Routine and Enhanced Intact Mass Analysis without Compromise on TripleTOF® Systems

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The goal of any pharmaceutical organization is to get the best possible product quality in the shortest time possible. In biopharma, being able to routinely and accurately measure intact protein mass from early development to QC is important as a step for monitoring product quality. Time-of-Flight systems operate at high resolution and have both an unrestricted mass range, as well as a wide dynamic range to simultaneously detect low and high abundance isoforms. These attributes have made TOF MS platforms the analytical system of choice for biotherapeutic development and production. In this study, the capabilities of the SCIEX TripleTOF 6600 system are demonstrated, which introduces the next generation analytical technology for intact mass analysis of biologics.

One of the most important characteristics when selecting a mass spectrometry platform for intact protein determination is the interscan linear dynamic range. TripleTOF Systems do not sacrifice dynamic range for resolution, maintaining a dynamic range of 4 to 5 orders of magnitude. This allows the detection of a wide variety of low abundance isoforms at the same time as major isoforms, with no loss of fidelity.

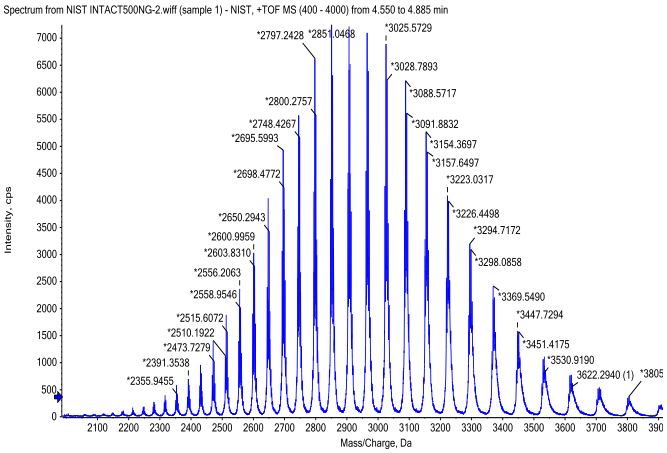

Figure 1: Intact Electrospray Ionization mass Spectrum of a NIST mAb over a Wide m/z Range. The multiplicity of charge states makes this data challenging to process visually.

Key Feature of TripleTOF® Systems

- High dynamic range of TripleTOF Systems can capture both high- and low-abundance molecular isoforms.
- BioPharmaView™ Software balances power and simplicity; bringing routine molecular weight analysis of proteins by mass spectrometry to all laboratories.
- Customizable processing parameters can be used to optimize analysis based on heterogeneity and complexity of the molecules.
- Simple and intuitive display shows the right amount of detail with visual simplicity for the analyst.

Deconvolution and Addressing High Mass

It is impossible to directly measure accurate mass based on the carbon 12 isotope of large, multiple-charged ions when the dynamic range between and the isotopes of lowest and highest intensity are beyond the limits of detection. For larger, heterogenous species, generally 15 kDa or above, average molecular weight is often reported. Almost all software deconvolution tools today use a system of iteration to determine when the modeled peak shape fits the raw data, and compares the end result to the original spectra to determine the number of optimal “iterations.” Additionally, software deconvolution has

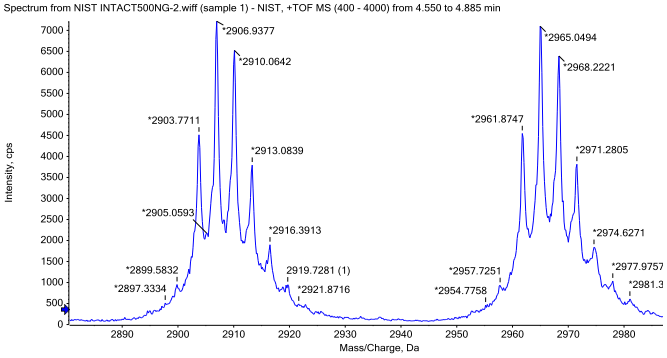

Figure 2: Zoomed View of the Intact NIST mAb. All major isoforms are visible, as well as a number of smaller isoforms from the non-processed data.

