Transferring a Small Molecule Quant Method to Skyline

J. Will Thompson
Duke Proteomics and Metabolomics Shared Resource
Duke School of Medicine
Durham, NC

Transfer of a Quantitative Small Molecule Quant Method to Data Analysis in Skyline

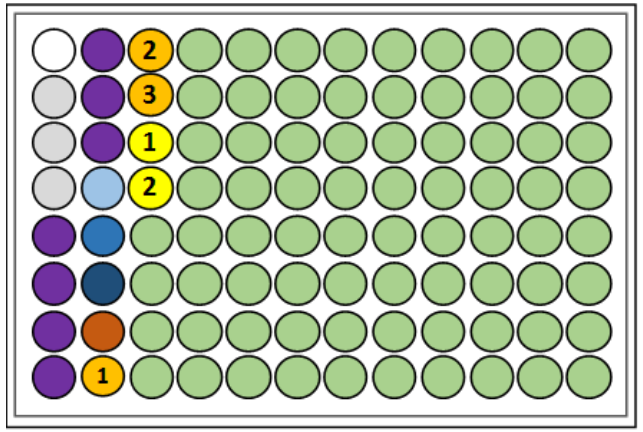
- Targeted Quantification based on TQ-MS, out of crashed plasma
- Starting from a method you may already be running (e.g. PK)

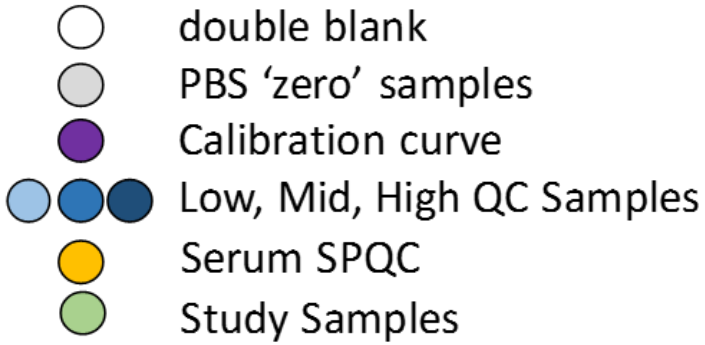
In the analysis of this dataset you will learn

- Insertion of simple set of known transitions
- Data Analysis and peak integration for small molecules
- Small Molecule Quantification workflow in Skyline

