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

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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.[1,2] 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.[3,4] 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.
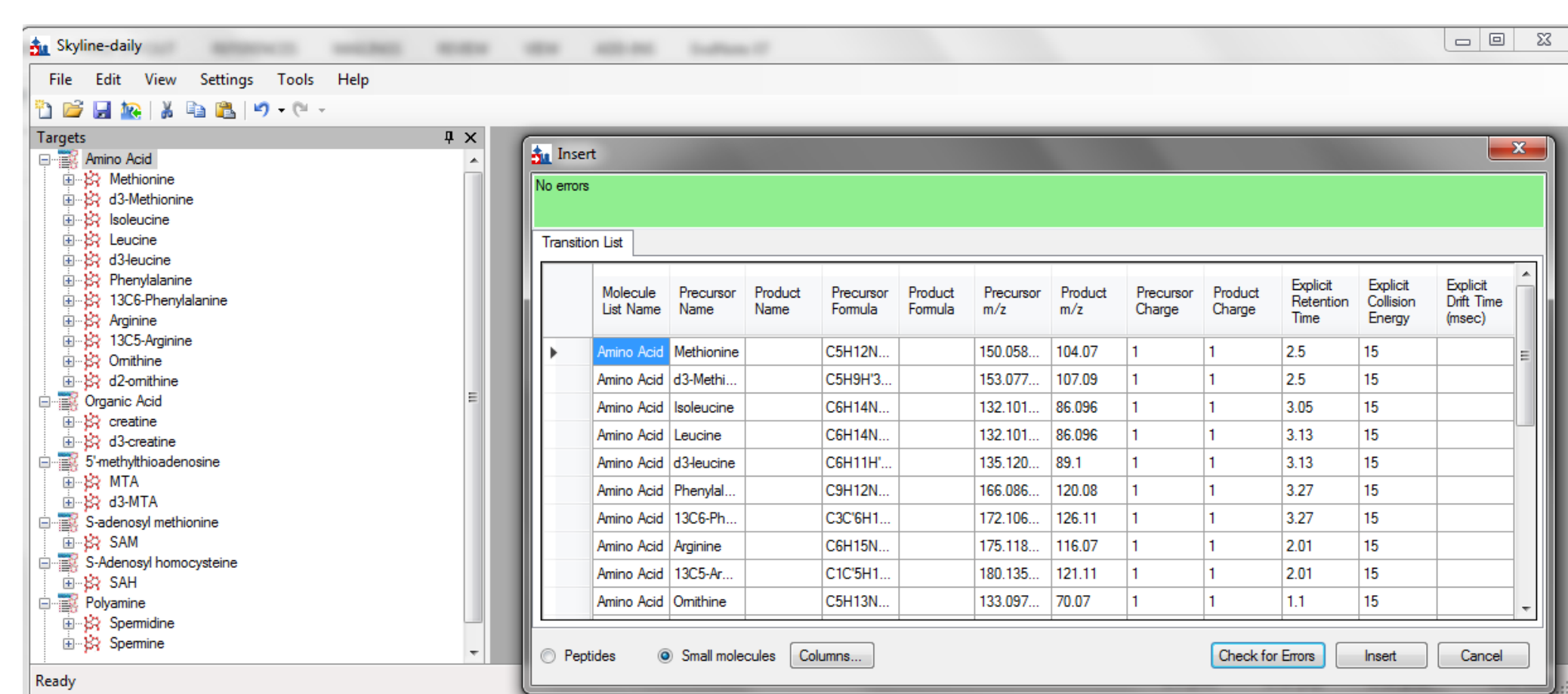
1. MacLean B, Tomazela DM, Shulman N, et al. Bioinformatics. 2010 Apr 1; 26(7):966-8.
2. Chambers MC, Maclean B, Burke R, et al. Nat Biotechnol. 2012 Oct; 30(10):918-20.
3. Smith CA, Want EJ, O'Maille G et al. Anal. Chem. 2006 Feb 1; 78(3):779-87.
4. Paglia G, Angel P, Williams J et. Al. Anal. Chem. 2015 Jan 20;87(2):1137-44.

