Increasing Coverage of Host Cell Proteins by FAIMS Depletion Using Low, High, and Combined Edge Retention Times
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ABSTRACT
Purpose: Time dependent FAIMS compensation voltages (CV) have been proven to deplete the major peptide in a tryptic map to increase coverage of host cell proteins (HCP). Here, the CV found from the low, high and a combination of the two is compared.

Methods: Tryptic maps of Trastuzumab were run at CV voltages from 0 to 80 volts in 5 volt increments. Plotting of the major tryptic peptid’s signal intensity vs. CV allows the determination of the low and high edge CV. These CV will be built into a retention time dependent DDA method to fragment and identify lower level background proteins. Proteome Discoverer 3.3 was used to compare the coverage of the data from the low edge, high edge and a combination of the two files.

Results: The high edge CVs increased coverage by 54%, the low edge increased 29%, and the combined search of the two files increased coverage by 66%.

INTRODUCTION
Peptide mapping of protein based drugs is one of the most used methods for characterization of background copurified host cell proteins (HCPs) which may cause undesirable effects or responses in patients. In trapping type instruments, intense digested peptides of the main protein often causes injection times to drop and block HCPs from being included in the trapped ions. High field asymmetric ion mobility spectrometry (FAIMS) uses a small DC bias potential called the compensation voltage which is normally chosen for optimized transmission. In this work, Time Dependent FAIMS Compensation Voltages (CV) were used to deplete the major peptides in a tryptic map to increase coverage of host cell proteins (HCP). Here, the CV found from the low, high and a combination of the two is compared.

MATERIALS AND METHODS
Sample Preparation
Trastuzumab (Anti-Human HER2, Humanized Antibody, MedChemExpress) was digested using the Thermo Scientific™-in-Solution Tryptic Digestion kit (P4658E) and was diluted to 5 pmol/ul. Two microliter injections (10 pmol) was used for all LCMS experiments.

Instrument Methods
The digested samples were separated on a Thermo Scientific™ Vanquish™ Halo UPLC-optimized column with a 1 mm x 15 cm Thermo Scientific™ Acclaim™ PepMap 100 C18 column with RSLC medium and a low flow rate of 0.25 mL/min. The low flow rate was used to minimize splitting of the sample. The digests were then injected sequentially with FAIMS, using compensation voltages from 0 to 80 volts. An Xcalibur™ processing method was used to integrate the base peak intensity (BPI) of each peptide at a particular charge state. The peak area of the peptides was processed and plotted vs. compensation voltage. The voltage chosen was just higher than the voltage that caused a significant drop in the height (the high edge of the curve) and just lower than the voltage that caused a significant drop in the area (the low edge of the curve). These digestion voltages were used to build a retention window around the top thirty peaks in the data dependent method. The samples were run in triplicate for the earlier non-FAIMS data. The resulting data was searched along with the initial non-FAIMS data in Thermo Scientific™ Protosure Discoverer™ 3.0 using the SwissProt database.

RESULTS
CV Determination and Instrument Method Building
The base peak plots of the Trastuzumab digest are shown in Figure 1 from 0 to 80 volts. The data was searched using Bioharma Finder 5.1 and showed 100% sequence coverage including all expected glycosylation sites. The processing method was run, and each individual retention was evaluated for proper start and stop points. The peak areas were exported to Excel and plotted vs. compensation voltage. An example plot is shown in Graph 1.

The values from the lower edge for each peptide was copied into a retention time file table. Modeling of CV Data
FAIMS Compensation Voltage
The CV high and low values for the top thirty peaks were graphed vs. charge state to determine a possible model for predicting FAIMS depletion voltages in the future. Lines were fitted, as shown in Graph 2 to simplify experiments.

CONCLUSIONS
• Method building with peptide dependent CVs increases the chance of identifying coeluting peptides. The high edge CVs increased coverage by 54%, the low edge increased 29%, and the combined search of the two files increased coverage by 66% total.

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