

MassWorks™

BEST SCAN_sCLIPS™

Scan-by-Scan Spectral Accuracy with HiRes MS

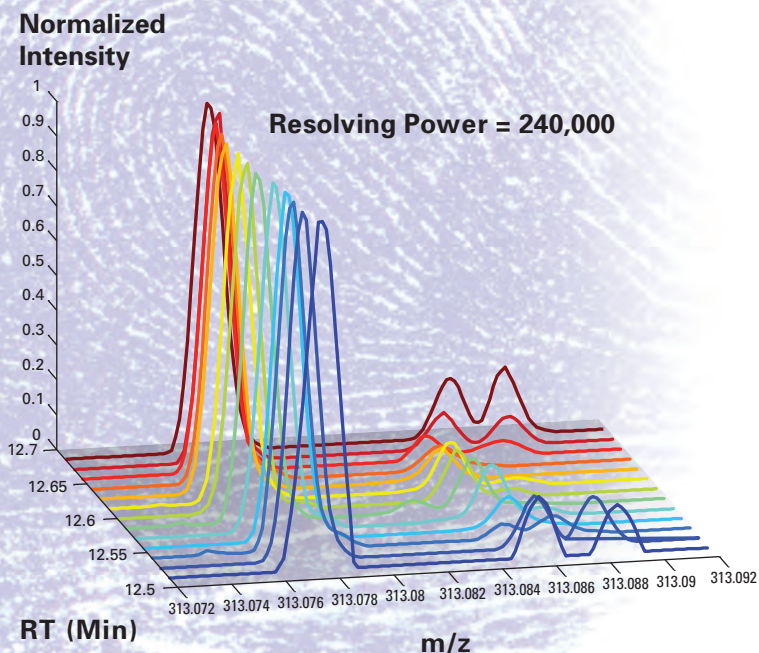
- ✓ Variable fine isotope measurements across chromatographic peak
- ✓ Scan-by-scan Spectral Accuracy evaluation
- ✓ Unknown ID: Orbitrap and FT ICR MS at $\geq 200,000$ resolving power
- ✓ Relative quantitation of known ion mixtures: any HiRes including TOF or Orbitrap

Spectral Accuracy analysis of fine isotopes from ultra high resolution MS to help achieve unique Formula ID

Mass accuracy of 1-2ppm or even sub-ppm can now be readily achieved on higher resolution MS systems such as Orbitrap or FT ICR MS to meet the needs of elemental composition determination of unknown compounds. For most applications, however, this level of mass accuracy alone would not be sufficient to achieve unique elemental composition determination with confidence. Accurate isotope measurement and exact mathematical modeling can help eliminate up to 95% of incorrect formula candidates. Experimental results have shown that measured isotope fine structures can vary significantly and systematically from scan to scan within a chromatographic peak, especially under ultra-high resolving power of $\geq 240K$, raising the interesting question of which scan to use for accurate isotope analysis of an unknown.

Best Scan sCLIPS takes into consideration scan-by-scan variations to turn this frequently observed phenomenon into additional information to help achieve unique elemental composition determination. With the changes in ion population across a chromatographic peak, isotope fine structures may appear and then disappear due to space charge effects, making some MS scans more spectrally accurate than others. Each MS scan contains a well resolved monoisotope peak, a measurement of the actual MS peak shape. A peak shape calibration is performed to transform the actual peak shape into a given statistical distribution which is applied to the rest of the isotope clusters including all measurable fine isotopes. Such calibrated MS scan is then quantitatively compared to the theoretical MS calculated for each candidate elemental composition using the same peak shape distribution, in an already available MassWorks process called sCLIPS (self Calibrated Line-shape Isotope Profile Search). A Spectral Accuracy metric can be calculated for each scan and each candidate elemental composition. The scan corresponding to the highest attainable Spectral Accuracy will be selected and the possible elemental compositions reported by this fully automated software feature.

Different points (scans) across a chromatographic peak provide a series of MS measurements at varying ion populations - Spectral Accuracy serves as an objective metric in selecting the most spectrally accurate scan to help achieve unique elemental composition determination of true unknowns.



A+2 fine isotopes as measured across a chromatographic peak | **Best Scan sCLIPS** evaluates Spectral Accuracy for each scan to provide an objective answer

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HOW DO I APPLY Best Scan sCLIPS TO MY DATA?

Similar to sCLIPS, performing a Best Scan sCLIPS search is quick and simple.

- Simply acquire the data using your current MS data system and directly read native data from major MS vendors into MassWorks
- Mark the chromatographic Retention Time (RT) window for the analyte of interest
- Click on the monoisotopic peak of the unknown ion and select "Best Scan sCLIPS" from the drop down menu.
- Set your elements to search, the mass tolerance and click "Search"

That's it! Within a short period of time, the formula list based on the mass tolerance is created, the line-shape calibration created and applied to each scan within the RT window, Spectral Accuracy calculated for each scan and formula, the best scan identified, and the formula list displayed according to Spectral Accuracy in a fully sortable sCLIPS report. You can also inspect various formula candidates by graphically exploring the Spectral Accuracy variations. The reports can be printed or exported via the Windows clipboard.

WHAT OTHER BENEFITS CAN Best Scan sCLIPS PROVIDE

In spite of the best efforts at controlling ion populations through modern instrument tools such as automatic gain control, the scan-to-scan fine isotope spectral variations are difficult to predict beforehand and hard to evaluate afterwards, especially for a true unknown. Best Scan sCLIPS automatically determines the scan with the highest Spectral Accuracy during the same unknown elemental composition determination process, saving you valuable R&D time while avoiding expensive, embarrassing and sometimes dangerous formula mis-identification.

