Metabolomic Profiling of Small Molecule Ion Mobility Assisted Data Independent Acquisition Data Using Skyline

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https://skyline.ms

Overview:

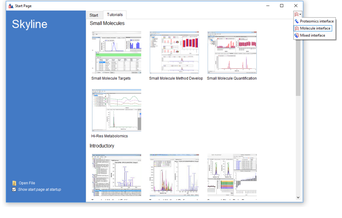
- The Skyline Targeted Mass Spectrometry Environment has distinguished itself as a reliable tool for chromatography-based quantitative proteomics. Skyline supports selected reaction monitoring (SRM) as well as full-scan methods including MS1 filtering, parallel reaction monitoring (PRM), and data independent acquisition (DIA) including the approaches popularized as SWATH and ion mobility assisted MSE.
- Skyline continues to evolve to meet the changing needs of researchers working with all kinds of biological molecules, including a new selectable user interface for small molecule users. Skyline is free and open source.
- Ion mobility separation (IMS) support is a particularly important feature of Skyline for small molecule work, as will be demonstrated.

Introduction:

Ten Years of Skyline

The Skyline project began in 2008 as an effort to create a new instrument-vendor-neutral software tool designed specifically for targeted proteomics. Most other tools in this area had been vendor-specific and adapted from small molecule quantitative software. With the support of many MS manufacturers and with the help of its large and active user community, Skyline has undergone continuous development and has become a sophisticated tool that directly interacts with equipment and native data formats from all major vendors for rapid and convenient targeted method creation and refinement.

Figure 1: Skyline start page showing available UI modes.



In recent years and by popular demand Skyline has evolved to work with non-proteomic molecules. Early adopters led the way by artfully constructing peptide modifications that resulted in the chemical formula of interest [1,2]. Today, Skyline supports direct specification of chemical formulas and a full range of adducts (e.g. "[M+Na]", "[2M+Hac-H]"). Most capabilities that were originally proteomics-only such as CE optimization and iRT are now available for all molecules.

Recently, considerable effort has gone into making the Skyline user interface support these non-proteomic capabilities in a much more user friendly manner.

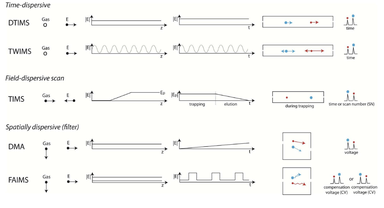
New Small Molecules User Interface

As of the latest Skyline-Daily release, users can now choose between the traditional "Proteomics" UI and the new "Molecules" UI, which omits proteomics-specific features and presents terms like "molecule" instead of "peptide" and "molecule list" instead of "protein".

Advances in Ion Mobility

Ion mobility separation (IMS) 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 *m/z* range of many precursor targets is relatively narrow. IMS technology continues to mature and we see increasing use of collisional cross section (CCS) as a molecular property that can be used to predict ion mobility, as highlighted by a recent review recommending on the reporting IM measurement values [3]. Skyline can both use and derive IMS library data, including CCS, when dealing with suitably equipped mass spectrometers from all major manufacturers.

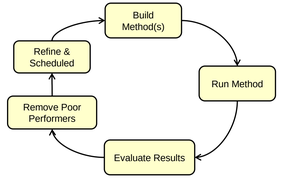
Figure 2: Classification of IM measurement principles. Adopted from [3].



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.

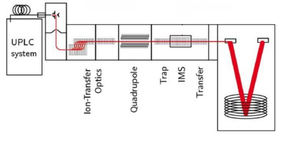
Figure 3: Targeted mass spectrometry method refinement cycle.



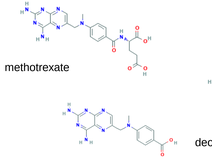
Methods:

Data Collection

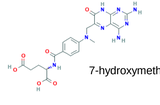
Data independent analysis (DIA) LC-IM-MS data were acquired on a TWIMS enabled hybrid quadrupole oa-ToF mass spectrometer shown below. The instrument was operated in positive ESI mode using 100 ms scans from *m/z* 50 to 1200.



The LC conditions employed a 2.5 min reversed-phase gradient using a 1 x 50 mm 1.8 µm C18 column to provide high throughput chromatographic separations. A ten rat urine sample set, obtained from five vehicle dosed control and five treated animals following a single oral dose of methotrexate, was used for evaluation. In addition, control samples were spiked with methotrexate (MTX), 7-hydroxymethotrexate, and deoxyaminopteroic acid to investigate quantitative response.



