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

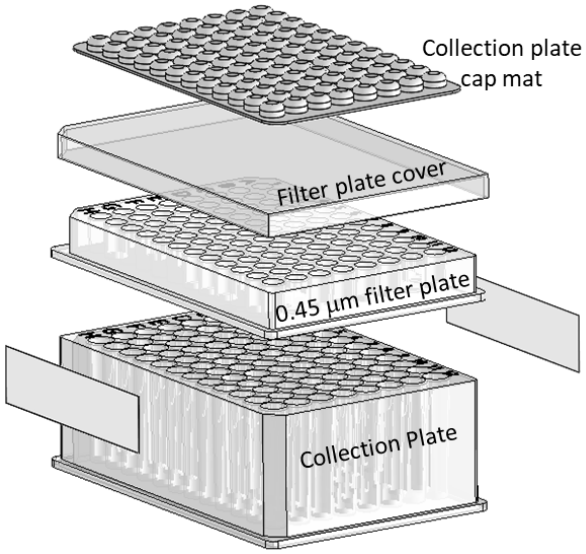
Sam Stewart, Erin Redman, J. Will Thompson

Research and Development, 908 Devices Inc.

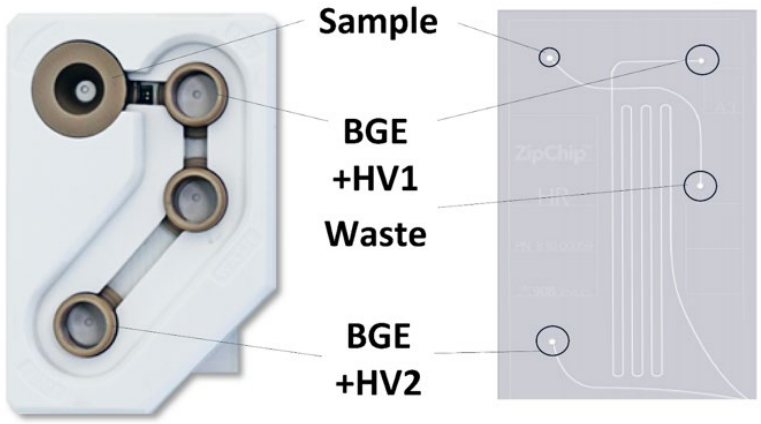
Metabolomics with ZipChip (microchip CZE-MS)

