Recent Advances in Skyline: Small Molecule Targets and Ion Mobility Filtering
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Overview:
The Skyline Targeted Proteomics Environment has become a powerful tool for targeted proteomics with the ability to search for and identify specific proteins. The Skyline Targeted Proteomics Environment utilizes a focused clinical or diagnostic ionization method to achieve high sensitivity and specificity for targeted proteomics. skyline is a comprehensive, easy-to-use platform that enables users to quickly and efficiently process and analyze large amounts of high-quality proteomics data. In recent years, there has been a significant increase in the use of Skyline for targeted proteomics, and the software has become widely adopted in various research settings. Skyline is a tools that provides a streamlined approach for targeted proteomics experiments, and it is able to support filtering in the lane dimension for instruments that support mobility separation.

Introduction:

Skyline History
The Skyline project began in 2008 as an effort to create a completely new environment for protein software tool, designed specifically for targeted proteomics, where most other tools in this area had been vendor-specific and adapted from small molecule software. With the generous support of many mass spectrometry vendors and other helpful members of the larger Skyline community, Skyline has undergone continuous development and has become a sophisticated tool that directly interfaces with equipment from all major mass spectrometry vendors for rapid and convenient targeted proteomics method creation and refinement.

Targeted Mass Spectrometry Basics
The process typically begins with a large list of likely precursors and fragments of interest (the ‘library’), which Skyline then filters down to a much smaller list of targets to refer to an optimal method or test list. The predictable results of mass spectrometry can be used to generate a library of known proteins. The ionization and chromatographic data allow Skyline to provide excellent automation for creation of initial methods from peptide search results.

Methods:

Specifying Generalized Small Molecule ions in Skyline

Because there are many mass spectrometers, Skyline requires an ion molecule formula that is robust for multiple instruments. The Skyline formula is robust enough to take into account variations in mass, charge and other factors.

Targeted Mass Spectrometry in Skyline

Tools are used to annotate the properties of small molecules targeted at specific targets that do not require the high performance of some products or the extra computational burden on small molecules.

Ion Mobility Filtering

Ion mobility technology provides additional degree of separation that is useful for reducing peaks. At one time, protein mass range of most common proteins is relatively narrow but with its ability to produce highly accurate mass values, this can be used and combined to improve identification limits for Agilent, Waters and UPLC for enhanced sensitivity. Support for SCIEX SelectScribeTM has been recently added as well.

Figure 1: Targeted Mass Spectrometry method refinement cycle.

Figure 2: Tandem mass spectrometry method refinement cycle.

Figure 3: Skyline small molecule transition list insert form, showing the ability to easily edit and generate a list of instrument methods which can be downloaded for convenient target or species-specific precursors.

Figure 4: Specifying Generalized Small Molecule ions in Skyline.

Figure 5: The Skyline small molecule library information to improve this initial step.

Figure 6: Specifying Generalized Small Molecule ions in Skyline.

Figure 7: Ion mobility filtering.

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

Results:

Beyond the specification of the initial targets, operation of Skyline for targeted metabolomics or lipids is a complex process in itself. Results of the above demonstration is related to using small molecule library information to improve this initial step. The neutral mass of fragmentation is actually determined by the two steps above. In the example shown, it is added to calculate a scan by a product ion for small molecules, as given by COMBOS.

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

Conclusions:

• Many researchers are using Skyline for proteomics as it is a very powerful and flexible tool that allows for targeted proteomics experiments. The software is a tool that enables users to quickly and efficiently process and analyze large amounts of high-quality proteomics data. It is a tool that provides a streamlined approach for targeted proteomics experiments.

• The existing target proteomics capabilities of Skyline are well beyond the capabilities of current workflows and provide a wealth of additional capabilities.

• These additional capabilities include support for targeted metabolomics experiments.

• Skyline is a tool that enables researchers to perform targeted metabolomics experiments with ease.

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Figure 9: Chromatograms extracted from the same dataset from the left with (left) and without (right) ion mobility filtering.