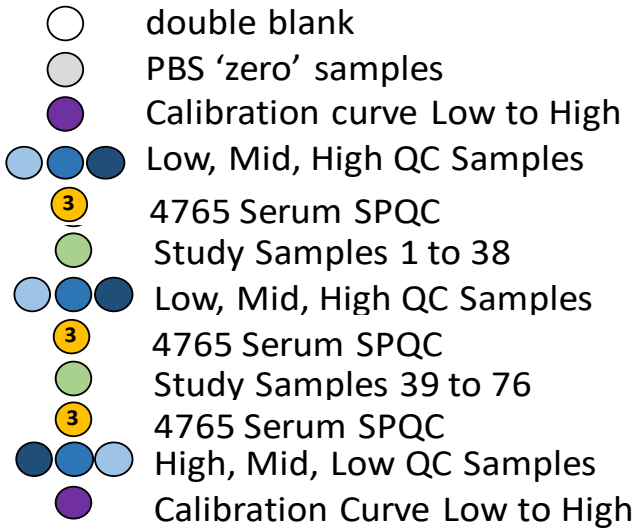
Experimental Layout

96-well plate layout



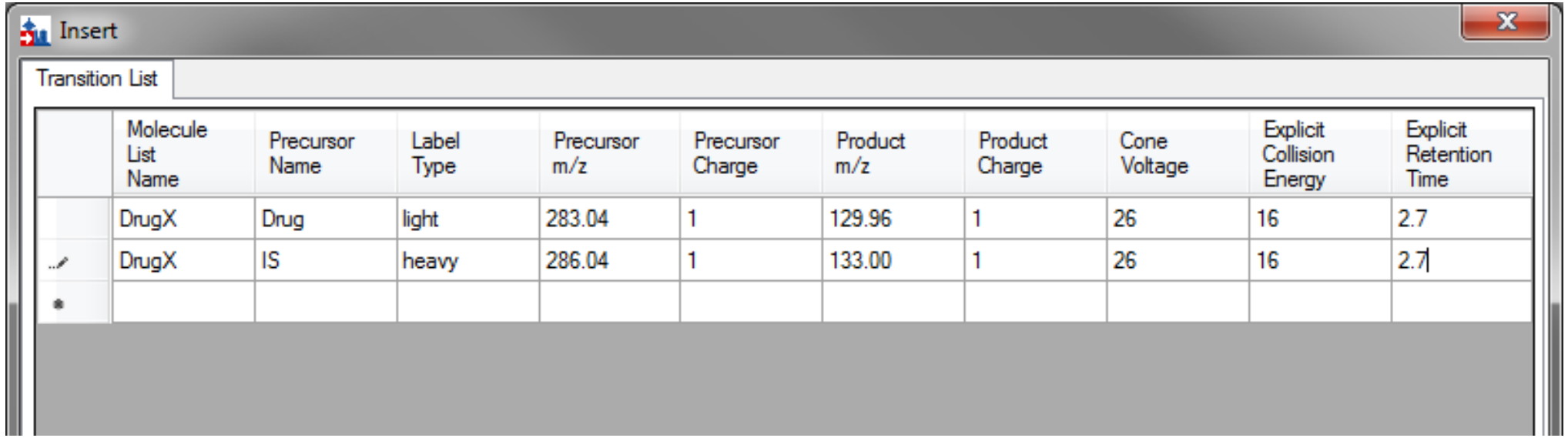


Serum Sample Injection Sequence

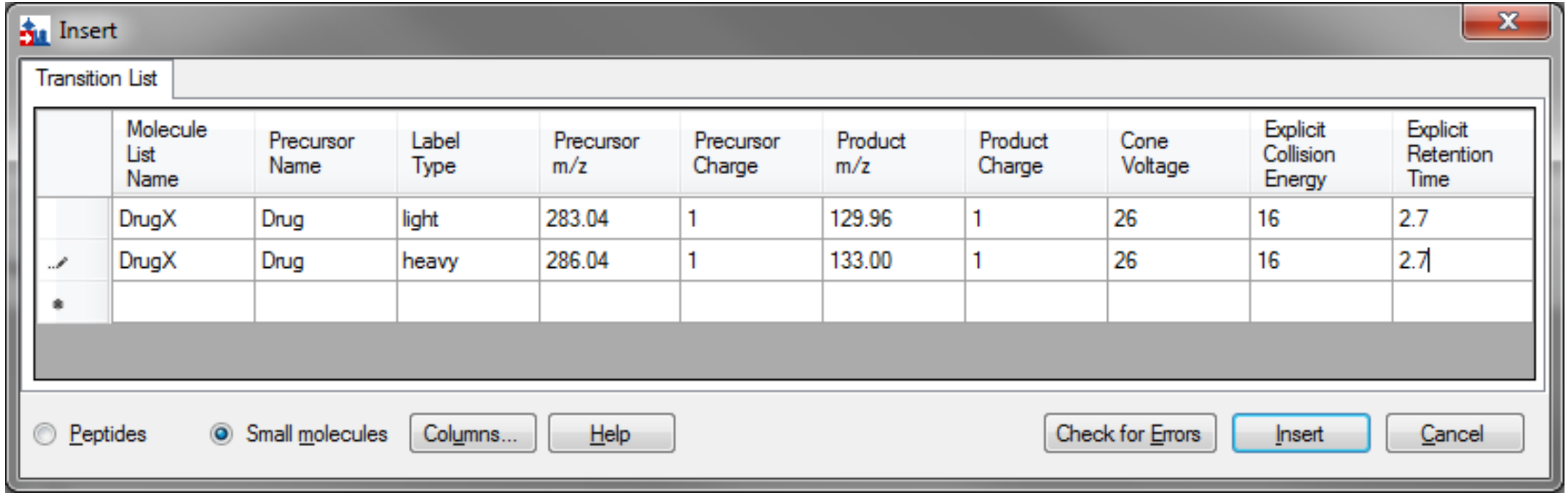


Create a Transition List From Scratch

- Small transition lists can be typed into Skyline Directly. Edit/Insert/Transition List.
- Select “Small Molecules”. Choose fields and enter data as below.



Alternative, define light/heavy within one molecule type.



Modify as shown, then click “insert”.

Method Development and CE Optimization for Small Molecules in Skyline

Development of a Method for Selected Energy Metabolites on LC-MS/MS (Triple Quad)

J. Will Thompson

Duke Proteomics and Metabolomics Shared Resource

Duke University School of Medicine

Durham, NC

Multiplexed Method Optimization of Small Molecules in Skyline

- Targeted Quantification Workflows based on TQMS
- Starting from a Publication including a transition list of putative molecules of interest, then using Skyline to perform multiplexed optimization of CE and RT scheduling.

