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PROTEIN QUANTITATION ACROSS BROAD MASS RANGES IN FTMS: HANDLE WITH CARE!

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FTMS enables protein analysis across a broad mass range. Quantitation often relies on deconvolved intensities or extracted ion current areas, assuming signal scales linearly with the number of detected ions. In reality, raw intensity and signal-to-noise ratio (SNR) depend on detection period and are shaped by isotopic beating and processing choices, yielding mass-dependent optimal transient lengths [1, 2]. Thus, in broadband spectra with markedly different protein masses, apparent abundance ratios can vary with transient length. Here, we quantify these effects using simulations and biotherapeutics-focused experiments and propose a strategy for fair cross-mass comparisons.

IgG1 monoclonal antibodies were obtained from collaborators or commercial sources. Subunits were generated by disulfide reduction with or without IdeS/KGP digestion. Data were acquired on a Q Exactive HF Orbitrap (BioPharma option) using native SEC-MS and denaturing RPLC-MS. Time-domain transients were captured with an external acquisition system (FTMS Booster X2, Spectroswiss) and processed in Peak-by-Peak with user-defined truncation and Fourier transform. Frequency-specific transient processing and FTMS Simulator datasets supported benchmarking and validation.

Protein intensity and SNR showed the expected nonlinear dependence on detection period in Orbitrap/ICR FTMS, driven by isotopic beat patterns, finite coherence, and FT processing. In co-acquisitions of a 25 kDa light chain, 50 kDa heavy chain, and 150 kDa intact mAb, using a single fixed transient length, biases inferred abundance ratios, typically inflating the apparent abundance of larger species at common SNR levels. Simulations reproduce these trends. Although correct ratios are obtained when all species are fully isotopically resolved, this is often impractical within LC time constraints. For resolution-matched comparisons, we apply frequency-specific transient processing approach. This stabilizes mAb:heavy-chain:light-chain ratios versus conventional single-window processing. The simulation framework also supports an analytical route to correct ratios from short detection periods. Other examples of wide mass range protein analysis of antibody-epitope peptide complexes, which is employed to estimate the epitope' binding strengths in the gas phase.

References

1. Nagornov et al., J. Am. Soc. Mass Spectrom. 2020, 31, 9, 1927–1942
2. Nagornov et al., J. Am. Soc. Mass Spectrom. 2022, 33, 7, 1113–1125