Skyline for small molecules: a flexible tool for cross-platform LC-MS/MS method creation and data analysis for metabolomics.

J. Will Thompson¹, Brian Pratt², Max Horowitz-Gelb², Laura G. Dubois¹, Lisa St.John-Williams¹, Giuseppe Astarita³, M. Arthur Moseley¹, Michael MacCoss², and Brendan MacLean²

¹Duke Proteomics and Metabolomics Core, Center for Genomic and Computational Biology, School of Medicine, Duke University, Durham, NC; ²Department of Genome Sciences, School of Medicine, University of Washington, Seattle, WA; ³Waters Corporation, Milford, MA

Abstract

The Skyline software package is a powerful open-source and vendor-neutral software tool which has built a strong reputation for promoting collaboration and cross-platform validation of targeted (SRM) and high-resolution proteomics analysis. The software allows direct import of raw mass spectrometry data from all major instrument vendors, speeds method development by allowing direct export of native instrument methods or transition lists, and performs peak integration with a flexible data reporting environment. While each instrument vendor does provide data analysis tools for quantitative analysis of small molecules by liquid chromatography – tandem mass spectrometry (LC-MS/MS), there is no software platform for small molecule analysis which allows cross-vendor method creation and data analysis. This poster demonstrates the initial implementation of the Skyline software package (v3.1) for the creation of custom LC-MS/MS methods for several classes of metabolites, using a custom targeted LC-MS/MS assay for the methionine pathway as an example. The software includes the ability to define precursor and product ions based on empirical formula or m/z, define collision energy specifically by molecule or based on a linear equation, and to define expected retention time. Additionally, we demonstrate the use of Skyline along with retention time and accurate mass lipid libraries for the quantification of lipid species from unbiased high-resolution lipidomics datasets, as an alternative to standard metabolomics workflows. Using Skyline for small molecule method creation and data analysis fills a computational gap by easing the translation and validation of targeted metabolomics methods between instruments and laboratories.

Methionine Pathway

The goal for this experiment was to generate a targeted MRM assay for several metabolites in the methionine pathway, along with internal standards for quantification based on stable-isotope dilution. The pathway was of interest because depletion of methionine had shown a unique gene expression signature in BT474 breast cancer cell line.

Verification of High-Resolution Differential Metabolomics Results in Skyline

High Resolution Differential Lipidomics analysis of a cancer cell line under drug treatment was performed using UPLC coupled to Synapt G2 HDMS system. Five biological replicates of each were performed, and the data was analyzed in the software package Progenesis QI.

Workflow for Using Skyline for Targeted Small Molecule Analysis

1. Flat File Containing Molecules of Interest
2. Import Into Skyline, building an analysis template
3. Export an instrument acquisition method or MRM list
4. Import Raw data

Conclusions:

- Skyline provides a seamless way to share MRM metabolomics data and methods, improving transparency in metabolomics experiments.
- The software eases cross-laboratory verification experiments by using a common data analysis pipeline and method development tool.
- Skyline can currently be used to perform novel MRM metabolomics experiments or to perform targeted interrogation of high-resolution metabolomics data.