
Brendan MacLeod1, Daniel T. Tempaz1, Susan E. Abbatellio2, Steven A. Carr2, and Michael J. MacCoss1

1. Department of Genome Sciences, University of Washington, Seattle, WA, USA.
2. Broad Institute of MIT and Harvard, Cambridge, MA, USA.

† These authors contributed equally to this work.

Abstract:

By simulating a differential proteomics experiment, wherein the only difference between groups of samples is the CE optimization, we have shown that utilizing the ability to predict non-inferior CE settings that work well with the broad-spectrum of peptide-ion types. For this reason, we characterized the impact of using single linear equations to predict the collision energy (CE) for peptide signal intensity and compared it with the empirically optimized CE for each peptide and transition individually.

We had derived optimized linear equations for predicting CE values that are different from the empirically optimized CE values obtained during the CE optimization study. The dashed lines represent the plots for their respective peptide and transition across multiple instrument platforms used in our analysis.

In these experiments we have used a fully automated workbench, implementing in version 1.6 of the Skyline open-source software. We have shown that using a simple linear equation to predict CE based on the peptide precursor mass can be a reasonable approximation for peak area,

1. Department of Genome Sciences, University of Washington, Seattle, WA, USA.

Results:

Multiple Reaction Monitoring (MRM) are being used to target large numbers of protein candidates in complex mixtures. At present, instrument parameters are often optimized for each peptide, a time and resource-intensive process. Large SRM experiments require careful planning, and the ability to predict non-inferior CE settings that work well with the broad-spectrum of peptide-ion types. For this reason, we characterized the impact of using single linear equations to predict the collision energy (CE) for peptide signal intensity and compared it with the empirically optimized CE for each peptide and transition individually.

We had derived optimized linear equations for predicting CE values that are different from the empirically optimized CE values obtained during the CE optimization study. The dashed lines represent the plots for their respective peptide and transition across multiple instrument platforms used in our analysis.

In these experiments we have used a fully automated workbench, implementing in version 1.6 of the Skyline open-source software. We have shown that using a simple linear equation to predict CE based on the peptide precursor mass can be a reasonable approximation for peak area.

A comparison of the detailed and optimized linear equations for 4 selected peptides, acquired on a ThermoFinnigan TSQ Ultra mass spectrometer (MS/MS) on transition 360.5–>291.5, shows that the linear regression equation is not optimal for the selected peptides and precursors. The solid line represents simple linear regression of the experimental data. The optimized CE equation results in an improved linear regression equation obtained for data acquired (Table 1). The same plots were generated for each of the instrument platforms used in our analysis.

2. Methods

2.1. Datasets

The datasets were acquired as described in our previous work. The collision energy optimization parameters were set to use 5 steps on either side of the value predicted by the default optimization. Consecutive mass variations of one hundredth of a mass unit were used for each fragment ion as a vendor-neutral method. The optimum CE was determined on the basis of the maximum peak area.

B. Methods

In these experiments we have used a fully automated workbench, implementing in version 1.6 of the Skyline open-source software. We have shown that using a simple linear equation to predict CE based on the peptide precursor mass can be a reasonable approximation for peak area.

Complementary strategies for determining the CE value that produced the maximum total peak area for the current precursor in the training set or the CE value that produced the maximum peak area for the current transition in the training set. The training set was used to derive “optimal” CE values from a single measurement. In these cases, peptides were not mixed, and instrument parameters were not altered.

Figure 1 shows the reproducibility of the relative peak areas, calculated by integrating the TIC area for each peptide used in the optimization study. The areas obtained at each voltage step were normalized within each technical replicate, and the percent change from the area obtained at the predicted CE was calculated for each peptide.

Figure 2: Comparison of the detailed and optimized linear equations for 4 selected peptides, acquired on a ThermoFinnigan TSQ Ultra mass spectrometer (MS/MS) on transition 360.5–>291.5, shows that the linear regression equation is not optimal for the selected peptides and precursors. The solid line represents simple linear regression of the experimental data. The optimized CE equation results in an improved linear regression equation obtained for data acquired (Table 1). The same plots were generated for each of the instrument platforms used in our analysis.

Figure 3: Comparison of the detailed and optimized linear equations for 4 selected peptides, acquired on a ThermoFinnigan TSQ Ultra mass spectrometer (MS/MS) on transition 360.5–>291.5, shows that the linear regression equation is not optimal for the selected peptides and precursors. The solid line represents simple linear regression of the experimental data. The optimized CE equation results in an improved linear regression equation obtained for data acquired (Table 1). The same plots were generated for each of the instrument platforms used in our analysis.

Complementary strategies for determining the CE value that produced the maximum total peak area for the current precursor in the training set or the CE value that produced the maximum peak area for the current transition in the training set. The training set was used to derive “optimal” CE values from a single measurement. In these cases, peptides were not mixed, and instrument parameters were not altered.

Figure 1: Collision energy (CE) optimization reproducibility for the doubly charged peptide YLLGYLEQLLR in n=150. The solid line corresponds to the final (i.e. when the last observation was made) optimal predicted CE value and CE values obtained during the optimization range. The red line corresponds to the CE value calculated using the moment-based (complexity) regression (TSQ Ultra, Thermo-Finnigan). The Table 1’s summary is that we had derived optimized linear equations for predicting CE values that are different from the empirically optimized CE values obtained during the CE optimization study. The dashed lines represent the plots for their respective peptide and transition across multiple instrument platforms used in our analysis.