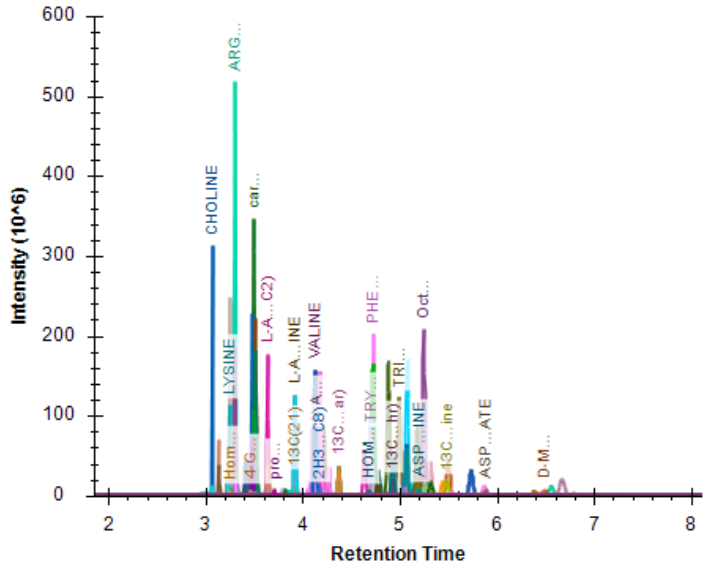
EXTRACTION

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

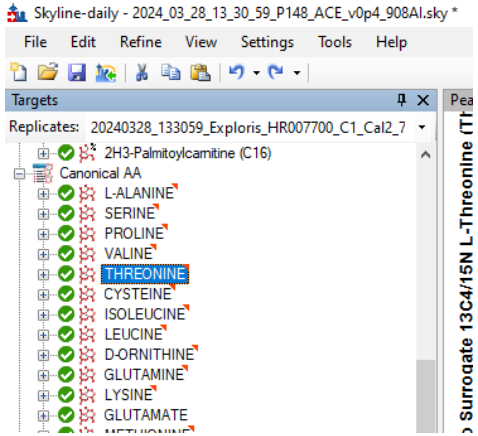


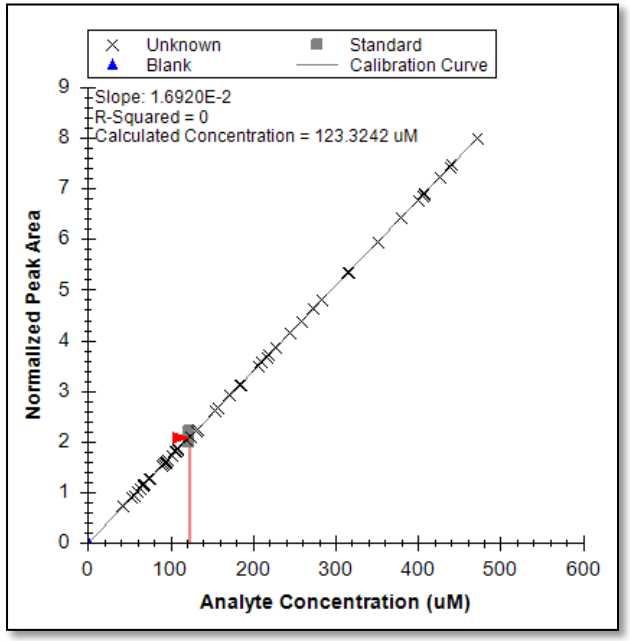
mCE-HRMS





TARGETED QUANT





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

System Suitability

Before Data Collection

Data Quality Check

Immediately After Data Collection

Quantification

Post-Analysis

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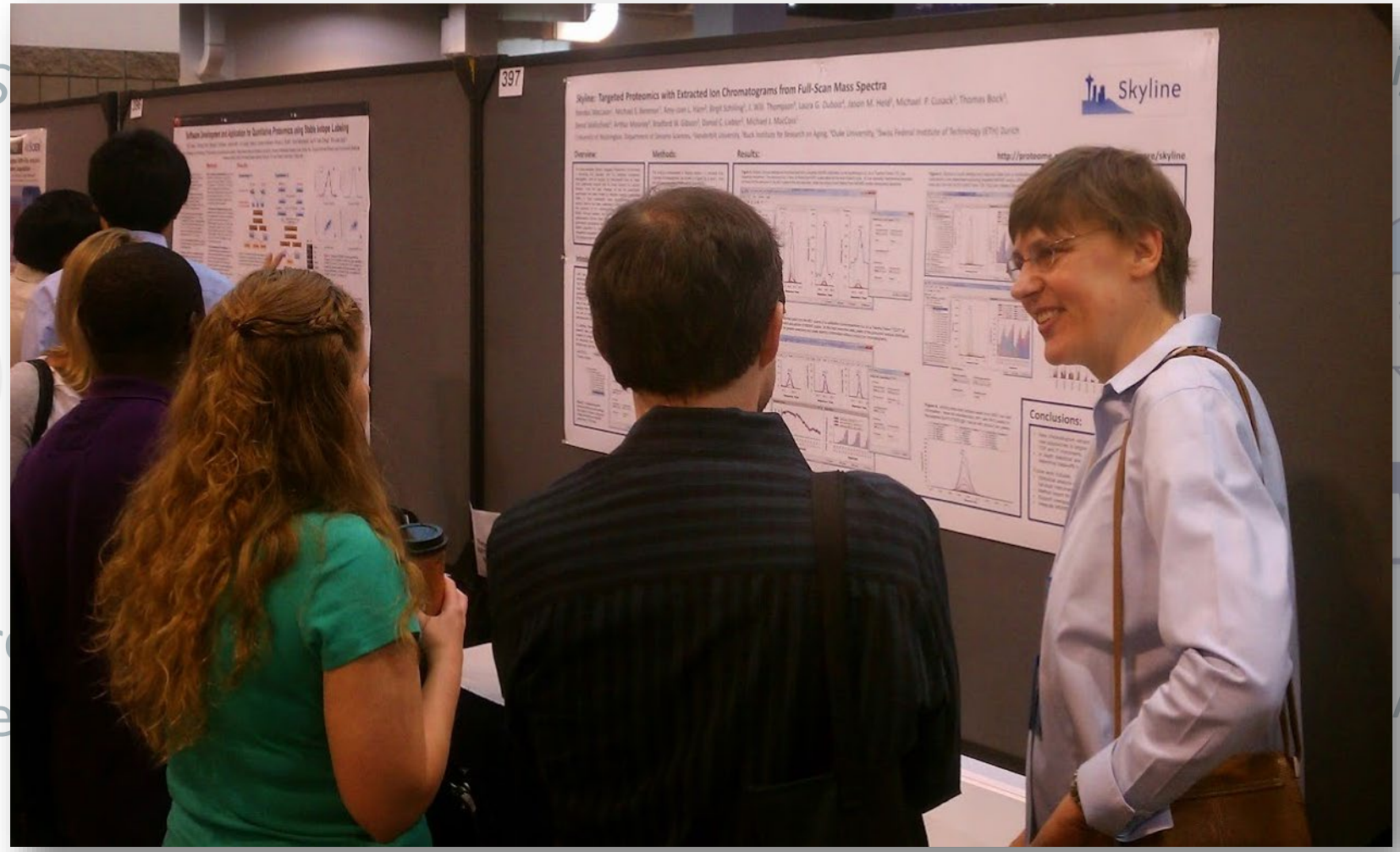
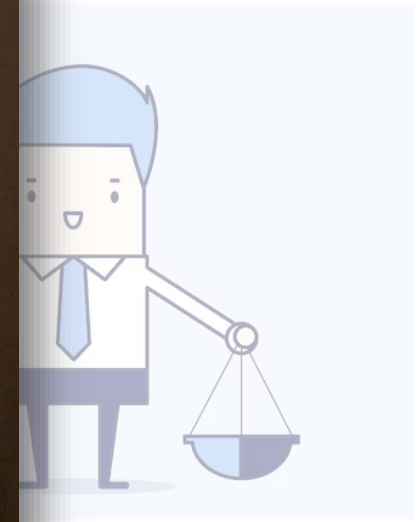
Areas we are Utilizing **Skyline** in our Software Pipeline