In the analysis of this dataset you will learn:

- Building a Skyline method from a simple transition list from a publication
- Scheduling RT and optimizing collision energies (CE) (different instrument platform)

A little work in Excel to start...

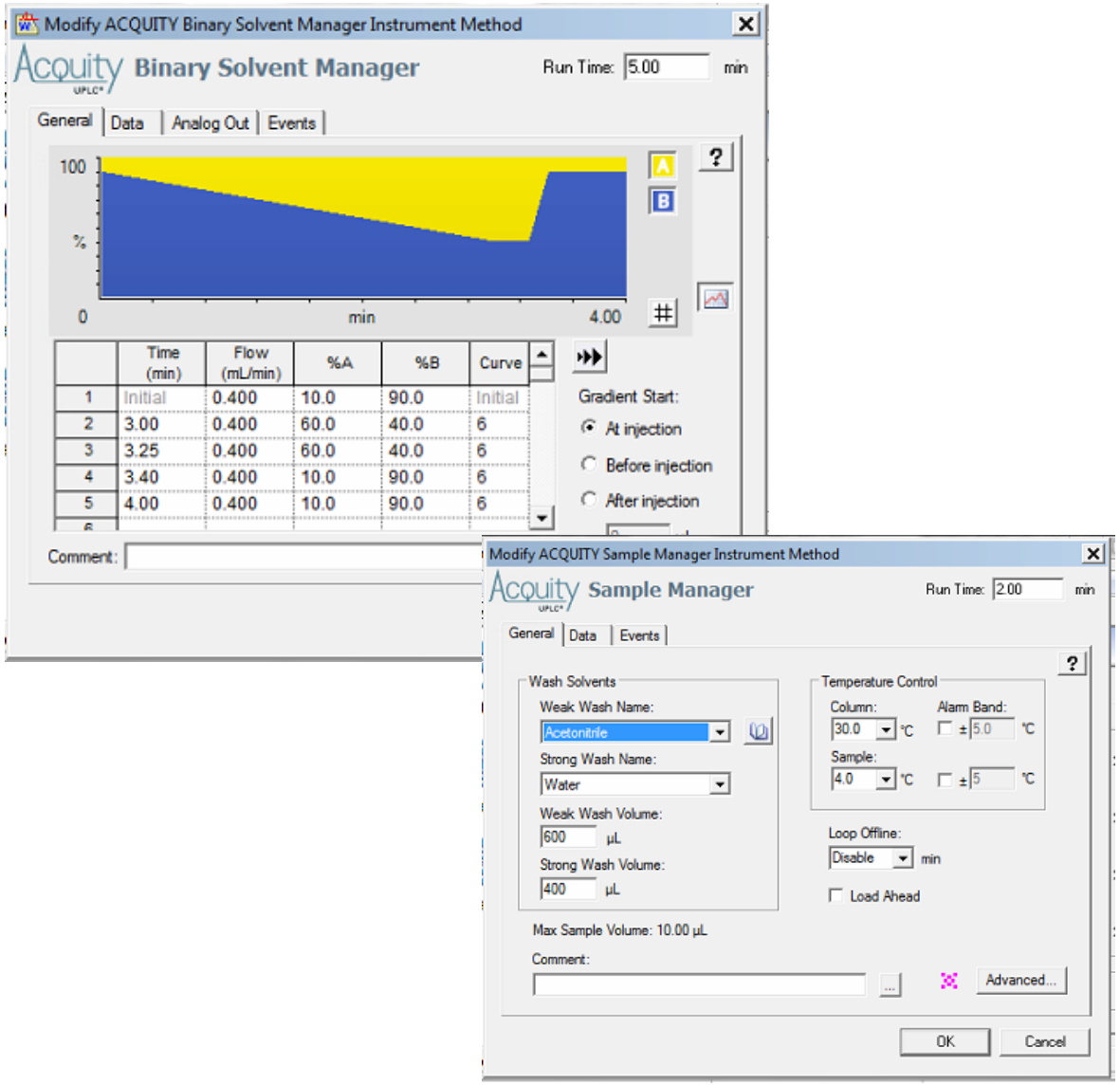
Published Transition List...

