



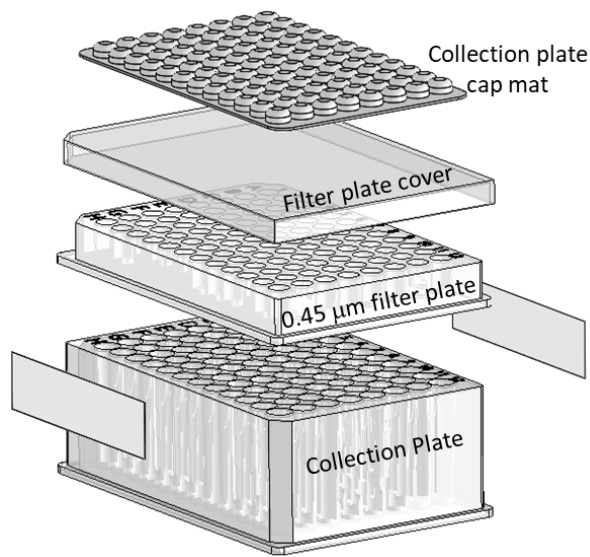
Utilizing Skyline in Automated System Suitability Testing, Data QC, and Metabolite Quantification for Microchip CE-MS Analysis

Sam Stewart, Erin Redman, J. Will Thompson
Research and Development, 908 Devices Inc.

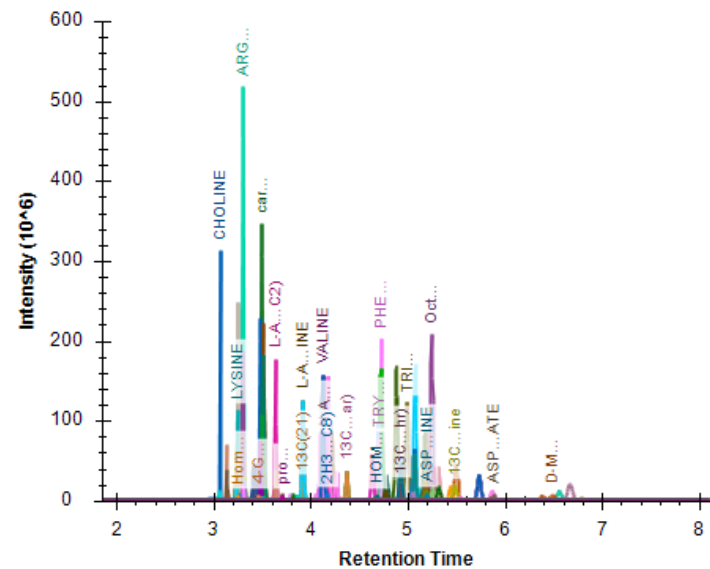
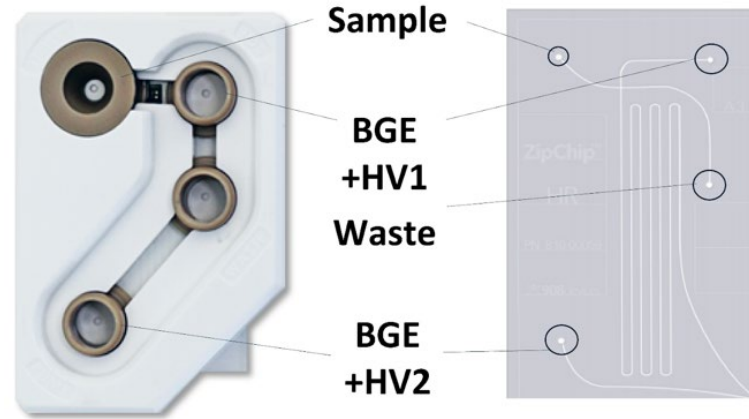
Metabolomics with ZipChip (microchip CZE-MS)

EXTRACTION

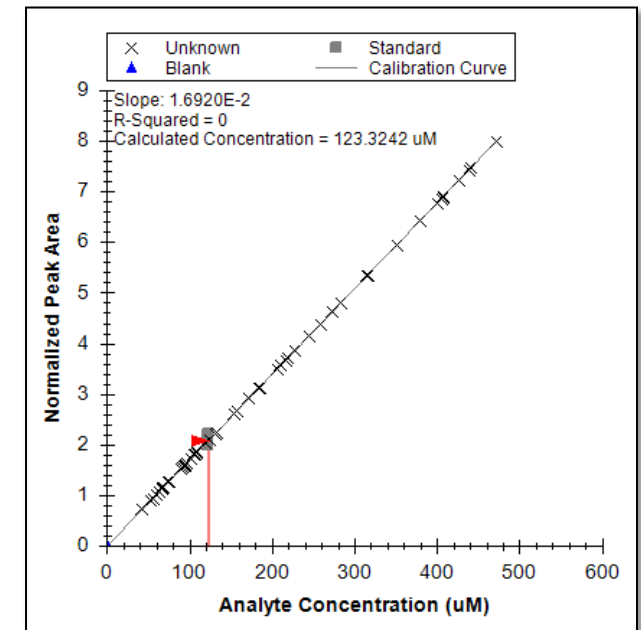
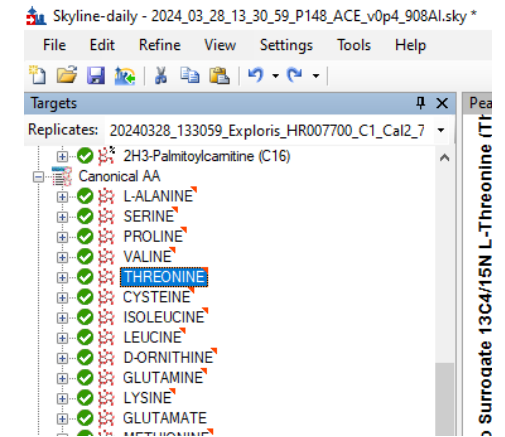
20 uL sample
140 uL MeOH+IS
40 uL Ammon. Acetate



mCE-HRMS



TARGETED QUANT



Areas we are utilizing **Skyline** in our software pipeline

System Suitability



Before Data
Collection

Data Quality Check



Immediately
After Data
Collection

Quantification



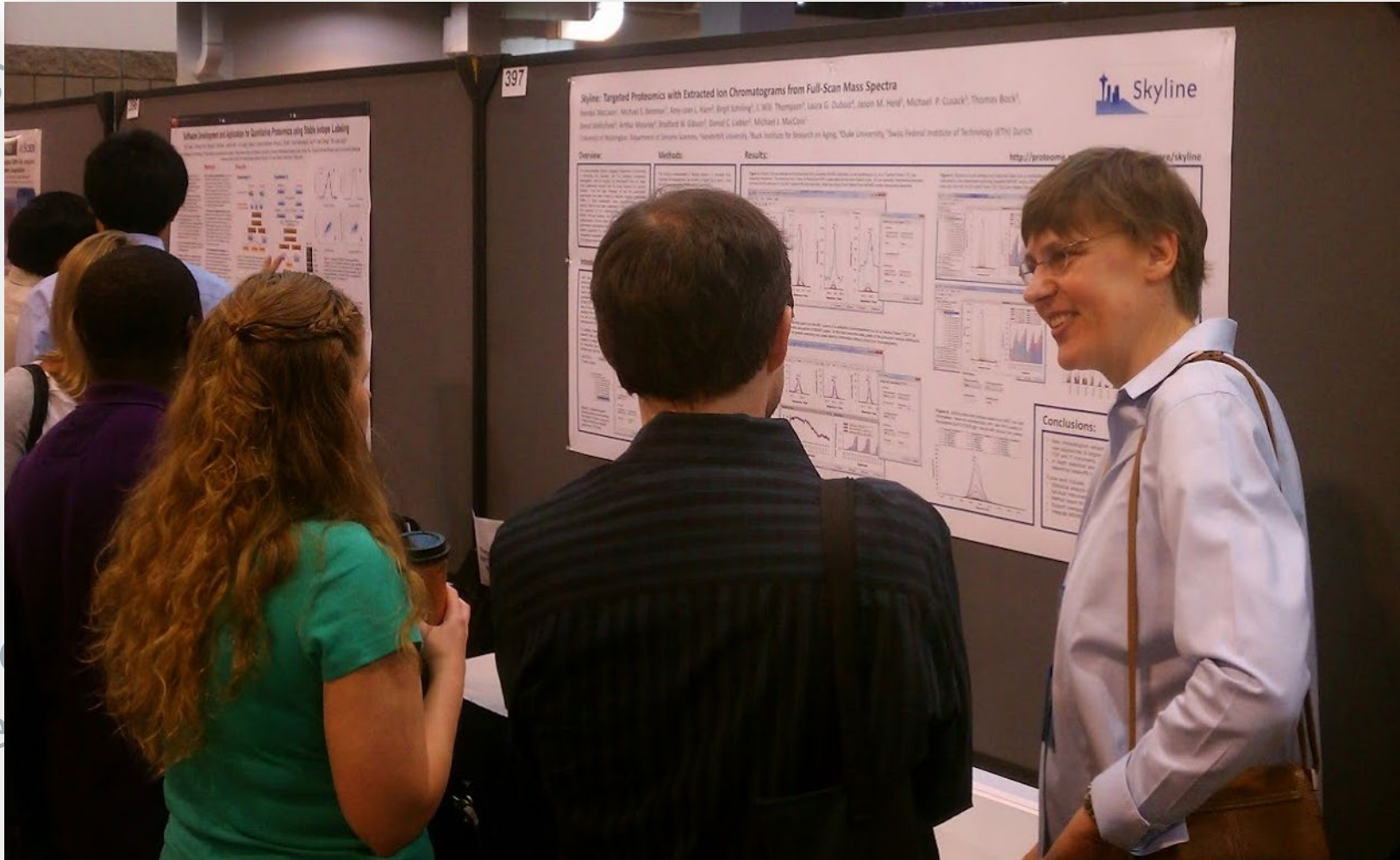
Post-Analysis

Areas we are Utilizing **Skyline** in our Software Pipeline

System S

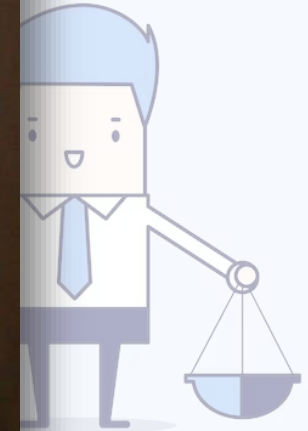


Before
Colle



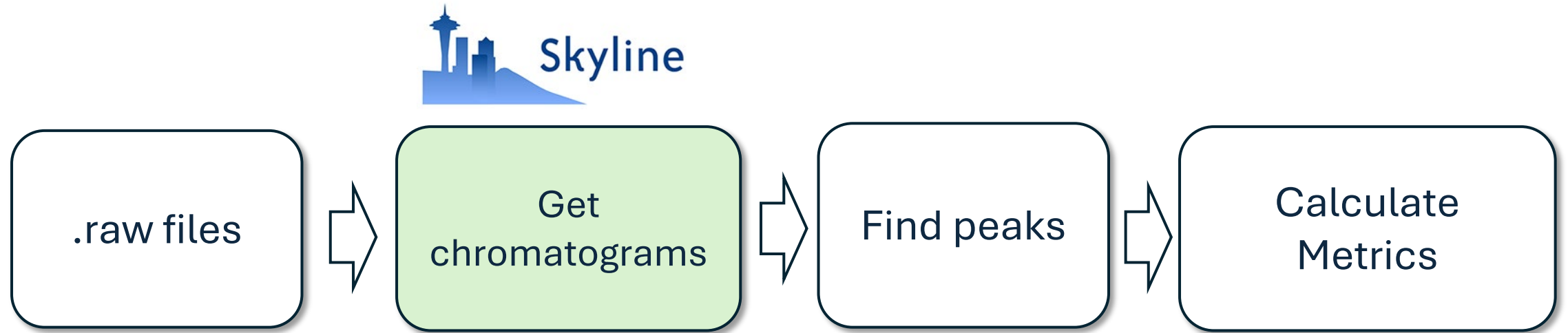
Birgit Schilling, ASMS 2011

ification



Analysis

How we interact with Skyline (SST and Data Check)



For our Product, **Skyline** Provides:

- Template/Library for Compounds
- Raw Data Access/Peak Extraction
- Raw Data Visualization (as desired... “White Box”)

