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应用纪要

MassLynx-Skyline Interface (MSI) – A New Automated Tool to Streamline MRM Method Development and Optimization for Large Molecule Quantification

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For research use only. Not for use in diagnostic procedures.

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief highlights the benefits of the MassLynx Skyline interface which automates and simplifies the process of generating MRM methods for quantification of peptides/proteins.

Benefits

Introducing a new tool that automates and simplifies multiple reaction monitoring (MRM) method development for peptides, digested proteins (therapeutics/biomarkers), or targeted proteomic LC-MS/MS assays using MassLynx and Skyline on tandem quadrupole instruments.

Introduction

Proteins and peptides are increasingly becoming routine analytes in laboratories performing quantification using LC-MS/MS. The fundamental differences between small molecules and peptides/proteins makes multiple reaction monitoring (MRM) method development and optimization for these larger molecules more challenging. Skyline is a freely available software tool created by the University of Washington, and is widely used in targeted proteomics workflows, but is now increasingly used to aid method development of large molecule bioanalytical assays that support drug discovery and development. Current compatibility between MassLynx and Skyline enables the user to easily create acquisition methods, review data, and optimize collision energy to generate a final MRM method. Although this process is significantly simpler than trying to develop these methods manually without the aid of Skyline, it requires manual intervention to create and export acquisition methods (Skyline), acquire data (MassLynx), import, review and filter the data (Skyline), and generate a final acquisition method.

Here, we describe a new software tool, MassLynx Skyline Interface (MSI), which automates the workflow described above to make for a simpler user experience with minimal intervention.

Results and Discussion

The established workflow for therapeutic peptides and digested proteins to ensure systematic and robust method development consists of four steps (Figure 1). Step 1 performs a precursor - precursor acquisition to determine the retention time of the most intense precursor for each peptide present in the sample. Step 2 performs a precursor - product acquisition to pick the most sensitive precursor/product pairs for every peptide in the sample using a default calculated collision energy. In Step 3, the 3–5 most sensitive MRM transitions for every peptide are retained and the collision energy optimized for each one. Step 4 generates a final acquisition method using the most sensitive MRM transitions and optimized collision energies. For each of the steps, an acquisition method is exported by the user from Skyline, a sample list is set up and data acquired in MassLynx, and then imported back into Skyline and filtered to give the final method. Previously, the user had to manually switch between the two software platforms multiple times to arrive at the final method.

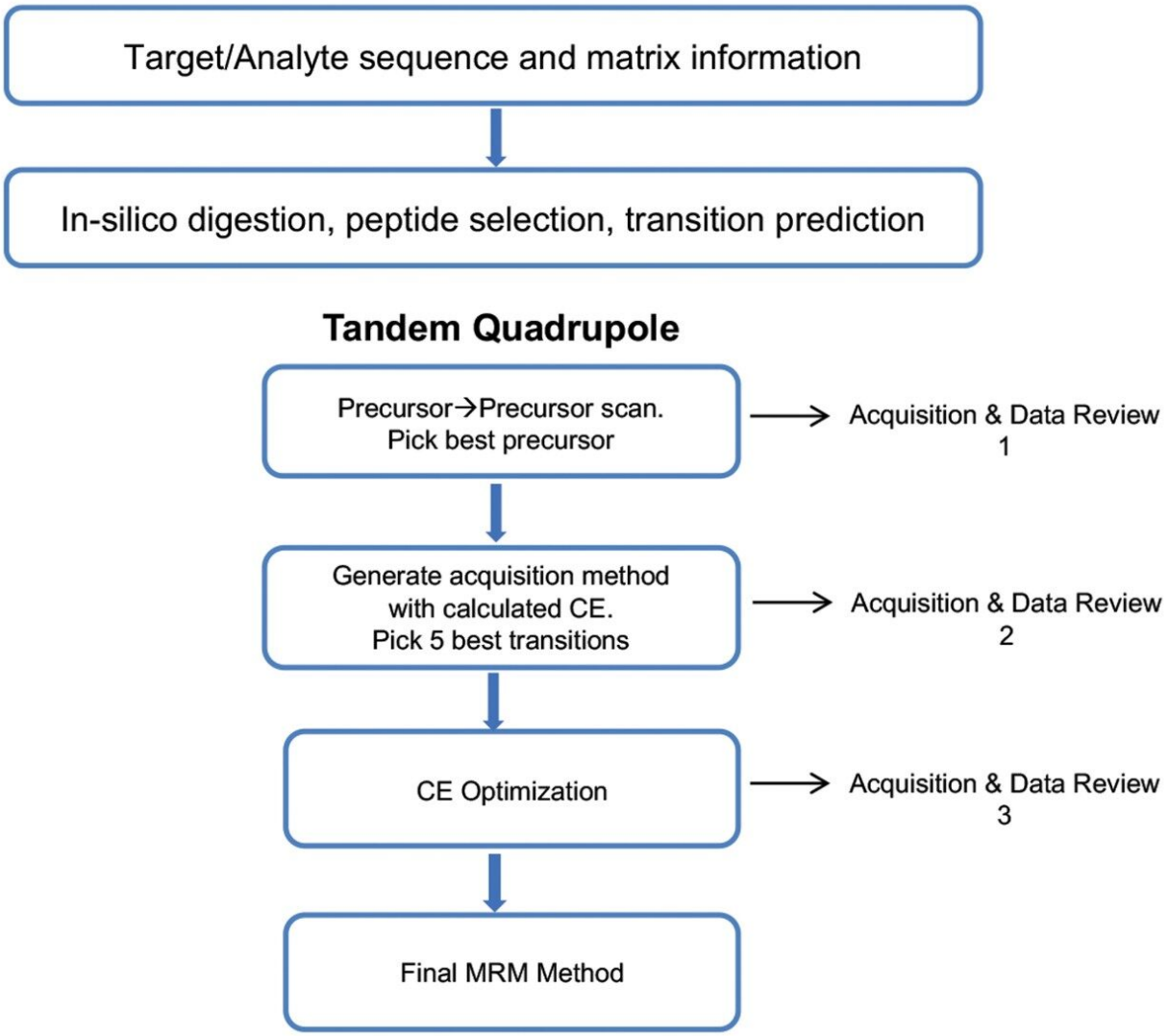

Figure 1. Recommended systematic workflow for development and optimization of an MRM method for peptides and proteins using MassLynx and Skyline.