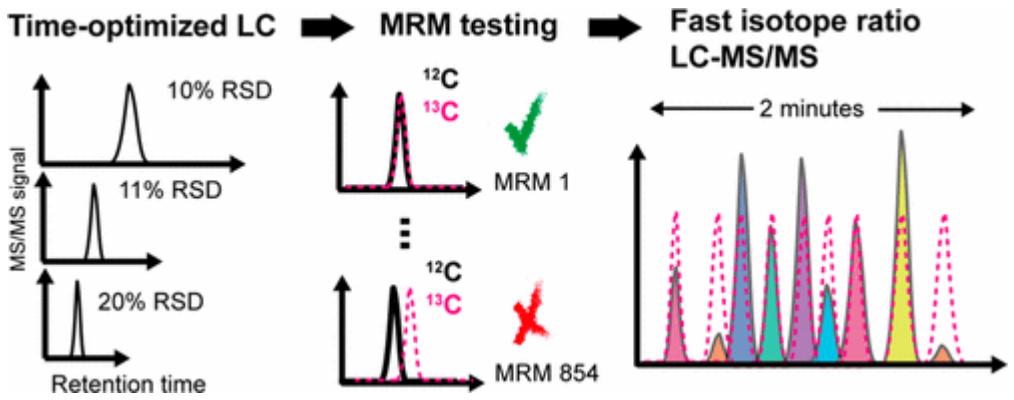
Functional Category	Metabolite	KEGG id	Q1 -12C	Q3-12C	Q1-13C	Q3-13C	CE	Mode
Central Metabolism	a-Ketoglutaric acid	C00026	145	101	150	105	5	Negative
Central Metabolism	Phosphoenolpyruvate	C00074	167	79	170	79	29	Negative
Central Metabolism	Dihydroxyacetone-P	C00111	169	79	172	79	29	Negative
Central Metabolism	Pentose-P	C00199	229	79	234	79	45	Negative
Central Metabolism	Hexose-P	C01094	259	79	265	79	53	Negative
Central Metabolism	Seduheptulose 7-P	C05382	289	97	296	97	17	Negative
Central Metabolism	Fructose-1,6-Bisphosphate	C00354	339	241	345	247	16	Negative
Central Metabolism	UDP-N-acetyl-D-Glucosamine	C00043	606	385	623	394	29	Negative
Central Metabolism	Acetyl-CoA	C00024	808	408	831	418	37	Negative
Cofactor metabolism	NAD	C00003	662	540	683	555	20	Negative
Cofactor metabolism	NADP	C00006	742	620	763	635	20	Negative
Nucleotide metabolisn	Orotate	C00295	155	111	160	115	9	Negative
Nucleotide metabolisn	Dihydroorotate	C00337	157	113	162	117	5	Negative
Nucleotide metabolisn	UDP	C00015	403	79	412	79	69	Negative
Amino acid metabolisn	GABA	C00334	104	69	108	73	37	Positive
Amino acid metabolisn	Phenylpyruvic acid	C00166	165	95	174	101	13	Positive
Amino acid metabolisn	Diaminopimelic acid	C00666	191	128	198	134	13	Positive
Central Metabolism	D-Alanyl-Alanine	C00993	161	44	167	46	13	Positive
Cofactor metabolism	D-Pantothenic acid	C00864	220	90	229	93	13	Positive
Cofactor metabolism	Oxidized glutathione	C00127	613	355	633	365	25	Positive
Nucleotide metabolisn	Hypoxanthine	C00262	137	55	142	57	37	Positive
Nucleotide metabolisn	Guanine	C00242	152	110	157	114	21	Positive
Nucleotide metabolisn	UMP	C00105	325	97	334	102	17	Positive
Nucleotide metabolisn	cAMP	C00575	330	136	340	141	29	Positive
Nucleotide metabolisn	AMP	C00020	348	136	358	141	21	Positive
Nucleotide metabolisn	ADP	C00008	428	136	438	141	37	Positive
Nucleotide metabolisn	UTP	C00075	485	97	494	102	21	Positive
Nucleotide metabolisn	ATP	C00002	508	136	518	141	37	Positive

Open “EnergyMet_TransitionList.xlsx”

