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

ABSTRACT

We demonstrate the use of CESI-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 digest with digested protein standards over a concentration range of ppb to parts per thousand (ppth) concentrations. mAb and HCP proteins at ppth concentrations were identified with a CESI-MS information-dependent acquisition (IDA) run to generate an ion library for proteins/peptides. Identical CESI 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-indicative peptides and ions were then used to generate calibration curves for the HCPs. HCPs were quantified down to the low ppm range and in some cases even into the high ppb range, representing detection of HCPs over three orders of magnitude.

INTRODUCTION

Host cell proteins are undesired impurities in biologic preparation processes and can negatively affect biotherapeutic quality, potency, 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-million (ppm) range. The integration of capillary electrophoresis (CE) and electrospray ionization (ESI) into one process (CESI) presents the possibility to improve the sensitivity of HCP quantitation 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 1 mg/mL using a 4-hour digestion protocol with RapiGest, DTT, iodoacetamide, and trypsin. The digested standard protein mixture was spiked into the digested mAb throughout the parts per thousand, ppm, and ppb range to represent contaminant host cell proteins. The peptide preparations were then diluted to 250 µg/mL in 125 mM ammonium acetate, pH 4.

CESI Conditions:
CESI experiments were carried out with a SCIEX CESI 8000 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 employed as the background electrolyte (BGE) and conductive liquid. After pressure injection of ~25 ng peptides, sample stacking was performed using transient isotachophoresis (t-ITP). CESI separations were performed at 20 kV.

MS/MS Conditions:
A SCIEX TripleTOF® 5600+ system with a NanoSpray® III source and CESI adapter controlled by Analyst® TF 1.7 Software were used. HCP identification was performed with information dependent acquisition (IDA) with 15 MS/MS cycles (100 and 50 ms accumulation times for MS and MS/MS, respectively). HCP detection and quantification were performed using data-independent SWATH™ acquisition with 30 constant window width scans (30 m/z with 1 m/z overlaps) 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™ softwares.

RESULTS

The first step for a host cell protein 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. CESI-MS IDA runs allow for identification of peptides within representative host cell proteins which yield good MS/MS spectra at lower concentrations. These peptide fragment ions are evaluated as good quantitative representatives of a HCPs concentration.

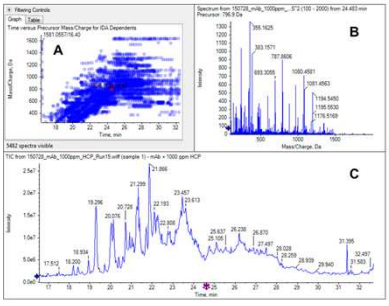


Figure 1. Raw data from a CESI-MS IDA run on the 1 parts per thousand standard protein and mAb sample preparation. The raw data is represented as (A) a heat map of the peptides selected for MS/MS, (B) a representative peptide MS/MS spectra, and (C) a total ion chromatogram (TIC) of the CESI peptide separation.

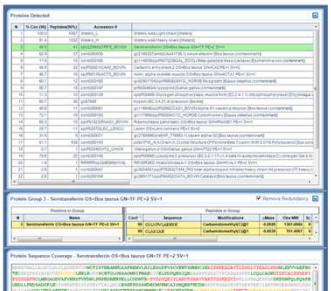


Figure 2. Identification of representative mAb and HCPs from CESI-MS IDA runs using ProteinPilot™. Peptides shown in green within the protein sequence represent the best candidates for quantitation using SWATH™ acquisition since they have the best MS/MS signals and are imported into the PeakView® SWATH microapp.

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 CESI-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 yeast lysate. The other variable window configuration we evaluated was generated from the replicate CESI-MS IDA runs from the HCP/mAb preparation as shown in Figure 1. Both variable window configurations are shown in Figure 2.

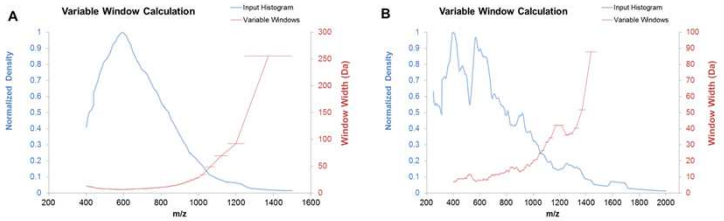


Figure 3. Calculation of variable SWATH™ acquisition window widths based on IDA runs of a (A) yeast lysate and (B) 1 parts per thousand HCP/mAb preparation.

The same CESI separation conditions are used for IDA identification and SWATH™ acquisition quantitation runs. Figure 3 shows some representative total ion electropherograms from quantitation runs.

