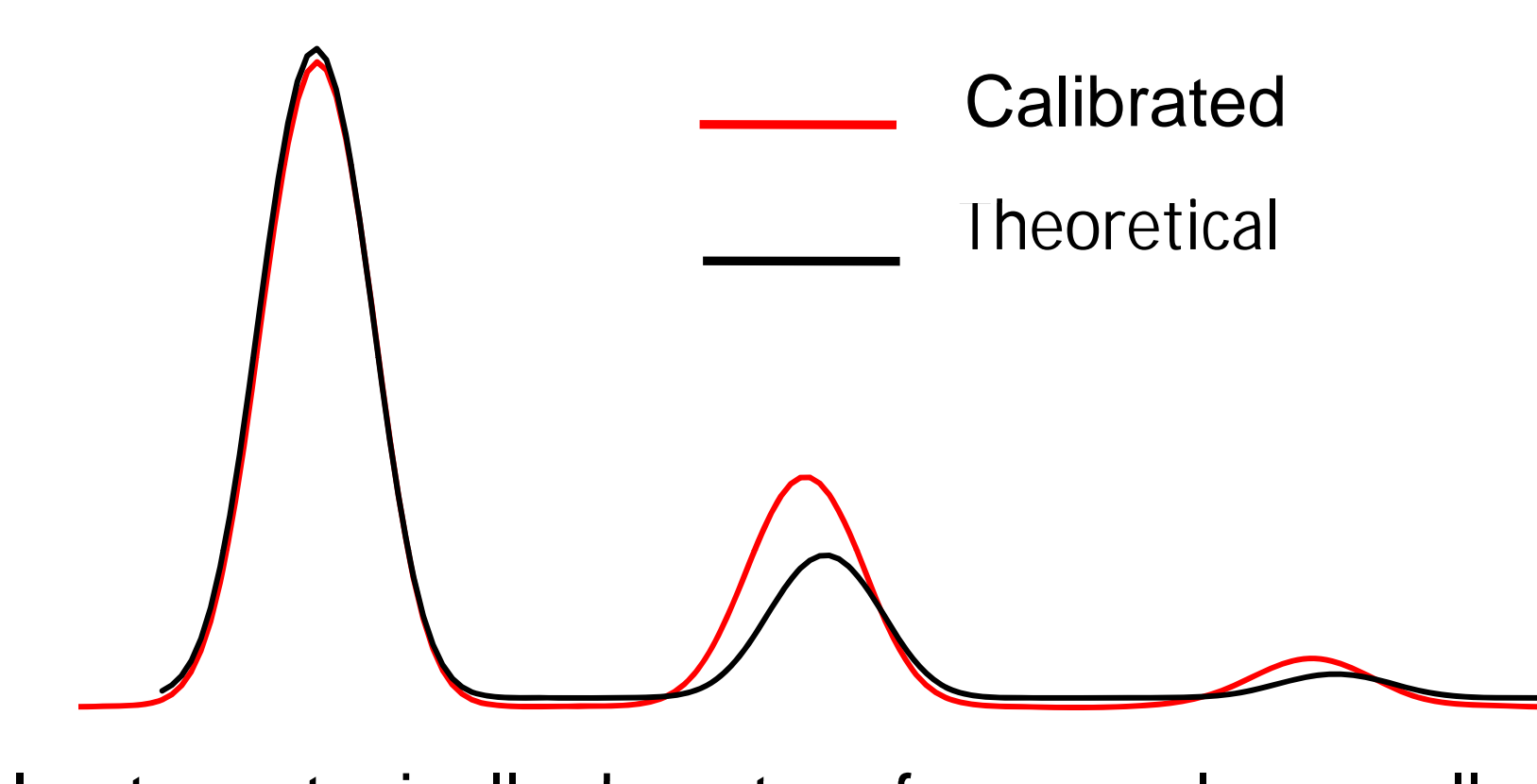
Results and Discussion

Quadrupoles



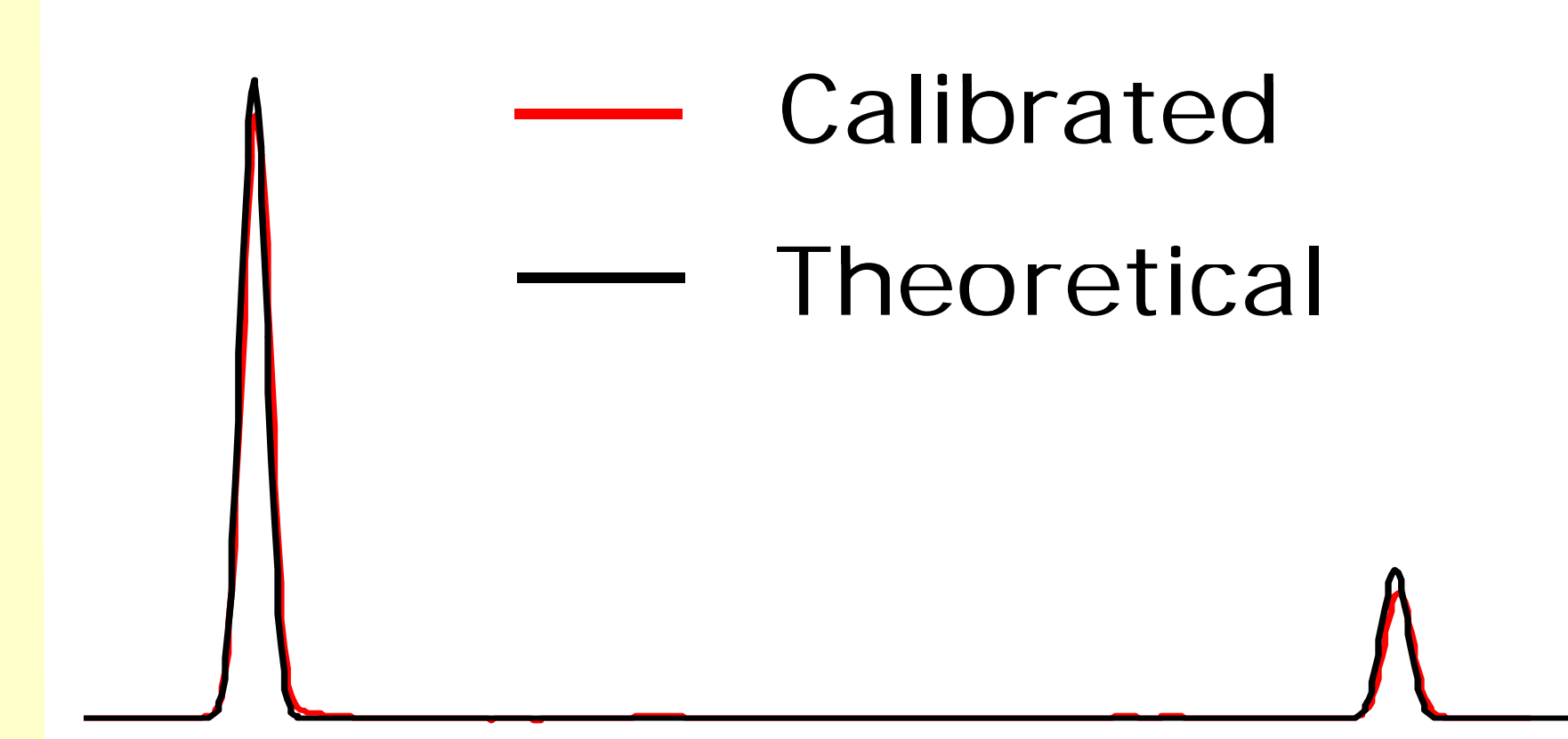
Most Quads produced excellent Spectral Accuracy results enabling highly confident formula ID even with unit resolution data and modest mass accuracy (+/- 10mDa). Some systems exhibited limited dynamic range or instrumental artifacts which could compromise results. Both GC and LC high and low resolution systems produced Spectral Accuracy values as high as 99.5%, provided there was adequate signal-to-noise. The fitting process also can also readily account for either known interferences or common fragmentation interferences such as coexisting M-H, M, or M+H as shown above (M-H from GC/MS).

Ion Traps



Ion traps typically do not perform nearly as well as quads for producing good Spectral Accuracy. The plot above of zoom mode data shows large errors in both the isotope peak intensity and mass position. The large errors are attributed to space-charge effects. Careful control of injection time and ion density in the trap can lead to acceptable results. Trap designs are also a factor.

FT ICR and Orbitrap



Both FT ICR and Orbitrap instruments produced Spectral Accuracy values adequate to consistently place the correct formula among the top matches. The absolute spectra accuracy values were not as good as for quads and TOF instruments, especially for weaker ions. It is suspected that low ion thresholding internal to some instruments is the primary cause. This is illustrated in the above plot (M+2 and M+3 shown) which shows the smaller isotope peaks produce lower intensities than theoretical predictions. It is expected that relatively minor modifications to the underlying instrument processing could significantly improve the Spectral Accuracy allowing these instruments to unambiguously ID unknown formula. Despite this, the Spectral Accuracy rankings provide very significant improvements in formula ID.