A	B	C	D	E	F	G	H	I	J	K
Molecule List Name	Precursor Name	Label Type	Precursor m	Precursor C	Product m/	Product Ch	Cone Volta	Explicit Colli	Explicit Retention Time	
Amino acid metabolism	Diaminopimelic acid	light	191	1	128	1	25	13		
Amino acid metabolism	Diaminopimelic acid	heavy	198	1	134	1	25	13		
Amino acid metabolism	GABA	light	104	1	69	1	25	37		
Amino acid metabolism	GABA	heavy	108	1	73	1	25	37		
Amino acid metabolism	Phenylpyruvic acid	light	165	1	95	1	25	13		
Amino acid metabolism	Phenylpyruvic acid	heavy	174	1	101	1	25	13		
Central Metabolism	Acetyl-CoA	light	808	-1	408	-1	25	37		
Central Metabolism	Acetyl-CoA	heavy	831	-1	418	-1	25	37		
Central Metabolism	a-Ketoglutaric acid	light	145	-1	101	-1	25	5		
Central Metabolism	a-Ketoglutaric acid	heavy	150	-1	105	-1	25	5		
Central Metabolism	D-Alanyl-Alanine	light	161	1	44	1	25	13		
Central Metabolism	D-Alanyl-Alanine	heavy	167	1	46	1	25	13		
Central Metabolism	Dihydroxyacetone-P	light	169	-1	79	-1	25	29		
Central Metabolism	Dihydroxyacetone-P	heavy	172	-1	79	-1	25	29		
Central Metabolism	Fructose-1,6-Bisphosphate	light	339	-1	241	-1	25	16		
Central Metabolism	Fructose-1,6-Bisphosphate	heavy	345	-1	247	-1	25	16		
Central Metabolism	Hexose-P	light	259	-1	79	-1	25	53		
Central Metabolism	Hexose-P	heavy	265	-1	79	-1	25	53		
Central Metabolism	Malate	light	133	-1	115	-1	25	9		
Central Metabolism	Malate	heavy	137	-1	119	-1	25	9		
Central Metabolism	Pentose-P	light	229	-1	79	-1	25	45		
Central Metabolism	Pentose-P	heavy	234	-1	79	-1	25	45		
Central Metabolism	Phosphoenolpyruvate	light	167	-1	79	-1	25	29		
Central Metabolism	Phosphoenolpyruvate	heavy	170	-1	79	-1	25	29		
Central Metabolism	Seduheptulose 7-P	light	289	-1	97	-1	25	17		
Central Metabolism	Seduheptulose 7-P	heavy	296	-1	97	-1	25	17		
Central Metabolism	Succinate	light	117	-1	73	-1	25	13		
Central Metabolism	Succinate	heavy	121	-1	76	-1	25	13		
Central Metabolism	UDP-N-acetyl-D-Glucosamine	light	606	-1	385	-1	25	29		

High Speed HILIC method, based on Guder et al, Anal Chem. 2017 Feb 7;89(3):1624-1631.





Column	Acquity BEH Amide								iHILIC-Fusion(P)								Zorbax				D. Hydride			
Dimension	30 x 2.1 mm								50 x 2.1 mm								30 x 2.1 mm				30 x 2.1 mm			
Particle size	1.7 µm								5 µm								1.8 µm				2.2 µm			
pH	acidic				basic				acidic				basic				acidic				acidic			
Run time (min)	3.8	2.5	2	1.5	3.8	2.5	2	1.5	3.8	2.5	2	1.5	3.8	2.5	2	1.5	3.8	2.5	2	1.5	3.8	2.5	2	1.5
Flow rate (mL min^{-1})	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5
Median RSD	10	9	12	19	11	11	11	11	10	12	12	14	8	10	10	14	11	11	12	13	12	10	13	14
#of metabolites RSD<20%	27	26	25	14	34	30	29	23	11	15	23	19	26	31	34	27	23	27	24	24	25	25	22	21

Sample Used for Method Development:

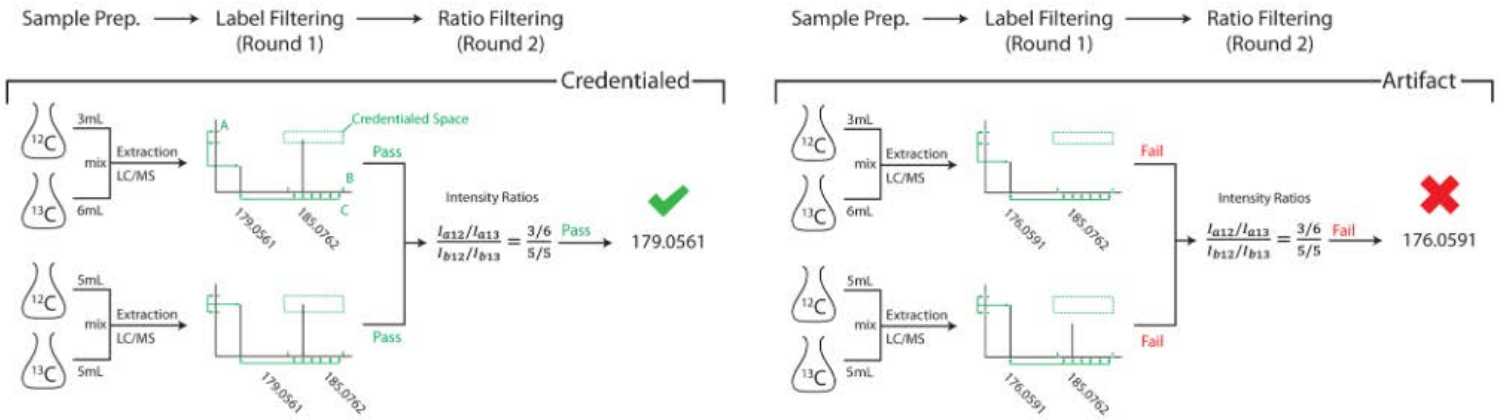
Credientialed E.Coli Lysate (Cambridge Isotope Laboratories)

