LC/MS Identification and Relative Quantitation of Singly Charged Monomer and Multiply Charged Multimer Yongdong Wang, Don Kuehl & Stacey Simonoff **Cerno Bioscience, Las Vegas, NV, USA Sunday ACS Poster: P147**

Mass Spectral Accuracy and Analysis of Spectrally Overlapped Ion Species

LC/MS is an effective and broadly applicable analytical tool for chemicals, polymers, and biomolecules Using a set of MS calibration standards (e.g., MS tune standard solutions), it is possible to both acquire

due to its highly specific detection of these molecules based on their m/z and respective isotope distributions. For this same reason, ions with their m/z close to each other, e.g., those produced by a singly charged monomer and a doubly charged dimer or multiply charged multimer, present an interesting analytical challenge due to their mutually overlapping mass spectral signals, especially if these ions are formed inside the ion source region of the MS system and therefore are not chromatographically separable from each other. In this paper, we present a novel approach for both the identification and relative quantification of these ion species even with a conventional unit mass resolution LC/MS system. and calibrate MS data in the raw profile mode so as to obtain spectrally accurate MS profile for a mixture of the monomers and any number of multiply charged multimers. At the same time, it is possible to accurately compute the discrete isotope distributions for each and every one of these possible ion species, which can then be converted into profile mode theoretical mass spectra conforming to the same calibrated MS peak shape. A least squares regression between the acquired and calibrated MS profile of a mixture and those calculated for each and every one of these possible components will allow both the detection for the presence of these possible components and their relative concentrations. The spectral accuracy metric could be used as an indication of the goodness of the fit, i.e., whether the correct set of possible components have been included for confident identification and/or analysis.

MS TrueCal[™]: 100x Better Mass Accuracy and High Spectral Accuracy

As outlined in a front cover feature article (Ref 1), acquiring profile mode (raw scan vs centroid) MS data is key to preserving all critical information about elemental compositions and any measured profile mode MS data are composed of two parts:

- Discrete isotope distributions arising from elemental composition(s)
- MS instrument- and tune-specific peak shape

In order to achieve truly accurate MS analysis, it is critically important to perform a new type of MS calibration by involving both the mass position and the MS peak shape (TrueCal). This could be achieved on lower resolution MS system via the use of known standards or on higher resolution system via the use of monoisotopic peak of the test compound itself.



LowRes TrueCal + CLIPS Formula ID

By calibrating both m/z position and MS peak shape through either internal or external standards of known elemental compositions, it is possible to achieve 100x better mass accuracy on a single or triple quad MS system, from 0.x to 0.00x mass accuracy. When combined with the unparalleled spectra accuracy attained during the same calibration process, it becomes practically feasible to determine elemental compositions of unknown compounds under typical GC/MS or LC/MS operating conditions via CLIPS (Calibrated Lineshape Isotope Profile Search).

With HiRes TOF or Orbitrap MS data, it is possible to perform a peak-shape-only calibration to transform the actual measured peak shape into a known/perfect mathematical function by using the measured monoisotope peak of the unknown compound itself as the standard, in a self-calibration process called (sCLIPS).

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Calibrating mass spectral profile mode data into spectrally accurate full profile data not only enables accurate mass elemental composition determination for the unknown analysis with even a single or triple quadrupole MS at unit resolution, it also allows for complex ion series analysis including the identification and analysis of additional ions created inside the ion source, including GC EI source or LC ESI source which we tested.

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References

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