Expanding Skyline's Capabilities to Small Molecule Data Analysis

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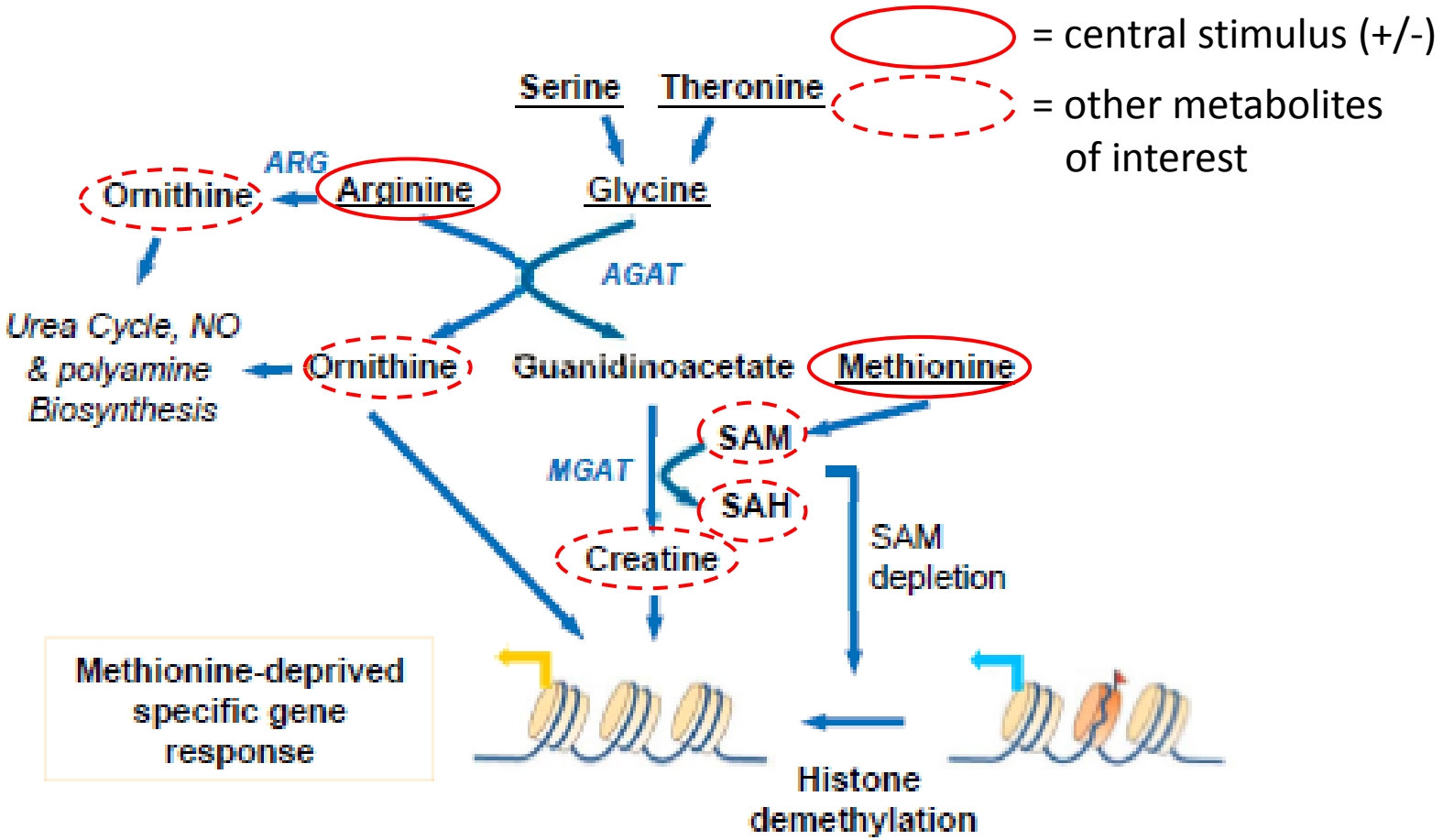
Goals and Objectives

- Growth of Skyline utilization in our lab
- How to create a Skyline document for small molecule applications
- Exemplar small molecule workflows
- Skyline – future additions and features to aid metabolomics community

Skyline Utilization

- Duke Proteomics Core began using Skyline for targeted proteomics in mid-2010
  - Backbone for all of our SRM and PRM pipelines
- Routinely use it as a system suitability/QC tool on all mass spectrometers
- Early 2013 - development of metabolomics platforms, and deployment soon thereafter
  - Positive ion and negative ion lipid profiling, **Met-Pathway analysis**, oxylipins, **Biocrates p180 panel**, **fatty acid analysis**, bile acid panel, and **hydroxycholesterol analysis**

Targeting a Metabolite Pathway of Interest: Methionine



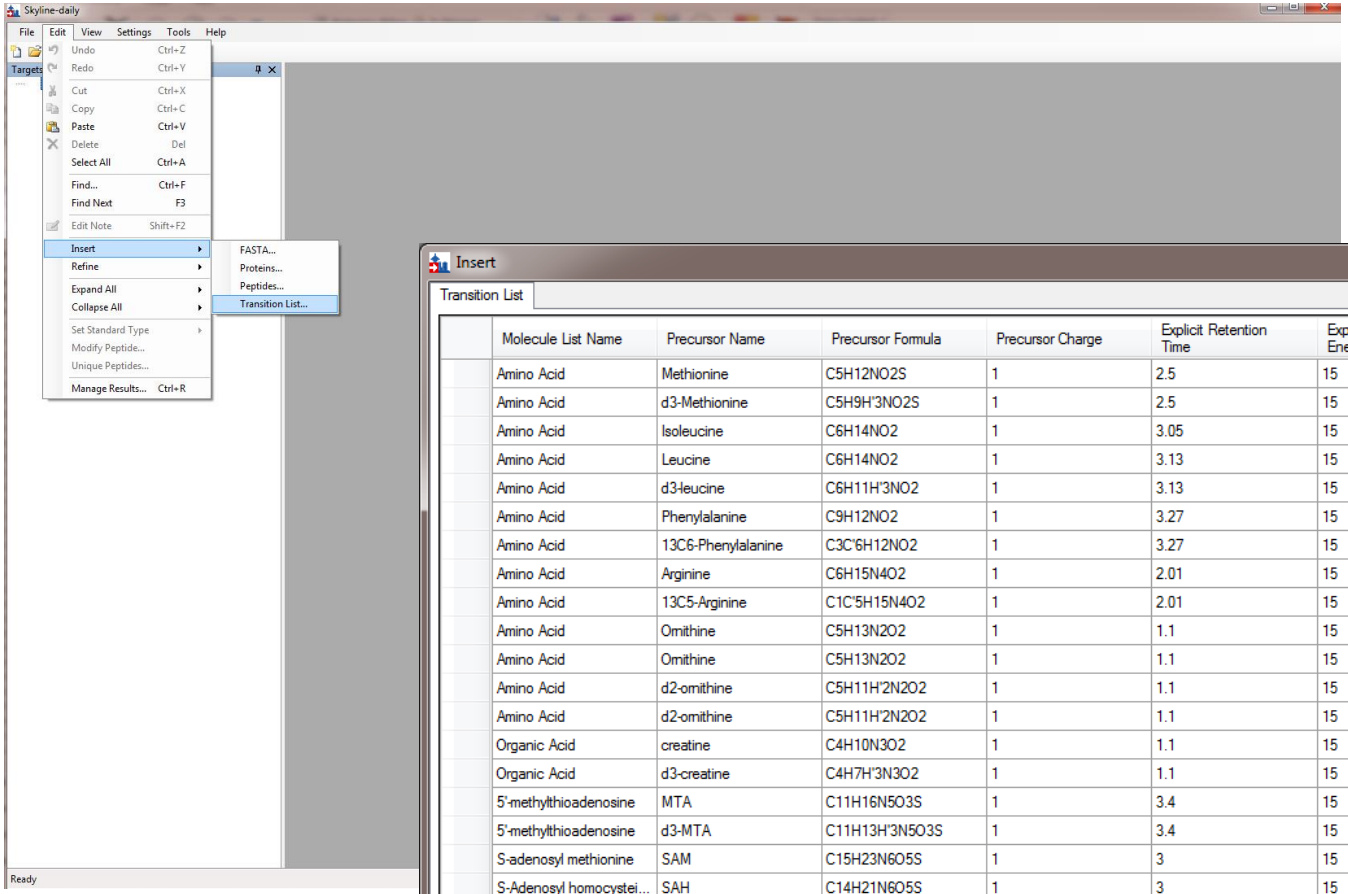
Polar Metabolites: Methionine Pathway Analysis

