Recent Advances in Skyline: Small Molecule Targets and Ion Mobility Filtering

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Overview:

The Skyline Targeted Proteomics Environment has distinguished itself as a reliable and useful tool for chromatography-based quantitative proteomics. From its initial focus on selected reaction monitoring (SRM) to its current support for full-scan methods including MS1 filtering, parallel reaction monitoring (PRM) and data independent acquisition (DIA – including the approach popularized as SWATH) Skyline has continuously evolved to meet the changing needs of proteomics researchers.

Now this popular and freely available tool has been extended to support metabolomics and other generalized small molecule targeted mass spectrometry experiments, and also to support filtering in the drift-time dimension for instruments that support ion mobility separation.

Introduction:

Skyline History

The Skyline project began in 2008 as an effort to create a completely new instrument vendor-neutral software tool, designed specifically for targeted proteomics, where most other tools in this area had been vendor-specific and adapted from small molecule quantitative software.

With the generous support of many mass spectrometer vendors and with the help of the large and active Skyline user community, Skyline has undergone continuous development and become a sophisticated tool that directly interacts with equipment from all major mass spectrometer vendors for rapid and convenient targeted proteomics method creation and refinement.

Recently, attracted to Skyline's ease of use and vendor independence, researchers in other "omics" fields have found ways to use Skyline despite its proteomics-centric design [1,2]. While they found that the masses of non-proteomic molecules could be communicated to Skyline by means of clever peptide modifications, this was inconvenient and hard to integrate with existing workflows. Also, the lack of support for negative ions in peptide-focused Skyline limited the usefulness of this approach. With many labs now engaged in multiple "omics" using targeted mass spectrometry, properly embracing generalized small molecules is a logical and welcome next step in the development of Skyline.

Targeted Mass Spectrometry Basics

The process typically begins with a large list of likely precursors and fragments of interest (the "targets") which Skyline then helps iteratively refine to produce an optimal method or transition list. The predictable nature of peptide ionization, fragmentation and chromatography allows Skyline to provide excellent automation for creation of initial methods from peptide search results.

Figure 1: Targeted mass spectrometry method refinement cycle.

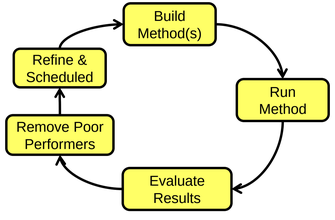
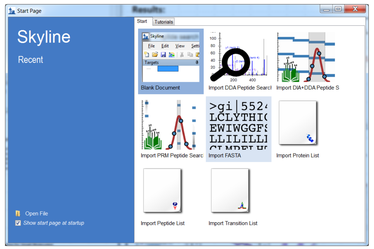


Figure 2: Skyline start page showing peptide search import wizards.



Metabolites, however, behave far less predictably. Protonation cannot be assumed. Fragmentation is difficult to model. Many instrument parameters that are calculable or constant for peptides must be experimentally determined and specified per target. Existing workflows may describe targets by any combination of chemical formula, mass, charge, or m/z value. Skyline is being adapted to these needs while retaining all the stability and ease of use that proteomics researchers have come to expect.

Ion Mobility Filtering

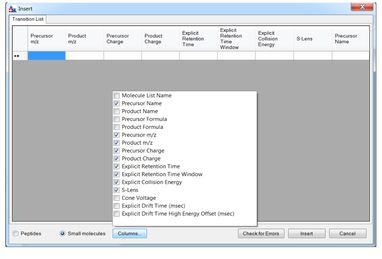
Ion mobility separation technology provides an additional degree of separation that is useful for reducing peak interference. This can be especially helpful in lipidomics and glycomics, where the mass range of many precursor targets is relatively narrow. Skyline can now use the ion mobility information found in Agilent, Waters and UIMF files for enhanced selectivity. Support for SCIEX SelexION™ has been recently added as well.

Methods:

Specifying Generalized Small Molecule Ions in Skyline

Because there are many means of ionization, Skyline requires an ion molecular formula rather than a neutral formula to derive mass and *m/z*. Targets can also be specified by mass or *m/z* only, though this makes it impossible to use ion isotope distributions.

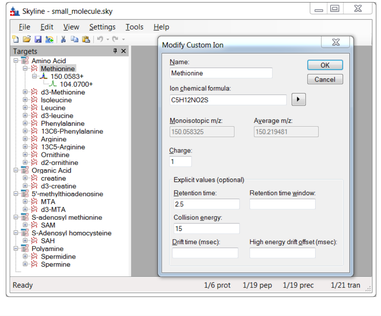
Figure 3: Skyline small molecule transition list insert form, showing the ability to explicitly specify certain values for small molecules which are calculated or treated as constants for peptides.



