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Ultrafast and Robust Optimization of Peptide MRMs Using a Fast-Scanning Triple Quadrupole Mass Spectrometer

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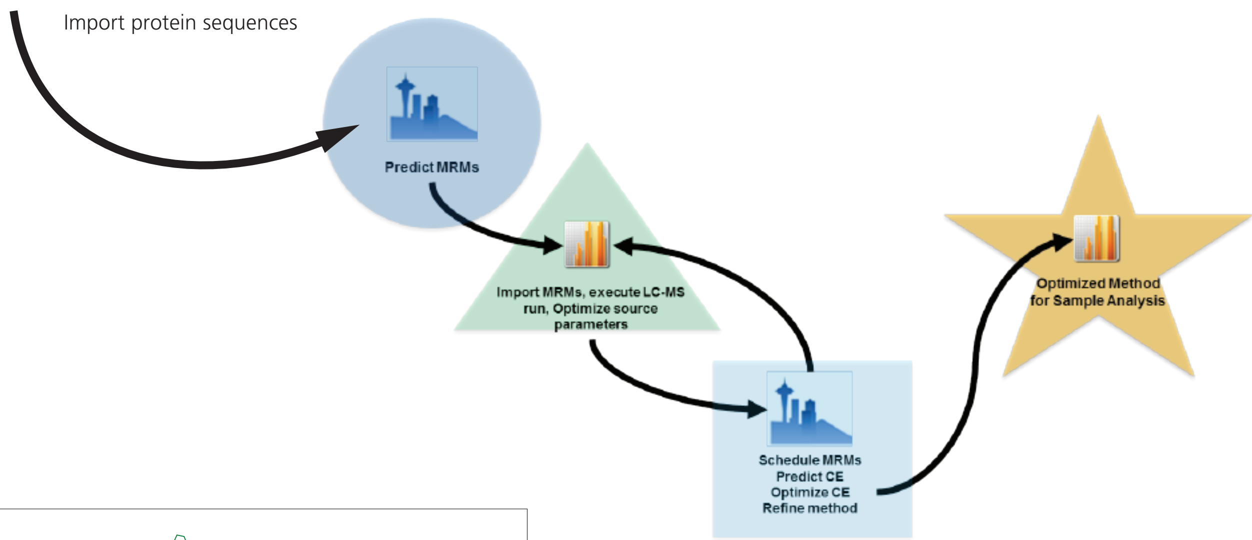
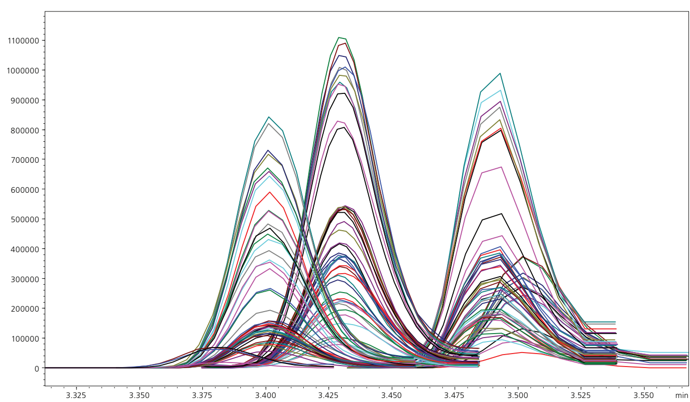
Introduction

Targeted proteomics methods may be composed of tens or hundreds of MRM channels corresponding to peptides from a wide variety of proteins of interest. Although precursor and product ions can be calculated in advance, the best ions for monitoring among all possible channels need to be selected and confirmed before analyzing a large or material-limited sample set. Similarly, collision energy and other instrument parameters may be estimated or modeled by software but require fine-tuning and verification to maximize instrument response for each channel. A fast-scanning triple quadrupole mass spectrometer has been used with proteomics software to verify and optimize large numbers of MRM transitions with a minimum number of optimization runs.





