Native ion exchange chromatography directly coupled to Orbitrap mass spectrometry allows surface charge discrimination and online detection of intact proteins

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ABSTRACT

We report significant performance advantages for our native ion exchange LC-MS method, which can be used to characterize complex intact monoclonal antibodies (mAbs) in a single chromatographic step. This improves mass accuracy, including the 3 most abundant glycoforms in both unmodified and deamidated forms. "Native" MS, utilizing aqueous buffers within physiological pH ranges, dramatically improves complex intact protein ESI spectra by reducing charge state values to simplify spectra. "Native" MS eliminates challenges in charge variant analysis of the commercially relevant isobaric species, such as antibody drug conjugate samples. Proteins have a net positive charge while glycosylated proteins have a net negative charge, which allows efficient separation by reversed phase chromatography. Both intact and modified versions of proteins can be separated by ion exchange chromatography. Native ion exchange chromatography method was developed to perform on-line fractionation and MS analysis of complex intact protein samples. Sample separation was achieved by a combination of size-exclusion and ion-exchange chromatography and then separated on the basis of hydrophobicity. Sample preparation and LC-MS analysis was performed using a BioPharma Option™ software. Peptide mapping data were analyzed using BioPharma Finder software. Averaged spectra corresponding to unique charge variants of Trastuzumab. Spectra are extracted chromatograms of deconvolved components, showing top 3 glycoforms in both deamidated and unmodified forms.

RESULTS

Nativation effects Unique and Potential Repercussions Made for Proteins

In the real sampling of NIST IgG1 monoclonal antibody (mAb) in a single chromatographic step. This improves mass accuracy, including the 3 most abundant glycoforms in both unmodified and deamidated forms. "Native" MS, utilizing aqueous buffers within physiological pH ranges, dramatically improves complex intact protein ESI spectra by reducing charge state values to simplify spectra. "Native" MS eliminates challenges in charge variant analysis of the commercially relevant isobaric species, such as antibody drug conjugate samples. Proteins have a net positive charge while glycosylated proteins have a net negative charge, which allows efficient separation by reversed phase chromatography. Both intact and modified versions of proteins can be separated by ion exchange chromatography. Native ion exchange chromatography method was developed to perform on-line fractionation and MS analysis of complex intact protein samples. Sample separation was achieved by a combination of size-exclusion and ion-exchange chromatography and then separated on the basis of hydrophobicity. Sample preparation and LC-MS analysis was performed using a BioPharma Option™ software. Peptide mapping data were analyzed using BioPharma Finder software. Averaged spectra corresponding to unique charge variants of Trastuzumab. Spectra are extracted chromatograms of deconvolved components, showing top 3 glycoforms in both deamidated and unmodified forms.

Orbitrap mass spectrometry to allow direct mass measurements without chromatographic separation. The results for BioPharma Finder peptide mapping and Sliding Window MS intact protein spectra using ReSpect deconvolution combined with Sliding Window algorithm are shown in Figure 8A. Despite the fact that conventional MS intact protein spectra can be very challenging due to the presence of isobaric species, our BioPharma Finder peptide mapping results show excellent performance. Figure 8B shows ReSpect deconvolution window of mass accuracy, including the 3 most abundant glycoforms in both unmodified and deamidated forms. "Native" MS, utilizing aqueous buffers within physiological pH ranges, dramatically improves complex intact protein ESI spectra by reducing charge state values to simplify spectra. "Native" MS eliminates challenges in charge variant analysis of the commercially relevant isobaric species, such as antibody drug conjugate samples. Proteins have a net positive charge while glycosylated proteins have a net negative charge, which allows efficient separation by reversed phase chromatography. Both intact and modified versions of proteins can be separated by ion exchange chromatography. Native ion exchange chromatography method was developed to perform on-line fractionation and MS analysis of complex intact protein samples. Sample separation was achieved by a combination of size-exclusion and ion-exchange chromatography and then separated on the basis of hydrophobicity. Sample preparation and LC-MS analysis was performed using a BioPharma Option™ software. Peptide mapping data were analyzed using BioPharma Finder software. Averaged spectra corresponding to unique charge variants of Trastuzumab. Spectra are extracted chromatograms of deconvolved components, showing top 3 glycoforms in both deamidated and unmodified forms.

Deconvolved spectrum of species identified in WCX-MS analysis of NIST antibody standard shows separation of known C-terminal lysine variants as well as acidic and basic amino acids. Table 1. Results for Peptide Analysis using Sliding Window algorithm.

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