The MassLynx Skyline Interface (MSI) automates this entire process, including acquisition method creation, data acquisition, and data processing to arrive at the final method. The user can choose between the peptide bioanalysis workflow which can be used to generate targeted MRM methods for therapeutic, and biomarker peptides or digested proteins for bioanalytical applications, or the targeted proteomics workflow more applicable for multiplexed biomarker quantification. The user creates a Skyline document with the peptide and transition settings appropriate for their workflow. Once this document is created, it can be imported into the MSI along with a template for the tune file, acquisition method, LC method, injection volume, and run length.

The tool then goes through the steps shown in Figure 2 in an automated, intelligent manner to fine-tune the best precursor/product pairs with the optimal collision energy to create the best final acquisition method. All the acquisition methods, Skyline documents, and data review documents generated throughout the process are systematically named and stored for review if needed.

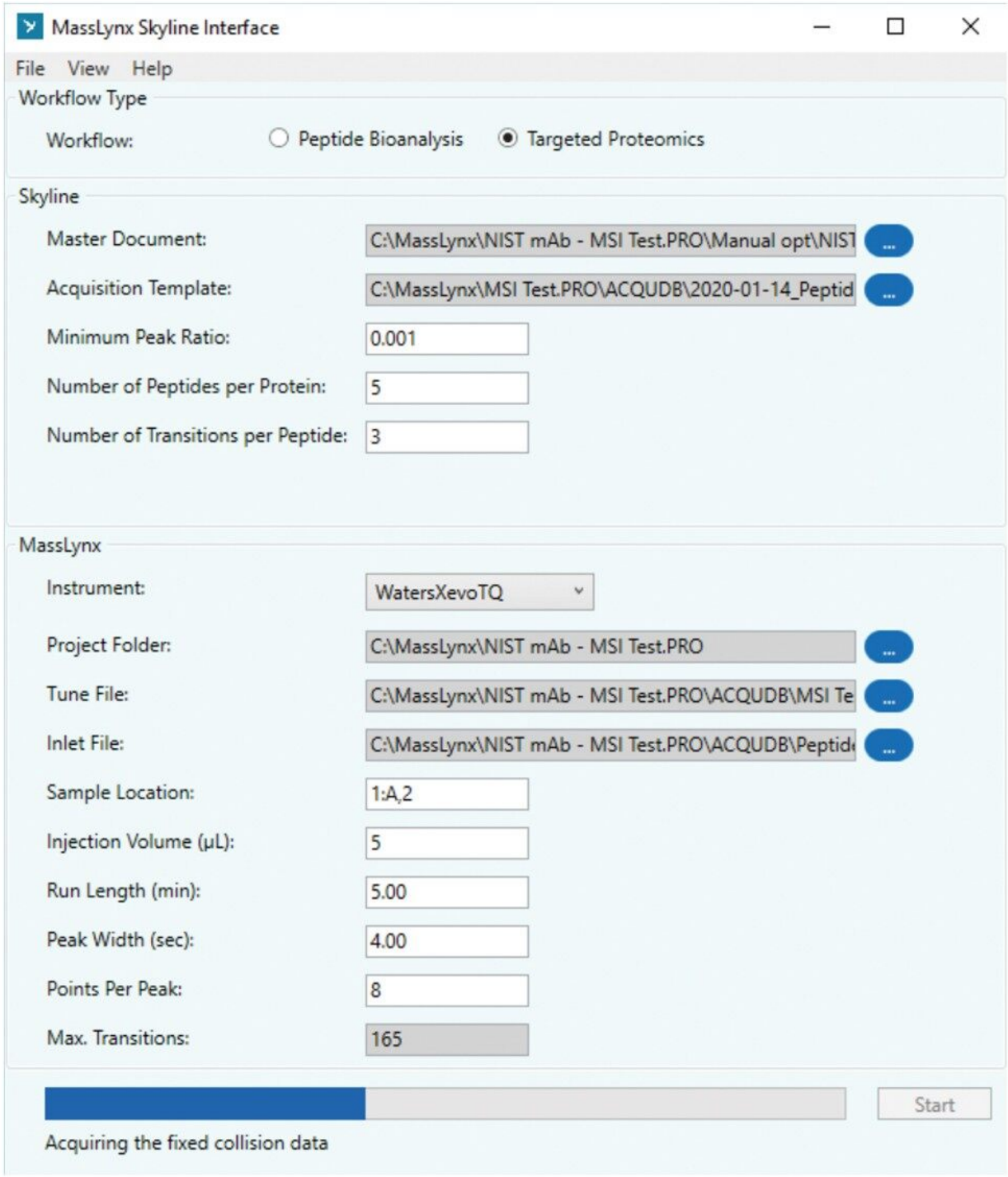

Figure 2. MassLynx Skyline interface.

Conclusion

The MassLynx Skyline Interface (MSI) automates the workflow for development and optimization of MRM methods for peptides and proteins using MassLynx and Skyline in an intelligent, systematic, and reproducible manner. It significantly simplifies the user experience, saves time, minimizes the number of times the user is required to switch between MassLynx and Skyline, and ensures consistent methods across different users.

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