View Larger Image

CREDENTIALED E. COLI CELL EXTRACT KIT (SOLUTION)

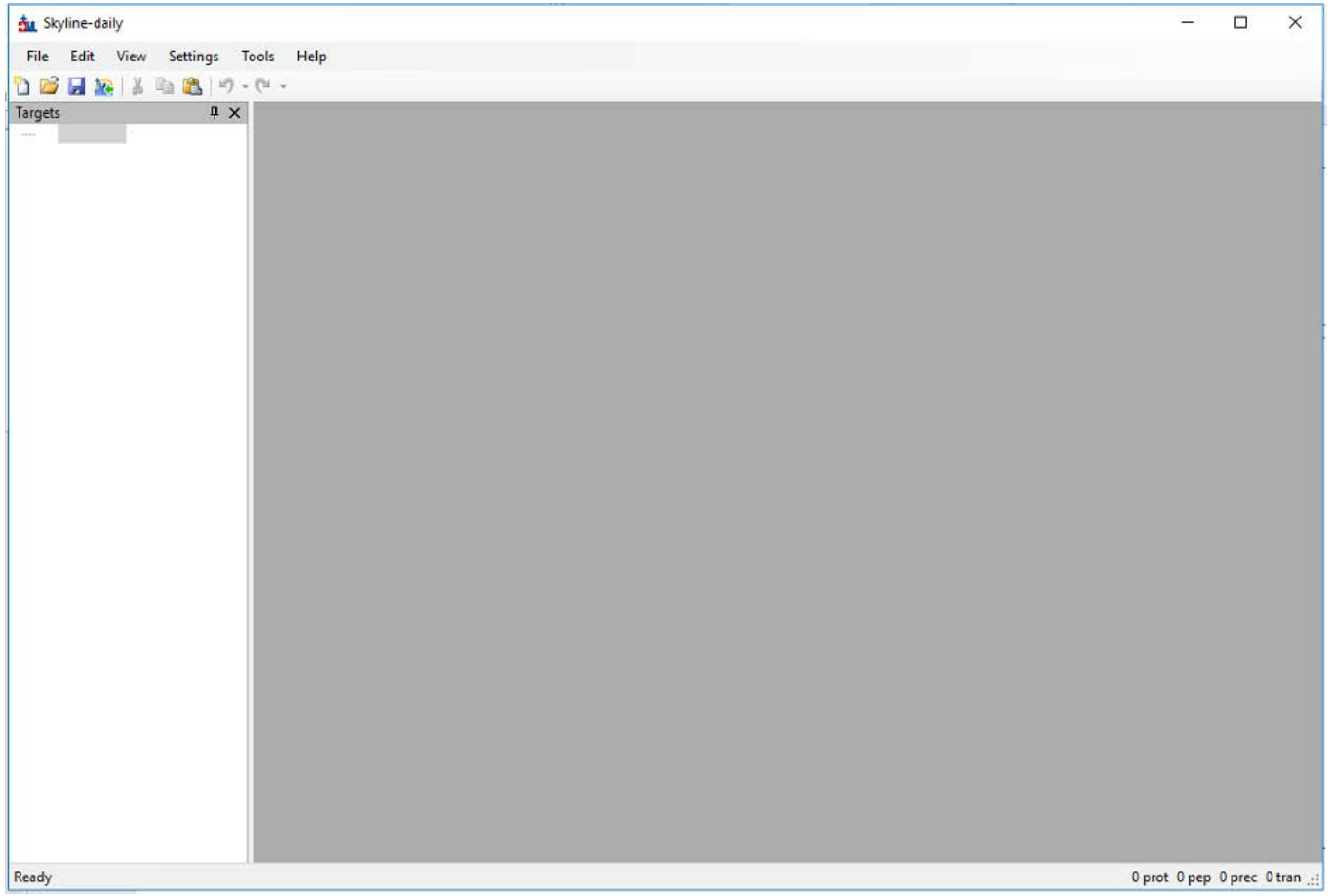
The kit contents are as follows: 13C-labeled E. coli cell extract (100uL solution); unlabeled E. coli cell extract (100uL solution); Detailed user manual with "Credentialing R" software. Note: the cells are E. coli K12 strain MG1655 and were extracted using a variation of the method described in PMID: 25160088. MUST SHIP ON DRY ICE

