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 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.

Methods: Tryptic maps of Trastuzumab were run at CV voltages from 0 to -80 volt in 5 volt increments. Plotting of the major tryptic peptide's signal intensity vs. CV allows the determination of the low and high edge CV. These CV values will be built into a retention time dependent DDA method to fragment and identify lower-level background proteins. Proteome Discoverer 3.0 was used 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 potential called the compensation voltage which is normally chosen for optimized transmission. In this work, compensation voltages that result in poor transmission of individually eluting major ions will be determined to effectively deplete the spectra of them. This will decrease the overall number of ions hence increasing the injection time to trap more HCP peptides.

Previous work has used a CV above the transmission range of the MAb peptide (**high edge**); here we compare the high edge CV to the CV below the transmission range of the MAb (**low edge**), and the results of both the high edge and low edge data files searched in a multi-consensus report (**combined edge**).

MATERIALS AND METHODS

Sample Preparation

Trastuzumab (Anti-Human HER2, Humanized Antibody, MedChemExpress) was digested using the Thermo Scientific™ In-Solution Tryptic Digestion kit (PN# 89895) and was diluted to 5 pmol/uL. Two microliter injections (10 pmols) was used for all LCMS experiments.

Instrument Methods

The digested samples were separated on a Thermo Scientific™ Vanquish™ Neo UHPLC optimized with a 1 mm x 15 cm Thermo Scientific™ Acclaim™ PepMap™ RSLC column using a one-hour total gradient from 2-40% acetonitrile with 0.1% formic acid in direct inject microflow mode. A data dependent method scanning from 300-2000 Th. at 120,000 resolution with HCD was used on the Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer.

FAIMS Instrument Methods

Apex peak detection with dynamic exclusion and charge state rejection of singly charged species was enabled with a total cycle time of 1.0 seconds. Quadrupole isolation with a width of 1.3 Th. was used along with normalized HCD energy of 30%. The resulting data was searched using Thermo Scientific™ BioPharma Finder™ 5.1 against the published sequence with mammalian glycosylations as variable modifications. No FAIMS was used.

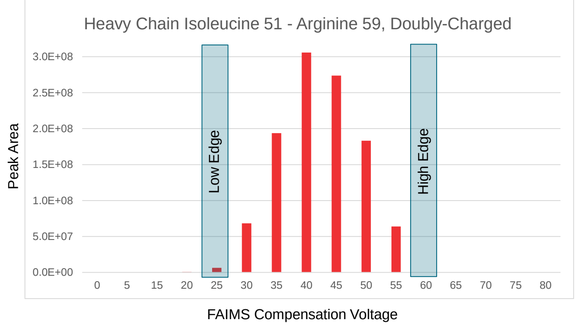
The digests were then injected sequentially with FAIMS, using compensation voltages from 0 to -80 volts. An Xcalibur™ processing method was built to integrate the Most Abundant Isotope (MAI) of each peptide at a particular charge state. The peak area of the peptides was processed and plotted vs. compensation 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 that caused a significant drop in the area (the low edge of the curve). These depletion 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 comparison with the earlier non-FAIMS data. The resulting data was searched along with the initial non-FAIMS data in Thermo Scientific™ Proteome 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 glycosylations. The processing method was run, and each individual integration was evaluated for proper start and stop end 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-based method shown in Figure 2. A separate method identical in nature was constructed using the high edge values. The samples were run in triplicate and searched via Proteome Discoverer. **The high edge CVs compared to no FAIMS in use, increased coverage by 54%, the low edge increased 29%, and the combined search of the two files increased coverage by 66% total against the digests were no FAIMS was used.**



Graph 1. Example edge plot of CV vs peak area for a single peptide. The plot allows the determination of the high and low edge values.

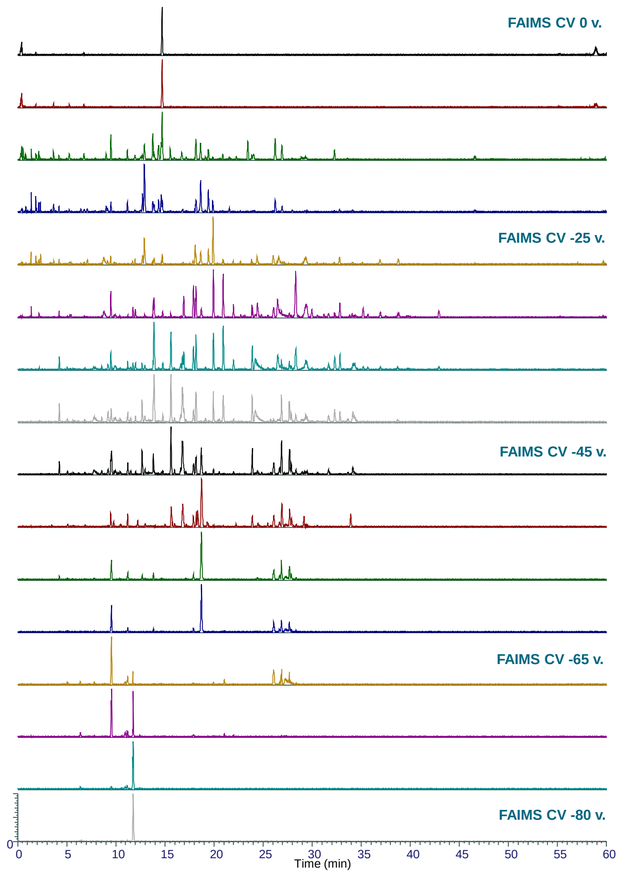


Figure 1. Trastuzumab peptide maps changing the CVs from 0 to -80 volts as base peak plots.

Modeling of CV Data

The CV high and low values for the top thirty peaks were graphed by 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.

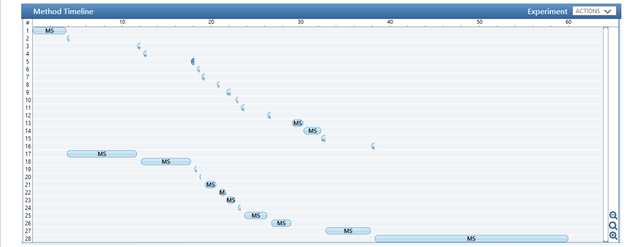
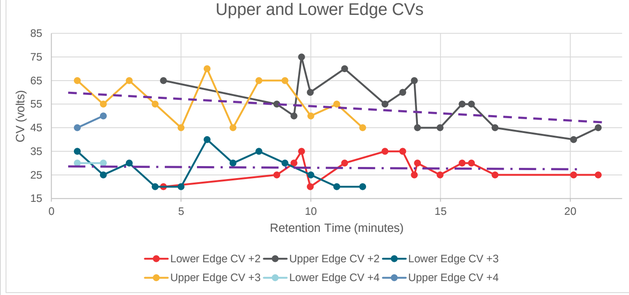


Figure 2. Example of building retention time based CV dependent DDA method. For ease of viewing this figure is shown with only fifteen of the thirty CVs used.



Graph 2. Plot of the thirty MAb peptides sorted by charge state against their retention times. Two purples dashed lines were fitted for the low and high edge.

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.
- A simple model for FAIMS depletion suggests that three injections: no FAIMS, CV of -30, and -50 would produce higher HCP coverage

TRADEMARKS/LICENSING

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