System Suitability in Mass Spectrometry 'Omics

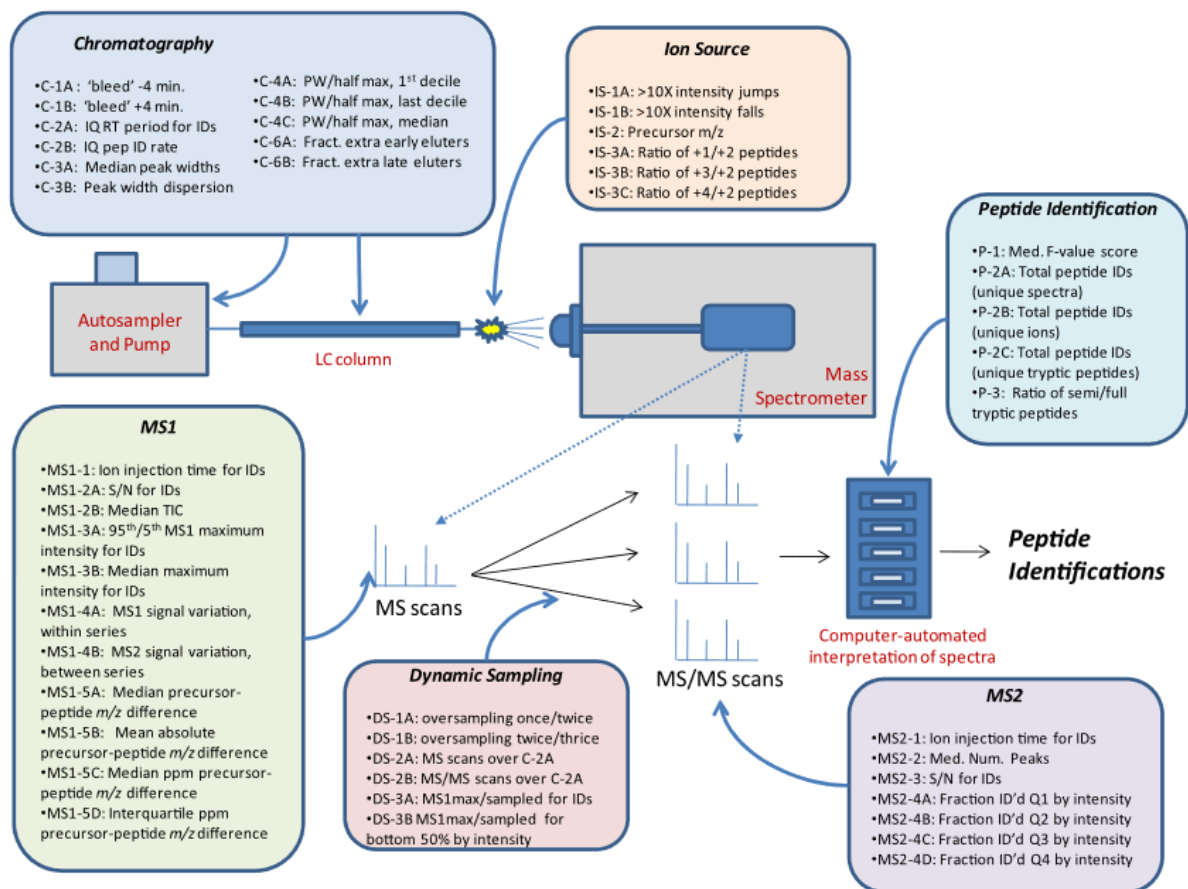
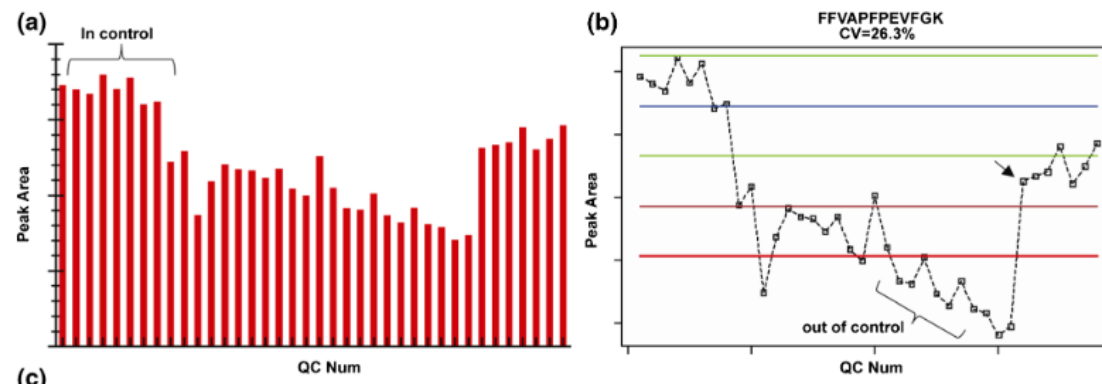


FIG. 1. Schematic representation of performance metrics mapped to LC-MS/MS system elements. PW, peak width; IQ, interquartile; pep, peptide; ID, identification; Med., median; ID'd, identified; Fract., fraction; Num., number.

Rudnick PA et al. *Mol Cell Proteomics*. 2010 Feb;9(2):225-41.

Bereman et al.: Statistical Process Control for Proteomics



Bereman et al. *J Am Soc Mass Spectrom*. 2014 Apr;25(4):581-7.

System Suitability in Mass Spectrometry 'Omics

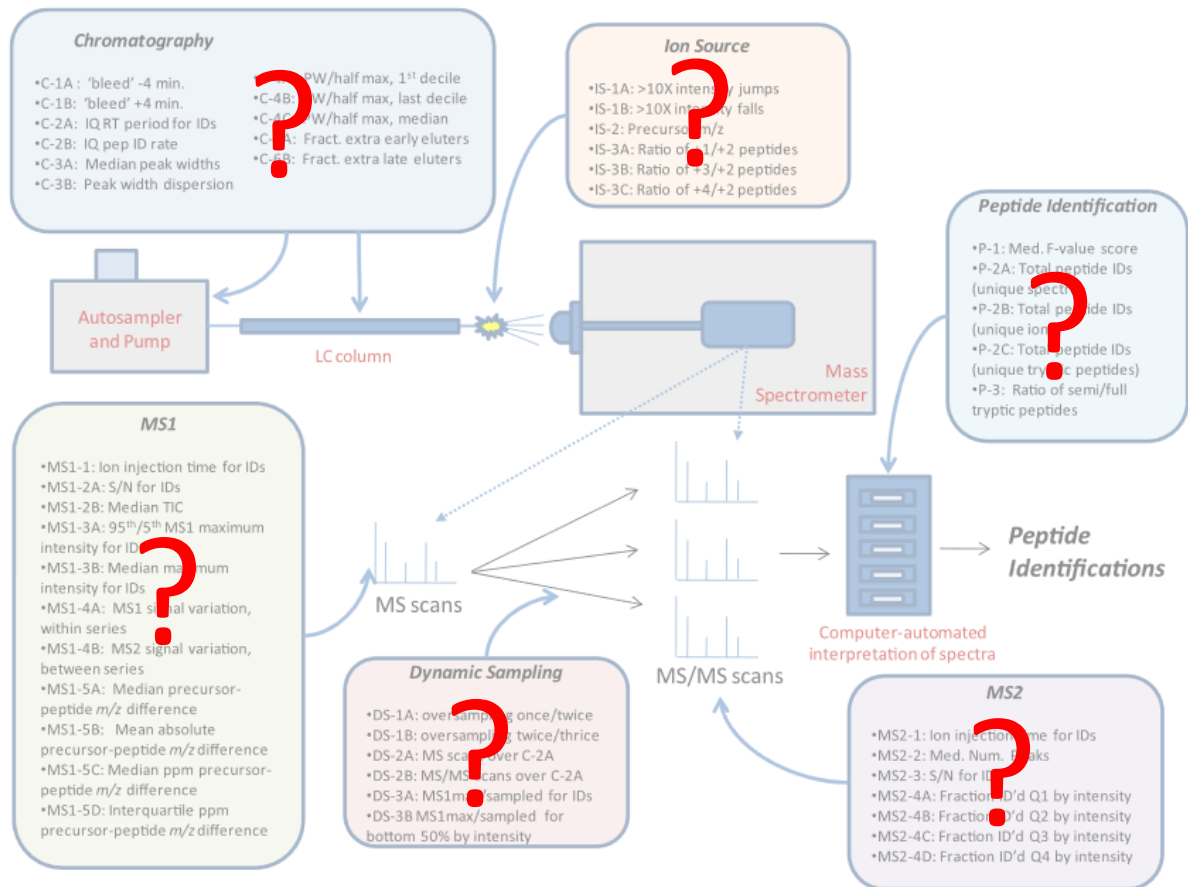
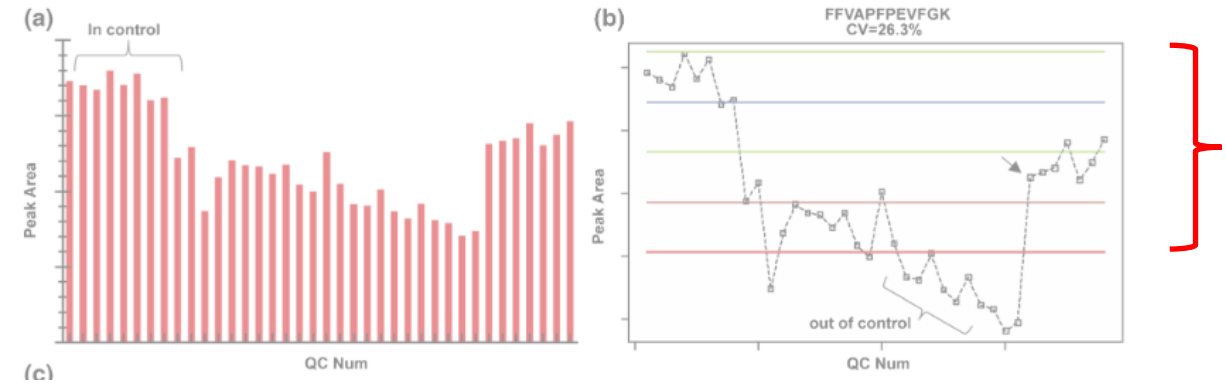


FIG. 1. Schematic representation of performance metrics mapped to LC-MS/MS system elements. PW, peak width; IQ, interquartile; pep, peptide; ID, identification; Med., median; ID'd, identified; Fract., fraction; Num., number.

Rudnick PA et al. *Mol Cell Proteomics*. 2010 Feb;9(2):225-41.

Bereman et al.: Statistical Process Control for Proteomics



Bereman et al. *J Am Soc Mass Spectrom*. 2014 Apr;25(4):581-7.

Key Issues:

1. There are too many possible **metrics**
2. Reference **ranges** are difficult to establish

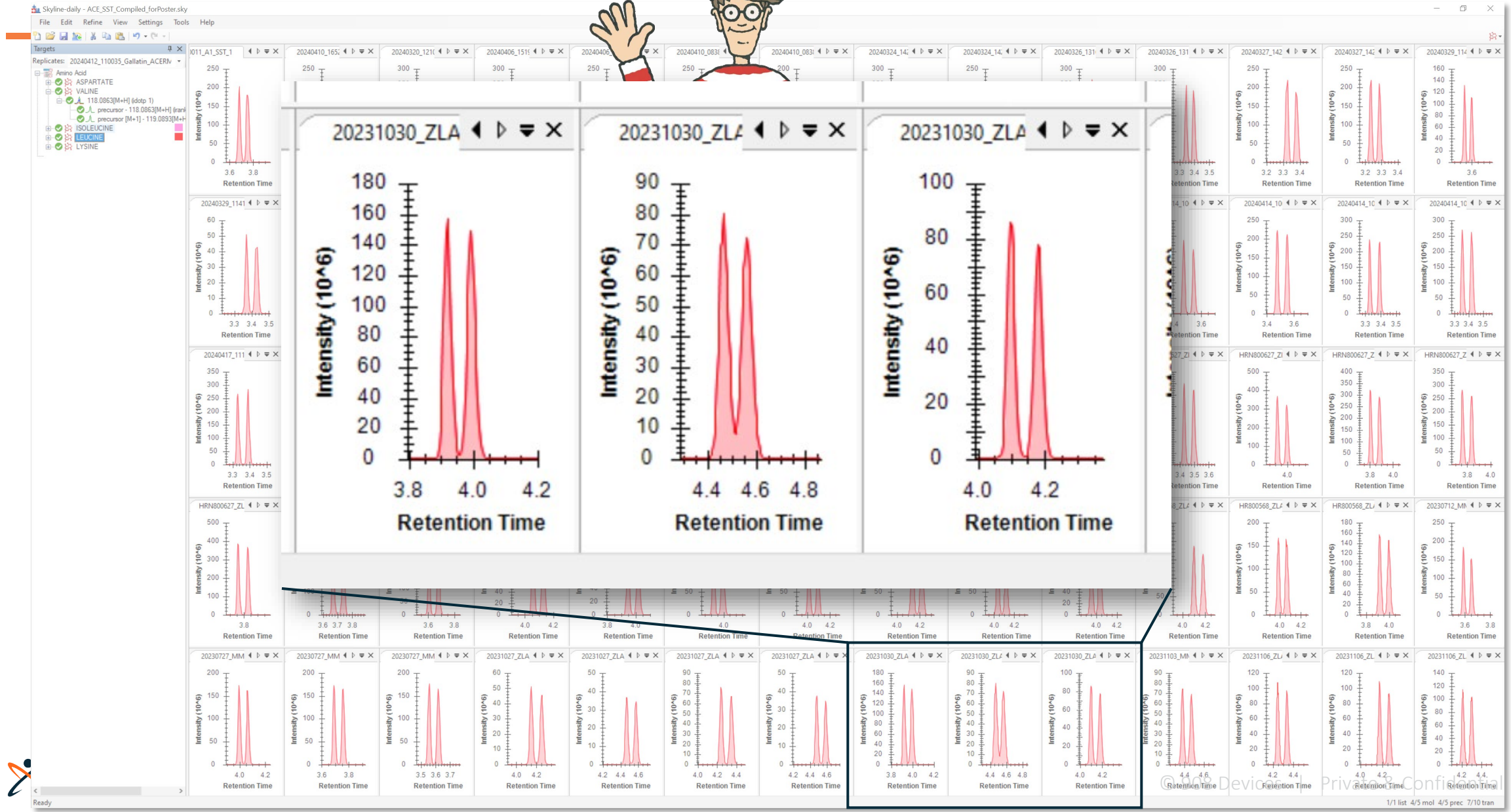
We should:

