Improving Selectivity in Quantitative Proteomics Using Targeted MS/MS/MS

Nicholas Shulman[1]; Lilian Heil[1]; Philip Remes[2]; Brendan MacLean[1]; Michael MacCoss[1]
[1]University of Washington, Seattle, WA; [2]Thermo Fisher Scientific, San Jose, CA

Introduction

Parallel reaction monitoring (PRM) is an increasingly popular alternative to selected reaction monitoring (SRM) for targeted proteomics. These data are commonly collected using accurate mass instruments (e.g. quadrupole-orbitrap or Q-TOF). Recently we showed that PRM using the linear ion trap (LIT) of an Orbitrap Eclipse was >2x more sensitive than using the orbitrap of the same instrument. In most cases, product ion transitions could be selected post-acquisition to overcome any limitations from the loss in specificity. That said, there are some peptides that had better sensitivity using the orbitrap mass analyzer because of its improved selectivity. To further improve this workflow, we have implemented support for MS/MS/MS (MS3) in Skyline to improve selectivity further.

Methods

In a first study, Pierce Retention Time Calibration (PRTC) standards were quantified in 0.2 ug/ul HeLa digest (Pierce). The MS2 fragments selected for simultaneous isolation and further activation were y-ions with m/z greater than the precursor m/z. The MS3 fragments monitored for quantitation were y-ions and their H2O and NH3 losses with m/z lower than the precursor m/z. In a second study, tandem-mass-tag (TMT) labeled yeast peptide samples were mixed in various ratios and added to a background of human peptides. The MS2 fragments with TMT-tags were isolated/activated and the resulting reporter ion chromatograms were extracted and quantified. In a third study, we compared the MS2 and MS3 quantitative figures of merit for a large set of peptides in plasma.

Results

Extracting chromatograms for internal MS3 fragment ions

SFANQPLEVVYSK
741.3854++

Fragmentation for MS/MS

y11	-ANQPLEVVYSK	1247.6630+
y10	-NQPLEVVYSK	1176.6259+
y9	-QPLEVVYSK	1062.5830+
y8	-PLEVVYSK	934.5244+

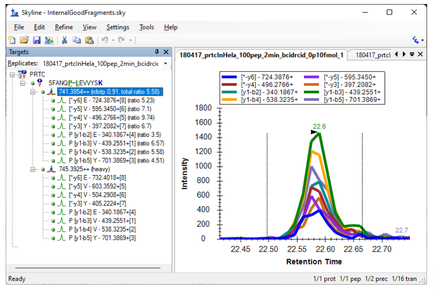
The PRM method told the mass spectrometer to isolate this MS1 precursor

The mass spectrometer did not collect an MS2 spectrum, but, instead isolated these product ions and fragmented them a second time

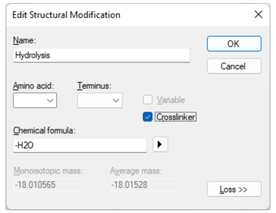
Fragmentation for MS/MS/MS

y6	-EVVYSK	724.3876+
y5	-VVYSK	595.3430+
y4	-VYSK	496.2766+
y3	-YSK	397.2082+
	-PLE-	340.1867+
	-PLEV-	439.2551+
	-PLEVV-	538.3235+
	-PLEVVY-	701.3869+

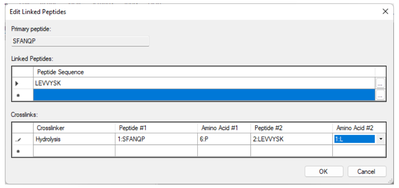
An MS3 spectrum was collected, and Skyline extracted chromatograms for these MS3 fragment ions

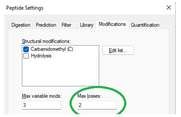


Using the crosslinked peptide feature to tell Skyline to extract chromatograms for internal fragment ions.



