A Software Tool to Automatically Evaluate Scan-by-Scan Spectral Accuracy of Ultra High Resolution LC/MS Data for Unique Elemental Composition Determination

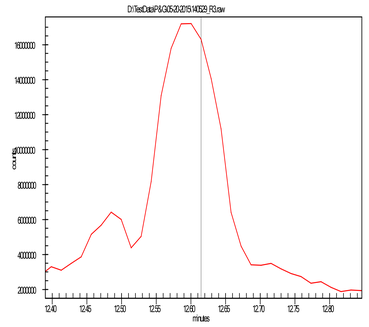
Jeff S. Andrews, Ming Gu & Yongdong Wang, Cerno Bioscience, Norwalk, CT, USA

The Objective: Holy Grail of Unknown ID?

High Mass Accuracy + **Fine Isotopes @ Ultra HiRes** → **Wide Open Search Conditions** → **Unique Elemental Composition Determination of True Unknowns**

Combining high mass accuracy with fine isotope measurement at ultra-high resolving power of ≥240,000, one would hope that the holy grail of unique elemental composition determination of a true unknown with reasonably open search conditions could be achieved, for small molecule ions with m/z ≥ 300.

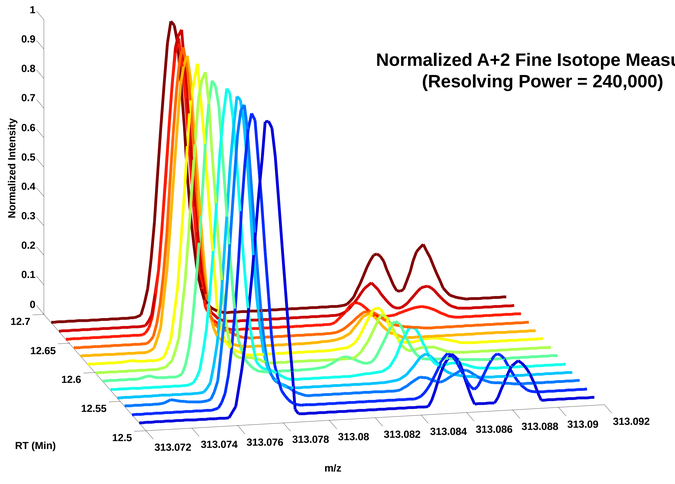
The Challenge: Varying Spectral Accuracy across Chromatographic Peak



HPLC Elution Profile under AGC

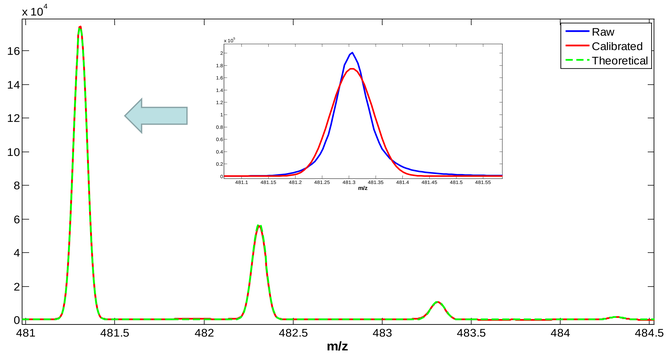
With the ion population control tool, Automatic Gain Control (AGC), available on the Orbitrap Elite system, the space charge effect (Ref 1) which could impact both mass accuracy and spectral accuracy is under management.

In spite of this effort, it is observed that the spectral accuracy does go through a systematic change across the full elution profile of the chromatographic peak, making it difficult to utilize the fine isotope information to aid in the elemental composition beyond the accurate mass measurement alone, as seen by the detail examination of the A+2 clusters shown below.



Normalized A+2 Fine Isotope Measured (Resolving Power = 240,000)

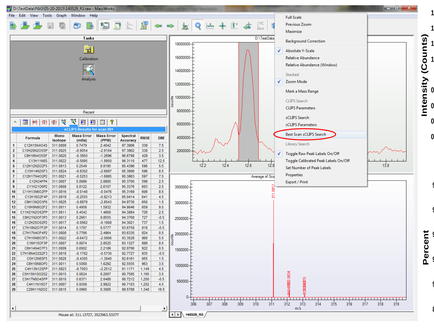
Solution I: sCLIPS for Spectral Accuracy