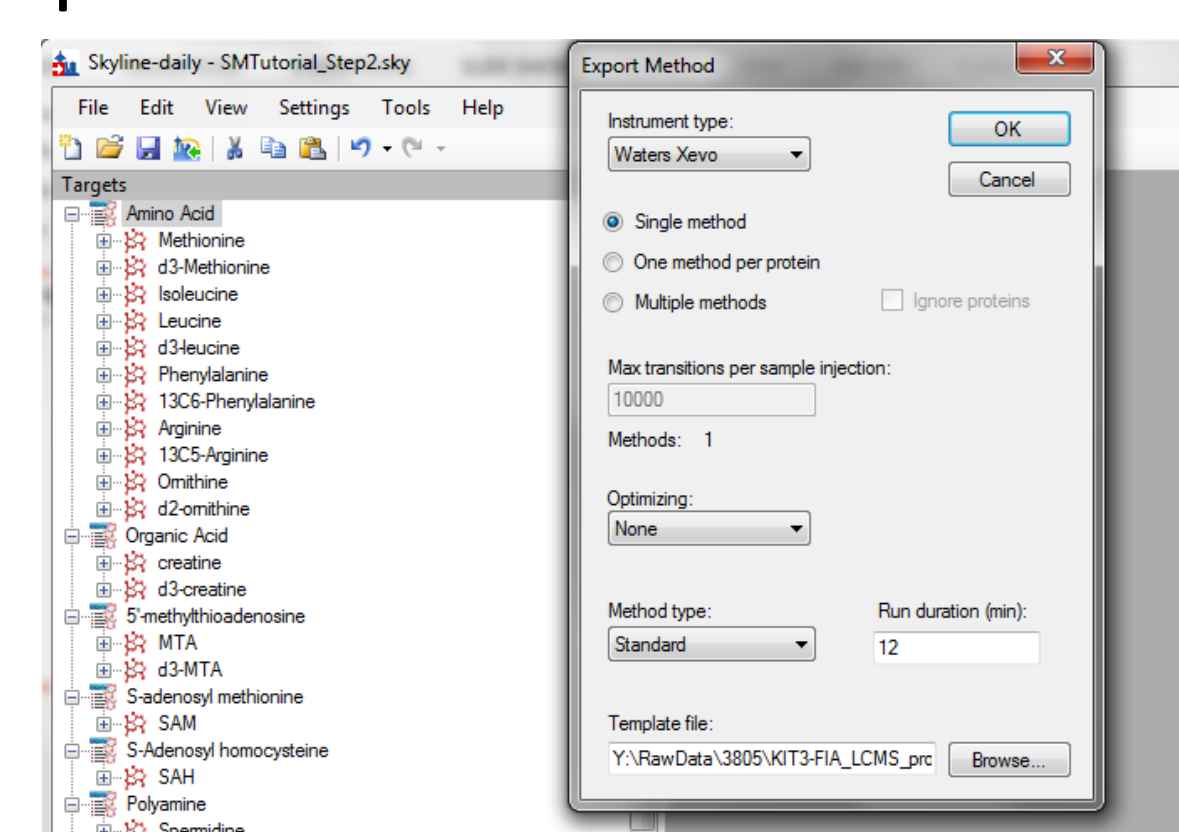## Workflow for Using Skyline for Targeted Small Molecule Analysis