Create an Excel Flat “Library” File

Molecule List Name	Precursor Name	Precursor Formula	Precursor Charge	Precursor RT	Precursor CE	Product m/z	Product Charge
Amino Acid	Methionine	C5H12NO2S	1	2.5	15	104.07	1
Amino Acid	d3-Methionine	C5H9H'3NO2S	1	2.5	15	107.09	1
Amino Acid	Isoleucine	C6H14NO2	1	3.05	15	86.096	1
Amino Acid	Leucine	C6H14NO2	1	3.13	15	86.096	1
Amino Acid	d3-leucine	C6H11H'3NO2	1	3.13	15	89.1	1
Amino Acid	Phenylalanine	C9H12NO2	1	3.27	15	120.08	1
Amino Acid	13C6-Phenylalanine	C3C'6H12NO2	1	3.27	15	126.11	1
Amino Acid	Arginine	C6H15N4O2	1	2.01	15	116.07	1
Amino Acid	13C5-Arginine	C1C'5H15N4O2	1	2.01	15	121.11	1
Amino Acid	Ornithine	C5H13N2O2	1	1.1	15	70.07	1
Amino Acid	Ornithine	C5H13N2O2	1	1.1	15	116.07	1
Amino Acid	d2-ornithine	C5H11H'2N2O2	1	1.1	15	72.07	1
Amino Acid	d2-ornithine	C5H11H'2N2O2	1	1.1	15	118.07	1
Organic Acid	creatine	C4H10N3O2	1	1.1	15	90.06	1
Organic Acid	d3-creatine	C4H7H'3N3O2	1	1.1	15	93.06	1
5'-methylthioadenosine	MTA	C11H16N5O3S	1	3.4	15	136.1	1
5'-methylthioadenosine	d3-MTA	C11H13H'3N5O3S	1	3.4	15	136.1	1
S-adenosyl methionine	SAM	C15H23N6O5S	1	3	15	250.11	1
S-Adenosyl homocysteine	SAH	C14H21N6O5S	1	3	15	136.08	1
Polyamine	Spermidine	C7H20N3	1	3.59	15	129.15	1
Polyamine	Spermine	C10H27N4	1	3.82	15	112.112	1

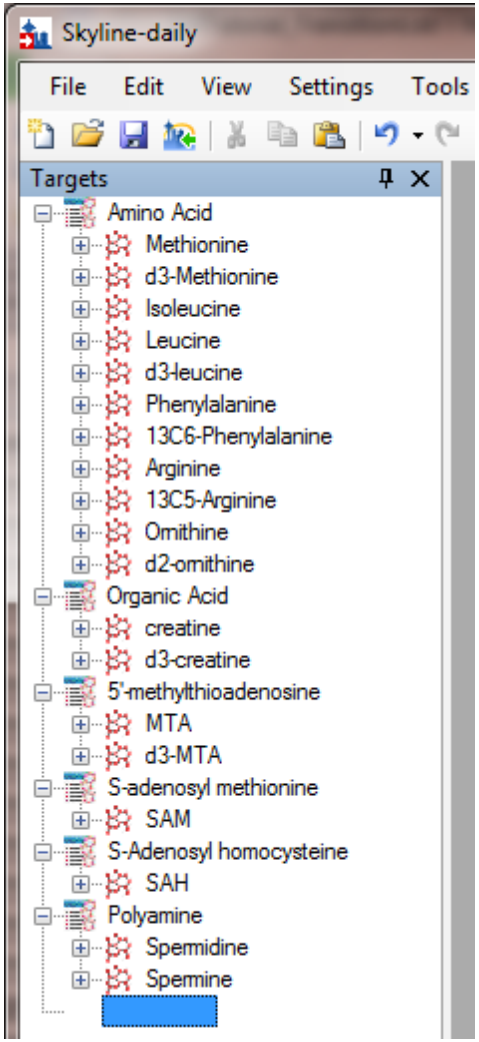
Skyline tutorial based on these data:
https://skyline.gs.washington.edu/labkey/wiki/home/software/Skyline/page.view?name=tutorial_small_molecule (April 2015)

