Simultaneous Quantitative Peptide Mapping and Host Cell Protein Detection in a Recombinant IgG1 Monoclonal Antibody Preparation using Data-Independent Acquisition

INTRODUCTION

Monoclonal antibodies (mAbs) are major target-orientated biotherapeutics used to treat an array of human diseases. mAbs are typically produced in biological systems such as Chinese hamster ovary (CHO) or other cell lines. Any heterogeneity in purified IgG1 protein products, for example post-translational modifications (PTMs), sequence variants, degradation products, and contaminants (such as host cell proteins) must be characterized completely to understand the purity, stability and potency of the mAb product, and to avoid immunogenicity. Mass spectrometry provides superior analytical capabilities for the characterization of mAbs, both at the intact protein level and for peptide digests of antibody preparations. Conventional data-dependent approaches to automatic analysis of peptides in LC-MS/MS experiments are fundamentally stochastic. This is not ideal for analysis of biotherapeutics, where analytical consistency and quantitative accuracy across samples are of critical importance. Here we explore the application of an unbiased and deterministic, data-independent method for mAb analysis using SWATH™. This approach provides substantial benefits over other MS strategies because it captures comprehensive, quantifiable MS and MS/MS information for every analyte in the sample, in every analytical run.

MATERIALS AND METHODS

Sample Preparation: IgG1 mAb was reduced/alkylated and trypsin-digested. A constant concentration of this digest was spiked with a range of Bovine Serum Albumin and AEFVEVTK(2+) concentrations.

Chromatography: Samples were analyzed using the Eksigent ekspert™ 425 System. Various amounts of digested were loaded onto a column (15 cm-ChromP C18-CL, 3µm, 120Å) Elution gradients of 3-35% acetonitrile (0.1% formic acid) in 30 min were run.

Mass Spectrometry: Unmodified and spiked mAb digests were analyzed using a TripleTOF® 5600 system. An information dependent acquisition (IDALCMS) method was used for initial peptide identification. SWATH™ data-independent acquisitions were subsequently performed in triplicate on each sample to obtain quantitative MS/MS chromatograms for every precursor ion between 400 and 1250 m/z, using a 20 Da Q1 isolation width and 30 ms Q1 and Q3 isolation on both quadrupoles. MRMHR, available on the 5600, still scans Q1 at a width of 0.7 Da and fragment ion chromatograms can be extracted from high-resolution (>30k) MS/MS scans at much narrower widths (0.07 Da) enabling higher selectivity. SWATH™ technology maximizes the use of MS/MS resolution, extracting narrow fragment ion chromatograms from fragmentations of a wide isolation (Q1: 250Da).

RESULTS

Peptide mapping sequence coverage was 100% for Light Chain and 99.5% for Heavy Chain. Several deamidation sites and oxidation sites were observed and quantified relative to the unmodified forms. Comparison of SWATH™ peak areas from replicate analyses revealed consistency typically within 5%CV (Figure 2). Critically, the data indicates that we can detect rare model “host cell proteins” at levels down to 0.01% contamination (wt/wt). Using the quantitative SWATH™ methodology, in a single sample run we can acquire MS/MS chromatograms on every fragment ion from every precursor peptide ion between m/z of 450 and 1250. By examining the data retrospectively, we can quantify the extent of PTM heterogeneity and host cell protein contamination with SWATH™ by fidelity and sensitivity, without any up-front method development or breakdown of the PTM or contamination. Additionally, a SWATH™ data file can be mined retrospectively if a contaminating protein is discovered in a subsequent lot.

CONCLUSIONS

MS/MS with SWATH™ Acquisition is a novel data-independent acquisition strategy that provides:

• Comprehensive high resolution MS/MS data for all detectable ions

• High quality quantitation similar to MRM with no method development

• Easy and retrospective data interrogation

• SWATH™ data can be processed by Peakview™ Software and MarkerView™ Software or extracted for use with 3rd party informatics tools

• SWATH™ Acquisition is ideal for quantifying Protein Contaminants in Biologic protein products.

• Quantitative sensitivity and fidelity rivaling ELISA without safety concerns of reagent preparation (not everything that produces a reaction in human produces a reaction in mAb)

• Captures a digital record of all fragments of all products in a protein product. This can be used to track changes over time and the data can serve as a digital archive of the current state of a sample at a given time. Can be retrospectively mined for any protein contaminant concerns in the future.

Using MarkerView™ software, trends in protein concentration changes can easily be visualized and tracked or extracted for use with 3rd party informatics tools. This can be used to track changes over time and the data can serve as a digital archive of the current state of a sample at a given time. Can be retrospectively mined for any protein contaminant concerns in the future.

REFERENCES


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