1. Pick the **simplest** set of metrics that work
2. Pick **fixed** threshold/cutoffs if we can

System Suitability Testing – Importance of Automation



System Suitability Testing – Importance of Automation

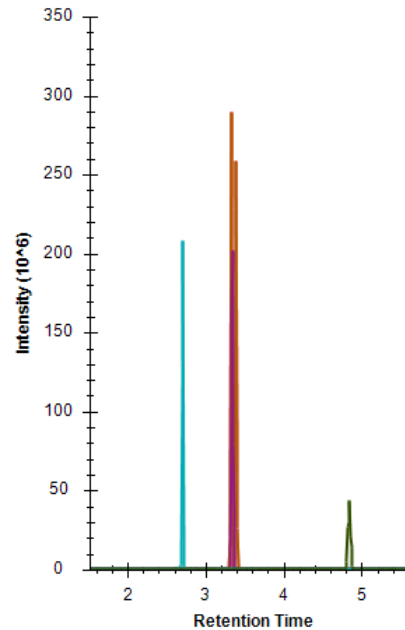
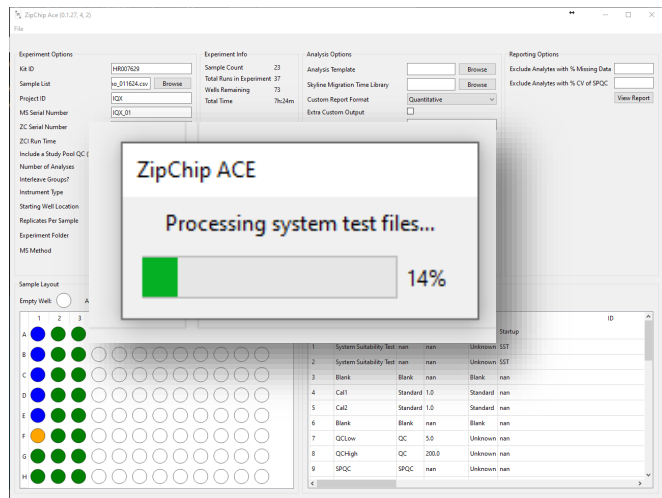


mCZE System Suitability Testing – Integration with Skyline

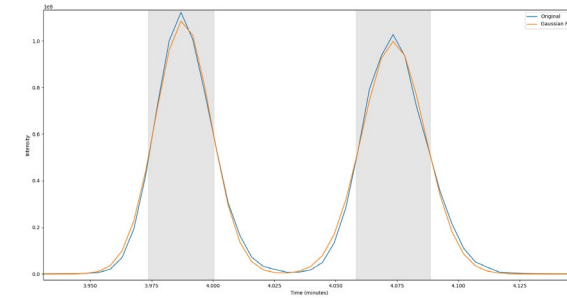
Single “Touchpoint” in ZipChip ACE App

Skyline Template for Extracting Chromatograms

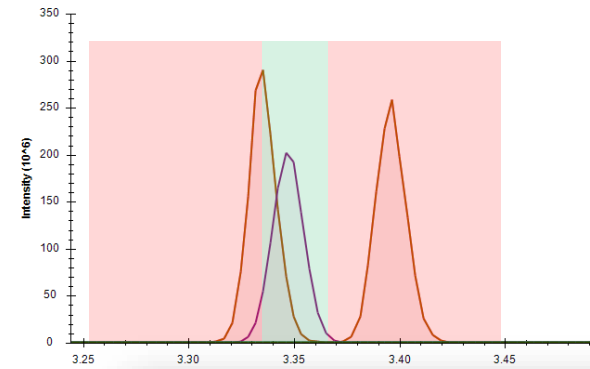
Calculate and Report Metrics



5 analytes, 4 m/z values
 ZipChip HR, Peptides BGE
 Exploris 240 MS
 10 uM Promega AA Mix



Resolution



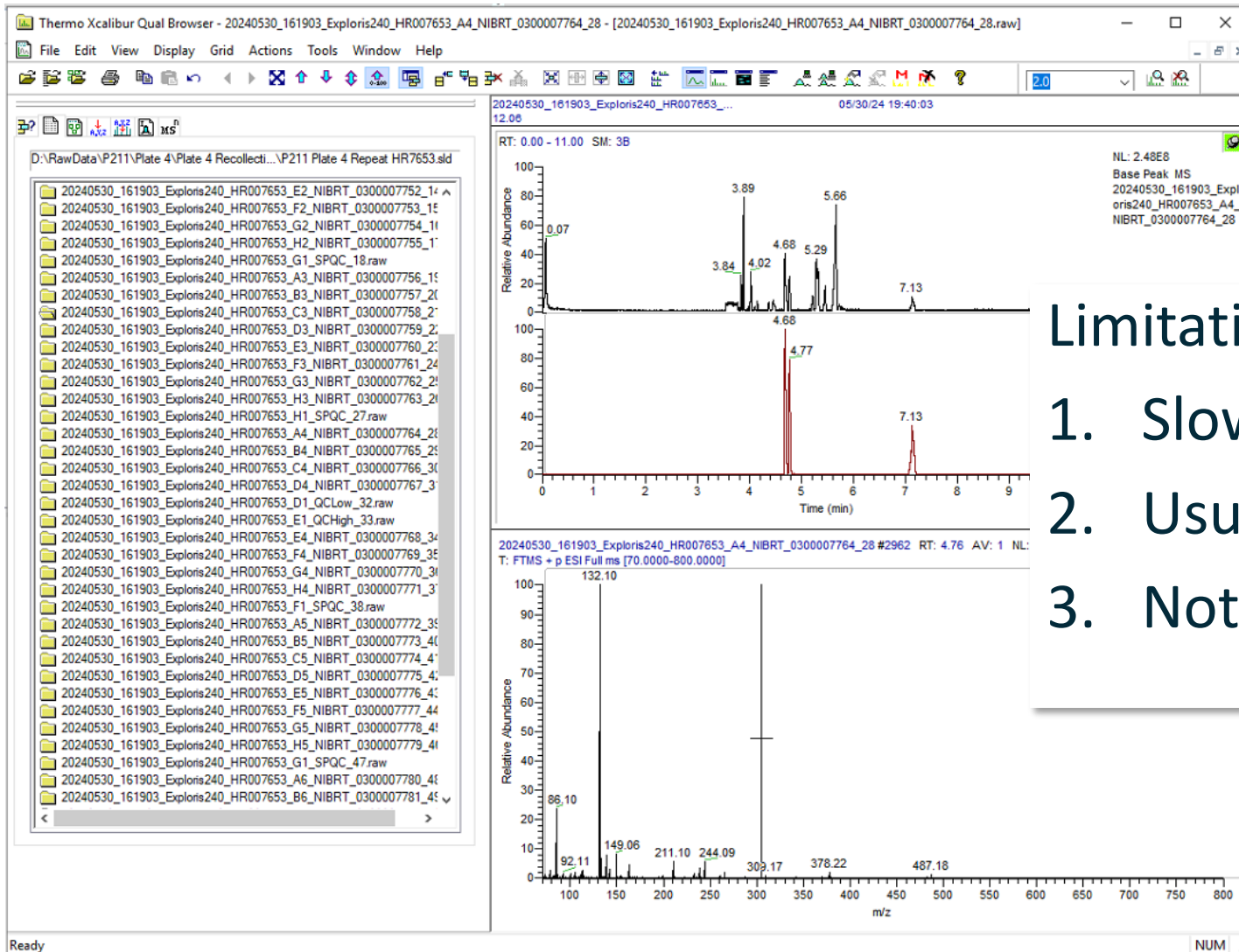
BGE “Quality”
 (effectively, pH)

ZipChip ACE RM system suitability test (SST) is complete and has passed with the following scores. Please proceed with your sample analysis. If samples are not analyzed within 4 hours, we recommend repeating SST before initializing ZipChip ACE RM sample set.

=====
 Resolution: 1.80, Bounds: [1.25, inf], Status: Passed.
 BGE Quality: 0.28, Bounds: [0.0, 0.5], Status: Passed.
 Lysine Migration Time (minutes): 2.91, Bounds: [2.5, 3.6], Status: Passed.
 Aspartate Migration Time (minutes): 6.03, Bounds: [2.5, 8.0], Status: Passed.

OK

Data Check Standard Practice: Manual Data “Check”

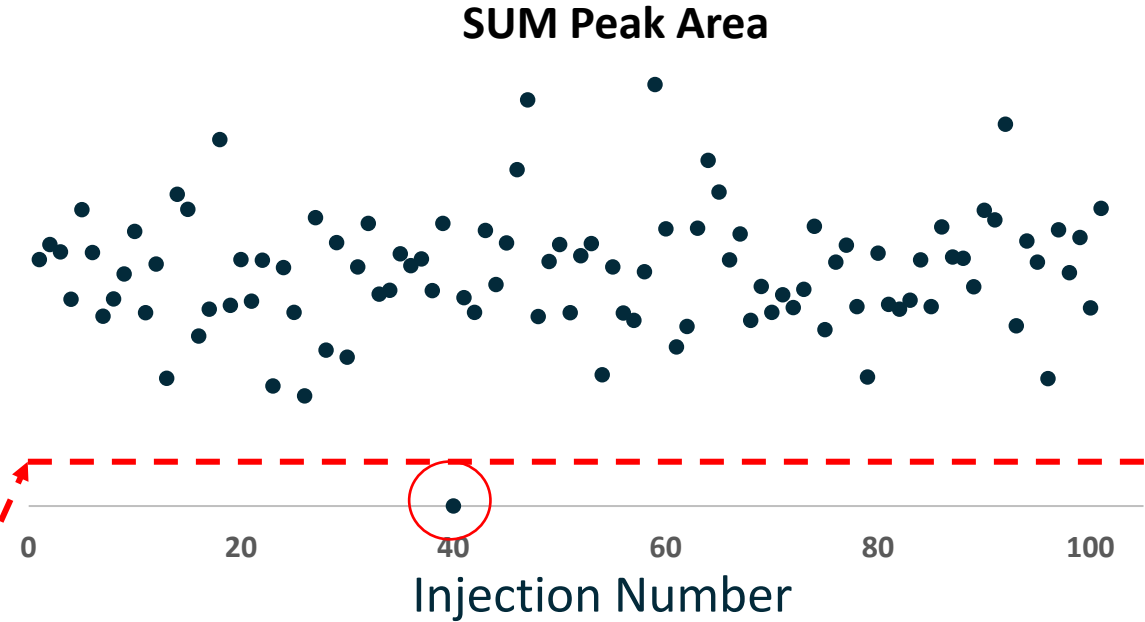
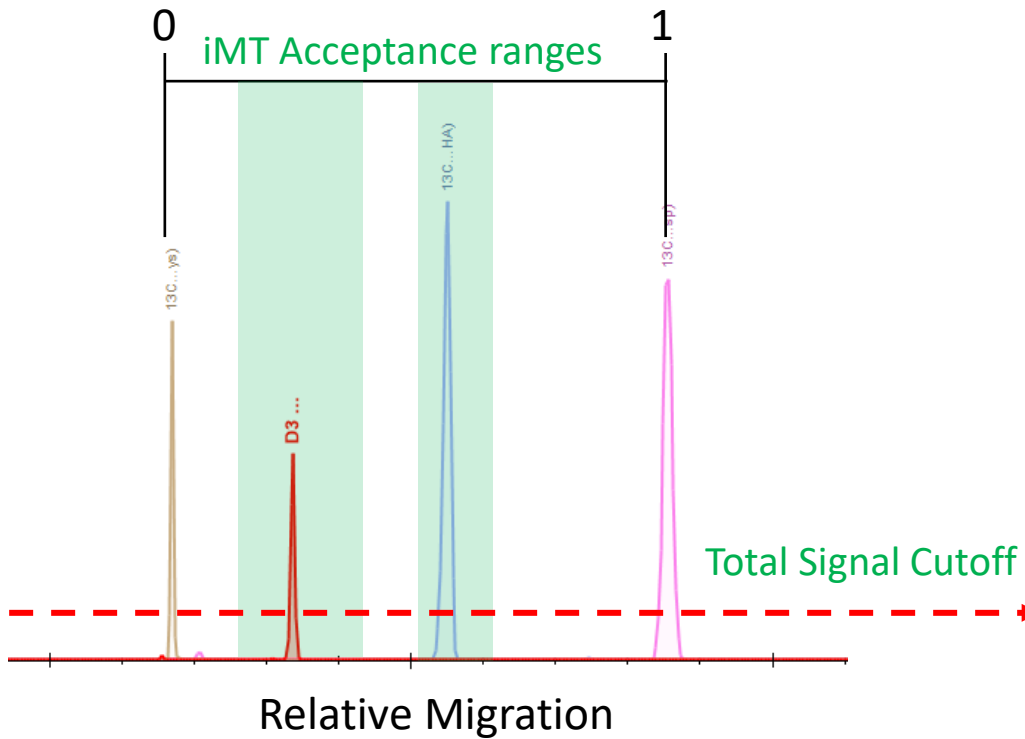