Import into Skyline – Edit→Insert→Transition List

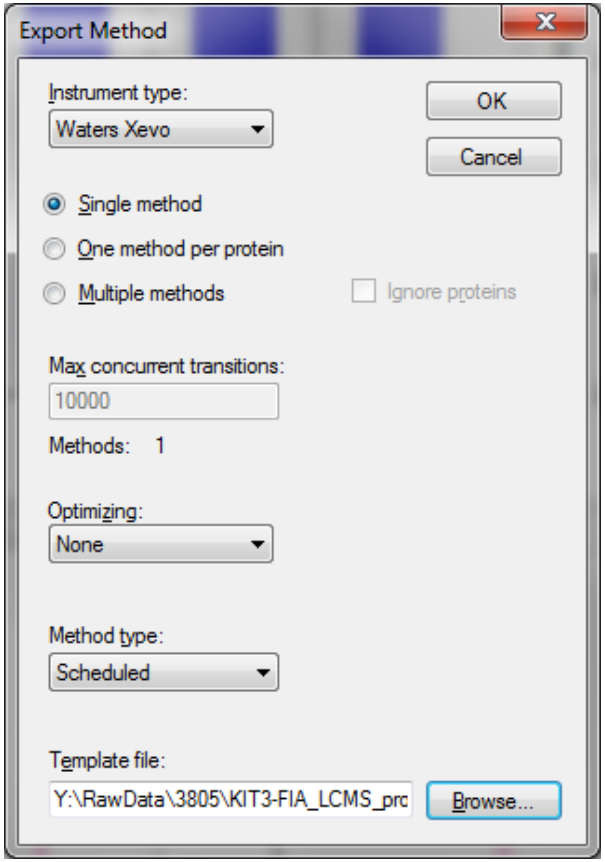


Met-Pathway in Skyline

Skyline Document

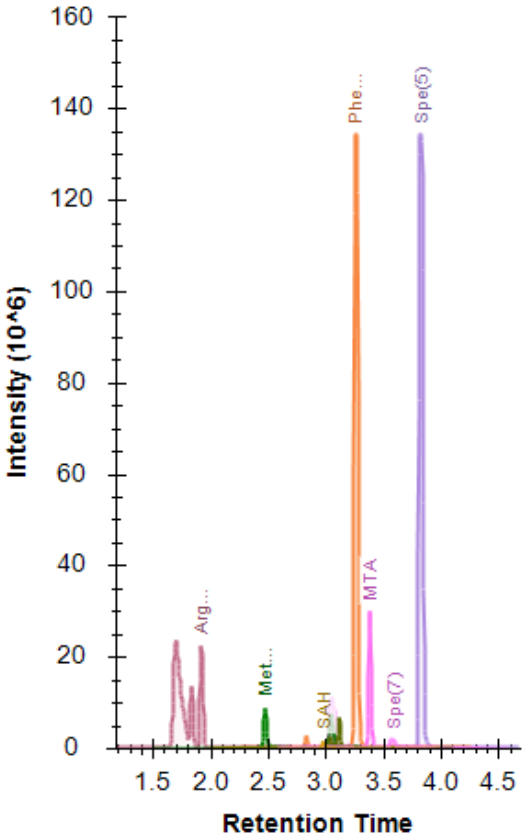


Export Transition List/Method

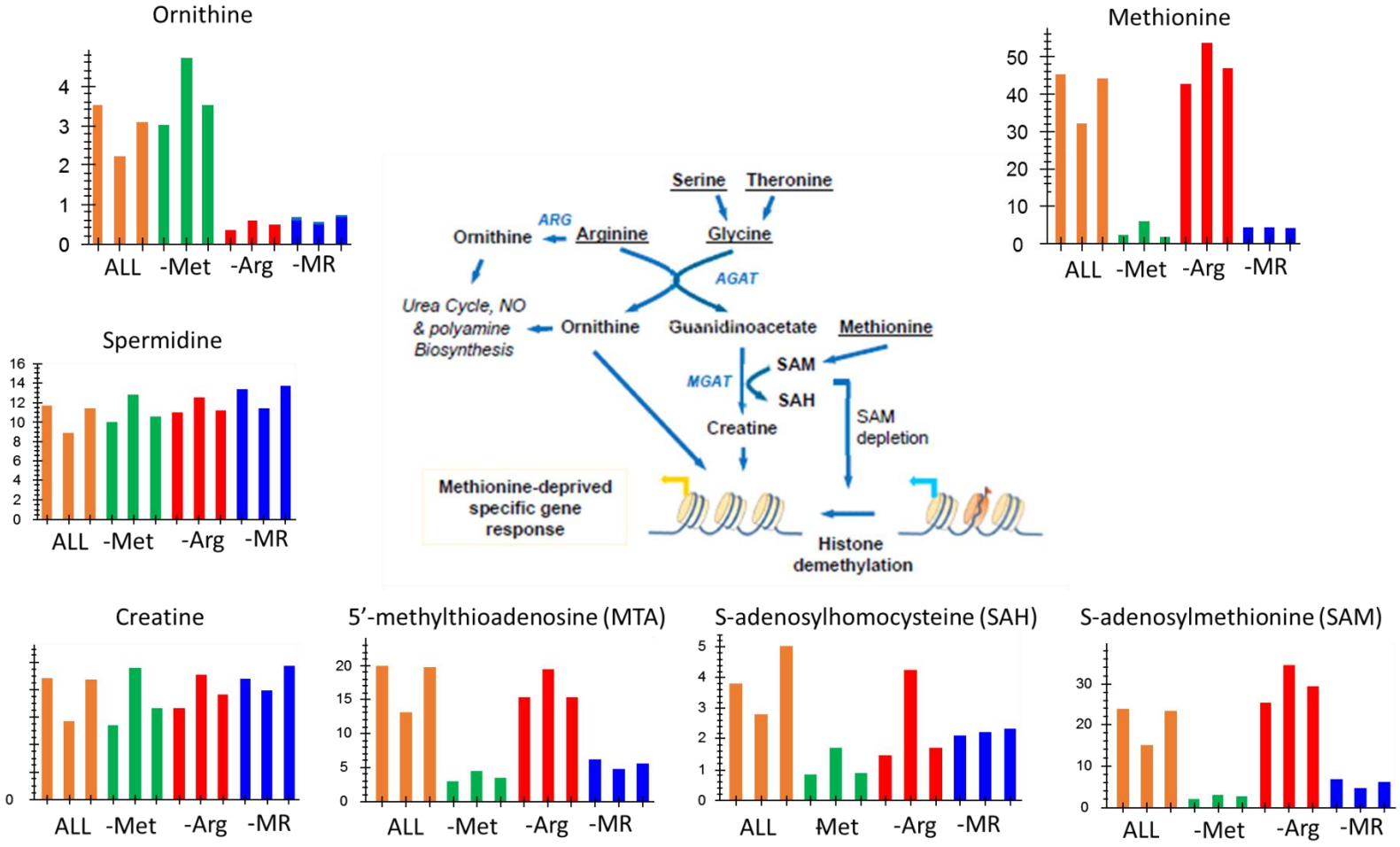


See Brian Pratt's poster #414 on Mon. for additional details

Import and Integrate Raw Data



Results – Met-Pathway Analysis with Amino Acid Depletion



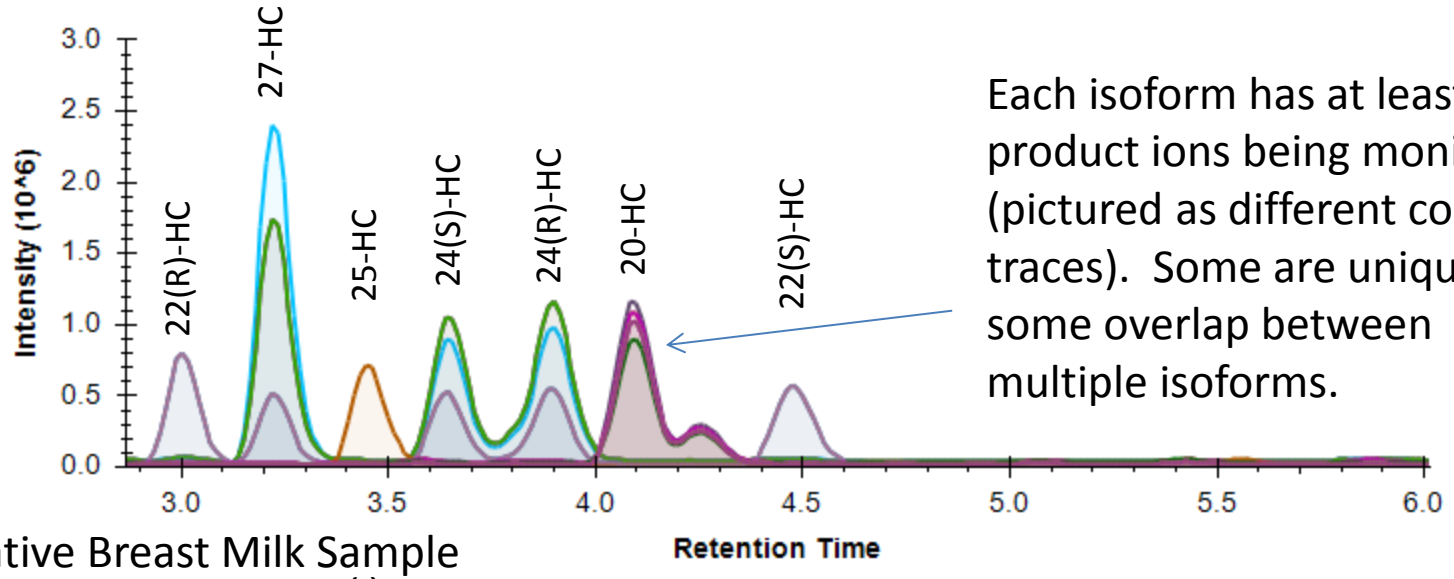
Tang X et al. (2015) Comprehensive Profiling of Amino Acid Response Uncovers Unique Methionine-Deprived Response Dependent on Intact Creatine Biosynthesis. PLoS Genet 11(4): e1005158. doi:10.1371/journal.pgen.1005158

Oxysterols

- Hydroxycholesterol method development in progress
  - Investigators interested in HCs over a range of applications (breast milk, ependymal cells, animal high-fat diet studies, engineered mammalian cells, etc.)
- Currently interested in 6 isoforms and cholesterol: 20-HC, 22(R)-HC, 22(S)-HC, 24(S/R)-HC, 25-HC, 27-HC
- Built a Skyline method that can be transferred to other labs, viewed by our PIs when reviewing data, and easily updated as development progresses (changes to chromatographic separation)

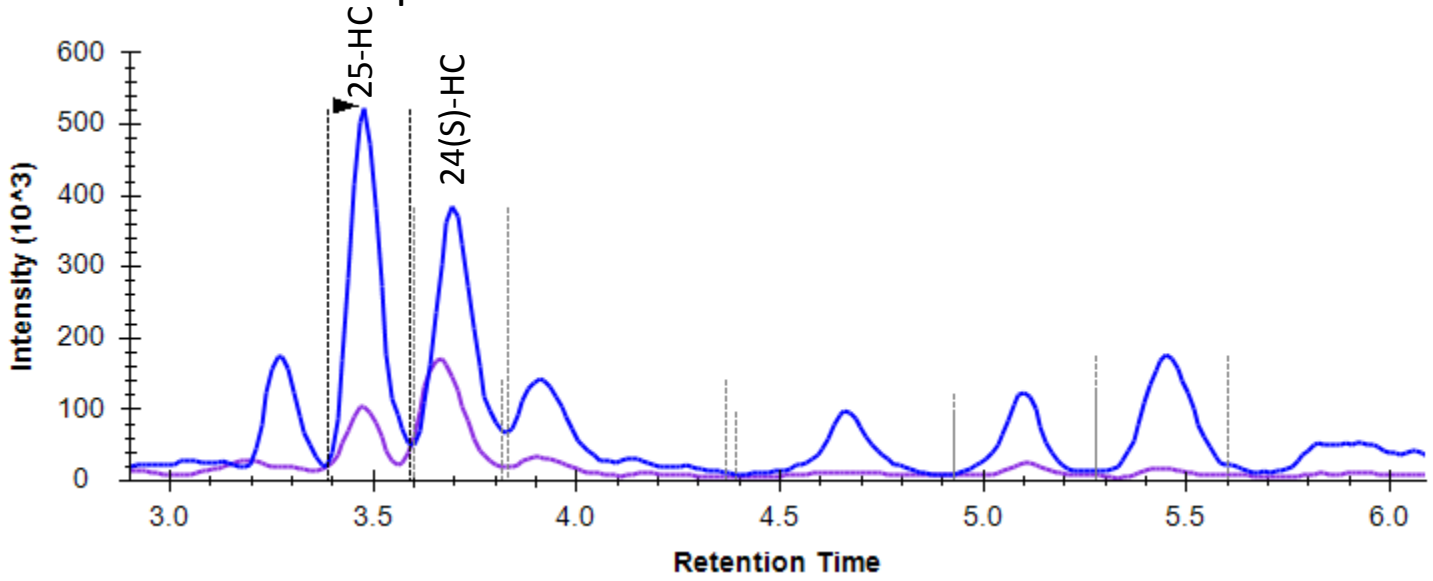
Presence of 24(S) and 25-HC in Breast Milk