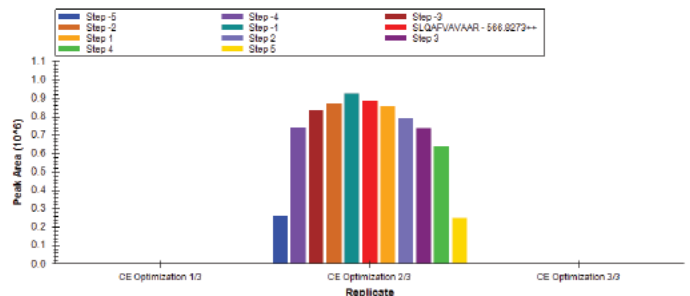


Figure 1 Peptide optimization workflow using Skyline and the LCMS-8050

Method

A commercially available peptide mix containing over 300 peptides representing a wide range of size, sequence, and hydrophobicity was used. A high sensitivity, fast scanning triple quadrupole mass spectrometer (LCMS-8050) was used with a Nexera UHPLC system. Skyline targeted proteomics software (MacCoss et al., University of Washington, Seattle Wash.) was used to predict precursor and product ions as well as collision energies and retention times using Shimadzu-specific models. A series of runs were created to select the best MRM transitions and optimize instrument settings. The methods were created in such a way as to minimize the total number of runs required for the optimization, utilizing the fast scan and MRM speeds of the instrument.

Table 1 Instrument parameters for peptide analysis

Parameter	Value	Parameter	Value
LC Column	: Aeris Peptide C18 (2.1×100 mm, 3 µm)	Interface Temp	: 400 °C
Mobile Phase A	: 0.1% Formic Acid	Nebulizing Gas	: 3 L/min
Mobile Phase B	: Acetonitrile	Drying Gas	: 10 L/min
Flow Rate	: 0.5 mL/min	DL Temp	: 150 °C
Probe Voltage	: 1.5 kV	Heat Block Temp	: 400 °C

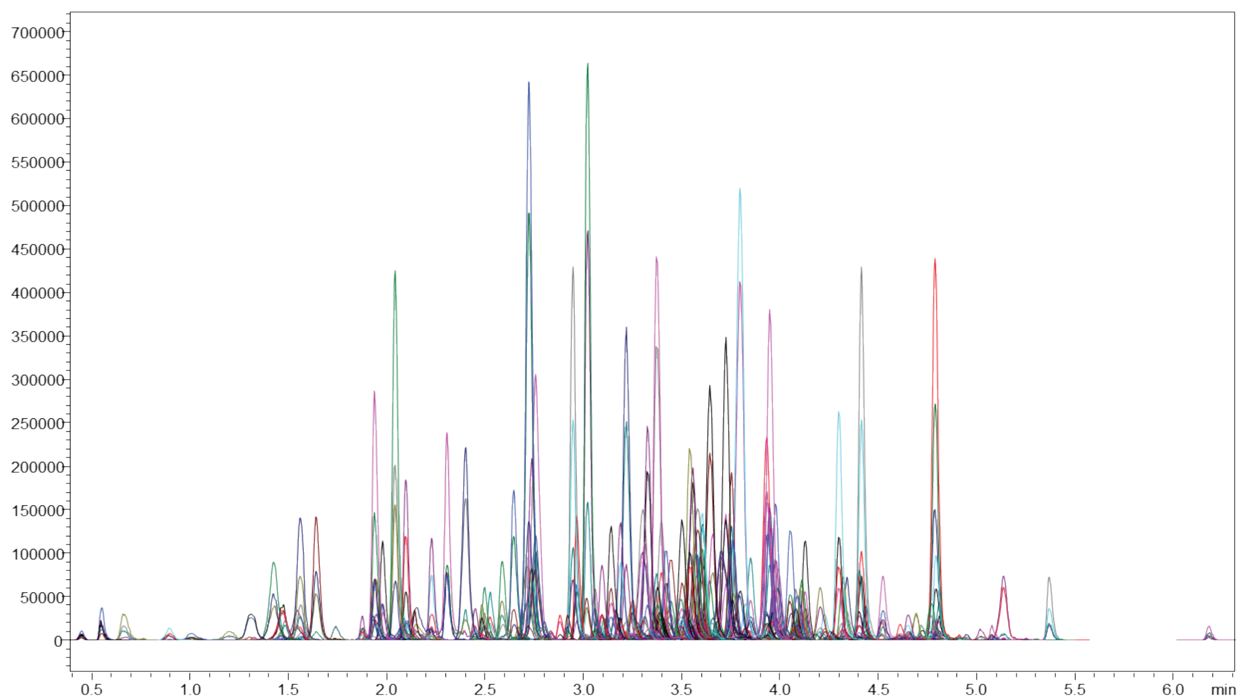


Figure 2 Representative chromatogram of peptide mix. 346 peptides were monitored over the run.