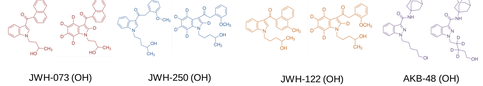
methotrexate



7-hydroxymethotrexate

deoxyaminopteroic acid

Additionally, eight hydroxylated synthetic cannabinoid metabolite standards, structures shown below, were supplied at a concentration of 100 ng/µL methanol. Four further standard solutions were obtained by diluting the initial stock of each metabolite into human urine. The final metabolite concentrations equaled 1 to 1000 ppb/µL urine, LC-IM-MS DIA data were acquired with the same equipment and conditions as described above, with the exception of a 10 min gradient.



JWH-073 (OH) JWH-250 (OH) JWH-122 (OH) AKB-48 (OH)

Data Analysis

Analysis was performed with Skyline-Daily 4.2.1.19095. Known $^{TW}CCS_{N2}$ reference data were obtained from publicly available resources and used for the qualitative profiling experiments. Ion mobility values derived by automated inspection in Skyline were used for the quantitative analyses of the data. In both cases, chromatographic peak finding was performed with and without ion mobility filtering for comparison purposes.

Results:

Ion Mobility Separation Reduces Chromatographic Peak Interference

Ion mobility separation allows large amounts of signal due to co-eluting species of similar *m/z* to be excluded from peak integration. Figures 4 to 6 illustrate the difference in either qualitative or quantitative results when signal is taken only from the ion mobility range indicated by the purple band in the (*m/z*, drift time, intensity) heat map (illustrated in Figure 5) or when IMS is or is not utilized in the analysis of the IM assisted DIA data.

Figure 4: Qualitative analysis rat urine with (top) and without IMS filtering (bottom), showing more conclusive detection with IMS of tentative metabolite identifications (co-eluting interference not detected and/or incorrectly integrated) and improved *S/N* (most noticeable for low abundant metabolites) followed by unsupervised PCA (areas summed intensity normalized, log transformed and Pareto scaled).

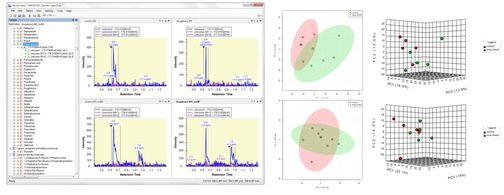


Figure 5: Multi-replicate MS2 data analysis of IM assisted DIA data in Skyline with and without IM filtering using quadratic curve fitting. The same data set was imported twice with filtering either enabled or disabled (data annotated as ''noIM'). Shown inset within the chromatographic pane is IM resolved MTX, illustrating that quantitation without IMS filtering could lead to overestimation of abundance and subsequently incorrect interpretation of metabolic response.

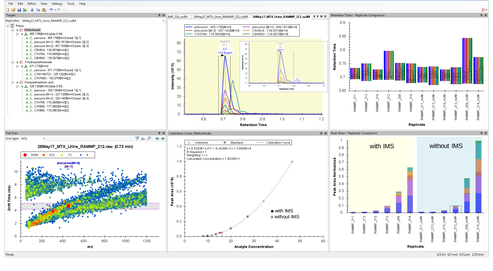
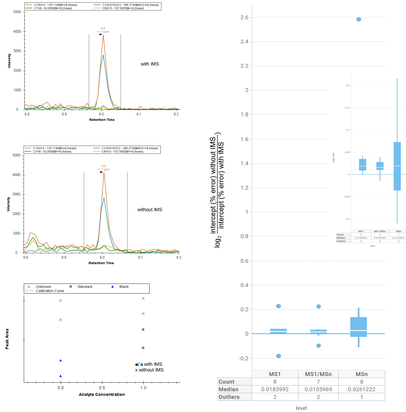


Figure 6: Quantitative synthetic cannabinoid MS2 data analysis illustrating reduced noise read-out with IMS, thereby improving detectability for low-level metabolites (left). This is quantitatively expressed by the ratio intercept error distribution values of the calibration curves for all eight metabolites without and with IMS incorporated in the quantitate analysis (right). Shown inset is a more detailed perspective with the outlier data removed from the experiment, illustrating greater relative intercept error without IMS employed during the analysis of the data



Conclusions:

- Skyline's support for IMS-capable mass spectrometers leads to reduced peak interference in chromatogram extraction and improved signal to noise ratios for quantitation.
- Now in its 10th year, Skyline continues to evolve to meet the needs of the targeted mass spectrometry community, and remains free and open source.
- Skyline's support for generalized small molecules continues to grow, notably including the addition of a long-anticipated user interface specific to non-proteomic work.

References:
[1] Hoofnagle, A., Skyline Users Group Meeting at ASMS 2013
[2] Liu, S. *et al*, Proteomics 14: 169–80.
[3] Gabelica, V. *et al*, Mass Spectrometry Review. February 2019: 1–30

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