Limitations to Manual Data Check

1. Slow
2. Usually a spot-check only
3. Not a quantitative assessment

“Data Check” Acceptance Criteria

Uses 4 Method-Specific Internal Standards



“Data Check” Acceptance Criteria

Uses

ZipChip ACE (0.1.27, 4, 2)

Experiment Options: Kit ID: HR007629, Sample List: jessing_ACEDemo_011624.csv, Project ID: IQX, MS Serial Number: IQX_01, ZC Serial Number: ZLA123, ZCI Run Time: 11.0, Include a Study Pool QC (SPQC): , Number of Analyses: 3, Interleave Groups?: , Instrument Type: Thermo, Starting Well Location: A1, Replicates Per Sample: 3, Experiment Folder: , MS Method: , Sample Layout: Empty Well:

Experiment Info: Sample Count: 23, Total Runs in Experiment: 37, Wells Remaining: 73, Total Time: 7h:24m

Analysis Options: Analysis Template: [Browse], Skyline Migration Time Library: [Browse], Custom Report Format: Quantitative, Extra Custom Output: , Bias of Calibration Points to Exclude: 20%, SST Status: Passed, File Check Status: Must Re-run, Failed Data Check: 1, Passed Data Check: 36, Process SST, Data Check, Process Samples, 908 AI

Reporting Options: Exclude Analytes with % Missing Data: [], Exclude Analytes with % CV of SPQC: [], View Report

The ZipChip ACE quality check has detected that a subset of your runs appear to have been missed injections. These runs have been annotated in your run list and a new run list has been exported containing the samples that should be re-analyzed.

Recollect Ignore Bad Runs

Filename	Injection Number
Human Serum Gender Pooled, PN HUMANSRM-0000351, Lot HMN1211904	B2
Human Serum Gender Pooled, PN HUMANSRM-0000351, Lot HMN1211904	G2
Human Serum Gender Pooled, PN HUMANSRM-0000351, Lot HMN1211904	D2
Human Plasma K2EDTA Gender Pooled Heat Inactivated, PN HUMANPLK2-0122691	E2
Human Plasma K2EDTA Gender Pooled Heat Inactivated, PN HUMANPLK2-0122691	F2
Human Plasma K2EDTA Gender Pooled Heat Inactivated, PN HUMANPLK2-0122691	G2
nan	F1
nan	D1
nan	E1
Human Plasma K2EDTA Gender Pooled Heat Inactivated, PN HUMANPLK2-0122691	H2
Human Plasma K2EDTA Gender Pooled Heat Inactivated, PN HUMANPLK2-0122691	A3
Human Plasma K2EDTA Gender Pooled Heat Inactivated, PN HUMANPLK2-0122691	B3
Human Urine, Single donor, Sampled 11/29/2023	C3
Human Urine, Single donor, Sampled 11/29/2023	D3
Human Urine, Single donor, Sampled 11/29/2023	E3
Human Urine, Single donor, Sampled 11/29/2023	F3
Human Urine, Single donor, Sampled 11/29/2023	G3