Results and Discussion

The UHPLC system rapidly separated the peptide mixture of over 300 peptides over a 6 minute gradient, creating a high density of peaks throughout the entire run. The fast scan and MRM performance of the mass spectrometer enabled the efficient detection and optimization of each peak despite the high signal density. In addition the UHPLC system with its small particle size column created very sharp peaks as narrow as 3 seconds each, facilitating the separation and increasing the signal intensity of each peak. In the present analysis, 1,000 MRMs were tested in each run, representing an unprecedented number of simultaneous optimizations. For each peptide, up to 5 candidate transitions were tested and more than 700 total MRM transitions were confirmed and selected for the final analysis. The predicted collision energies were fine-tuned for each transition with 1 volt resolution and in nearly all cases, the optimized value was within the ±5 volt optimization window. Predicted and optimized collision energies were also well correlated. This indicates excellent performance of the collision energy prediction as well as the effectiveness of the collision energy fine-tuning step. In addition to collision energy, optimum settings of other parameters including quadrupole prerod bias, QArray bias, and collision gas pressure were verified in a similar way. The final method was therefore created with fully optimized parameters using a minimum number of analytical runs and represents a rapid and robust way of creating and optimizing targeted proteomics runs. The combined application of a fast, high resolution chromatographic separation with a fast scanning triple quadrupole mass spectrometer and targeted proteomics software represents the most efficient and effective way to carry out high sensitivity targeted proteomics runs directed at a large number of targets.

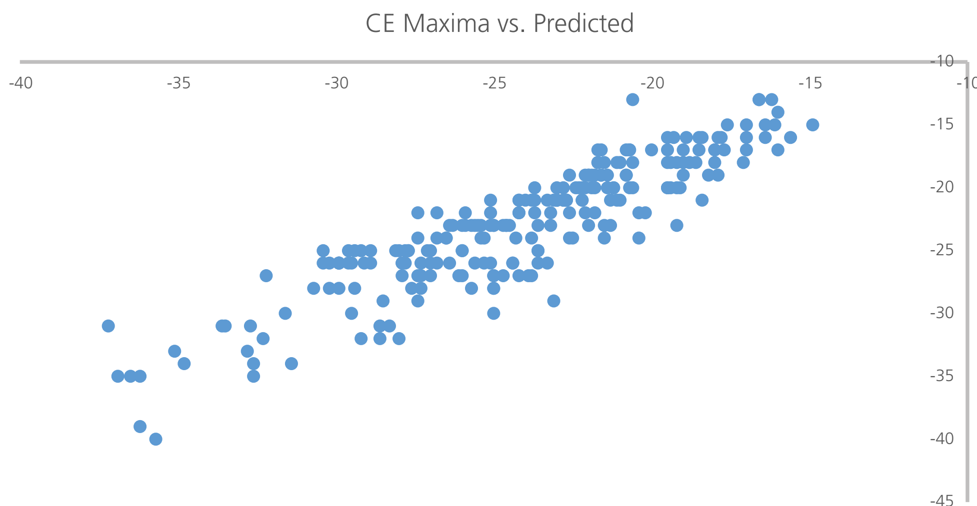


Figure 3 Optimum CE values predicted by the model vs. the optimum observed. The results agree closely with the predicted value.