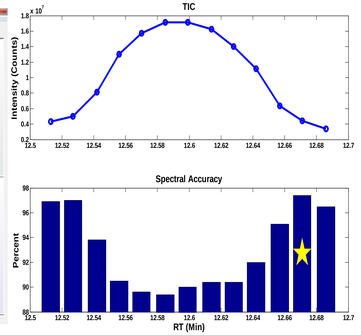


In order to take advantage of the rich yet varying spectral information across a chromatographic peak, an accurate and reliable isotope modeling approach is required. This is accomplished by first transforming the monoisotopic peak shape into a well defined mathematical function, through a novel peak shape calibration process which preserves both the m/z axis (no m/z adjustment) and the integrated peak area under the curve. This peak shape transformation (calibration) is then applied to the rest of the isotope clusters to convert the measured mas spec into a calibrated mass spec with well defined peak shape function, allowing for accurate spectral comparison between the calibrated and the theoretically calculated mass spec conforming to the same speak shape definition. The elemental composition whose theoretical mass spec provides the best spectral match to the calibrated mass spec (i.e., with the highest Spectral Accuracy, Ref 2-3) is the most likely candidate. This process is called self-Calibrated Lineshape Isotope Profile Search (sCLIPS™) and has been shown to help eliminate up to 99% of incorrect elemental compositions from consideration under moderately high resolving power of 7,500 or 15,000 (Ref 4).

Solution II: Automatic Search for the Most Spectrally Accurate Scan across the Chromatographic Peak

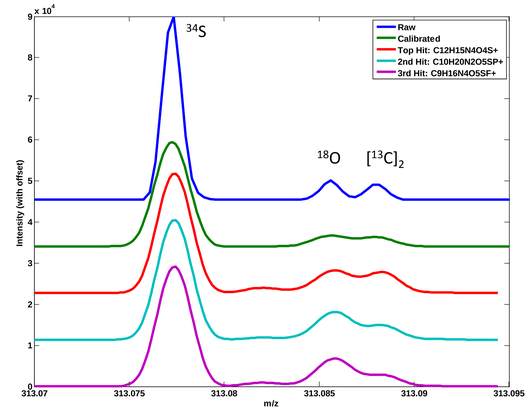
Conceptually, one could perform an sCLIPS one scan at a time or on the averaged mass spec within the RT window, with the former yielding different attainable spectral accuracy for each scan and the latter suffering from the varying spectral errors encountered by each scan. A new software tool, Best Scan sCLIPS™, implemented inside the commercially available MassWorks™ software (Cerno Bioscience, Norwalk, CT, USA) has been developed to perform this scan-by-scan analysis in a computationally efficient manner to automatically select the most spectrally accurate scan.





The Results: Towards Unique Elemental Composition

Rank	Elemental Composition	Exact Mass (Da)	Mass Error (mDa)	Mass Error (ppm)	Spectral Accuracy
1	C12H15N4O4S+	311.0809	0.7	2.4	97.39%
2	C10H20N2O5SP+	311.0825	-0.9	-2.9	97.39%
3	C9H16N4O5SF+	311.0820	-0.4	-1.3	96.68%
4	C13H11N8S+	311.0822	-0.6	-1.9	96.31%
5	C12H12N2O2F5+	311.0813	0.3	0.8	95.44%
6	C15H14N2SF3+	311.0824	-0.8	-2.7	95.39%
7	C12H17N4O2P2+	311.0821	-0.5	-1.7	95.39%
8	C12H24FP4+	311.0807	0.9	2.9	95.37%
9	C11H21O6P2+	311.0808	0.8	2.6	95.34%
10	C11H13N6O2FP+	311.0816	0.0	0.0	95.32%
⋮	⋮	⋮	⋮	⋮	⋮
29	C20H11N2O2+	311.0815	0.1	0.3	89.58%



The Best Scan sCLIPS search results from the most spectrally accurate scan. Under the generous yet reasonable search conditions of ±3ppm mass tolerance, even electron, double bound equivalence [-1 to 30], and possible elements of C [1-25], H [0-46], N [0-13],O [0-13], S [0-2], F [0-11],P [0-6], I [0-2], 29 candidate elemental compositions are found and ranked by Spectral Accuracy. Spectral differences as small as a few tenths of a percent can be discerned, narrowing down the candidates from 29 to 2 for further consideration, while automatically determining the best MS scan to use for reliable unknown identification.

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References

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