Ultra-sensitive host cell protein detection using C ESI-MS with SWATH® Acquisition

ABSTRACT
We demonstrate the use of C ESI-MS with SWATH® acquisition for the ultrasensitive detection of host cell proteins (HCPs) in a representative mAb preparation. To simulate HCPs, we spiked a mAb-digested digest with digested protein samples over a concentration range of pg to parts per thousand (ppt) concentration. mAb and HCP proteins at ppt concentrations were identified with a C ESI-MS information-dependent acquisition (IDA) routine that generated an ion library for proteins/peptides. Identical C ESI separations were performed with the HCP dilution series using SWATH® acquisition. The ion library and SWATH data were used to screen for peptides and fragment ions which best represent the HCP concentrations. The concentration-relevant peptides and ions were then used to generate calibration curves for the HCPs. HCPs were quantified down to the low ppt range and in some cases even into the high ppt range, representing detection of HCPs over three orders of magnitude.

INTRODUCTION
Host cell proteins are undesired impurities in biologic production processes and can negatively affect biologic potency, quality, purity, and safety. Identification and quantification of HCPs within therapeutic monoclonal antibody (mAb) preparations remain a challenge with a need for improved sensitivity and specificity. Mass spectrometry, particularly data-independent acquisition such as provided by SWATH® acquisition, provides a robust and sensitive means to quantify HCPs in the parts-per-billion (ppb) range. The integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into one process (C ESI) presents the possibility to improve the sensitivity of HCP quantification through reduced ion suppression and improved ionization efficiency at ultralow nanoliter per minute flow rates.

MATERIALS AND METHODS
Sample Preparation:
A representative mAb and 17 standard protein mixture were digested separately to peptides at 5 μg/mL, using a 4-hour digestion protocol with Trypsin, DTT, iodoacetamide, and tripsin. The digested standard protein mixture was spiked into the digested mAb throughout the parts per thousand, ppt, and ppb range to represent concentration of host cell proteins. The peptide preparations were then diluted to 250 μg/mL, in 125 mM ammonium acetate, pH 4.

C ESI Conditions:
C ESI experiments were carried out with a SCIEX C ESI 6500 system equipped with a temperature controlled autosampler and a power supply with the ability to deliver up to 30 kV. A commercial bare fused-silica capillary cartridge with a porous tip was used for infusion and peptide mapping experiments. Solutions of 10% acetic acid were used as the background electrolyte (BGE) and conductivity liquid (CEL) pressure of ~250 ng peptides, sample stacking was performed using transient isochromatography (tICP). C ESI separations were performed at 20 kV.

MS/MS Conditions:
A SCIEX TripleTOF® 6600+ system with a NanoSprayer® III source and C ESI adapter controlled by Analyst® TF 1.7 Software were used. HCP identification was performed with information dependent acquisition (IDA) with 10 MS/MS cycles (130 and 50 ms accumulation times for MS1 and MS/MS, respectively). HCP detection and quantification were performed using data-independent SWATH® acquisition with 30 constant window width scans (30 ms with 1 ms overlap), from 300 – 1200 m/z using 150 and 50 ms accumulation times for MS and SWATH scans, respectively.

Data Analysis:
High resolution MS and MS/MS spectra were analyzed using SCIEX ProteinPilot®, PeakView®, and MultiQuant® software.

RESULTS
The first step for a host cell experiment is identifying proteins with an IDA experiment while present at higher concentrations. In practice, this experiment would be performed on a partially purified mAb preparation. Here we simulated the partially purified preparation with protein standards spiked in at 1 parts per thousand by mass relative to the mAb. C ESI-MS IDA runs allow for identification of peptides within representative host cell proteins which yield good MS/MS spectra across all concentration levels. These peptide fragment ions are searched as good qualitative representatives of a HCP concentration.

SWATH® acquisition can be performed with different m/z window configurations to focus on information-rich regions of the m/z-domain. We investigated the use of constant and variable window widths on the detection of HCPs using C ESI-MS. Peptides are generally most abundant in the 400 – 800 m/z range, so one of the variable window configurations tested was generated from LC-MS IDA analysis of a common peptide digest from West Coast Labs. The other variable window configuration was generated from the peptide C ESI-MS IDA runs from the HCP/mAb preparation as shown in Figure 1. Both variable window configurations are shown in Figure 2.

CONCLUSIONS
An integrated C ESI-MS workflow is presented that facilitates the ultra-sensitive detection of representative host cell proteins in a mAb preparation. The combined use of C ESI with SWATH® acquisition expedites reduced ion suppression and data-independent analysis to create a powerful tool for host cell protein quantitation. This new application adds to the versatility and efficiency of open tube capillary electrophoresis for biologics characterization.

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