Analysis of Standards Mix



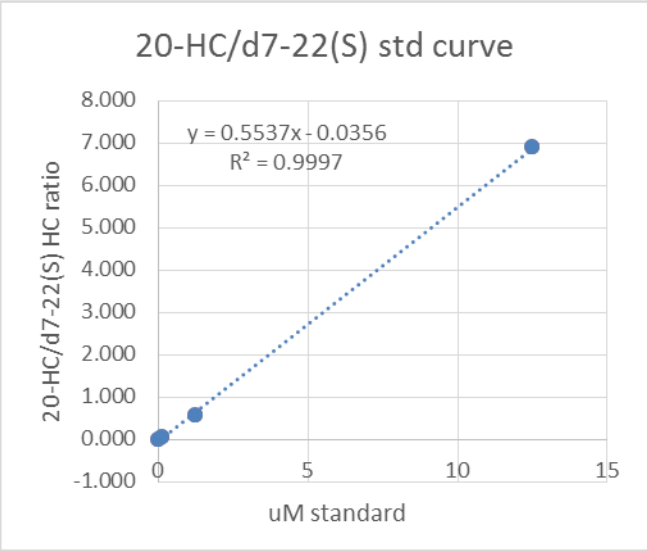
Each isoform has at least two product ions being monitored (pictured as different colored traces). Some are unique while some overlap between multiple isoforms.

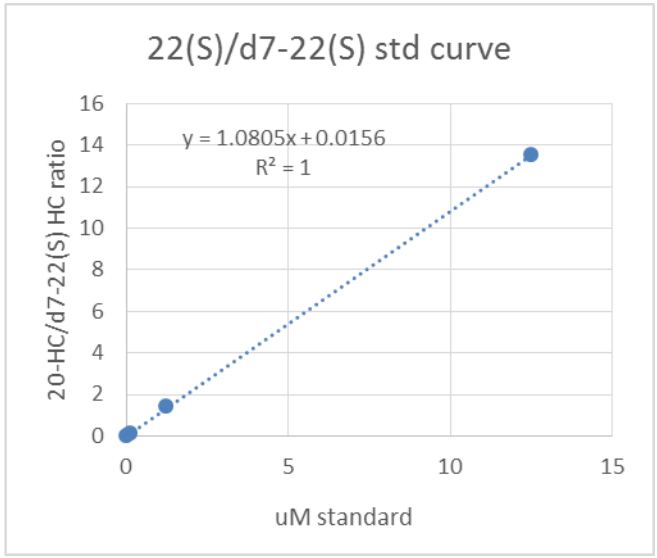
Representative Breast Milk Sample

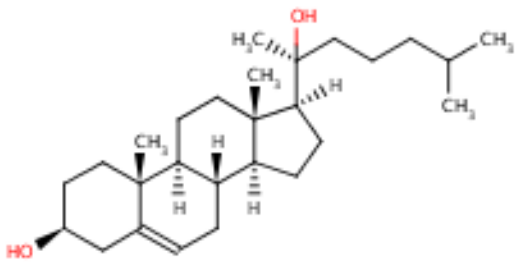


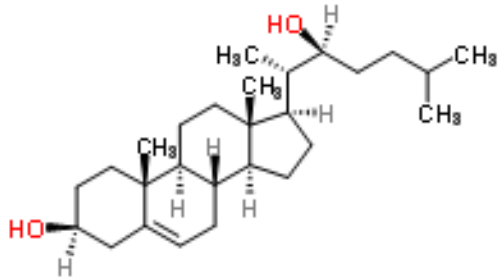
24(S)-HC across several sample types

Standard Curves







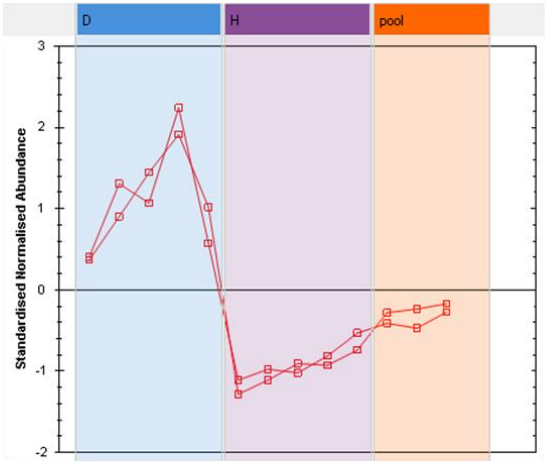


- Peak areas generated in Skyline but 3-point standard curves were generated from an export to Excel
  - ➢ Future Skyline feature

Using Skyline for Lipidomics Verification

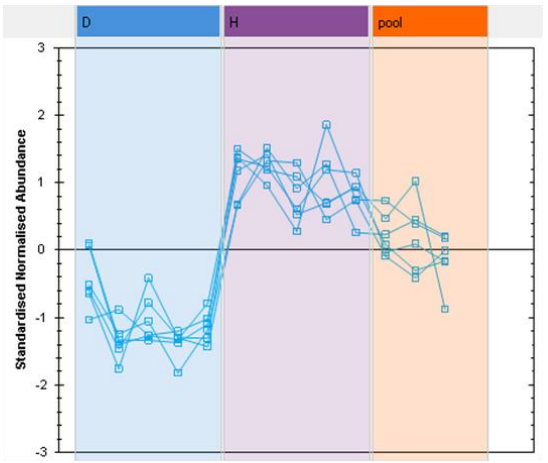
- Cancer cell line under drug treatment
  - UPLC coupled to Synapt G2 HDMS system for high res differential lipidomics analysis
  - 5 biological reps
  - Data analyzed in Progenesis QI
- A cluster of compounds was differentially expressed and putatively identified in Progenesis QI, showing several fatty acids:

Downregulated with Drug



Accepted Compound ID	Accepted Description
LMSP00000001	N-(tetradecanoyl)-deoxysphing-4-enine-1-sulfonate
LMGP02010302	PE(14:0/16:0)