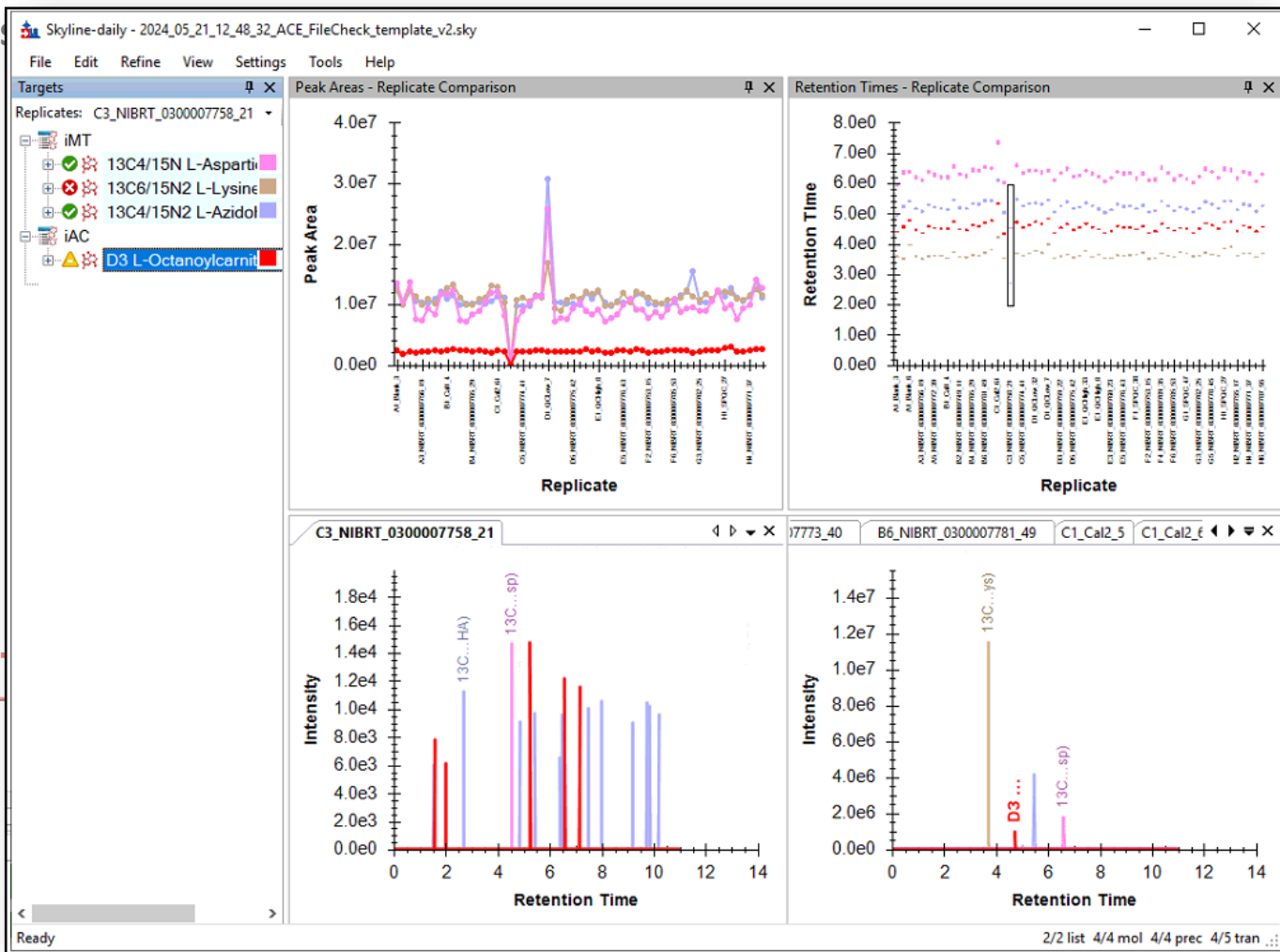
SUM Peak Area



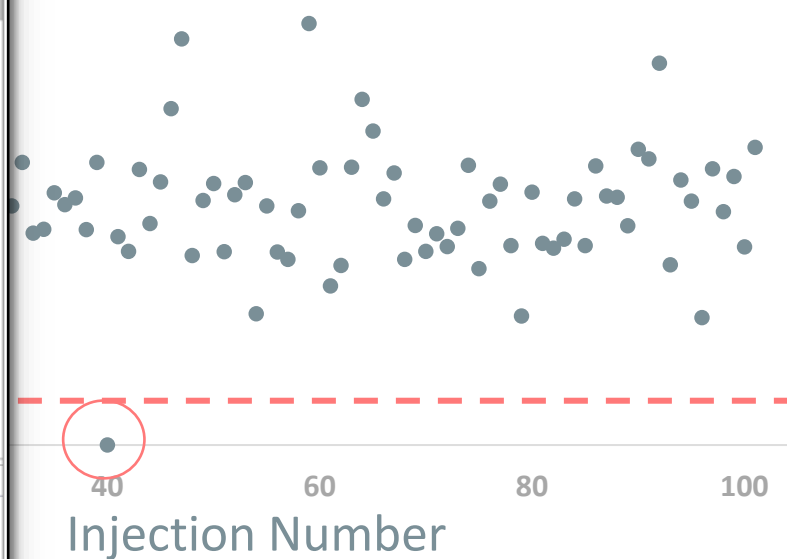
Injection Number

“Data Check” Acceptance Criteria

Use

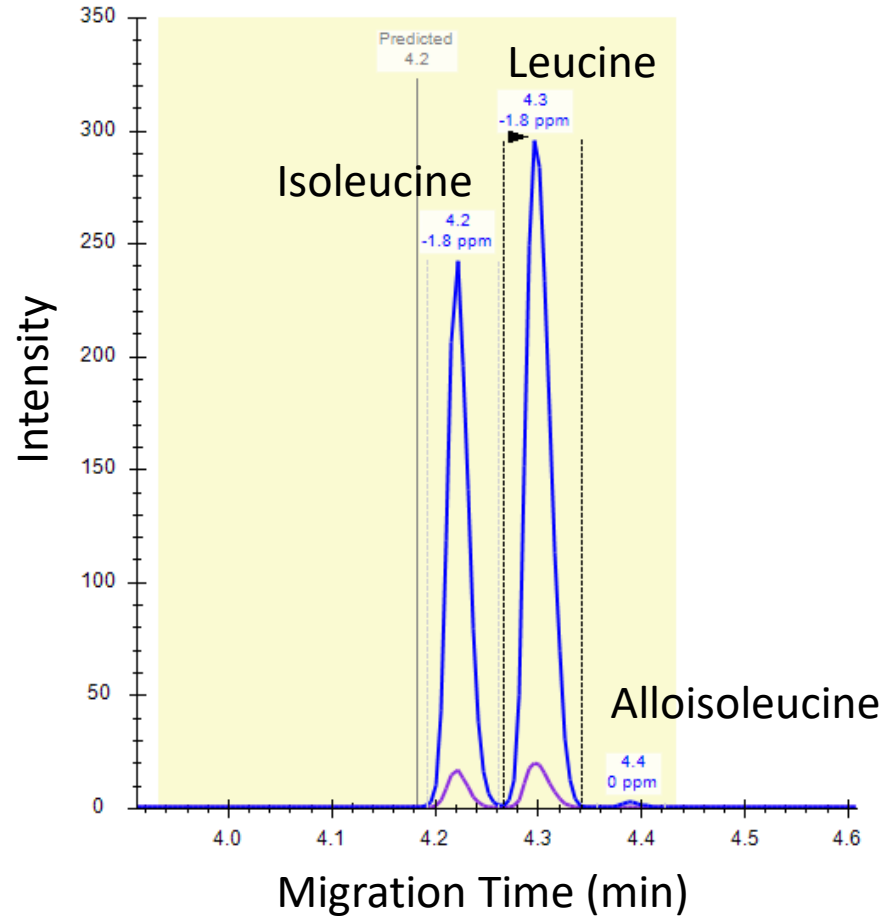


SUM Peak Area

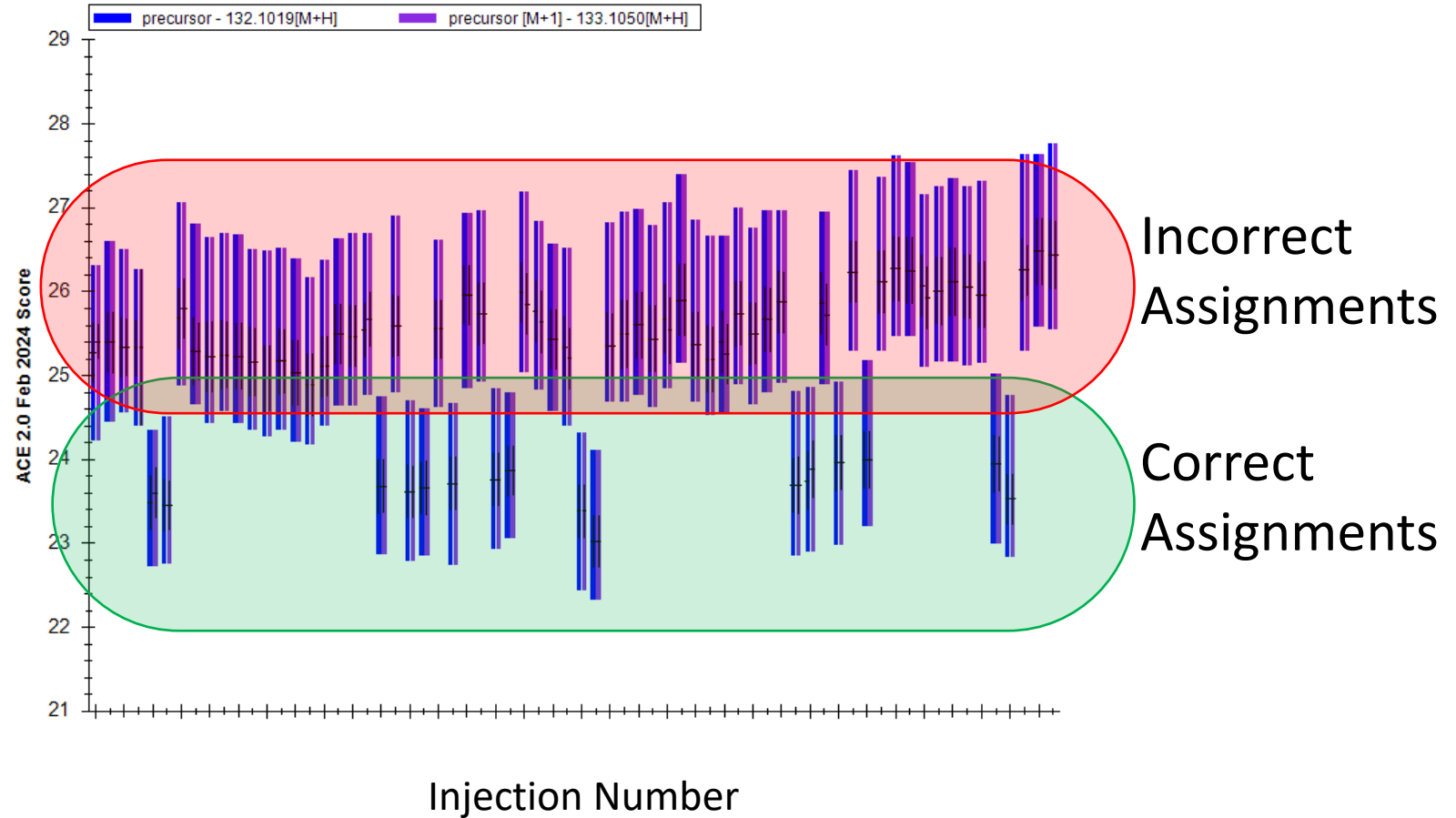
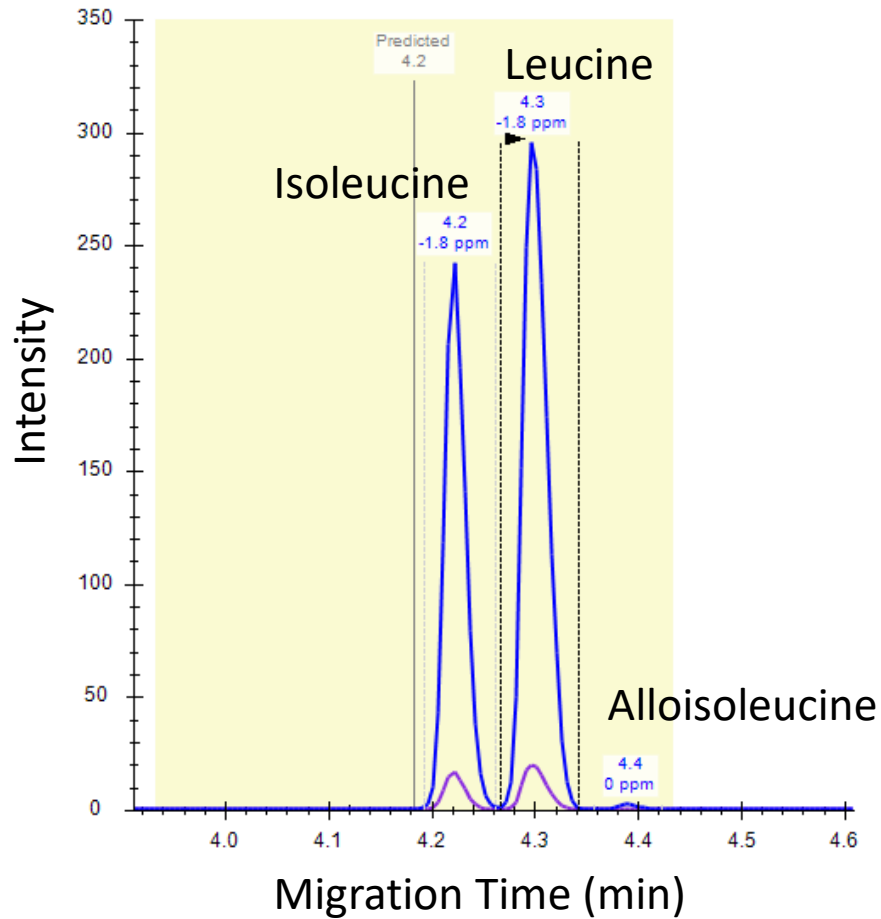


Injection Number

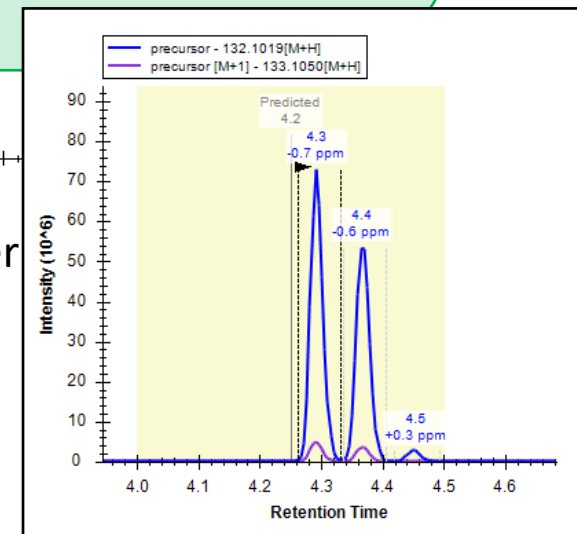
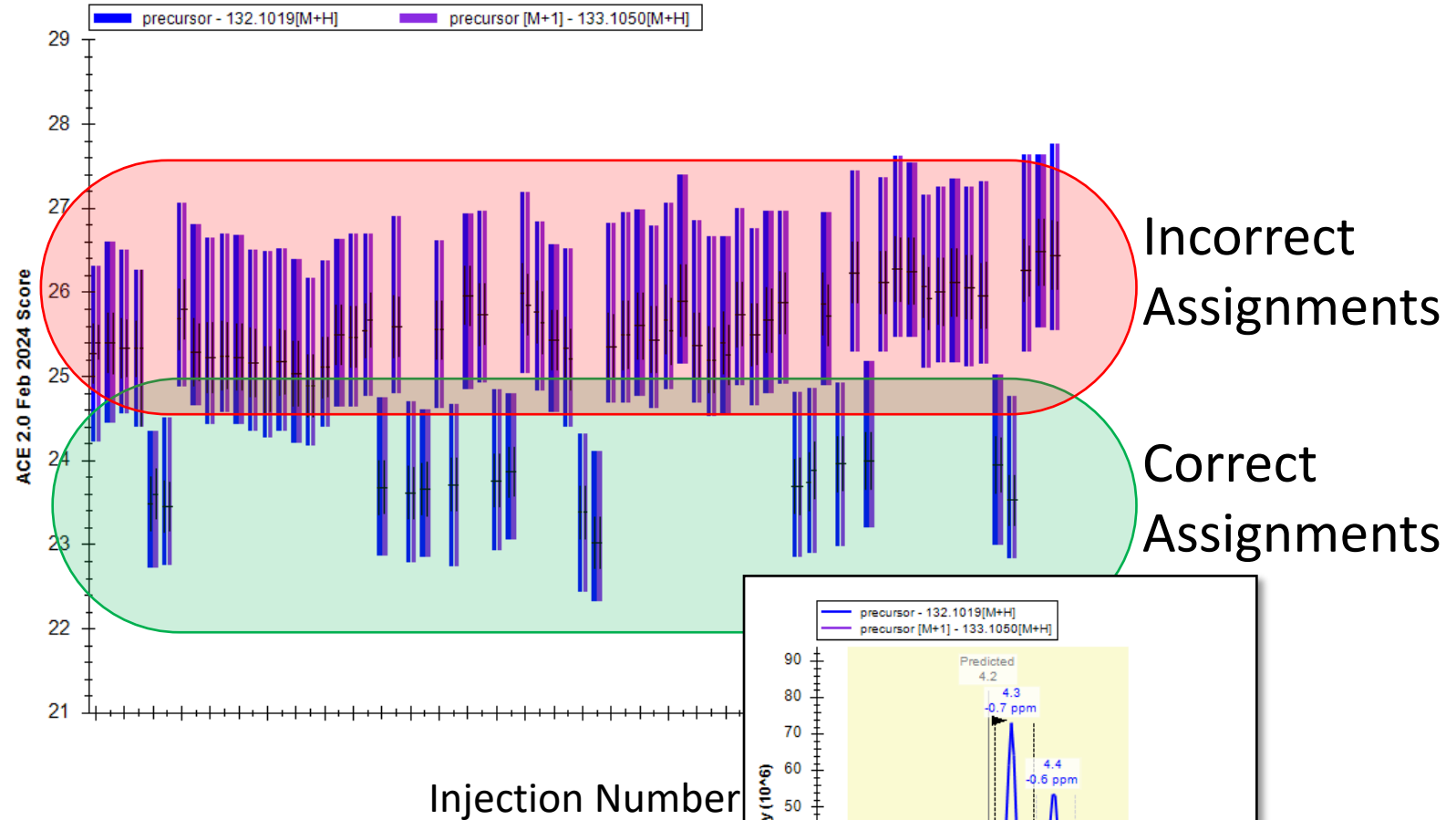
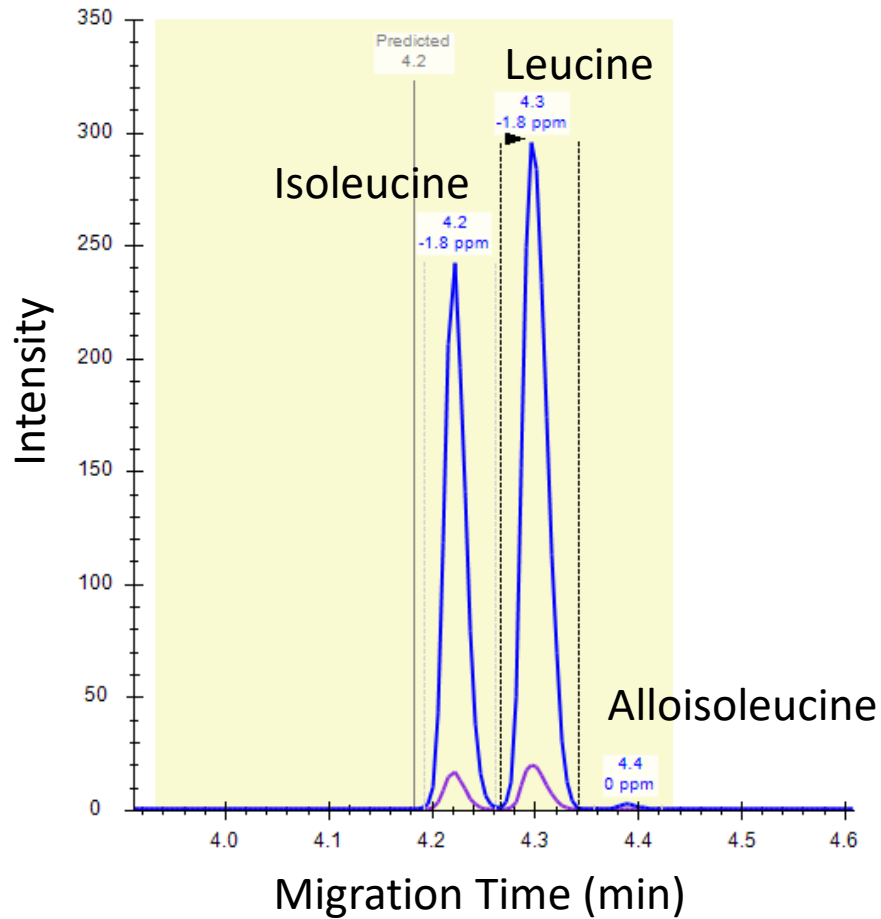
The Most Common and Tedious Problem In Targeted Metabolomics is Peak Assignment for Closely-Eluting, Isobaric Compounds