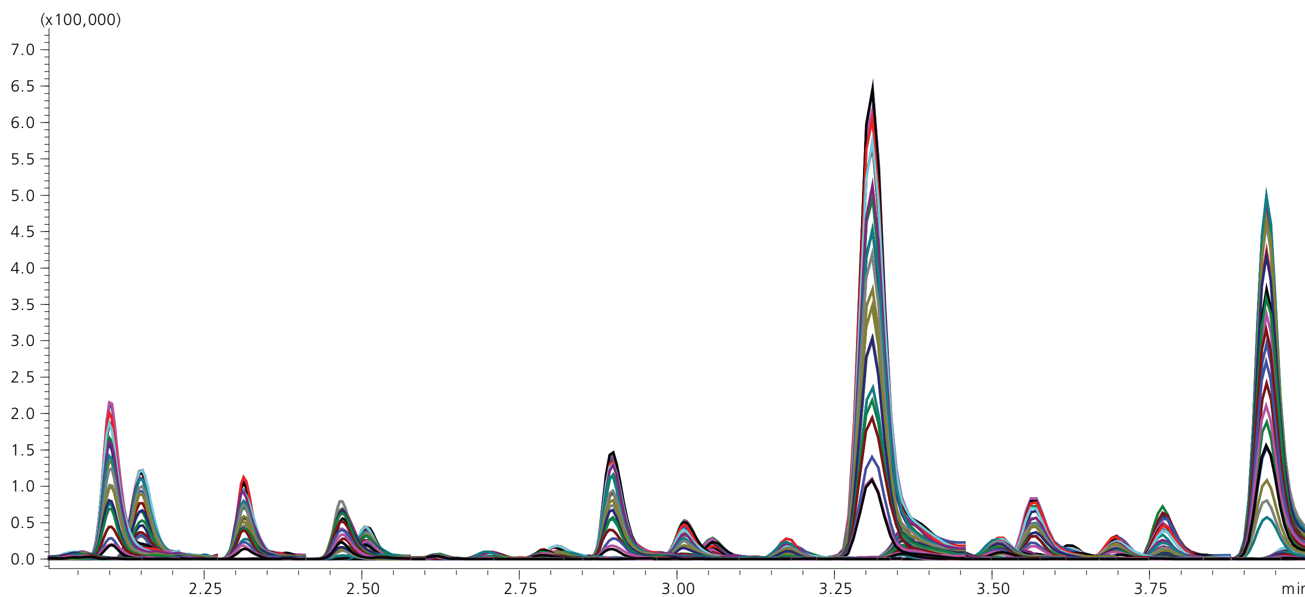


Figure 4 Detailed chromatogram view of CE optimization. A large number of peptides can be concurrently optimized due to the fast speed of the instrument.

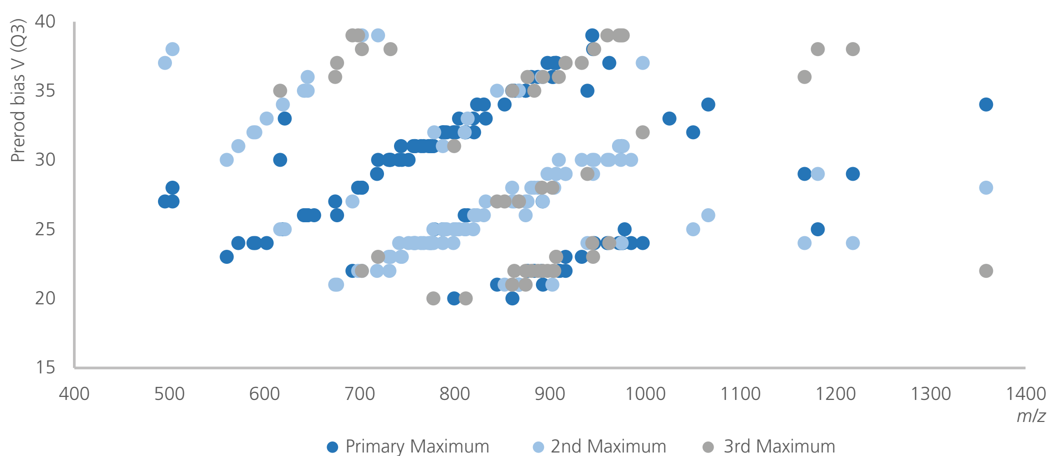


Figure 5 Q3 Prerod bias optimization result shows the several maxima can be accurately predicted on the basis of *m/z* alone. The optimum value corresponds to the tune file value at the primary maximum in nearly all cases.

