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Full Characterization of Heterogeneous Antibody Samples under Denaturing and Native Conditions on a Hybrid Quadrupole-Orbitrap Mass Spectrometer

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Abstract : Purpose: MS analysis of monoclonal antibodies (mAbs) at the protein and peptide levels is critical during development and production of biopharmaceuticals. The compositions of current generation therapeutic proteins are often complex due to various modifications which may affect efficacy. Intact proteins analyzed by MS are detected in higher charge states that also provide more complexity in mass spectra. Protein analysis in native or native-like conditions with zero or minimal organic solvent and neutral or weakly acidic pH decreases charge state value resulting in mAb detection at higher m/z ranges with more spatial resolution. Methods: Three commercially available mAbs were used for all experiments. Intact proteins were desalted online using size exclusion chromatography (SEC) or reversed phase chromatography coupled on-line with a mass spectrometer. For streamlined use of the LC- MS platform we used a single SEC column and alternately selected specific mobile phases to perform separations in either denaturing or native-like conditions: buffer A (20 % ACN, 0.1 % FA) with Buffer B (100 mM ammonium acetate). For peptide analysis mAbs were proteolytically digested with and without prior reduction and alkylation. The mass spectrometer used for all experiments was a commercially available Thermo Scientific™ hybrid Quadrupole-Orbitrap™ mass spectrometer, equipped with the new BioPharma option which includes a new High Mass Range (HMR) mode that allows for improved high mass transmission and mass detection up to 8000 m/z. Results: We have analyzed the profiles of three mAbs under reducing and native conditions by direct infusion with offline desalting and with on-line desalting via size exclusion and reversed phase type columns. The presence of high salt under denaturing conditions was found to influence the observed charge state envelope and impact mass accuracy after spectral deconvolution. The significantly lower charge states observed under native conditions improves the spatial resolution of protein signals and has significant benefits for the analysis of antibody mixtures, e.g. lysine variants, degradants or sequence variants. This type of analysis requires the detection of masses beyond the standard mass range ranging up to 6000 m/z requiring the extended capabilities available in the new HMR mode. We have compared each antibody sample that was analyzed individually with mixtures in various relative concentrations. For this type of analysis, we observed that apparent native structures persist and ESI is benefited by the addition of low amounts of acetonitrile and formic acid in combination with the ammonium acetate-buffered mobile phase. For analyses on the peptide level we analyzed reduced/alkylated, and non-reduced proteolytic digests of the individual antibodies separated via reversed phase chromatography aiming to retrieve as much information as possible regarding sequence coverage, disulfide bridges, post-translational modifications such as various glycans, sequence variants, and their relative quantification. All data acquired were submitted to a single software package for analysis aiming to obtain a complete picture of the molecules analyzed. Here we demonstrate the capabilities of the mass spectrometer to fully characterize homogeneous and heterogeneous therapeutic proteins on one single platform. Conclusion: Full characterization of heterogeneous intact protein mixtures by improved mass separation on a quadrupole-Orbitrap™ mass spectrometer with extended capabilities has been demonstrated.

Keywords : disulfide bond analysis, intact analysis, native analysis, mass spectrometry, monoclonal antibodies, peptide mapping, post-translational modifications, sequence variants, size exclusion chromatography, therapeutic protein analysis, UHPLC

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