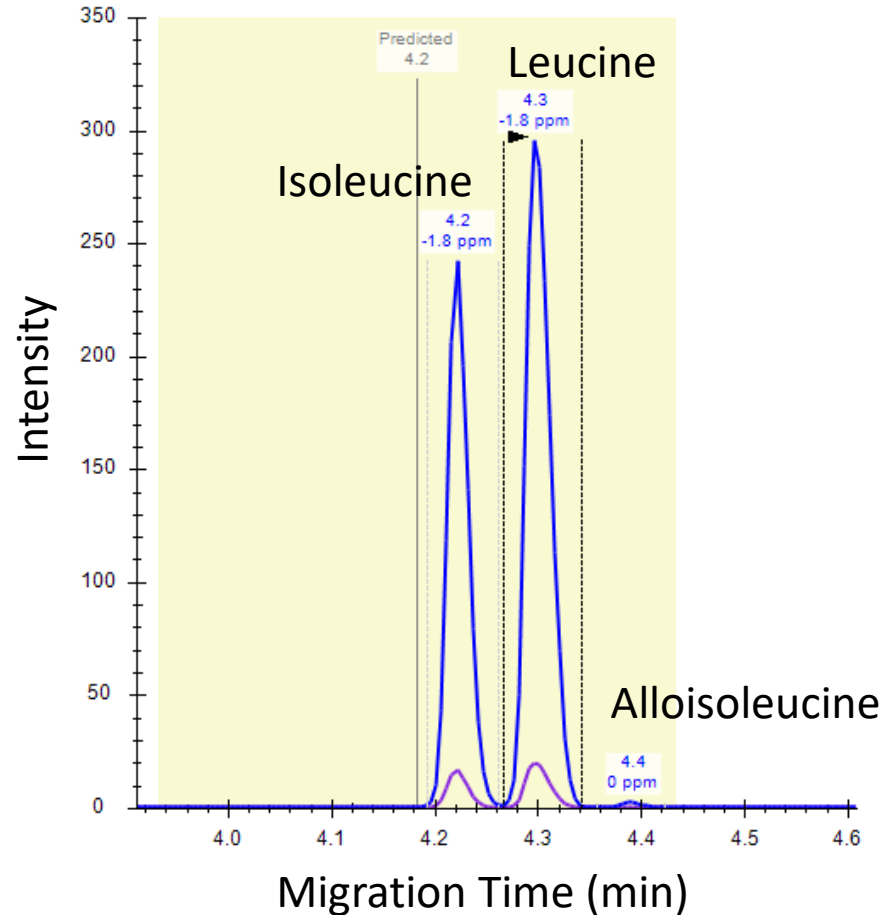
No Targeted Analysis Software is Immune, Including Skyline



No Targeted Analysis Software is Immune, Including Skyline



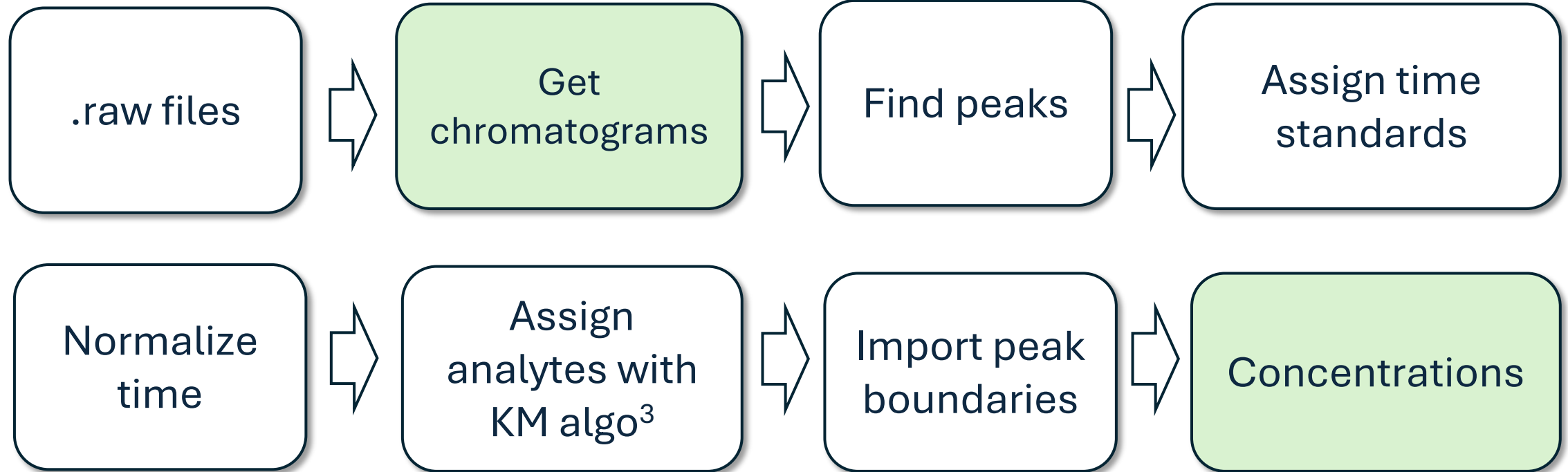
The Mis-assignments Are Made Largely for Two Simple Reasons



Skyline has No “Knowledge” of:

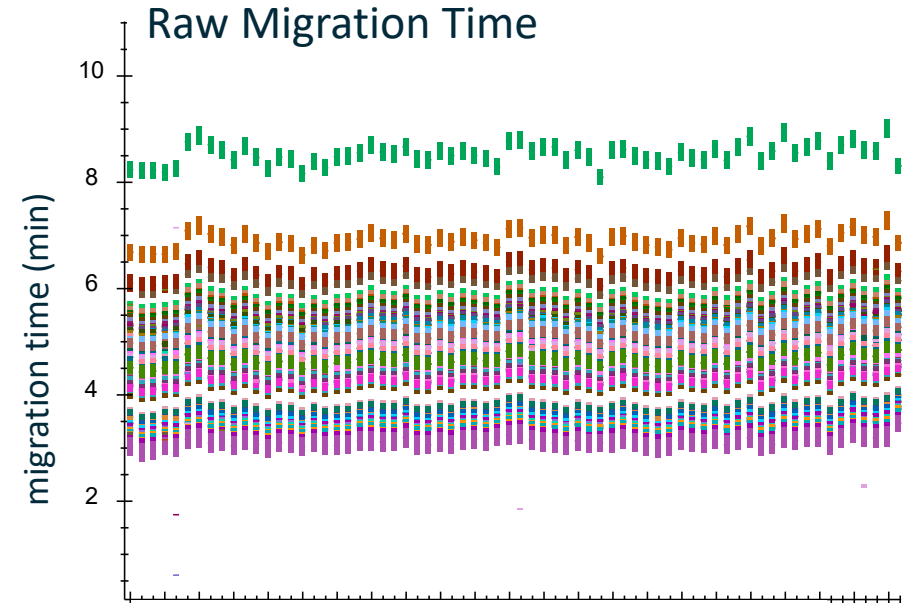
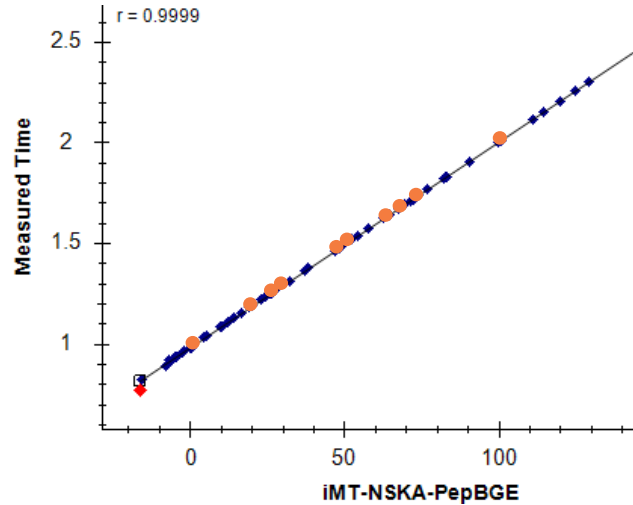
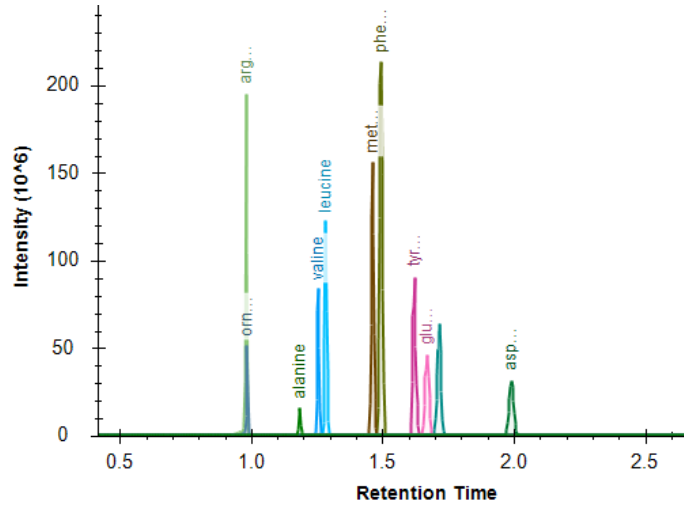
- 1. Relative elution** time of analytes
- Whether or not a peak has **already been assigned** to another analyte

How we interact with Skyline (Metabolite Quantification)

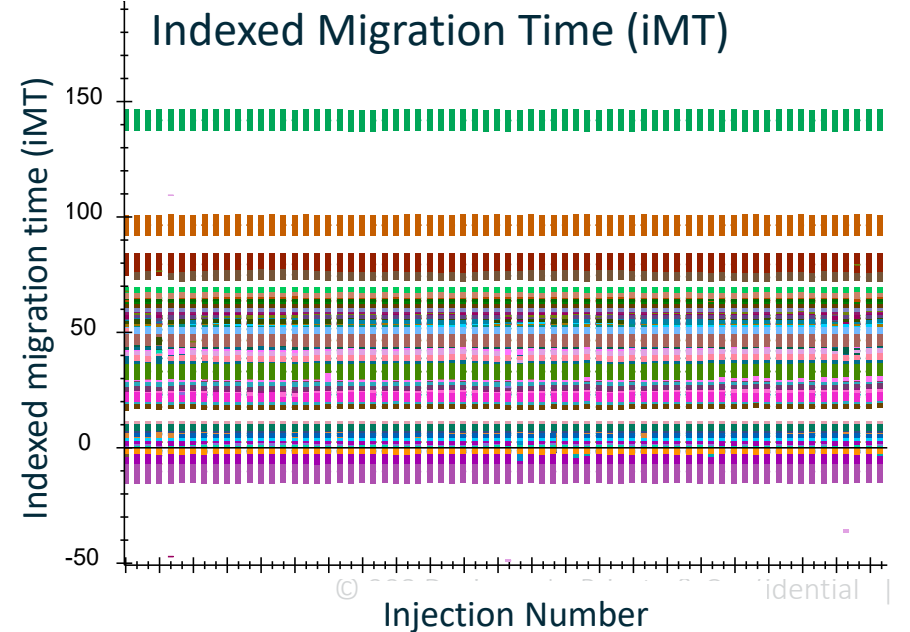
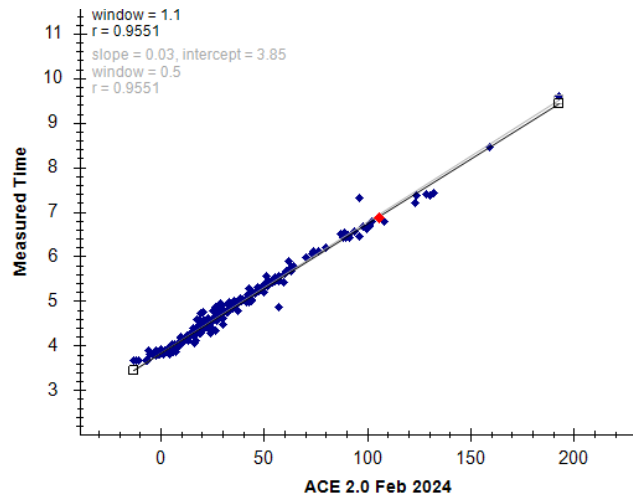
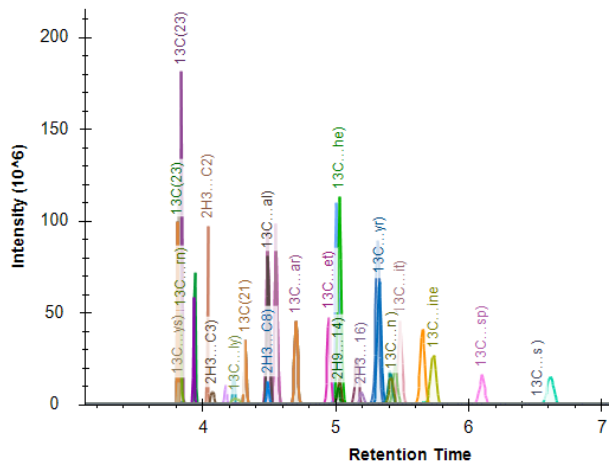