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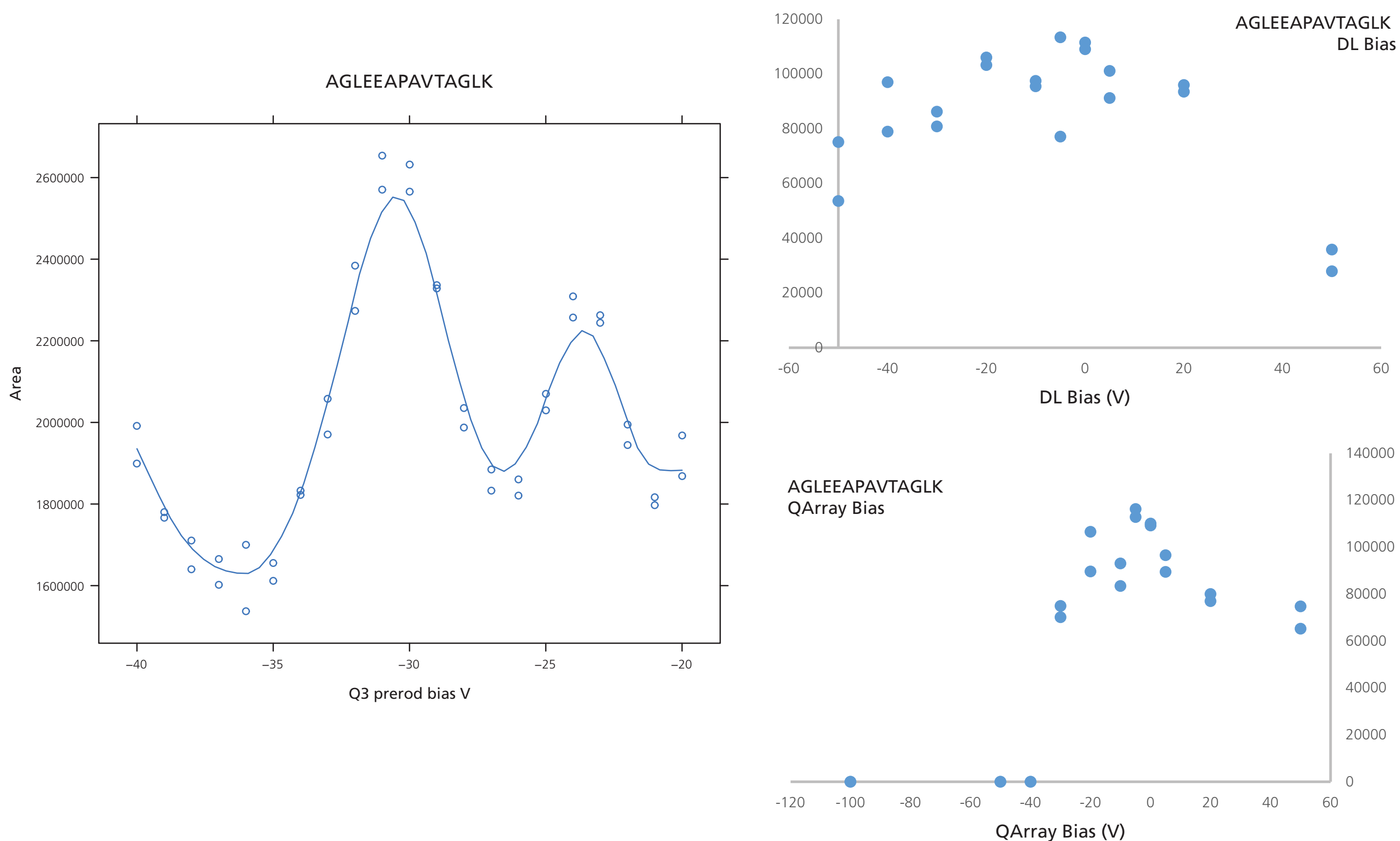

Figure 6 Representative Q3 prerod optimization result for the peptide AGLEEAPAVTAGLK (left). The DL bias and QArray bias result for the peptide is also shown (right top and bottom).