Item Number	MSK-CRED-KIT
Chemical Formula	
Unlabeled CAS#	
Labeled CAS#	
Molecular Weight	
Chemical Purity	98%

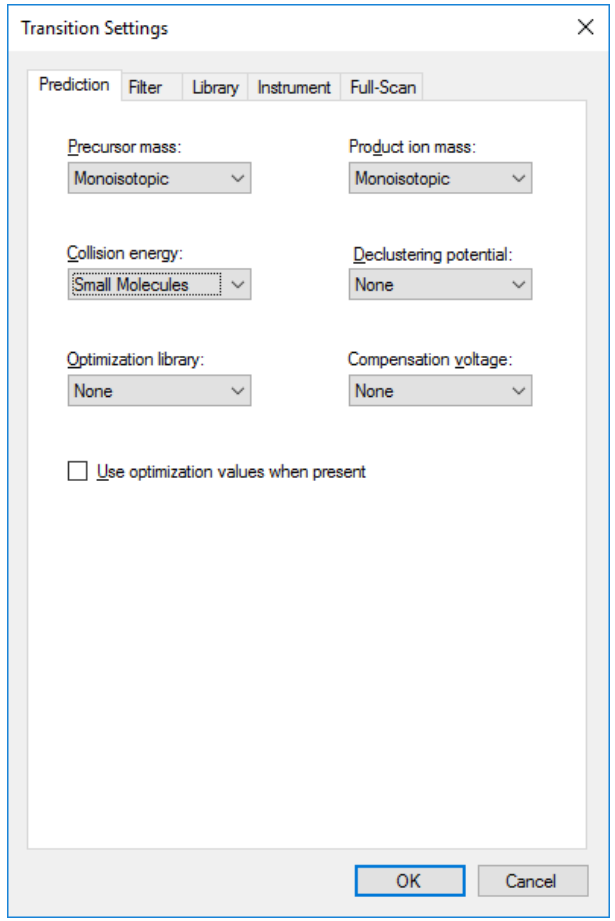


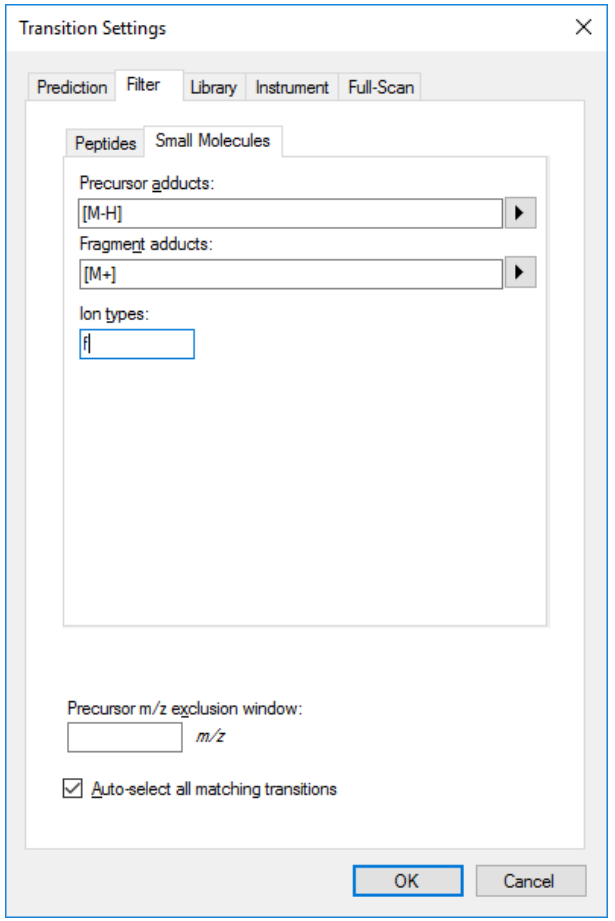
Mahieu NG1, Huang X, Chen YJ, Patti GJ. Credentialing features: a platform to benchmark and optimize untargeted metabolomic methods. *Anal Chem.* **2014** Oct 7;86(19):9583-9. doi: 10.1021/ac503092d. Epub 2014 Sep 22.