Start by adopting the iRT Approach for microchip CZE

CIL "NSK-A" as iMT Standards (2021) - ~60 library compounds



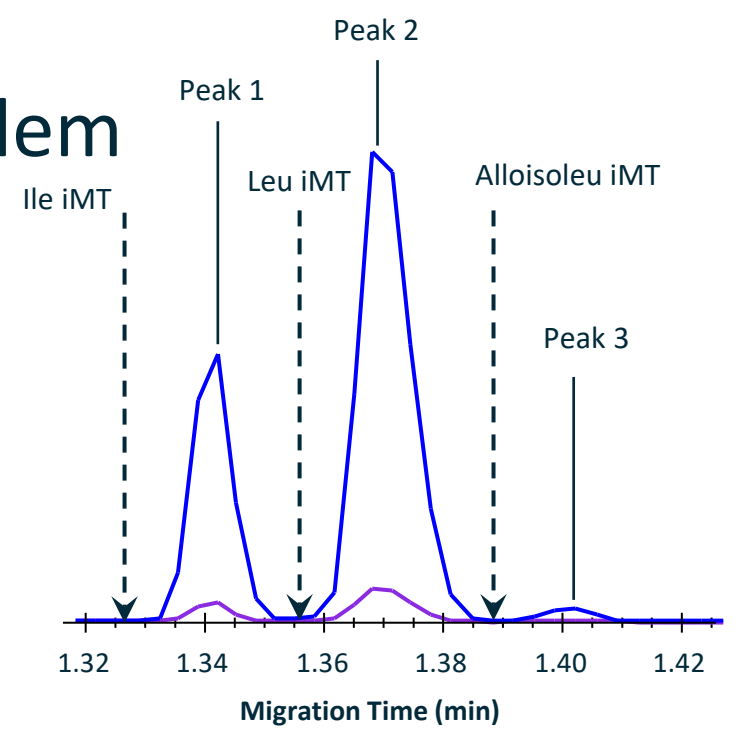
ZipChip ACE iMT Standards (2023) - ~325 library compounds



KM Algorithm³ for Linear Sum Assignment Problem

We Require Two Conditions Are Met:

1. We minimize the total distance between library and observed peaks
2. No two library analytes can be assigned to same observed peak



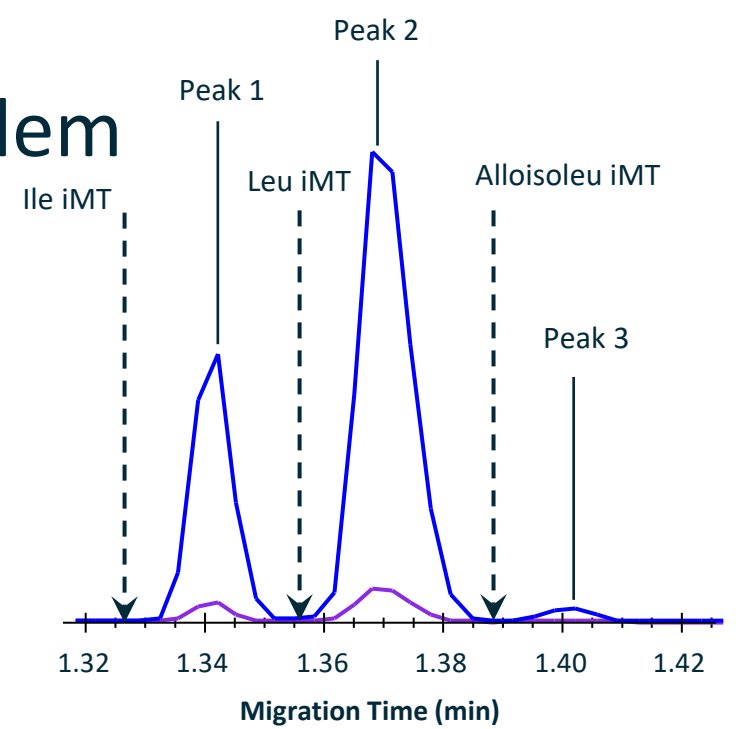
isoleucine	0.015 ✓	0.03	0.055
leucine	0.015	0.015 ✓	0.03
alloisoleucine	0.03	0.015 ✓	0.016
	Peak 1	Peak 2	Peak 3

Library List

KM Algorithm³ for Linear Sum Assignment Problem

We Require Two Conditions Are Met:

1. We minimize the total distance between library and observed peaks
2. **No two library analytes can be assigned to same observed peak**



isoleucine	0.015 ✓	0.03	0.055
leucine	0.015	0.015 ✓	0.03
alloisoleucine	0.03	0.015 ✗	0.016 ✓
	Peak 1	Peak 2	Peak 3

Library List

A green arrow points from the 0.015 value in the alloisoleucine row, Peak 2 column to the 0.016 value in the alloisoleucine row, Peak 3 column.

Approach for Comparison of **KM Algo** versus **Skyline**

- Goals

- > Benchmark KM algo performance in a relevant complex dataset
- > Understand where performing well and where needs work

- Approach

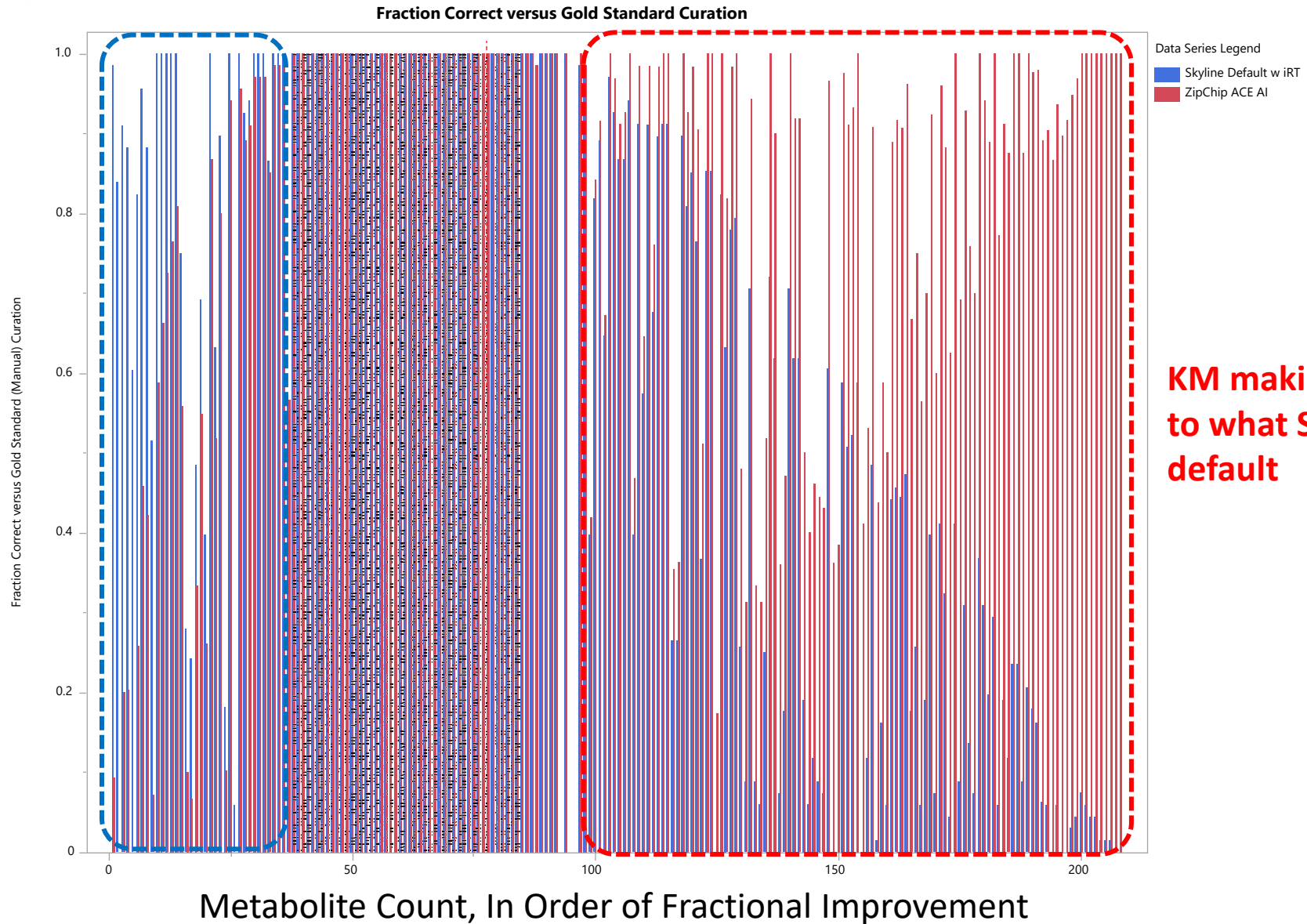
- > ZipChip ACE was run on a set of 52 Bioreactor media samples plus standards and QCs
- > Import Data into Skyline automatically using the ACE App
- > Compare the results of default Skyline processing to 908 Algo post-processing

- Method For Comparison

- > Compared the Apex Time for the “Gold Standard” curated dataset against Skyline Default and 908 Algo approaches.
- > Selection was considered “correct” if **retention time** of that selected was within 1% of gold standard peak selection

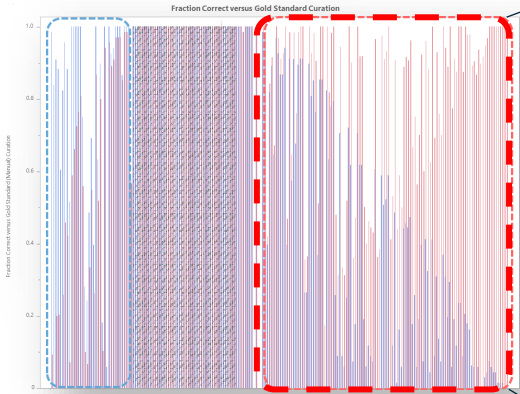
Metric For Comparison, Fraction “Correct” In Both Cases