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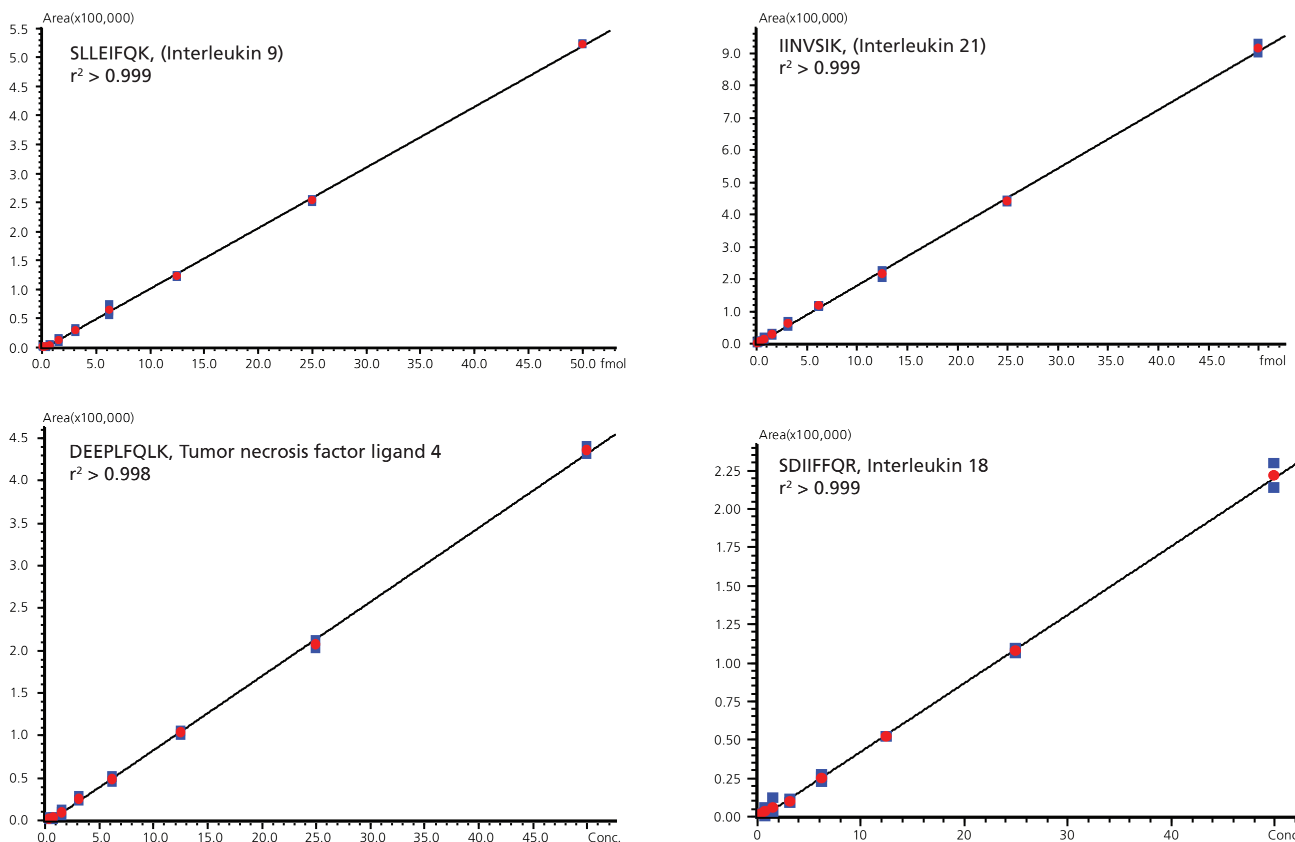

Figure 7 Calibration curves for selected peptides of interest.

Conclusion

UHPLC-MS-MS was used to verify and optimize a large number of peptide transitions with the minimum number of analytical runs. Using Skyline targeted proteomics software and the fast scanning LCMS-8050, more than 700 MRMs for more than 300 peptides could be refined into a final method for accurate and sensitive quantitative analysis. More detailed optimization presented here demonstrates that tune file settings for prerod, QArray, and DL biases are near-optimal and the CE prediction model is accurate. However any settings can be quickly and easily optimized when desired.

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