become critical to making the process routine and minimizing the parameters to optimize. Overall, greater application of smoothing, background subtraction, and a large number of iterations will lead to a more simplified visualization. This is likely to help the reviewer, but there can be a risk of obscuring low abundance isoforms. In this application, the capabilities of SCIEX TripleTOF 6600 system provide both levels of information without compromise. This capability also means that the user does not have to adjust resolution settings, or repeat analysis because all of the data is already available for processing.

Figures 1 and 2 show the raw data for a monoclonal antibody, with an intact molecular weight measurement of ~148 kDa. Figure 1 shows the mAb detected over a wide m/z range, with the multiplicity of charge states. Figure 2 is the zoomed in view of the non-processed raw spectra, displaying both the major isoforms as well as many of the minor species. The peak ratios are maintained, and relative proportions of the isoforms can be estimated. Figure 3 shows the same spectrum with baseline subtraction and Gaussian smoothing parameters applied. The

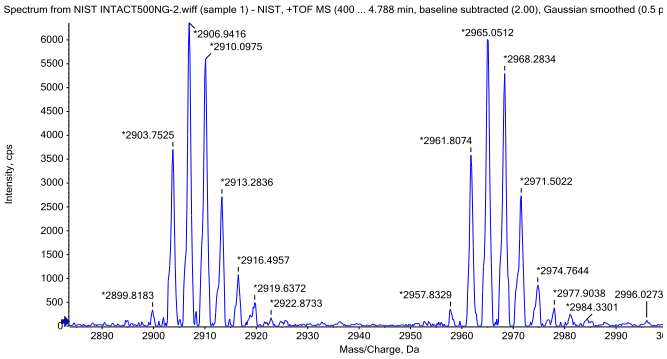

Figure 3: 'Heavily' Processed Intact NIST mAb Data to Simplify the Spectrum to the Major Species and Separate the Peaks as far as is amenable. This data processing is not generally used for average molecular weight reporting, but may be used to simplify visualization. It is clear that the relative ratios of some peaks are different from the unprocessed raw data.

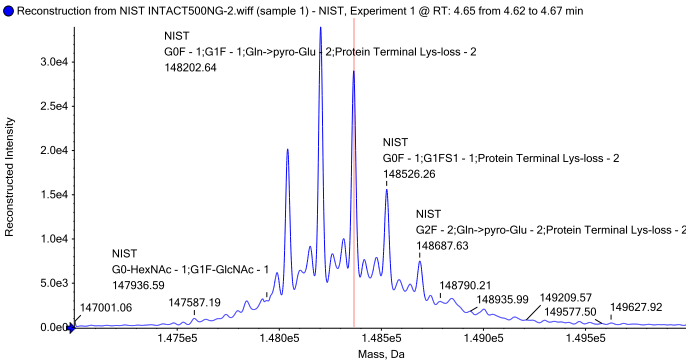

Figure 4: Deconvolution Spectrum of the Unprocessed Data. From Figures 1-2, the average masses of detectable isoforms of NIST mAb are shown. Note that many low-level isoforms are easily detectable.

heavily processed view is greatly simplified by comparison to the unprocessed view, as well as displaying a baseline separation. Although this might offer a simpler visual data representation, it runs the risk of omitting some minor details. However, under both conditions, the same average molecular weight determination can be made without recourse to changes in instrument settings (Figures 4-5). The data in this study was all acquired within the original run and without compromising quality. An organization can feel confident that they are capturing low-level isoforms with the wide dynamic range of the TripleTOF 6600 System. This additional dynamic range will allow an organization to see deeper into the structure of each potential therapeutic molecule at the intact mass level and allow additional screening capabilities before needing to resort to time consuming peptide mapping techniques. This assay can be linked to multiple separations techniques and the processing automated using BioPharmaView Software (Figure 6), allowing for fast, easy, and reproducible determination of molecular weight of both high and low abundance protein isoforms.