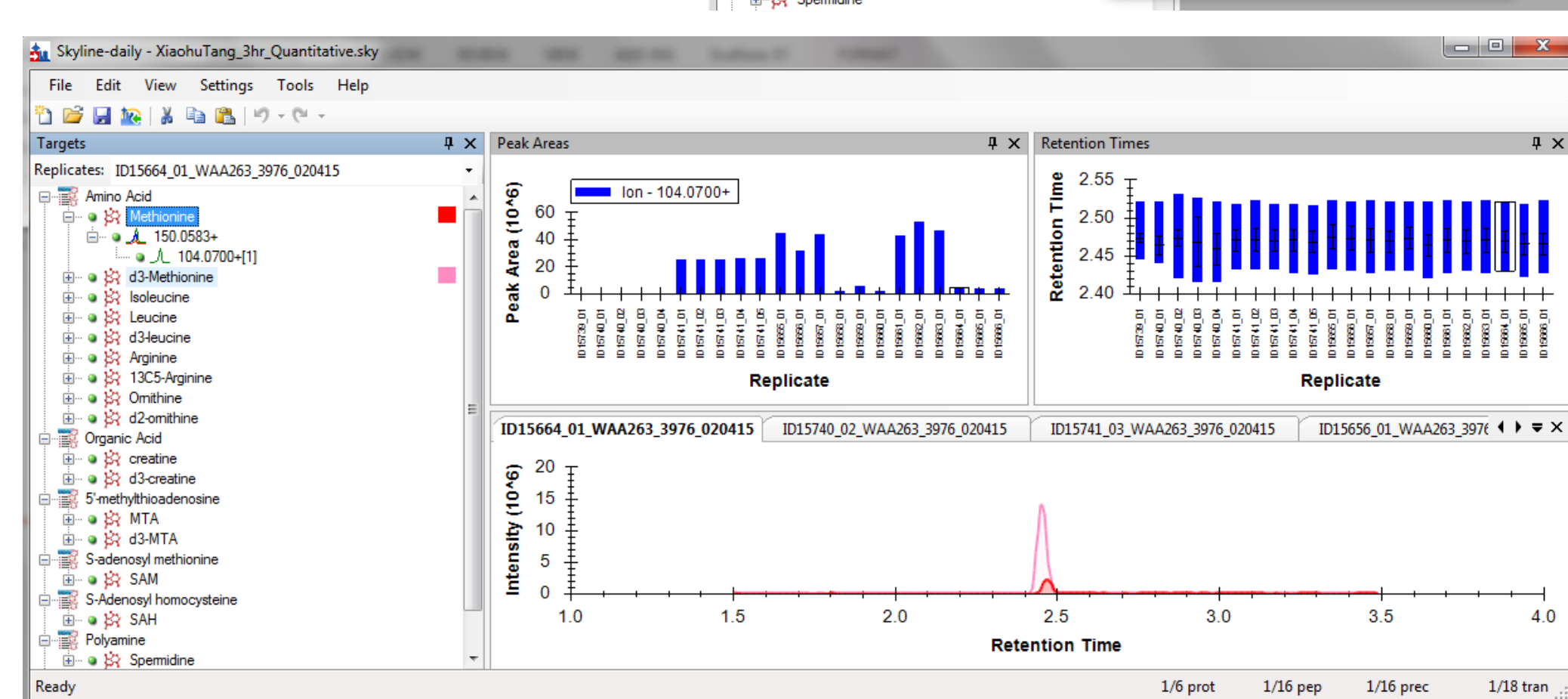### 1. Flat File Containing Molecules of Interest