Skyline typically only understands fragment ions which include the residue at the C or N terminus such as y or b ions. However, by telling Skyline that it is two peptides connected with a crosslinker, Skyline will allow you to effectively choose internal fragment ions.

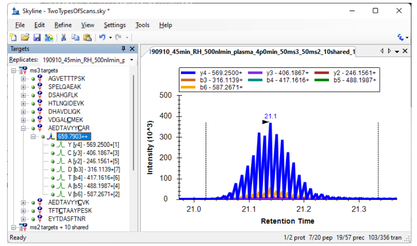




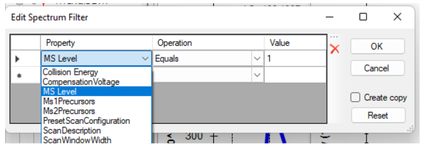
The "Max Losses" setting controls the number of fragmentation events that Skyline allows for fragment ions. When it is set to 1, only b or y ions attached to either nothing or the intact precursor of the linked peptide will be allowed. When it is set to 2, Skyline will look for y ions attached to b ions.

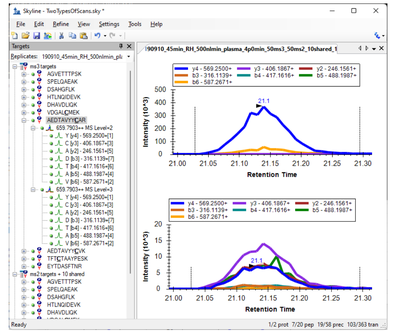
Jagged chromatograms when there are two kinds of spectra

The current version of Skyline (21.2) will extract a chromatogram point from every spectrum whose MS1 isolation window includes the precursor m/z. This can result in jagged chromatograms if there a multiple types of spectra which have the same isolation window, but which differ in other ways. In this example, there were MS2 and MS3 spectra which had the same MS1 precursor. The MS2 spectra had much higher intensities than the MS3 spectra which caused the chromatogram to be jagged.

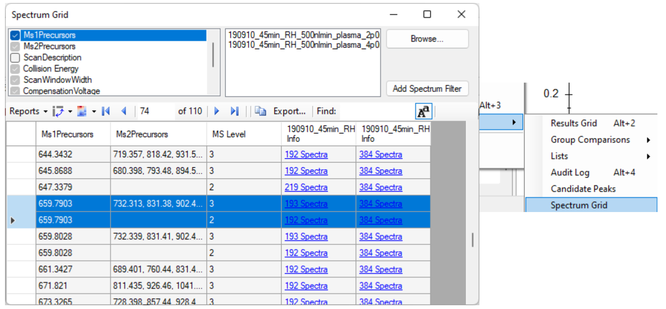


A future version of Skyline will allow specifying additional filtering to apply to spectra when extracting chromatograms.



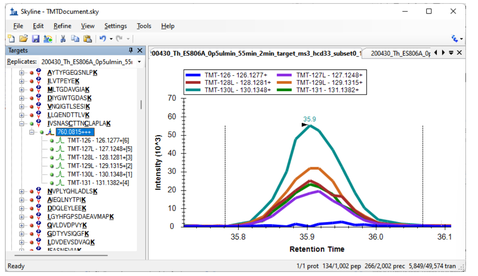


Using the Spectrum Grid to see what types of spectra are in raw files



The new Spectrum Grid will be a great way to quickly see all the different sorts of spectra that exist in a raw file, and what attributes they have which you might want to use to generate separate sets of chromatograms.

TMT tags



For this TMT-labeled peptide, the b2, b3, b4, b6, y2, y3, and y4 ions were selected for fragmentation and chromatograms for the TMT reporter ions were extracted from the MS3 spectra.

Conclusions

- Isolating specific fragments in MS2 and further fragmenting them results in cleaner chromatograms extracted from MS3 spectra
- A future version of Skyline will give users much more control over which spectra should contribute points to extracted chromatograms