Conclusions

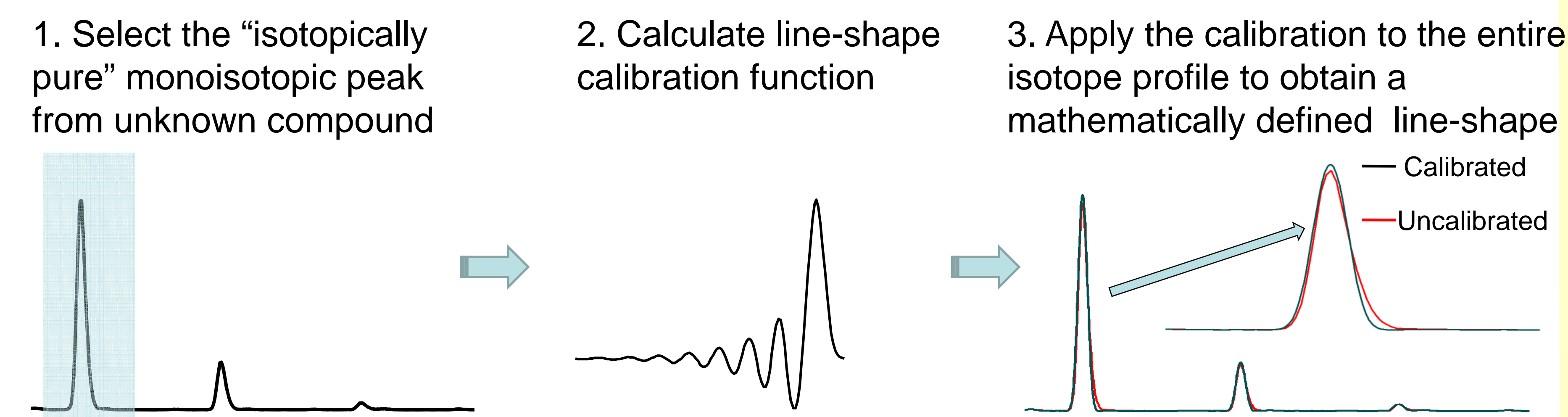
- Proper line-shape calibration allows precise matching of measured and theoretical spectra which can significantly improve formula ID results on high resolution instruments and enable formula ID on unit resolution instruments.
- Line-shape calibration exposes instrumental errors which have to be carefully considered to obtain good Spectral Accuracy.
- Future improvements to instrumentation hardware and firmware could allow even more accurate matching of theoretical and calibrated spectra.

References

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- 2) S. M. Rock Anal. Chem., 1951; 23(2); 261-268.
- 3) Roussis SG, Proulx R Anal. Chem., 75 (6), 1470 1482, 2003.
- 4) Evans, J. E.; Jurinski, N. B. Anal. Chem. 1975, 47, 961-963.
- 5) MassWorks software, Cerno Bioscience, Danbury, CT 06810

Figure 1. Line-shape Calibration

High Resolution



Unit Resolution

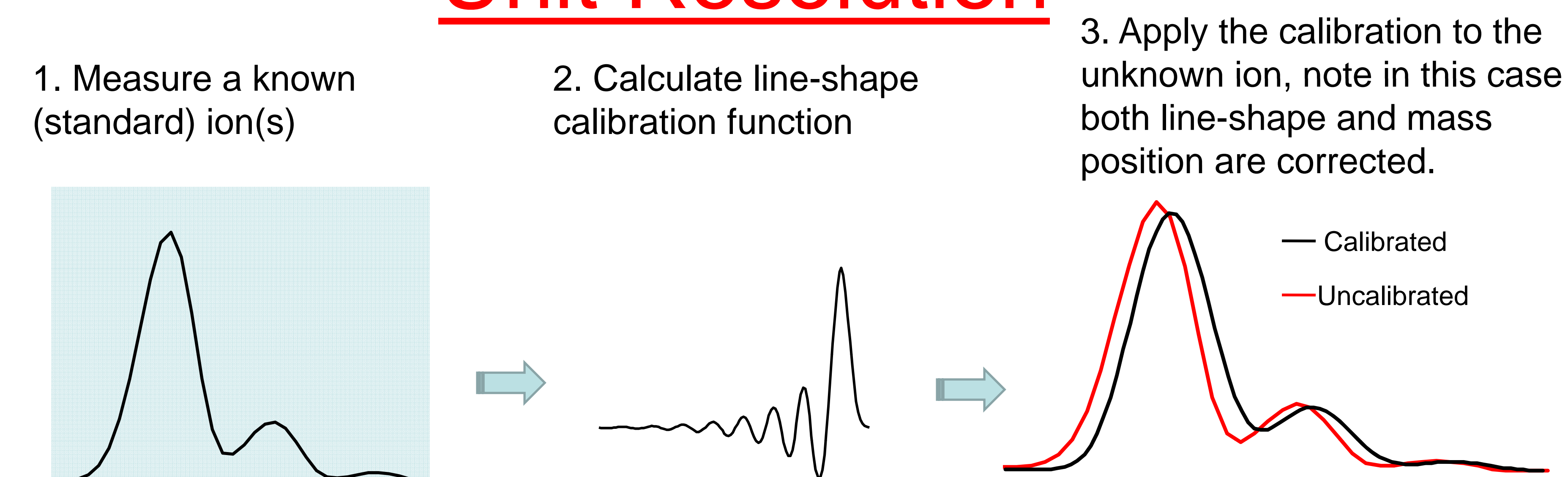


Figure 2. Calculate Spectral Accuracy