2. Import Into Skyline, building an analysis template

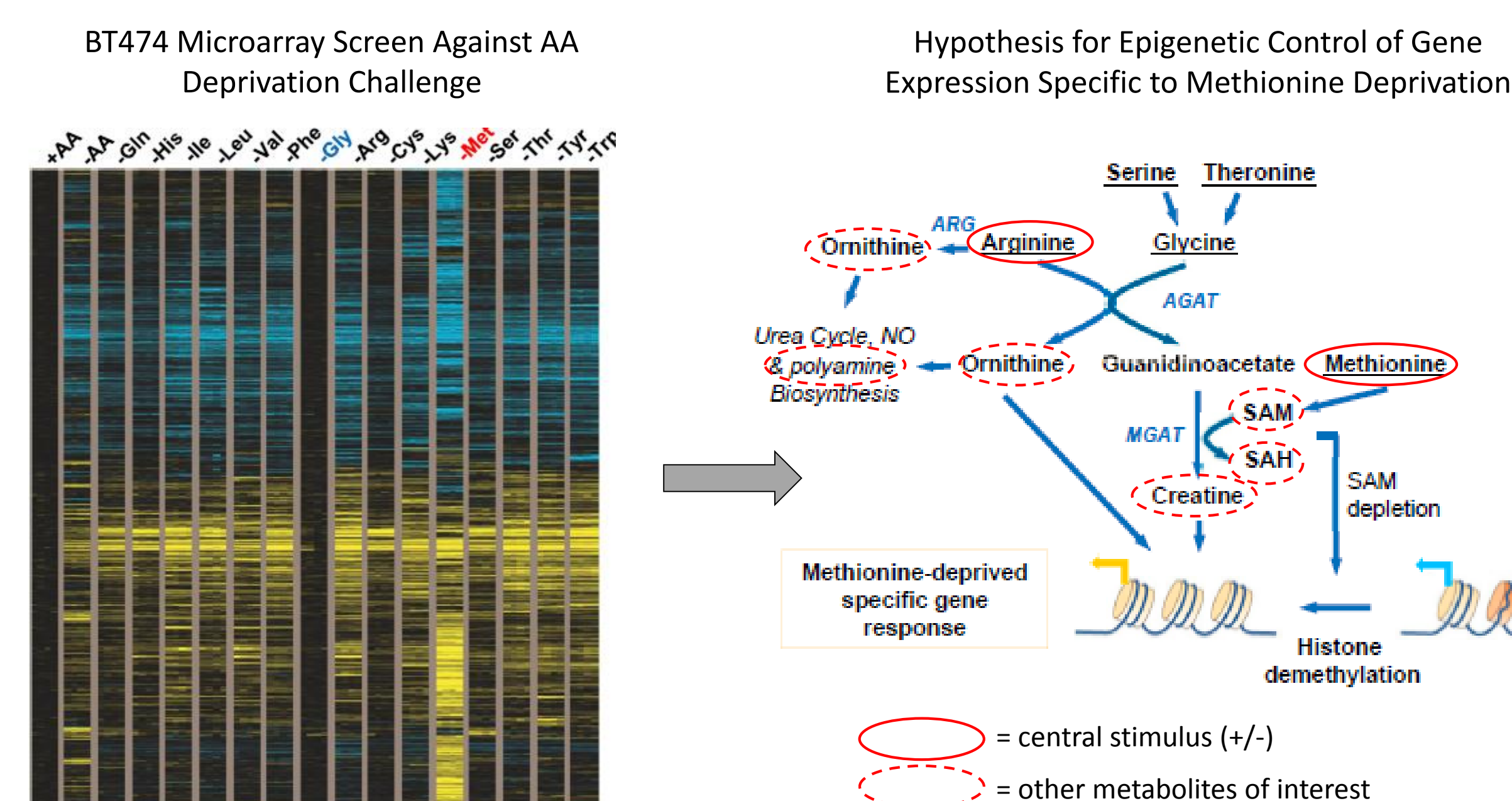### 3. Export an instrument acquisition method or MRM list

4. Import Raw data


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 very unique gene expression signature in BT474 breast cancer cell line.

Tang X et al "Comprehensive profiling of amino acid response uncovers unique methionine-deprived response dependent on intact creatine biosynthesis", *PLOS Genetics* **2015**.

BT474 Microarray Screen Against AA Deprivation Challenge

Hypothesis for Epigenetic Control of Gene Expression Specific to Methionine Deprivation