In experiments involving labeled isotopes such as hydrogen-deuterium exchange, ^{13}C -labeling (e.g., for metabolic flux analysis), or ^{14}C radio isotope labeling, the MS signals from these multiple ions would spectrally overlap with each other, when measured with conventional high resolution MS such as TOF. The MS signal thus measured may be compromised by the limited linear dynamic ranges, depending on the MS signal level which varies across a chromatographic peak. The Best Scan sCLIPS can be applied to automatically determine the most spectrally accurate scan for the accurate relative quantitation of these known ions through the powerful mixture search feature involving ion series. These are capabilities not possible with accurate mass measurements alone.

Rank	Elemental Composition	Exact Mass (Da)	Mass Error (mDa)	Mass Error (ppm)	Spectral Accuracy
1	$\text{C}_{12}\text{H}_{15}\text{N}_4\text{O}_4\text{S}^+$	311.0809	0.7	2.4	97.39%
2	$\text{C}_{10}\text{H}_{20}\text{N}_2\text{O}_5\text{SP}^+$	311.0825	-0.9	-2.9	97.39%
3	$\text{C}_9\text{H}_{16}\text{N}_4\text{O}_5\text{SF}^+$	311.0820	-0.4	-1.3	96.68%
4	$\text{C}_{13}\text{H}_{11}\text{N}_8\text{S}^+$	311.0822	-0.6	-1.9	96.31%
5	$\text{C}_{12}\text{H}_{12}\text{N}_2\text{O}_2\text{F}_5^+$	311.0813	0.3	0.8	95.44%
6	$\text{C}_{15}\text{H}_{14}\text{N}_2\text{SF}_3^+$	311.0824	-0.8	-2.7	95.39%
7	$\text{C}_{12}\text{H}_{17}\text{N}_4\text{O}_2\text{P}_2^+$	311.0821	-0.5	-1.7	95.39%
8	$\text{C}_{12}\text{H}_{24}\text{FP}_4^+$	311.0807	0.9	2.9	95.37%
9	$\text{C}_{11}\text{H}_{21}\text{O}_6\text{P}_2^+$	311.0808	0.8	2.6	95.34%
10	$\text{C}_{11}\text{H}_{13}\text{N}_6\text{O}_2\text{FP}^+$	311.0816	0.0	0.0	95.32%
⋮	⋮	⋮	⋮	⋮	⋮
29	$\text{C}_{20}\text{H}_{11}\text{N}_2\text{O}_2^+$	311.0815	0.1	0.3	89.58%

Table1 The Best Scan sCLIPS search results from the most spectrally accurate scan. Under the generous yet reasonable search conditions of $\pm 3\text{ppm}$ mass tolerance (accurate mass @ 311.0816), even electron, double bond 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.

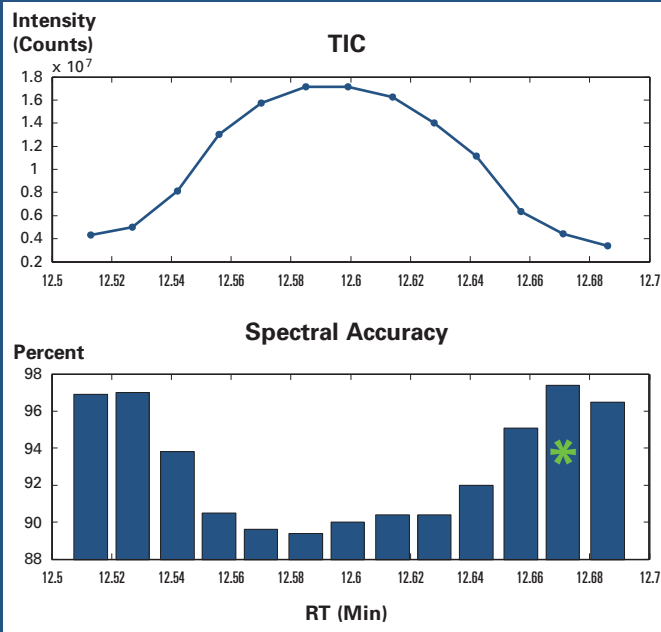


Figure 1. Total Ion Chromatogram (TIC) in the Retention Time (RT) window where the analyte of interest elutes (top) and the Spectral Accuracy attainable for each MS scan (bottom)

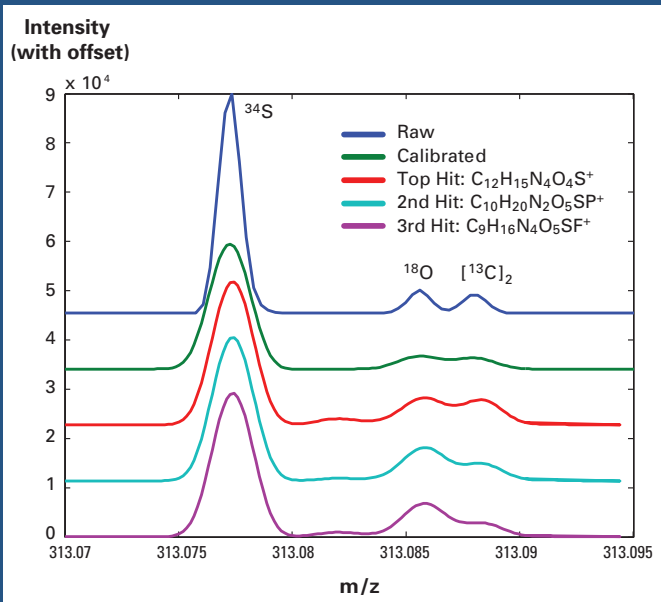


Figure 2. The sCLIPS line shape calibration and the Spectral Accuracy match from the top three elemental composition candidates

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