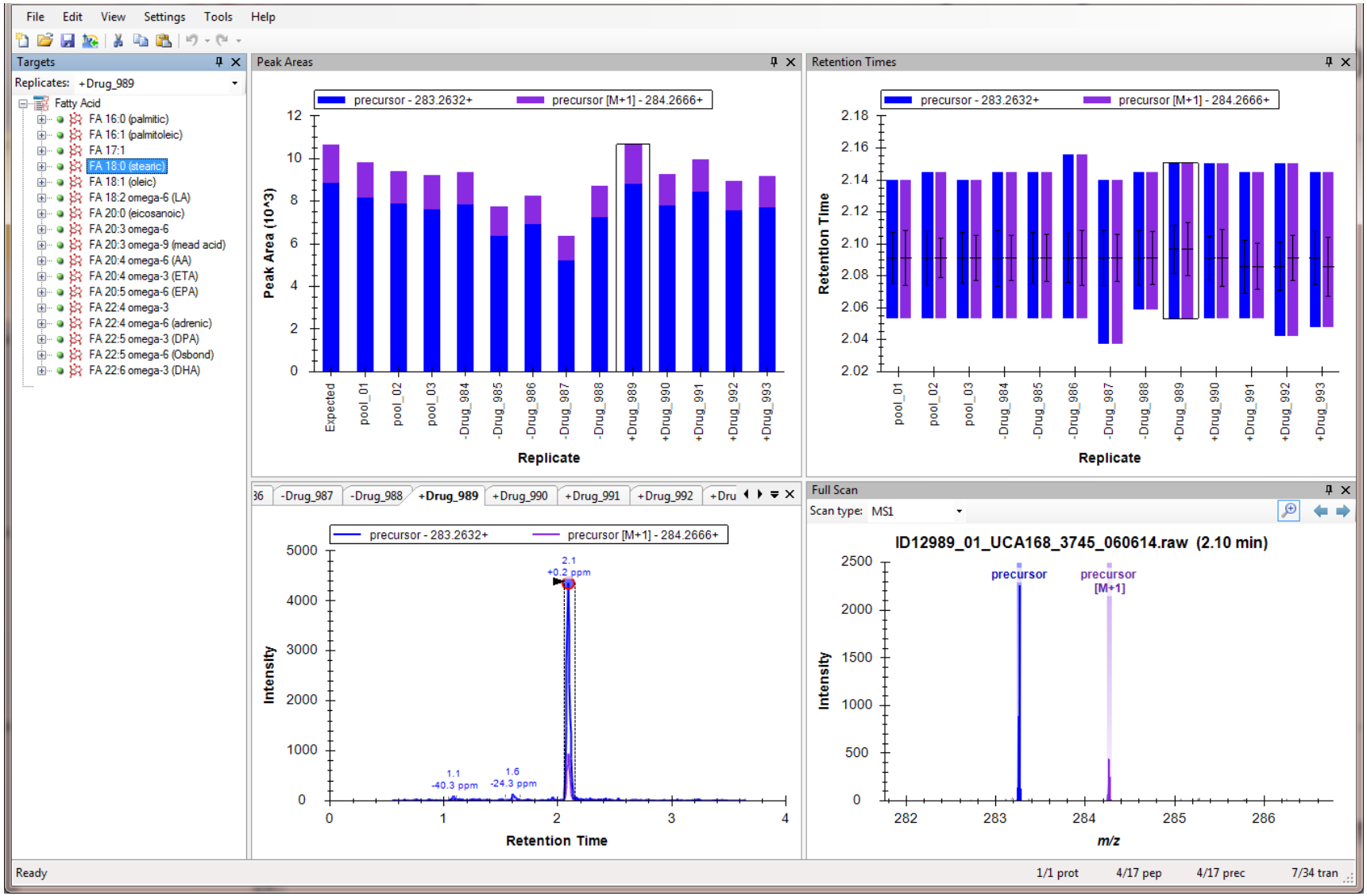
Upregulated with Drug



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))

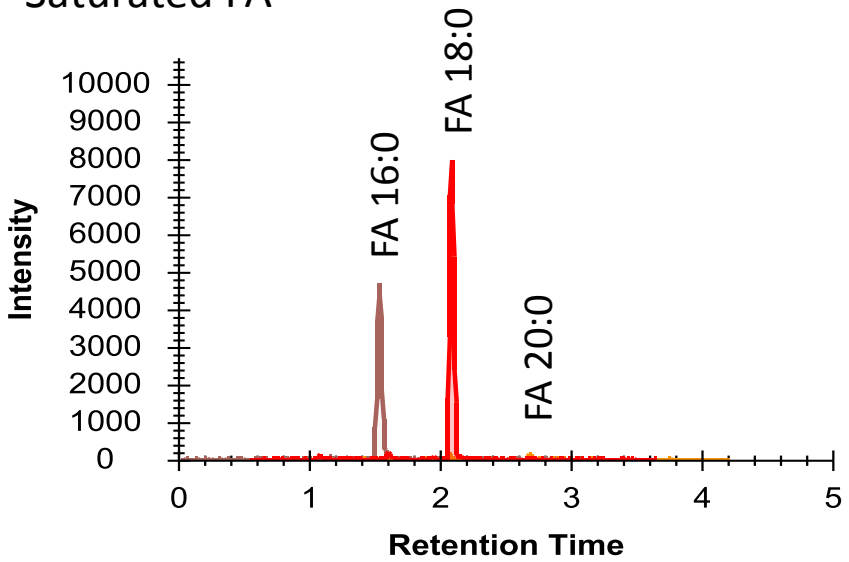
See Will Thompson's poster #251 on Wed. for additional details

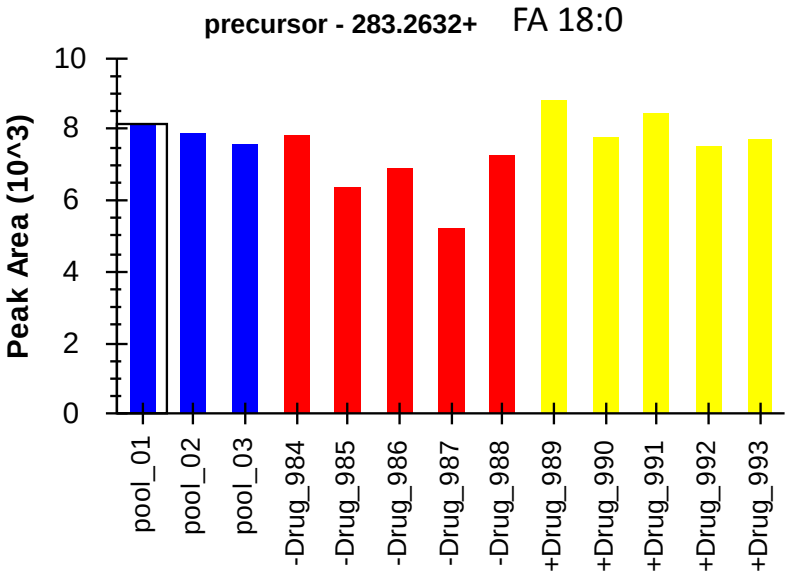
Full-Scan (MS1) Measurement – Method Setup