= central stimulus (+/-)
= other metabolites of interest

Example of High versus Low-Methionine Sample, Chromatograms in Skyline

(Method: 2.1 mm x 10 cm BEH C18 column with MPA 0.1% Formic acid/0.02% HFBA in water, MPB is 90/10 MeCN/IPA. F = 0.4 mL/min @ 35C. Acquity TQ-S in ESI+ MRM mode; full method and Skyline file available in Tang et al, *PLOS Genetics* **2015**)
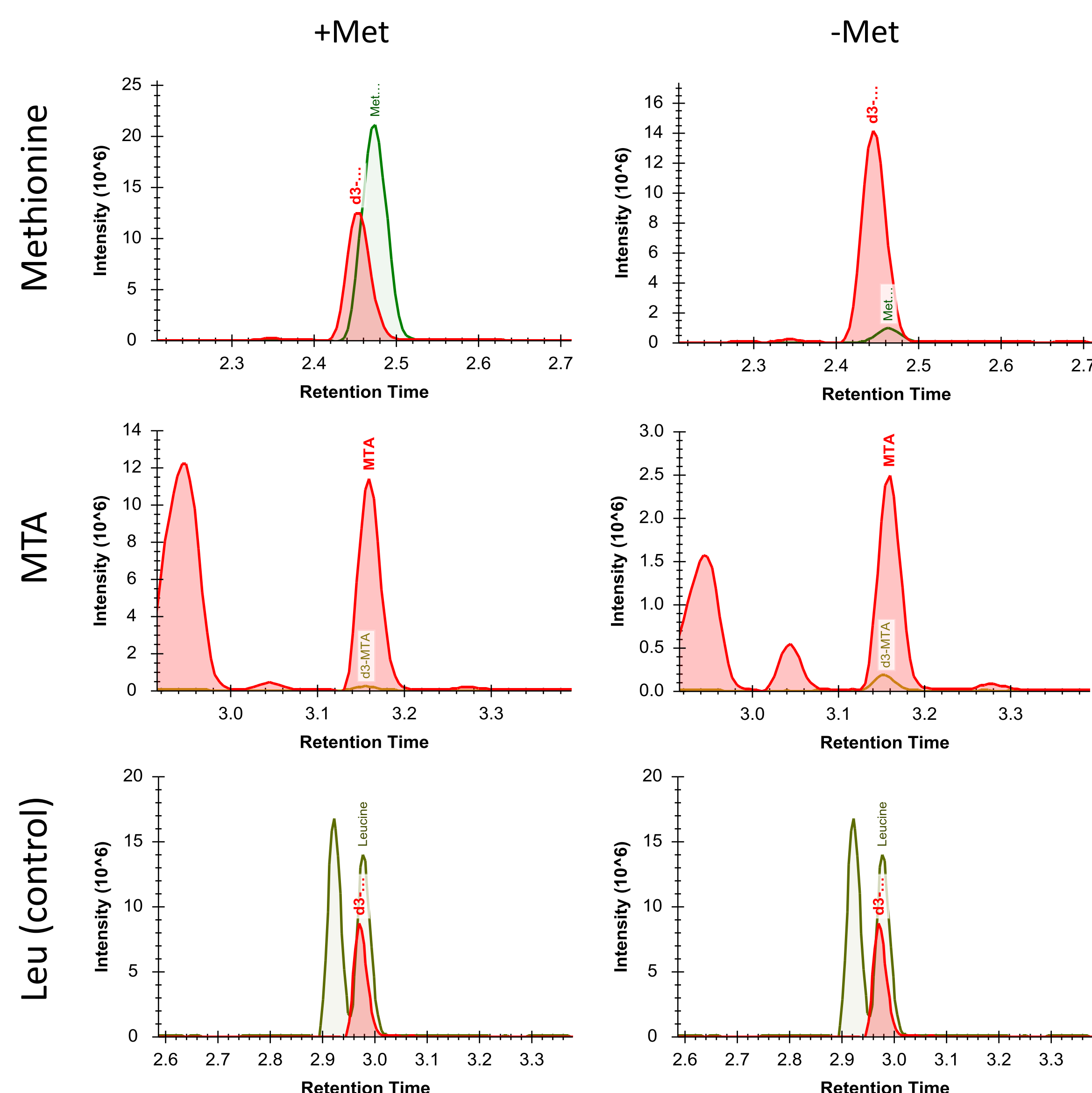

