Retention Time Based FAIMS Depletion of Protein Drugs in Peptide Mapping to Increase Coverage of Background Host Cell Proteins

Craig P Dufresne, Thermo Fisher Scientific, West Palm Beach, FL, United States and Scott M Peterman, Thermo Fisher Scientific, San Jose, CA, United States

ABSTRACT

Purpose: Time dependent FAIMS compensation voltages are used to deplete the major peptides in the tryptic map of a protein-based drug allowing more host cell protein contaminates to be identified.

Methods: Tryptic maps of the protein drug with run at different compensation voltages will be used to determine the best voltage for each major peptide that eliminates the peptide form the spectra. These CV values will be built into a time dependent DDA method to fragment and identify lower-level background proteins.

Results: Identifications of the host cell proteins increased by 52%.

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 be 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 and increase the injection time to increase background coverage.

MATERIALS AND METHODS

Sample Preparation

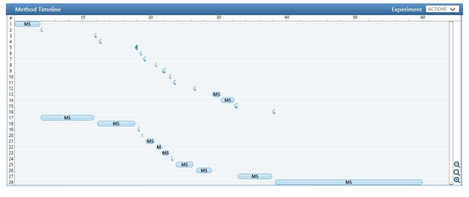
Ten 10 ug samples of NIST MAb RM 8671 was digested using the Thermo Scientific™ In-Solution Tryptic Digestion kit (PN# 89895) in parallel. The samples were individually quality controlled by LCMS peptide mapping. The samples were pooled and diluted to 1.25 pmol/uL.

Instrument Methods and Data Analysis

The digested samples were separated on a Vanquish Neo 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 ion trap detected CID tandem mass spectrometry was used on the Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer. Apex peak detection with dynamic exclusion and charge state rejection on singly charged species was enabled with a total cycle time of 0.7 seconds. Quadrupole isolation with a width of 1.3 Th. was used with a resonance excitation energy of 35%. The resulting data was searched using BioPharma Finder™ 4.0 against the published sequence with mammalian glycosylations as variable modifications. One hundred percent sequence coverage was obtained. The top ten peptides from the antibody sorted by spectral peak height were selected for depletion as a proof of concept (**Figure 1**).

The digest was injected sequentially using compensation voltages from -30 to -80 volts. The files were analyzed in FreeStyle™ 1.8 by creating extracted ion chromatograms of the top fifteen peaks. The peak area of the extracted ions was integrated 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). This depletion voltage was used to build a retention window around the top ten 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™ 2.5 using the SwissProt database.

Figure 1. The top half of the Fusion Lumos method editor is used to add the time-dependent scan events for each protein drug tryptic peptide being eliminated. Each gap between them is filled in with a full ms using the default CV setting of -55v.

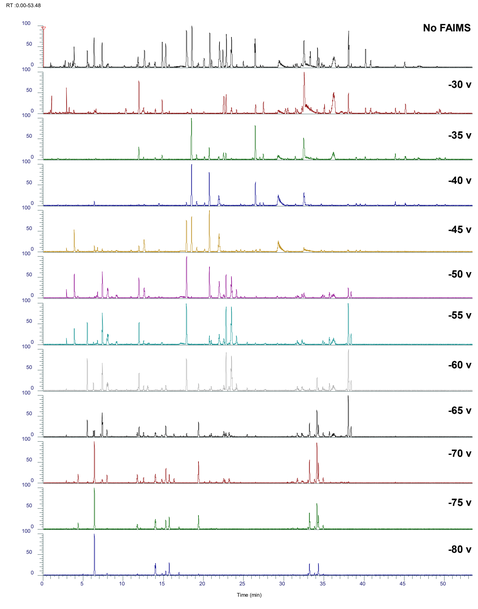


RESULTS

Protein Drug Peptide Map with and without FAIMS

Freestyle 1.8 SP2 was used to plot the base peak chromatogram of the tryptic digest peptide map without the FAIMS source and then with the FAIMS source at each different compensation voltage (**Figure 2**). While no single CV covers all the peptides, -55 volts was chosen as the default value to use during the gaps when no specific major tryptic peptide is eluting.

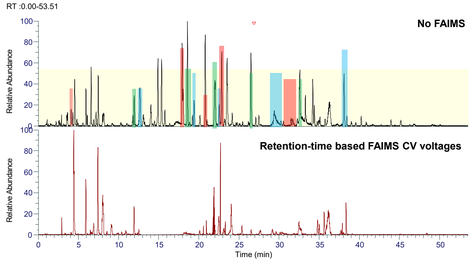
Figure 2. Base peak plots comparing no FAIMS to FAIMS changing the compensation voltage form -30 to -80 volts.



Peptides Chosen in Windows

The top fifteen peptides identified from the BioPharma Finder search sorted in descending order by intensity, were chosen to be the retention time windows for depletion in the study. Data from Figure 2 was used to choose the CV for that window.

Figure 3. Base peak plots comparing no FAIMS to retention-timed based FAIMS voltages. The red, green, and blue boxes represent the windows where the FAIMS CV was changed from the default setting of -55. The height of the box represents the CV as labeled using the Y-axis. The yellow box height represents the CV default voltage. The fifteen windows were chosen by sorting the m/z spectral peaks identified as either heavy or light chains of the antibody drug using Biopharma Finder.