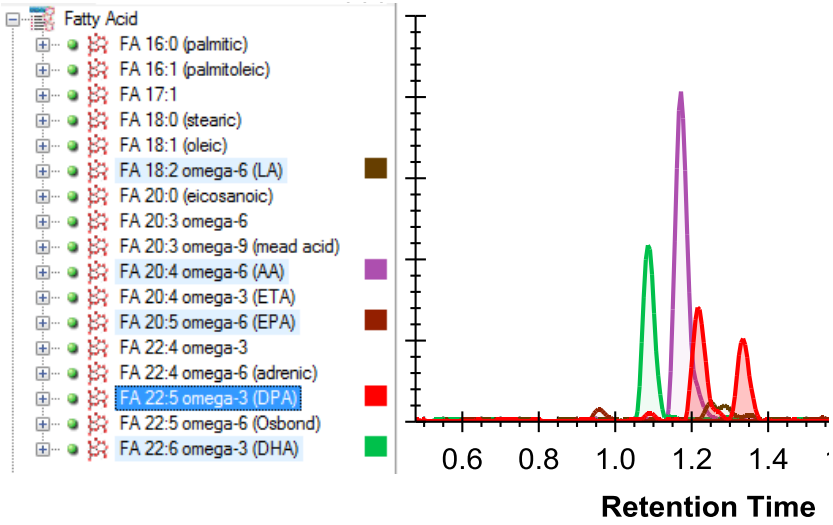
Validation in Skyline

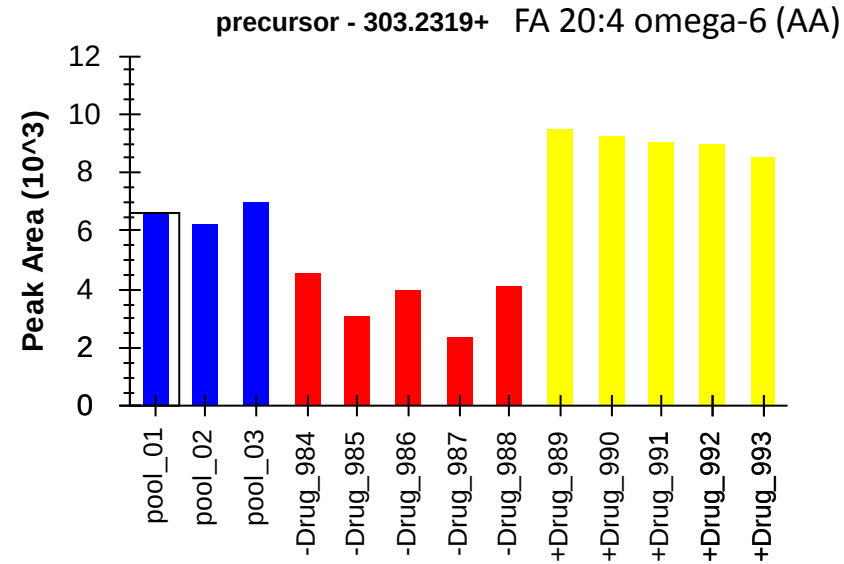
Saturated FA





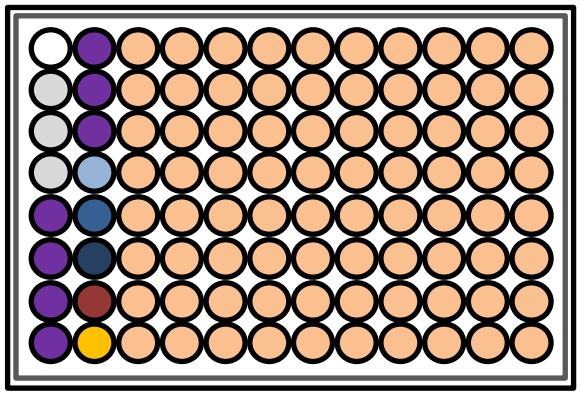
Unsaturated FA

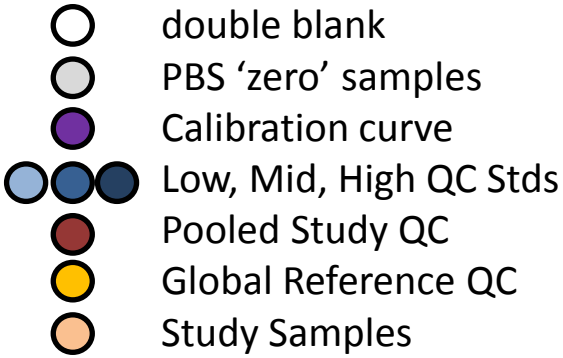


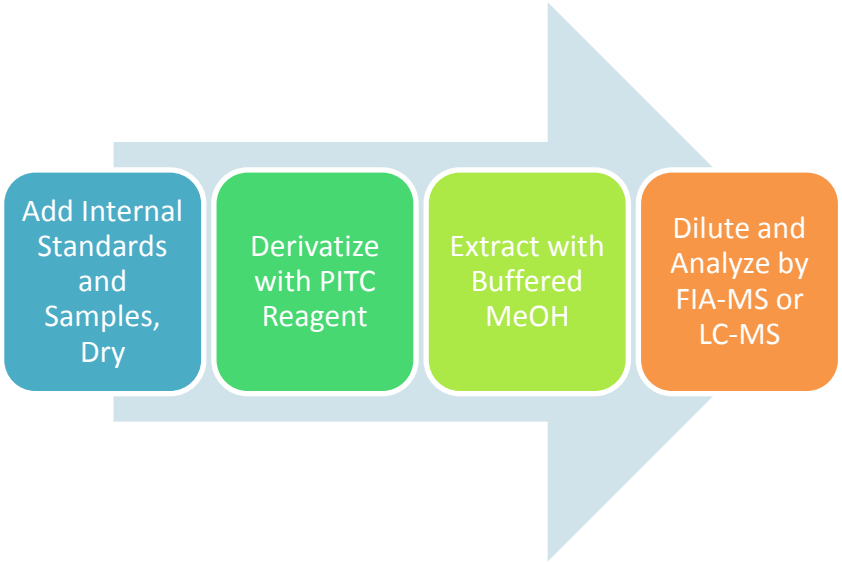


Biocrates Absolute IDQ p180 Assay Layout and Approach

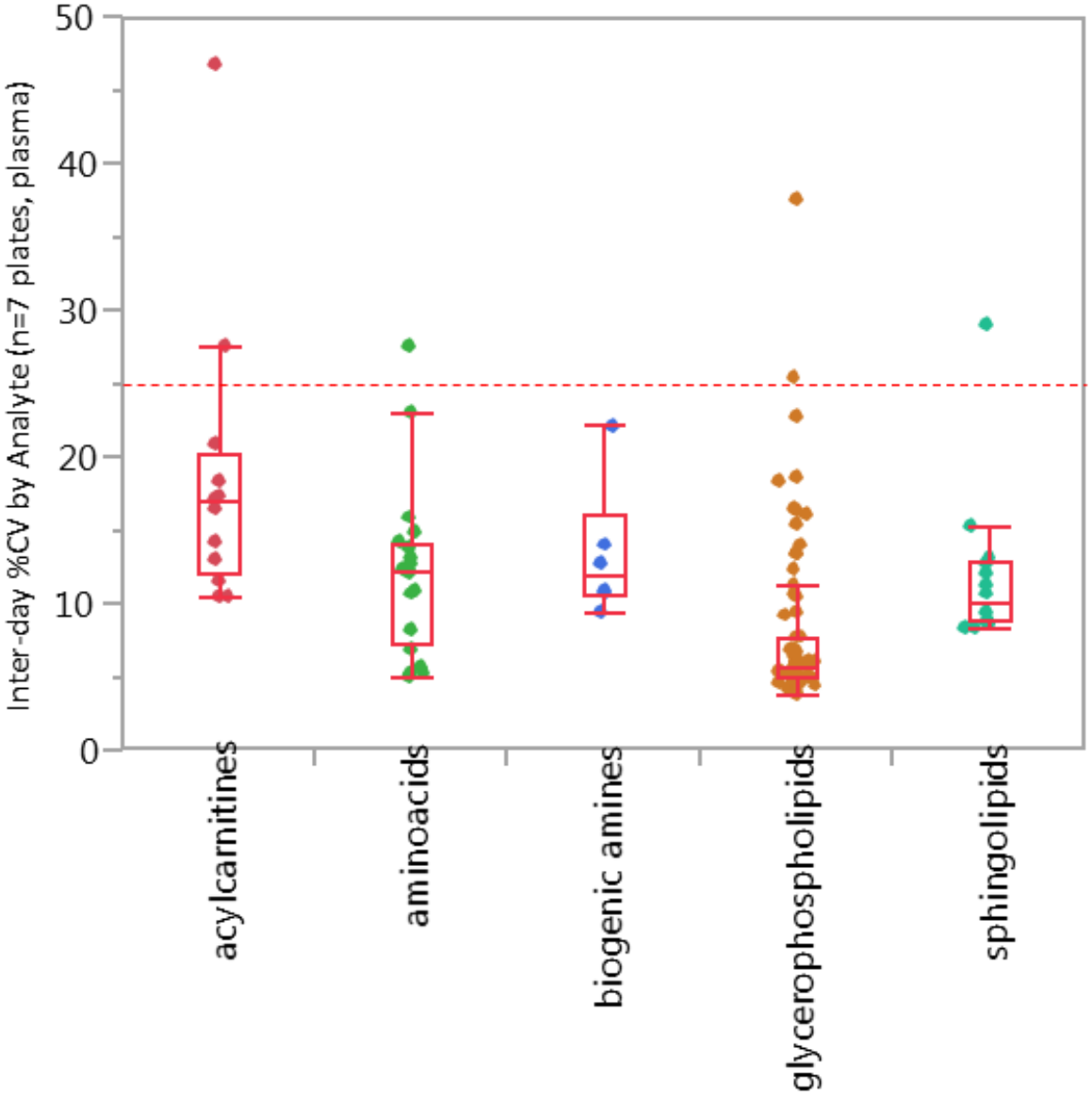
96-well plate layout







Inter-day reproducibility of Biocrates AbsoluteIDQ p180 kit GoldenWest Biologicals pooled serum reference sample on each plate

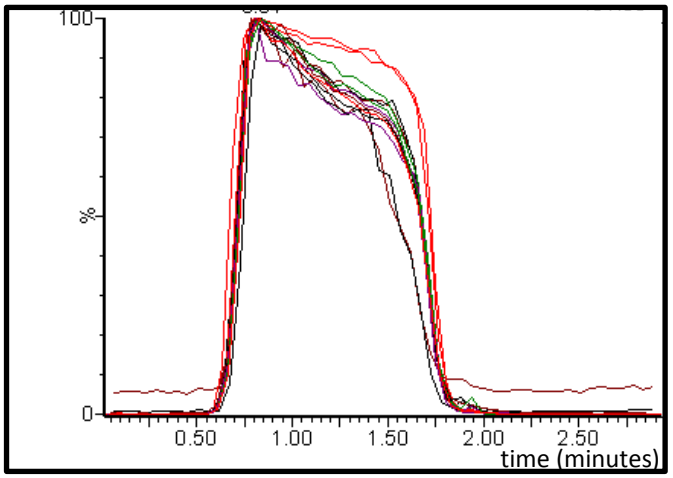


See Lisa St.-John Williams' poster #381 on Mon. for additional details

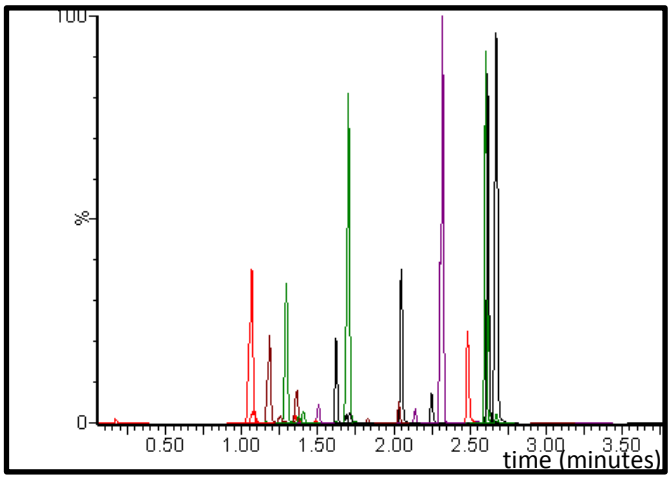
Biocrates Absolute IDQ p180

Quantitative Analysis of 5 Metabolite Classes

Flow-Injection Analysis



LC-MS/MS



156 analyte-specific MS/MS transitions
40 Acylcarnitines
15 Sphingomyelins
90 Phosphatidylcholines (PC) and Lyso-PC
11 stable-isotope internal standards