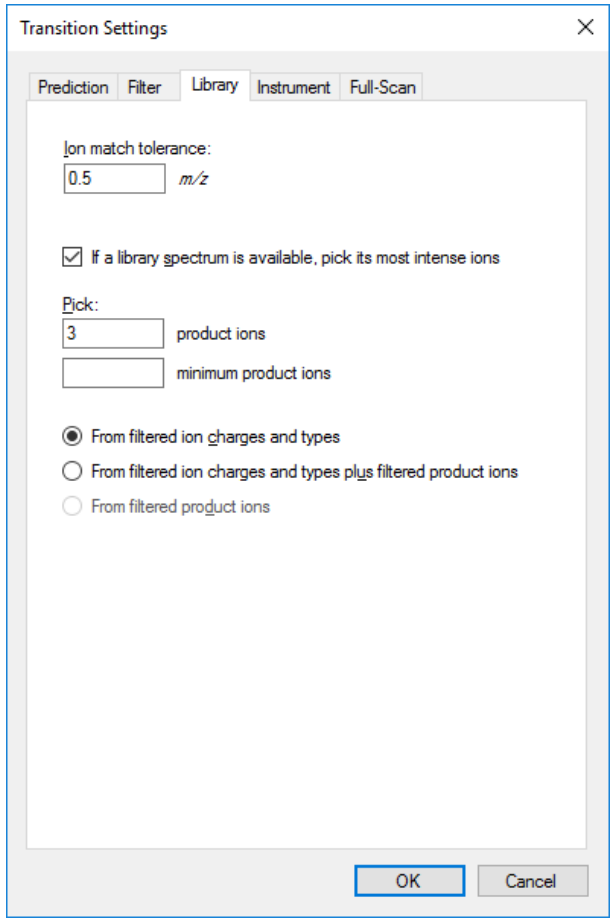
Start with a Blank Skyline Document. Save as “EnergyMet_demo.sky”.

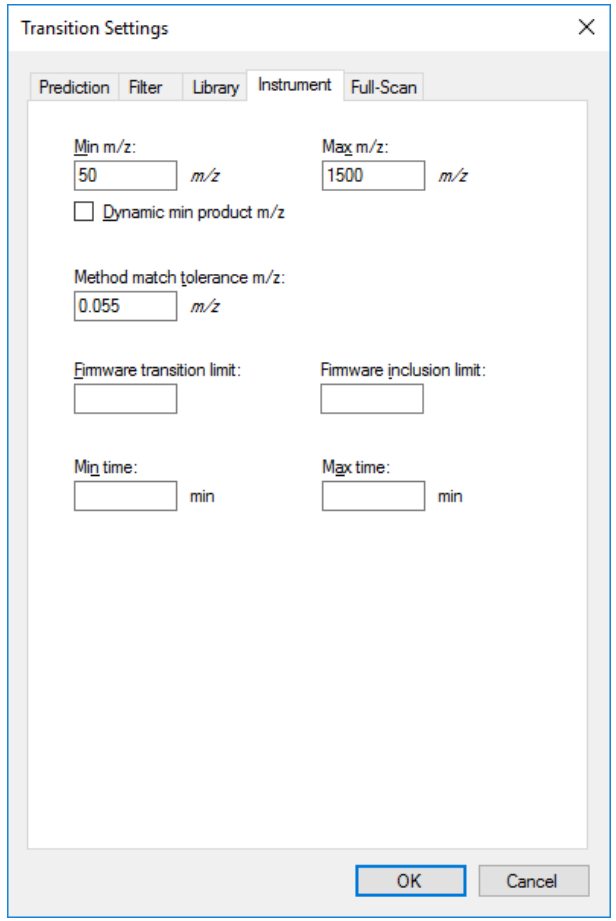


Document Setup for Instrument and Extraction Parameters (Xevo TQ-S triple quad, Waters) (Settings -> Transition Settings)









Edit/Insert/Transition List

Use “columns” button to select columns to match “EnergyMet_TransitionList.xlsx”
Copy/paste transition list into table and click “check”. If green, then click “insert”.