Metabolite overlays on the mechanism: Met depletion drives changes in central methyl donors (SAM), which then causes epigenetic changes in gene expression
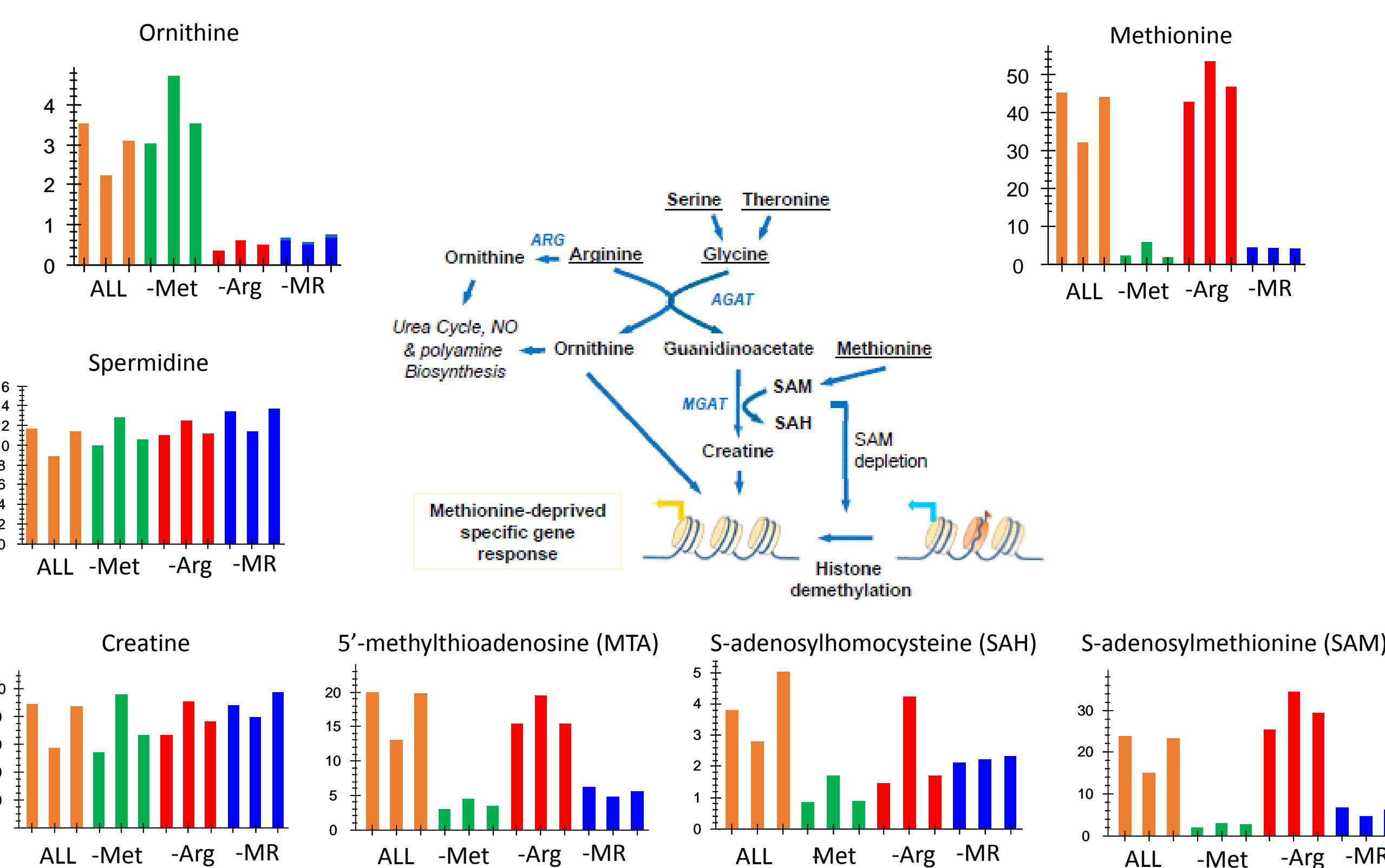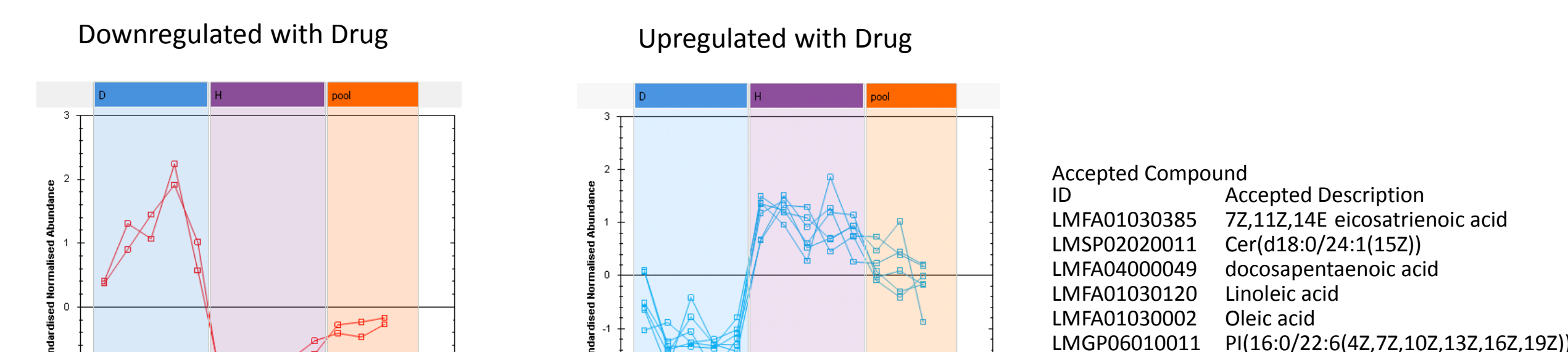