62 retention time scheduled transitions
22 amino acids
18 biogenic amines
20 stable-isotope internal standards

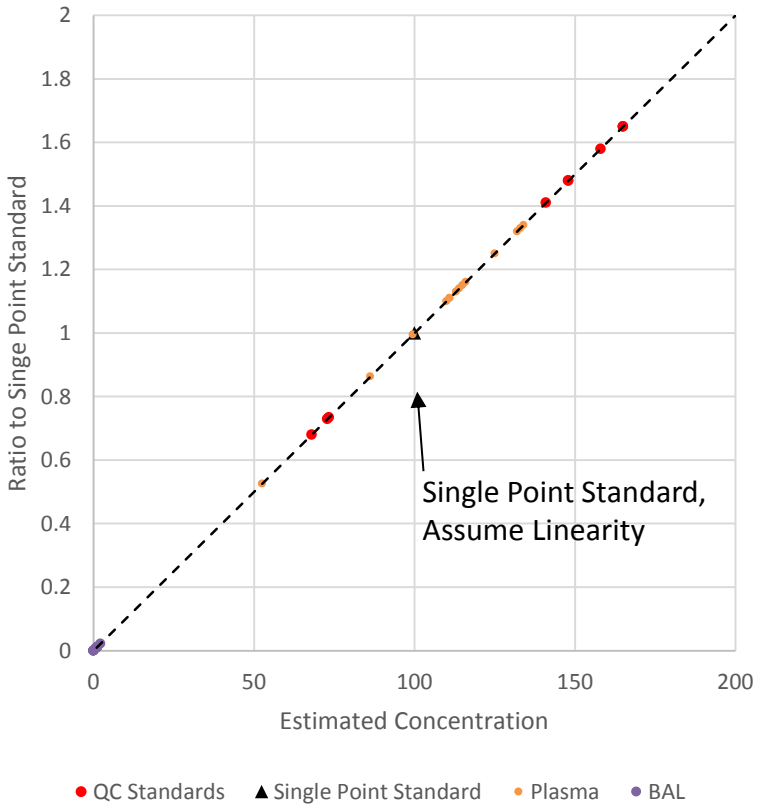
Standardize and Harmonize Across Experiments and Laboratories

Biocrates Absolute IDQ p180

Quantitative Analysis of 5 Metabolite Classes

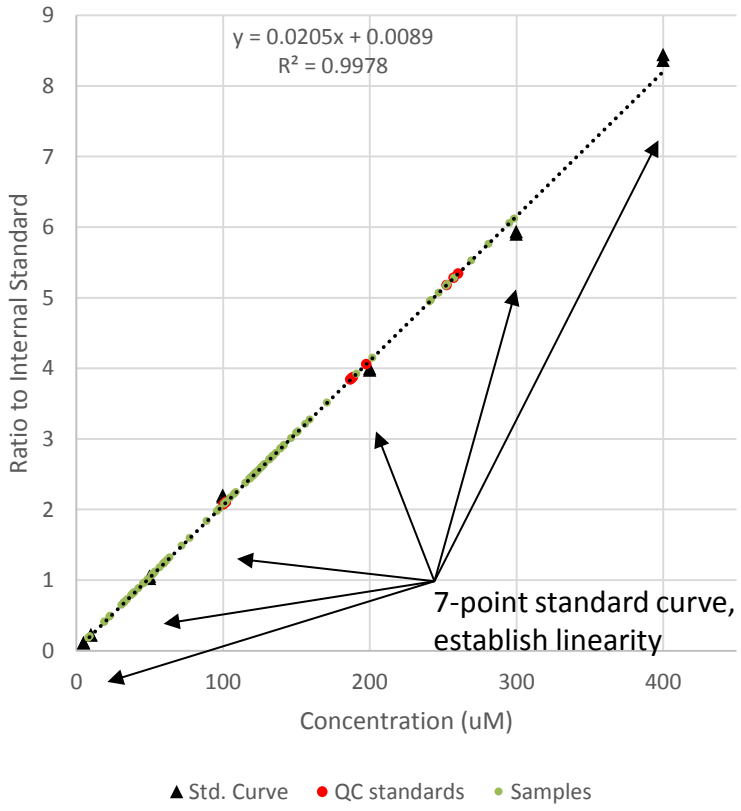
FIA-MS/MS

"Semiquantitative"

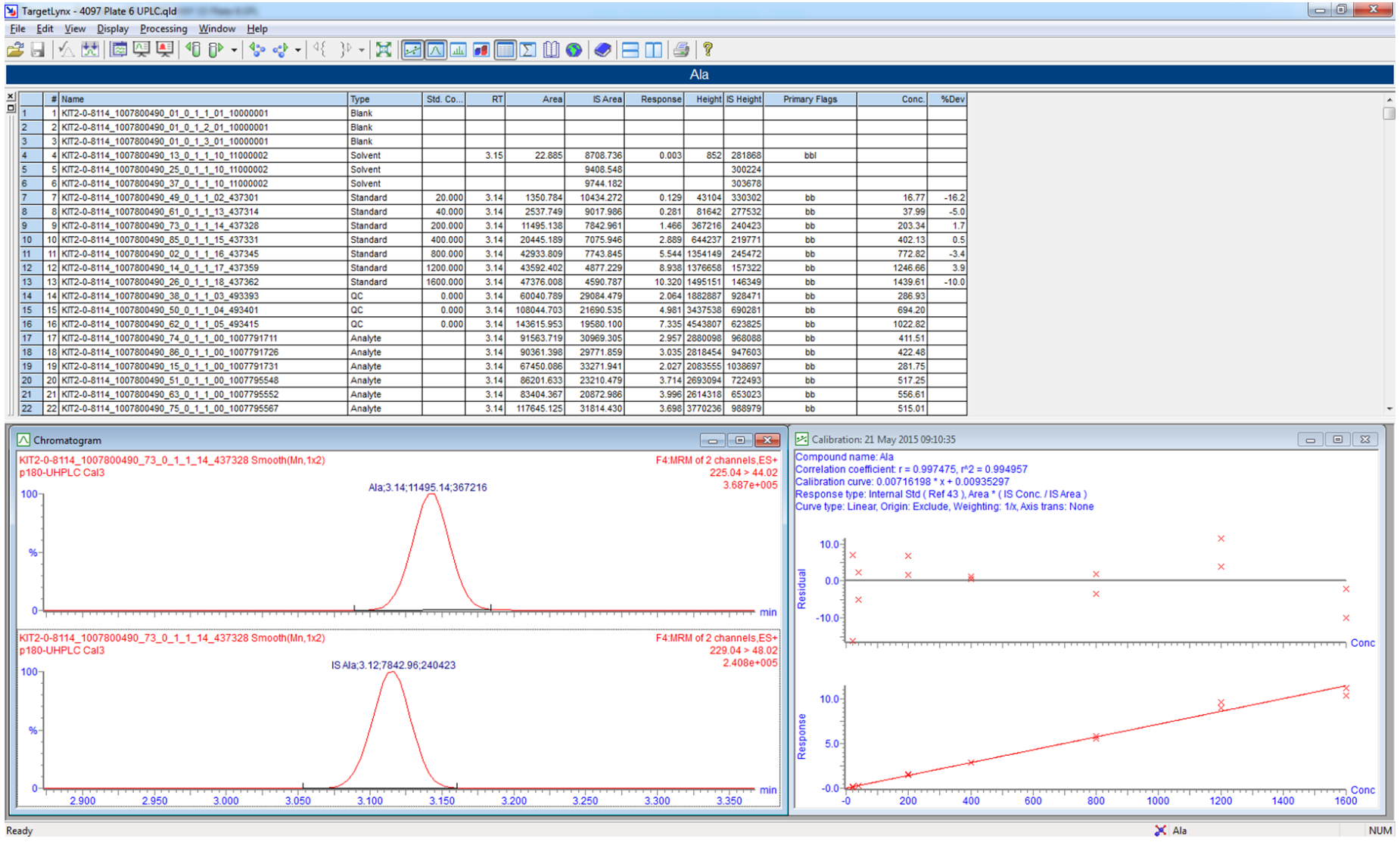


LC-MS/MS

“Quantitative”