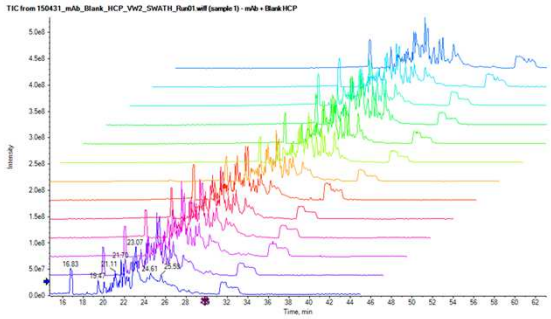


Figure 4. Representative total ion electropherograms (TIEs) from CESI-MS SWATH™ acquisition runs including blank and HCP-spiked runs in the ppb to ppth concentration range.

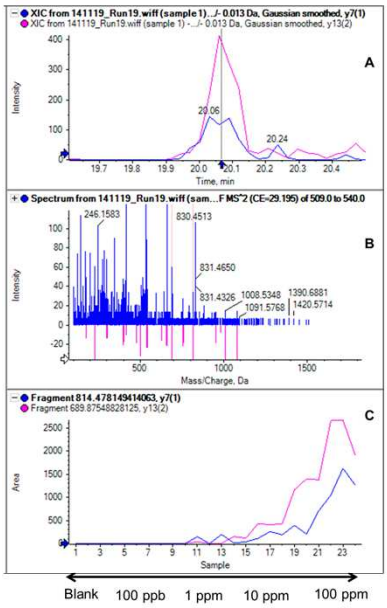
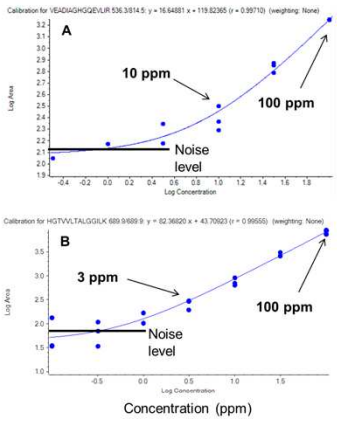


Figure 5. (A) Extracted fragment ion electropherograms for myoglobin peptide VEADIAGHGQEVLIR y7 and y13 ions. (B) Matching SWATH MS spectra (blue) to previously identified myoglobin peptide fragment ions (blue). (C) Fragment ion peak area plot based on HCP concentration.

Figure 6. Calibration curves from triplicate CESI-SWATH runs of myoglobin peptides (A) VEADIAGHGQEVLIR b2 ion and (B) HGTVVTALGGLK y7 ion.



Due to the very low concentrations of host cell proteins within therapeutic mAb preparations, the sensitivity of mass spectrometric methods are pushed, potentially into the low S/N levels. With the reduced ion suppression of CESI run at ultra-low flow rates (~20 nL/min), it's possible to extend the dynamic range and sensitivity of HCP detection. Figures 5 and 6 illustrated the common, expected sensitivities and dynamic ranges for the peptides identified. However, there were examples of peptides with even greater sensitivity and dynamic range in our analysis. To specifically show the S/N ratios for one of these quantified peptides we generated extracted fragment ion electropherograms for myoglobin peptide NDIAAK shown in Figure 6. Even at 0.1 ppm peptide NDIAAK has a S/N ratio of ~20, facilitating detection of this representative HCP over 3 orders of magnitude.

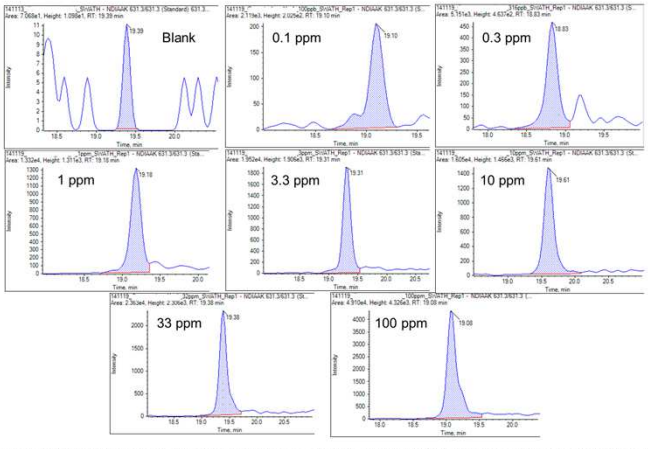


Figure 7. Extracted fragment ion electropherograms for myoglobin peptide NDIAAK in the concentration range from 0.1 ppm to 100 ppm using MultiQuant™.

CONCLUSIONS

An integrated CESI-MS workflow is presented that facilitates the ultra-sensitive detection of representative host cell proteins in a mAb preparation. The combined use of CESI with SWATH™ acquisition exploits 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.

TRADEMARKS/LICENSING