Increase in Signal to Noise Ratio from Depletion

The effective use of retention time-based windows is most observable with coeluting peptides near the spectral limits of Orbitrap detection. In **Figure 5** we see an example where a large peptide elutes with many smaller intensity species. In this case the ion routing multipole is Automatic Gain Control limited to the method assigned number of ions. Most of the ion population is consumed by the large main peptide from the heavy chain in this example, resulting in poor signal to noise capture of minor coeluting species (**Figure 4**). Depletion of the major peak using FAIMS allows the injection time to increase resulting in a higher signal to noise ratio as seen in **Figure 6**.

Figure 4. The peptide 'NQVSLTCLVK' (N364-K373) from the heavy chain of the antibody was used as an example. The doubly-charged species dominates the filling of the ion routing multipole leaving little room for other ions. The top panel shows the extracted ion chromatogram (XIC) of the major species without the use of FAIMS. The bottom XIC is a coeluting peptide without the use of FAIMS. The center panel shows the same coeluting peptide XIC with FAIMS using a CV of -30v. Notice the poor signal to noise ratio even though the peak height is ~30% higher.

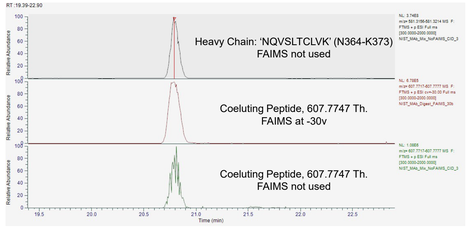
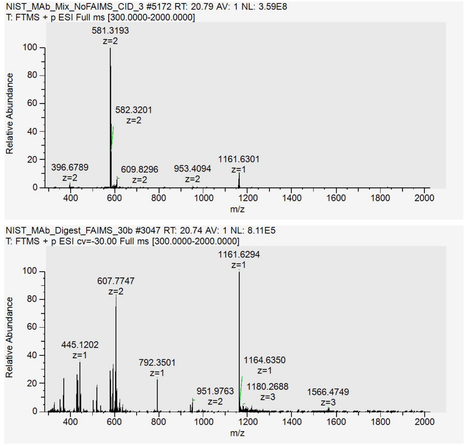


Figure 5. The top plot shows the scan at the apex of the chromatographic peak for the peptide NQVSLTCLVK form the heavy chain of the antibody. The doubly-charged species dominates the spectra resulting in poor signal to noise ratios for smaller coeluting species. The lower pane shows the spectra with a -30v CV applied using FAIMS. Many more coeluting species are observed with higher signal to noise ratios.



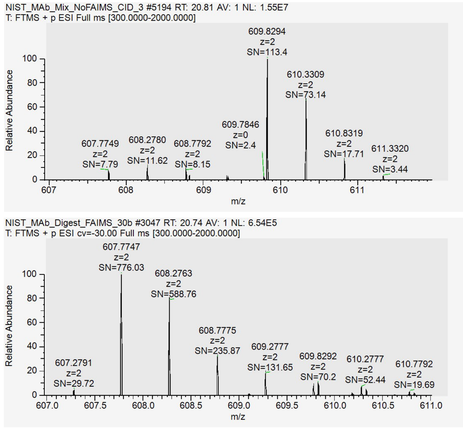
Advantages Obtained from Higher Signal to Noise Ratio Coeluting Species

The isotopic cluster shown in Figure 6 in the bottom panel illustrates the features increasing the signal to noise ratio of a coeluting peptide. The higher SN increases the chance the on-board computer interscan processing of the (n-1) full scan data will calculate the correct charge state. In addition, the high number of ions will result in a better isotopic fidelity increasing the chance that monoisotopic precursor selection (MIPS) will succeed. MIPS processing of the full scan data will result in more precursor ions being available for fragmentation and decrease the chance of dynamic exclusion repeating selection of the same precursor multiple times.

Search Results Using Retention Time Based CV Windows

The tryptic digest was searched using Proteome Discoverer against the mammalian SWISSPROT database. One hundred percent of the heavy and light chain sequence was covered using the one-hour gradient of the Vanquish Neo in microflow mode with direct injection. Seventy-seven host cell proteins were identified without the use of FAIMS. When the retention time-based CV windows were added, forty new host cell proteins were identified. This was an increase of 52%.

Figure 6. The top panel shows the signal to noise ratio (SN) of the coeluting peptide without the use of FAIMS. The peak at 607.7749 Th. has a signal to noise ratio of 7.79 in spectra. There are isotopes present that allow the on-board computer to calculate a charge state assignment, but the isotope ratios are not accurate due to the low ion abundance. When FAIMS is used for major peak depletion, the injection time increases filling the ion routing multipole with more ions of low abundance species. In this example, the coeluting peak at 607.7747 Th. has a signal to noise ratio of 776.03, with a complete isotopic cluster with accurate ratios. The signal to noise ratio has increased by almost one-hundred times.



CONCLUSIONS

- Retention time based FAIMS compensation voltages can be calculated for individual peptides of a tryptic digest easily.
- Method building with peptide dependent CVs increases the chance of identifying coeluting peptides. This results in a 52% increase in the number of host cell proteins identified.

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