Note that some of the low level isoforms are obscured by processing.

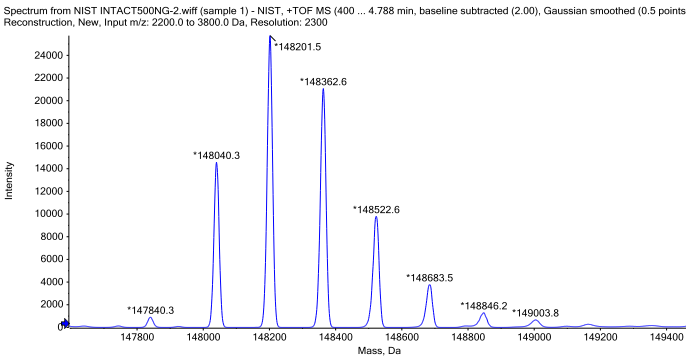

Figure 5: Deconvolution Spectrum from Processed Data. Deconvolution of the processed data from Figure 3, showing the average masses of many of the detectable isoforms of NIST mAb.

Conclusions

Screening of intact protein mass is a fast first step towards characterization and monitoring of biologics. Using the TripleTOF 6600 system for data acquisition gives high resolution, high sensitive data which has 4-5 inter-scan linear dynamic range, ensuring that the lowest abundant protein species are identified while maintaining mass accuracy on the major protein forms. Couple high resolution, mass accurate data with software like BioPharmaView Software, gives you fast, reproducible and accurate results from a single sample to a batch list into a final report.

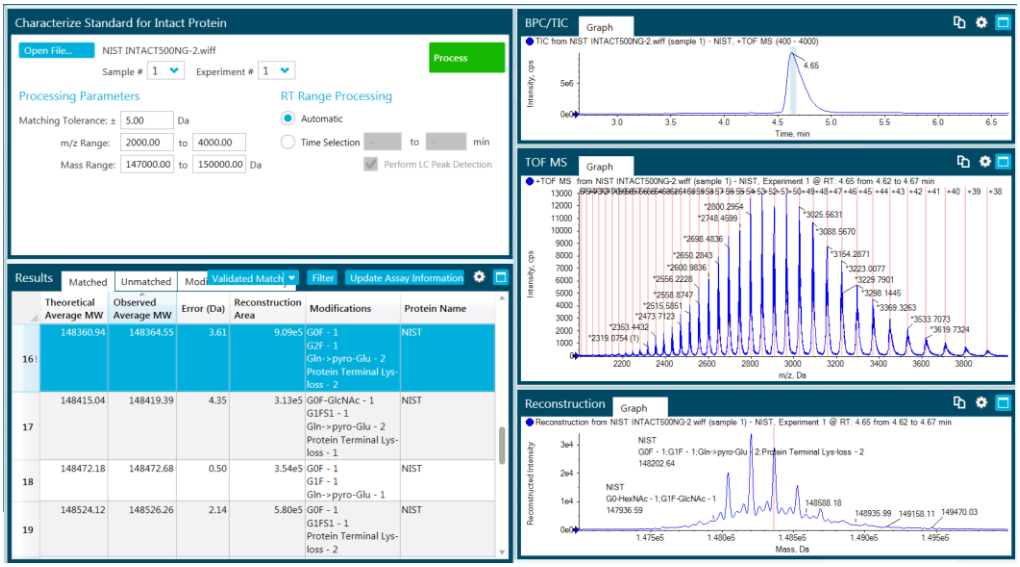

Figure 6: Deconvolution of the Unprocessed Raw Data using BioPharmaView Software. This automated solution allows for batch sample processing and sample comparisons, accelerating your analysis time.

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