Editing Small Molecule Ion Properties in Skyline

Skyline allows users to adjust the properties of small molecules targets in ways that peptides do not require – though editing of some per-target explicit values for peptides will be enabled in future releases.

Figure 4: Editing a small molecule transition in Skyline.



Results:

Beyond the specification of the initial targets, operation of Skyline for targeted metabolomics or lipidomics is virtually identical to that in proteomics. Much of the work that remains is related to using small molecule library information to improve this initial step.

Figure 5: Importing a small molecule transition list in Skyline. The neutral formula of Methionine is actually C5H11NO2. In this case a single Hydrogen atom is added to indicate ionization by protonation yielding the formula given as C5H12NO2S.

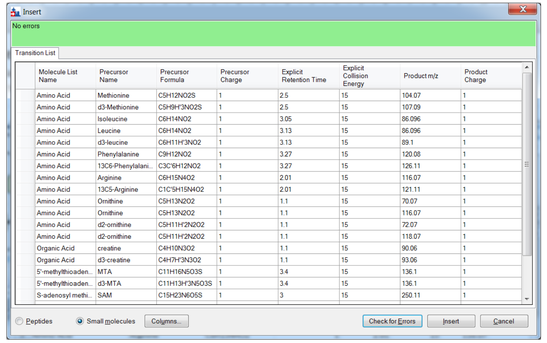


Figure 6: Targeted metabolomics method development in Skyline.

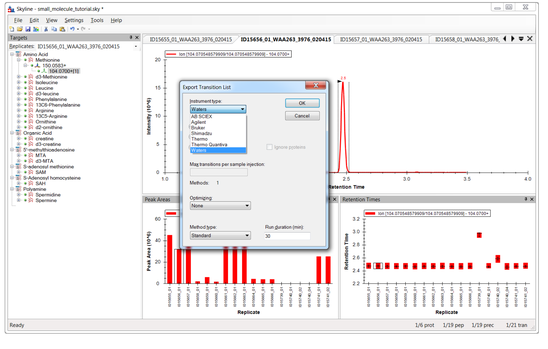


Figure 7: Ion mobility separation improves signal quality by separating ions of similar mass but different shape .The shaded horizontal band is the signal retained by the ion mobility filter for the target.

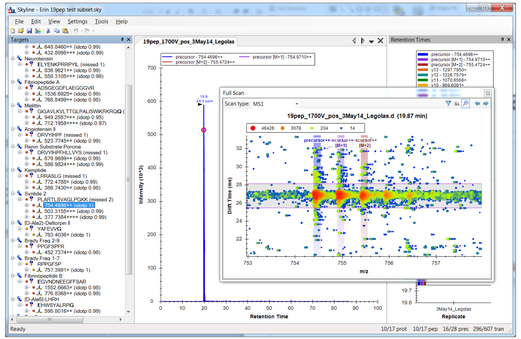
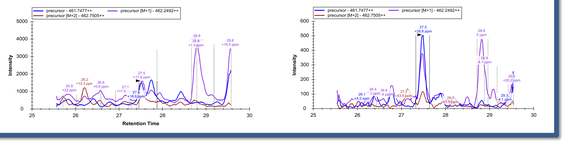


Figure 8: Chromatograms extracted for the same target from the same data without (left) and with (right) ion mobility filtering.



Conclusions:

- Many researchers using Skyline for proteomics are interested in using it for generalized small molecule work, and the Skyline team is working with them to make this happen.
- The existing targeted proteomics capabilities of Skyline adapt well beyond peptides, especially with the addition of negative charge state handling, description of targets by ion molecular formula, and ion mobility separation support.
- Skyline is being expanded to allow explicit per-transition settings such as retention time and collision energy, along with vendor specific values such as S-Lens (Thermo) and cone voltage (Waters). Others will be added as they are identified.

Future work includes:

- Library support for metabolites to speed method creation.
- Allow explicit setting of selected per transition properties for peptides, too.
- Less protein oriented user interface language when used for small molecules.
- Investigate GC-MS support for small molecules.

References: [1] Hoofnagle, A., Skyline Users Group Meeting at ASMS 2013
[2] Liu, S. et al, Proteomics 14: 169–80.