KM “Breaking” What Skyline Does by Default

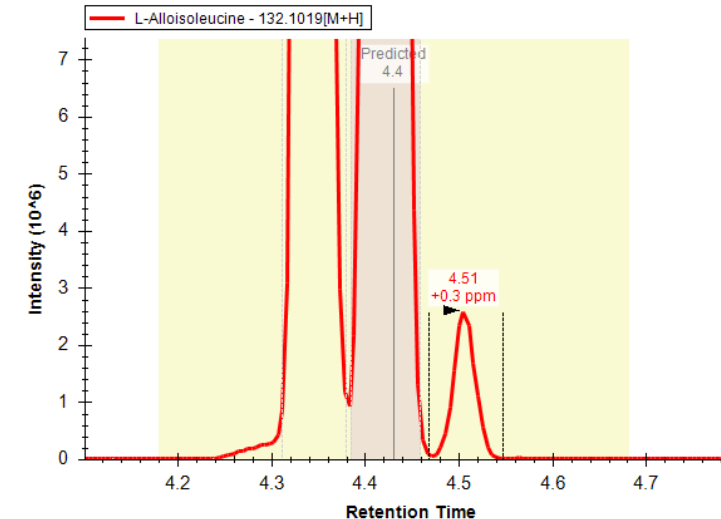
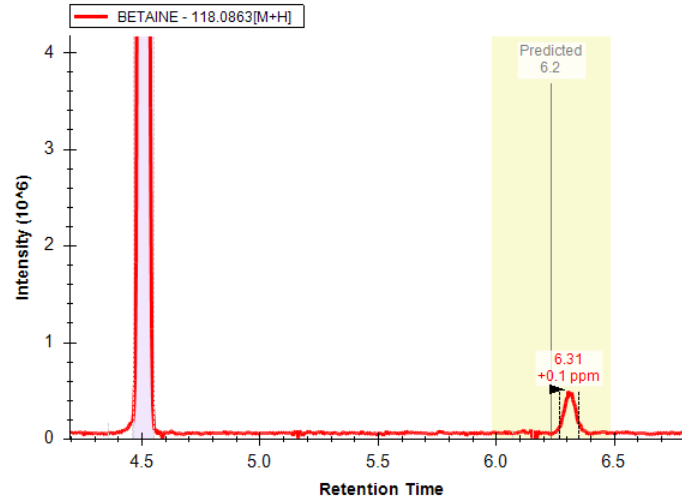
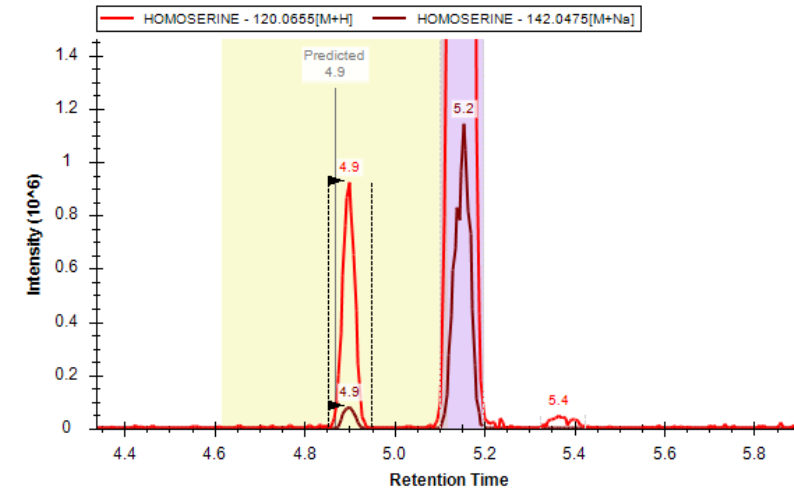


KM making Improvements to what Skyline does by default

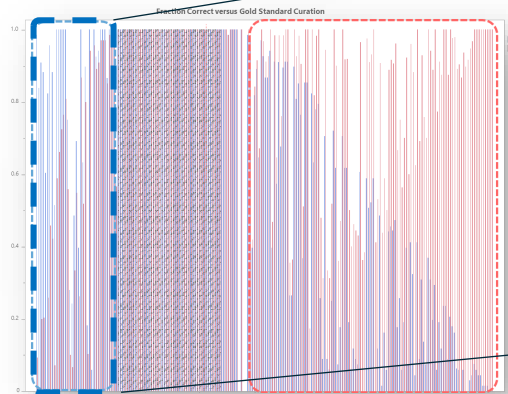
KM Algorithm is solving some time-intensive assignment problems



Molecule List Name	Molecule Name	% Correct Auto	% Correct 908AI	Improvement %
Acylcarnitine	Valerylcarnitine (C5)	19.7%	88.9%	69.2%
Dipeptide	Histidinyl-Valine	29.4%	100.0%	70.6%
Polyamine	N-ACETYLPUTRESCINE	5.9%	77.3%	71.4%
Vitamin	BETAINE	19.1%	91.2%	72.1%
Noncanonical AA	5-Methoxytryptophan	11.8%	87.5%	75.7%
IS	13C6/15N L-Leucine (Leu)	23.5%	100.0%	76.5%
Canonical AA	ISOLEUCINE	23.5%	100.0%	76.5%
Dipeptide	Alanylglutamic acid	8.8%	87.5%	78.7%
Noncanonical AA	2-Phenylglycine	20.6%	100.0%	79.4%
Dipeptide	Lysyl-Leucine	17.9%	97.7%	79.8%
Purine/Pyrimidine	3-METHYLADENINE	16.2%	97.9%	81.7%
Noncanonical AA	Homo-L-arginine	6.3%	89.1%	82.8%
Noncanonical AA	L-Alloisoleucine	5.9%	90.3%	84.4%
Other	5-AMINOPENTANOATE	0.0%	86.7%	86.7%
Noncanonical AA	CIS-4-HYDROXY-D-PROLINE	5.9%	93.7%	87.8%
Noncanonical AA	BETA-ALANINE	0.0%	89.7%	89.7%
Noncanonical AA	ALLOTHREONINE	0.0%	91.7%	91.7%
Dipeptide	Glycyl-Glycine	3.0%	94.7%	91.7%
Noncanonical AA	HOMOSERINE	4.4%	96.8%	92.4%
Dipeptide	Alanyl-Alanine	7.5%	100.0%	92.5%
Canonical AA	CITRULLINE	5.9%	100.0%	94.1%
Dipeptide	N-Methyl-L-proline	4.4%	100.0%	95.6%
Dipeptide	L-Phenylalanyl-L-proline-2	4.4%	100.0%	95.6%
Noncanonical AA	N-METHYLASPARTATE	1.5%	100.0%	98.5%
Dipeptide	Valyl-Glycine	1.5%	100.0%	98.5%
Dipeptide	Threoninyl-Leucine	1.5%	100.0%	98.5%
Dipeptide	Glycylvaline	0.0%	100.0%	100.0%
Dipeptide	Glycyl-Glutamate	0.0%	100.0%	100.0%

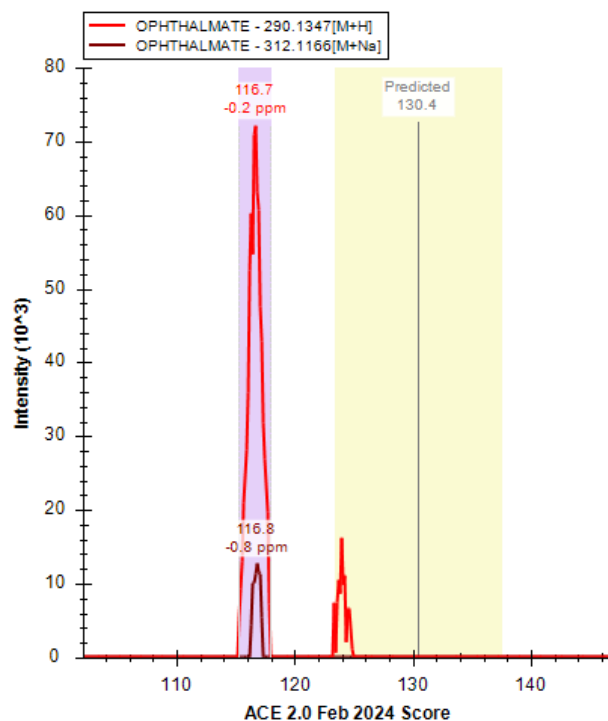


KM Algorithm needs 'tuning' for some cases

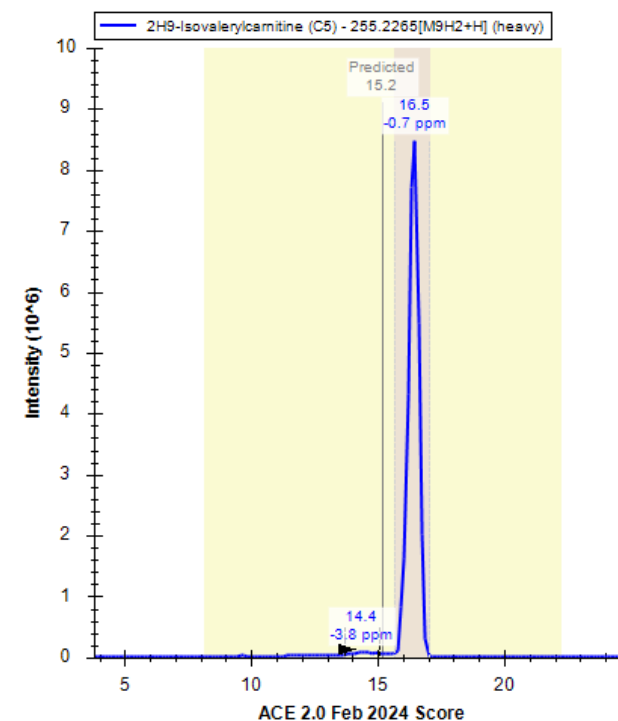


Molecule List Name	Molecule Name	% Correct Auto	% Correct 908AI	Improvement %
Dipeptide	epsilon-(gamma-Glutamyl)lysine	98.5%	9.3%	-89.2%
Dipeptide	N2-gamma-Glutamylglutamine	83.8%	0.0%	-83.8%
Vitamin	NICOTINAMIDE	90.9%	20.0%	-70.9%
Sugar	GLUCOSAMINE	88.2%	20.3%	-67.9%
Other	OPHTHALMATE	60.3%	0.0%	-60.3%
Purine/Pyrimidine	DEOXYCYTIDINE	82.4%	25.8%	-56.5%
Redox	GLUTATHIONE REDUCED	95.6%	45.8%	-49.8%
Noncanonical AA	N,N-DIMETHYLARGININE	88.2%	42.2%	-46.0%
Neurotransmitter/Modulator	ACETYLCHOLINE	51.5%	7.1%	-44.3%
IS	2H9-Isovalerylcarnitine (C5)	100.0%	58.8%	-41.2%
IS	2H9-Carnitine (CN)	100.0%	66.2%	-33.8%

Nonlinear iMT calibration



Noise filtering if # peaks > # targets



Conclusions and Future Directions

- Skyline provides a powerful tool to “build from” for commercial solutions
- KM algorithm has general applicability in quantitative mass spec
- We plan to publish and open-source our algo solution
- If you’d like to work with us or hear more, please contact me!

wthompson@908devices.com

Booth 159

MP405

TP423

THANK YOU!





Extra slides

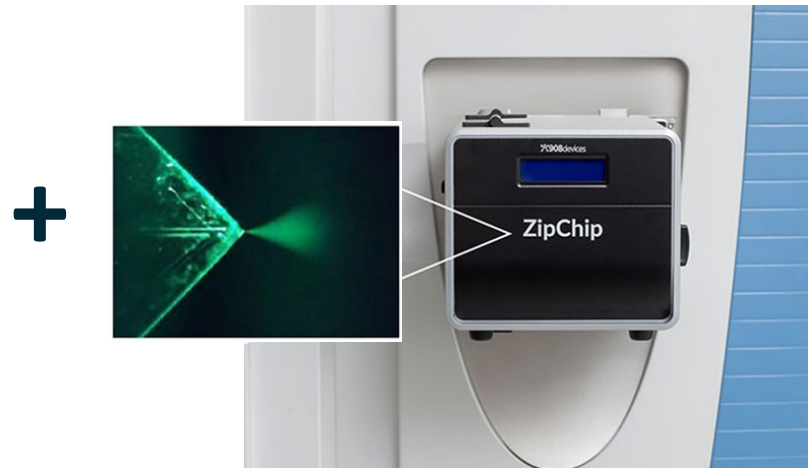
Metabolomics with ZipChip (microchip CZE-MS)

KIT



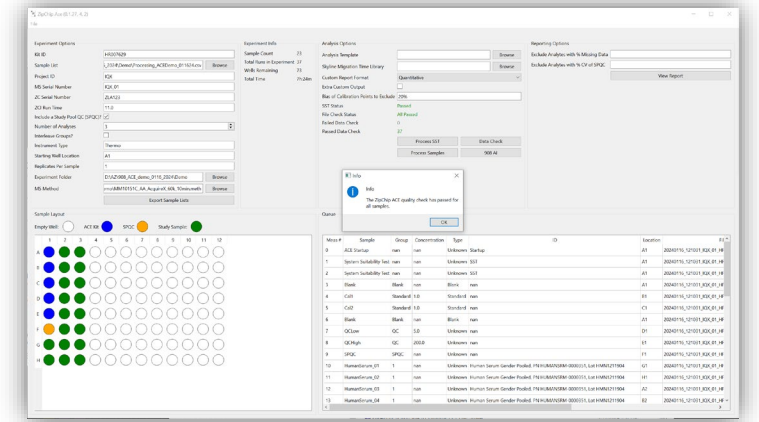
Reagents/Consumables

HARDWARE



ZipChip Interface, Separation Chip (908 Devices) and Orbitrap MS (ThermoFisher)

SOFTWARE



ZipChip ACE App