Verification of High-Resolution Differential Metabolomics Results in Skyline

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

Below shows the general informatics wokflow for unbiased lipidomics:
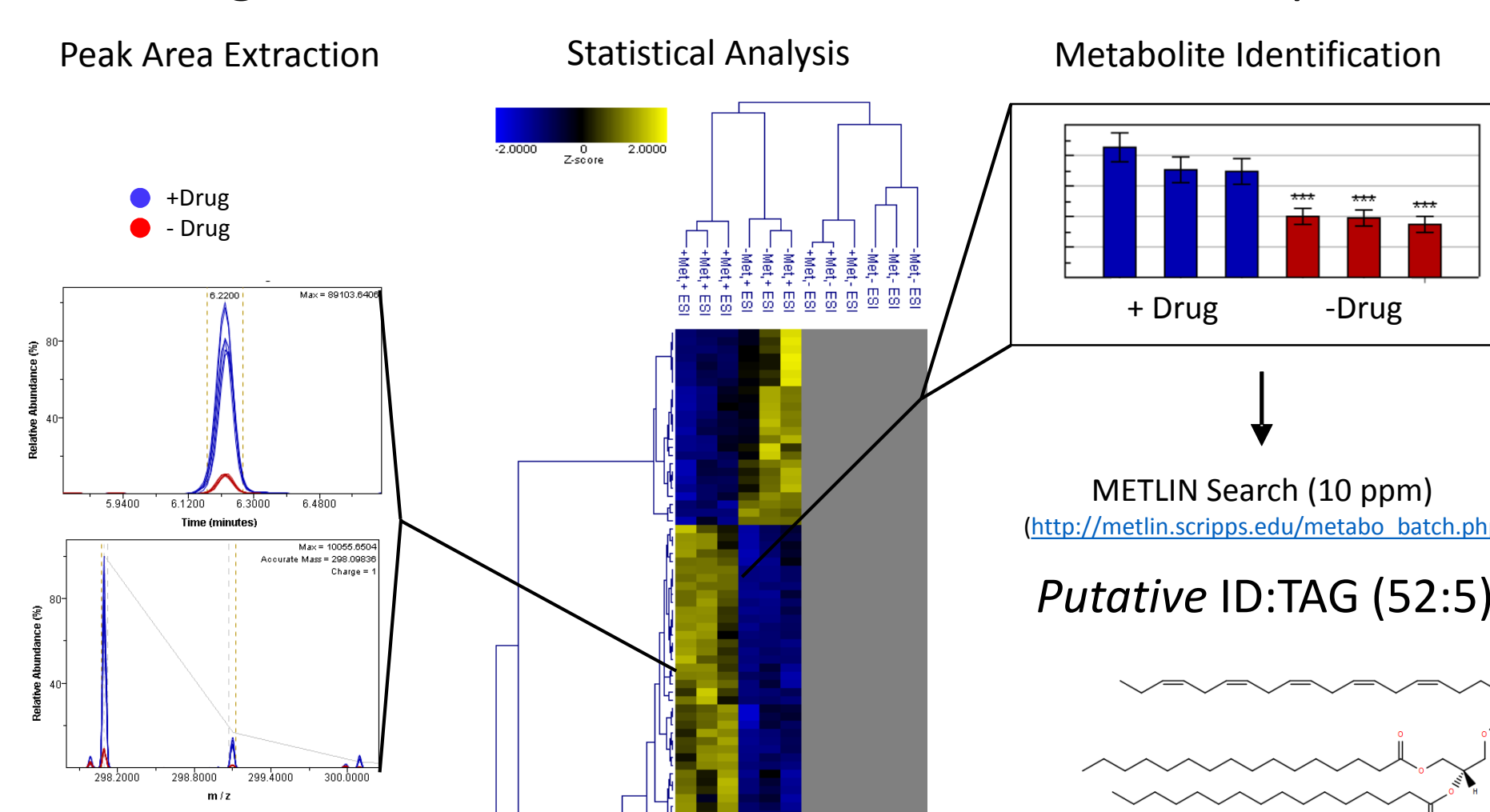
A cluster of compounds was differentially expressed and putatively identified in Progenesis QI, showing several fatty acids:

Accepted Compound ID	Accepted Description
LMFA01030385	7Z,11Z,14E eicosatrienoic acid
LMSP02020011	Cer(d18:0/24:1(15Z))
LMFA04000049	docosapentaenoic acid
LMFA01030120	Linoleic acid
LMFA01030002	Oleic acid
LMGP06010011	PI(16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))

A compound list including saturated, mono- and polyunsaturated Fatty Acids was generated in Skyline, and set to perform high-resolution MS1 extraction at 10,000 Rs.