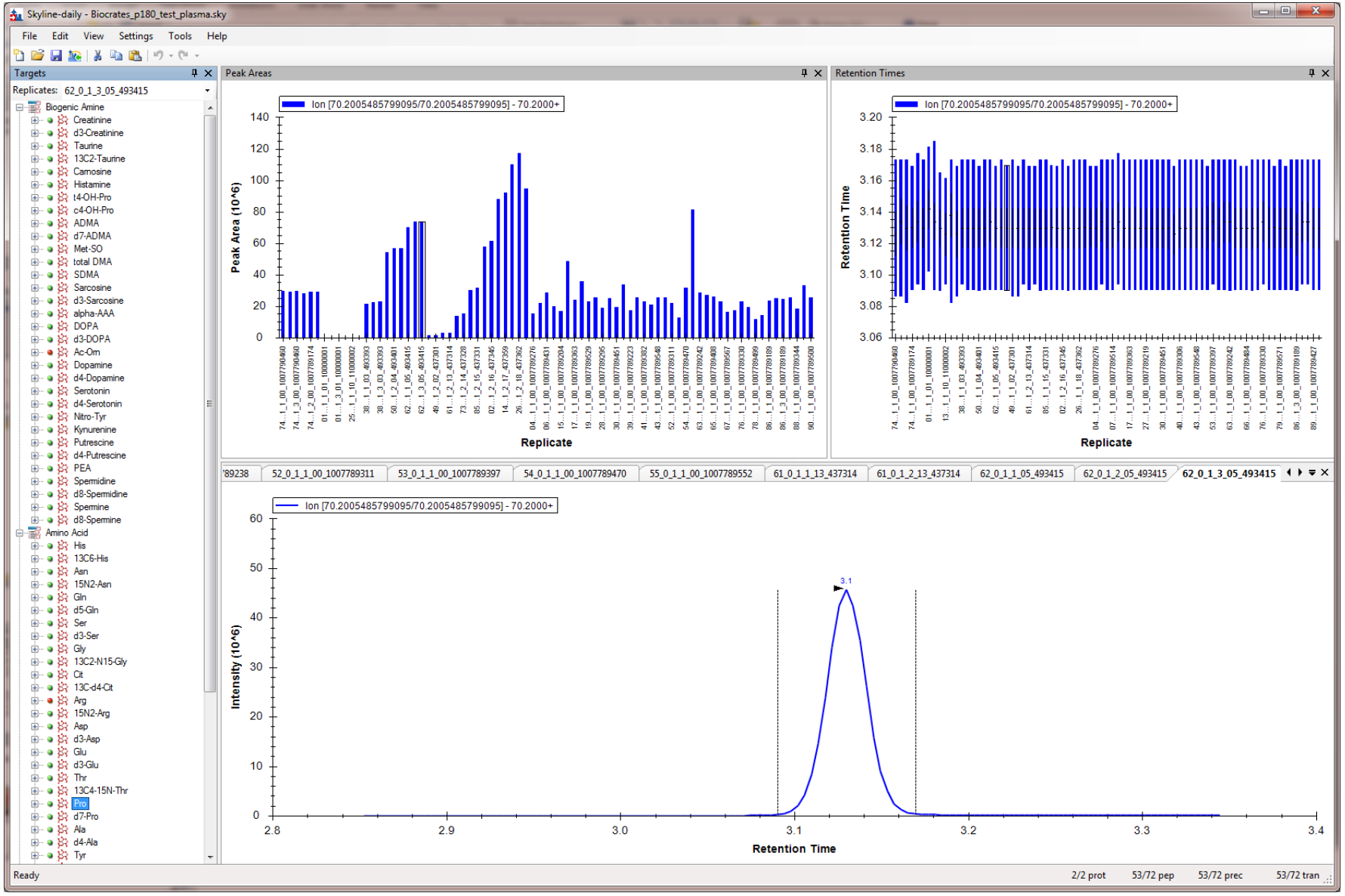


TargetLynx Peak Integration – Bottleneck for Biocrates p180 Analyses



	#	Name	Type	Std. Co...	RT	Area	IS Area	Response	Height	IS Height	Primary Flags	Conc.	%Dev
1	1	KIT2-0-8114_1007800490_01_0_1_1_01_10000001	Blank										
2	2	KIT2-0-8114_1007800490_01_0_1_2_01_10000001	Blank										
3	3	KIT2-0-8114_1007800490_01_0_1_3_01_10000001	Blank										
4	4	KIT2-0-8114_1007800490_13_0_1_1_10_11000002	Solvent		3.15	22.885	8708.736	0.003	852	281868	bbl		
5	5	KIT2-0-8114_1007800490_25_0_1_1_10_11000002	Solvent				9408.548			300224			
6	6	KIT2-0-8114_1007800490_37_0_1_1_10_11000002	Solvent				9744.182			303678			
7	7	KIT2-0-8114_1007800490_49_0_1_1_02_437301	Standard	20.000	3.14	1350.784	10434.272	0.129	43104	330302	bb	16.77	-16.2
8	8	KIT2-0-8114_1007800490_61_0_1_1_13_437314	Standard	40.000	3.14	2537.749	9017.986	0.281	81642	277532	bb	37.99	-5.0
9	9	KIT2-0-8114_1007800490_73_0_1_1_14_437328	Standard	200.000	3.14	11495.138	7842.961	1.466	367216	240423	bb	203.34	1.7
10	10	KIT2-0-8114_1007800490_85_0_1_1_15_437331	Standard	400.000	3.14	20445.189	7075.946	2.889	644237	219771	bb	402.13	0.5
11	11	KIT2-0-8114_1007800490_02_0_1_1_16_437345	Standard	800.000	3.14	42933.809	7743.845	5.544	1354149	245472	bb	772.82	-3.4
12	12	KIT2-0-8114_1007800490_14_0_1_1_17_437359	Standard	1200.000	3.14	43592.402	4877.229	8.938	1376658	157322	bb	1246.66	3.9
13	13	KIT2-0-8114_1007800490_26_0_1_1_18_437362	Standard	1600.000	3.14	47376.008	4590.787	10.320	1495151	146349	bb	1439.61	-10.0
14	14	KIT2-0-8114_1007800490_38_0_1_1_03_493393	QC	0.000	3.14	60040.789	29084.479	2.064	1882887	928471	bb	286.93	
15	15	KIT2-0-8114_1007800490_50_0_1_1_04_493401	QC	0.000	3.14	108044.703	21690.535	4.981	3437538	690281	bb	694.20	
16	16	KIT2-0-8114_1007800490_62_0_1_1_05_493415	QC	0.000	3.14	143615.953	19580.100	7.335	4543807	623825	bb	1022.82	
17	17	KIT2-0-8114_1007800490_74_0_1_1_00_1007791711	Analyte		3.14	91563.719	30969.305	2.957	2880098	968088	bb	411.51	
18	18	KIT2-0-8114_1007800490_86_0_1_1_00_1007791726	Analyte		3.14	90361.398	29771.859	3.035	2818454	947603	bb	422.48	
19	19	KIT2-0-8114_1007800490_15_0_1_1_00_1007791731	Analyte		3.14	67450.086	33271.941	2.027	2083555	1038697	bb	281.75	
20	20	KIT2-0-8114_1007800490_51_0_1_1_00_1007795548	Analyte		3.14	86201.633	23210.479	3.714	2693094	722493	bb	517.25	
21	21	KIT2-0-8114_1007800490_63_0_1_1_00_1007795552	Analyte		3.14	83404.367	20872.986	3.996	2614318	653023	bb	556.61	
22	22	KIT2-0-8114_1007800490_75_0_1_1_00_1007795567	Analyte		3.14	117645.125	31814.430	3.698	3770236	988979	bb	515.01	

Biocrates Analysis in Skyline



Conclusions

- Skyline currently being used across multiple metabolomics platforms in our lab
  - Enables method/data sharing and improves transparency of metabolomics experiments
- Ongoing discussions between Skyline and Biocrates teams to address the bottleneck in Biocrates analyses (beneficial for all vendor platforms) and the hurdle to import data into MetIDQ for qualification
- Skyline features requested and in progress:
  - MS2 library viewing, building, and matching (enabled for proteomics):
    - Building targeted MS2 methods
    - Verification in MSE analysis
  - Heavy/light isotope standard grouping for quantification (enabled for proteomics)
  - Improvements in peak integration
  - Quantification against calibration curves

Duke Proteomics and Metabolomics Core

http://www.genome.duke.edu/cores/proteomics/

Arthur Moseley

Will Thompson

Erik Soderblom

Matthew Foster

Lisa St. John-Williams

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