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Intact NIST Monoclonal Antibody Characterization – Proteoforms, Glycoforms – using CE-MS and CE-LIF

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ABSTRACT: Determining and linking the structural heterogeneity of recombinant antibodies to function is critical in the biopharmaceutical industry. We introduce a new microfluidic capillary electrophoresis - mass spectrometry (µCE-MS) approach to characterize intact monoclonal antibody (mAb) and simultaneously quantifying distinct variants. Our MS analysis of intact NIST monoclonal antibody (RM8671) shows 18 variants identified amongst proteolytic and glycolytic modifications with a range of relative abundances between 0.1 - 100%. In order to verify our quantitative MS results, we used an established system based on capillary electrophoresis - with laser induced fluorescence (CE-LIF) for profiling the N-glycans. All major glycans were identified and further substantiated by exoglycosidase digestion.

Interestingly, the μ CE-MS analysis of intact NIST monoclonal antibody consistently yielded higher amounts of G2FG2F-Hex glycoform (~3.4%), whereas the CE-LIF analysis indicates that only 1.4% of G2F-Gal is present. Therefore, the additional hexose adduct observed in μ CE-MS may have been the glycation product of the mAb. Further analysis of deglycosylated mAb with μ CE-MS made it possible to reveal the glycation with 10.5% of one hexose product and 4.9% of two hexose product in the intact deglycosylated antibody. An integrated solution using two orthogonal and complementary techniques for characterizing antibody glycosylation is provided here.

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