Targeted molecule extraction verified the statistical changes observed in Progenesis QI, and suggests a general trend towards no change in saturated FA, but an accumulation of essential omega-3 and omega-6 FA with drug Tx.
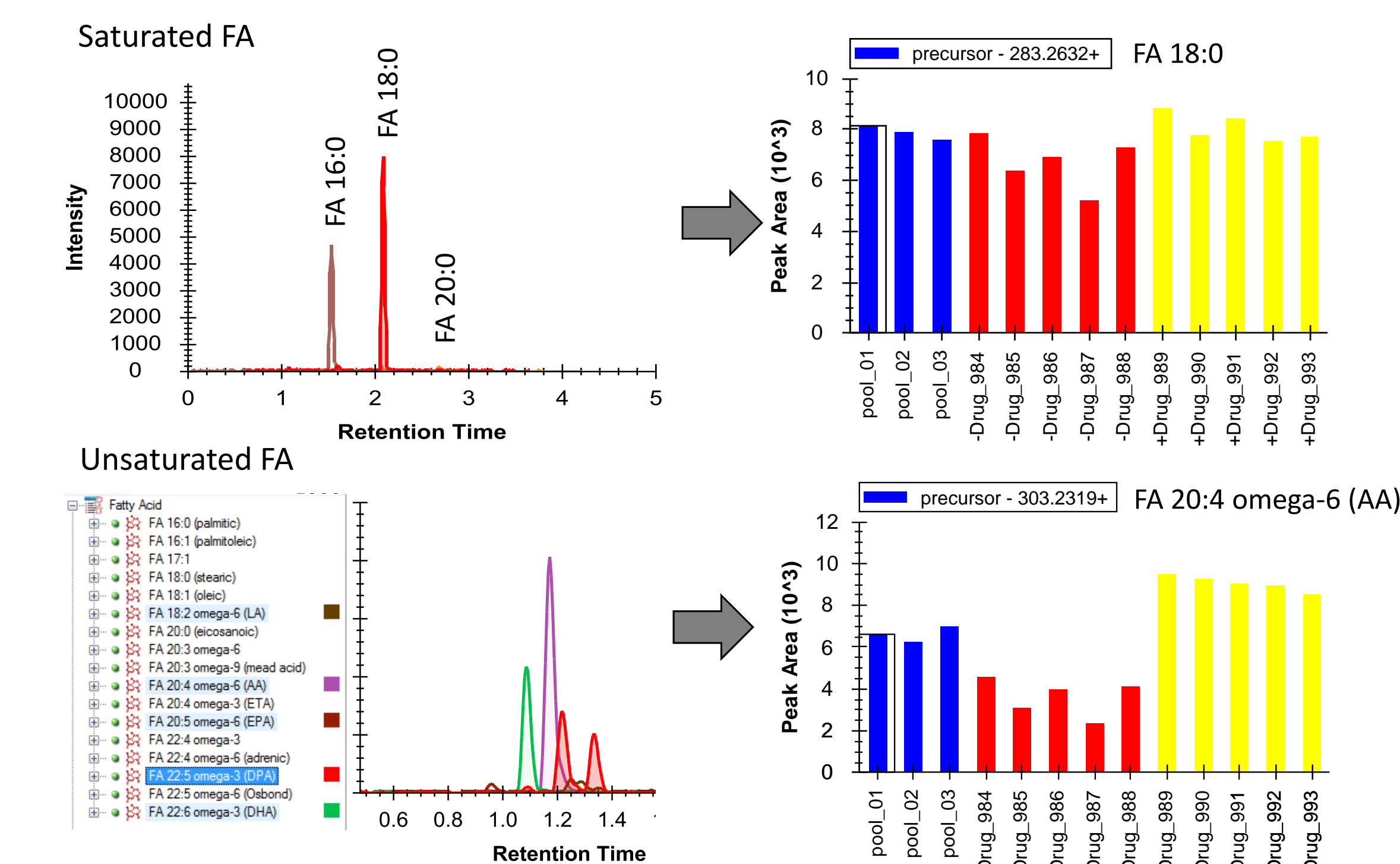
Conclusions:

- 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