System S

ification





Birgit Schilling, ASMS 2011

Before
Colle

Analysis

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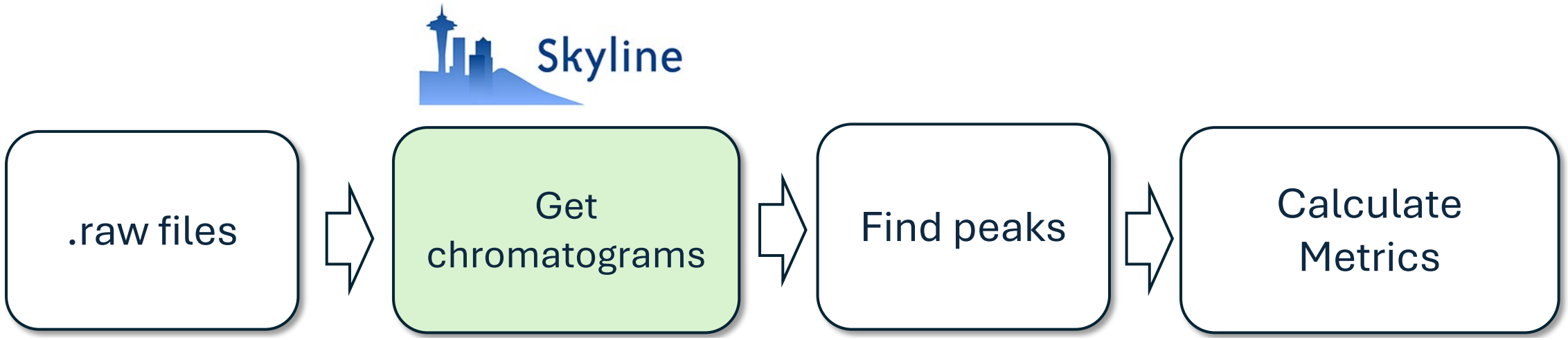
How we interact with Skyline (SST and Data Check)



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

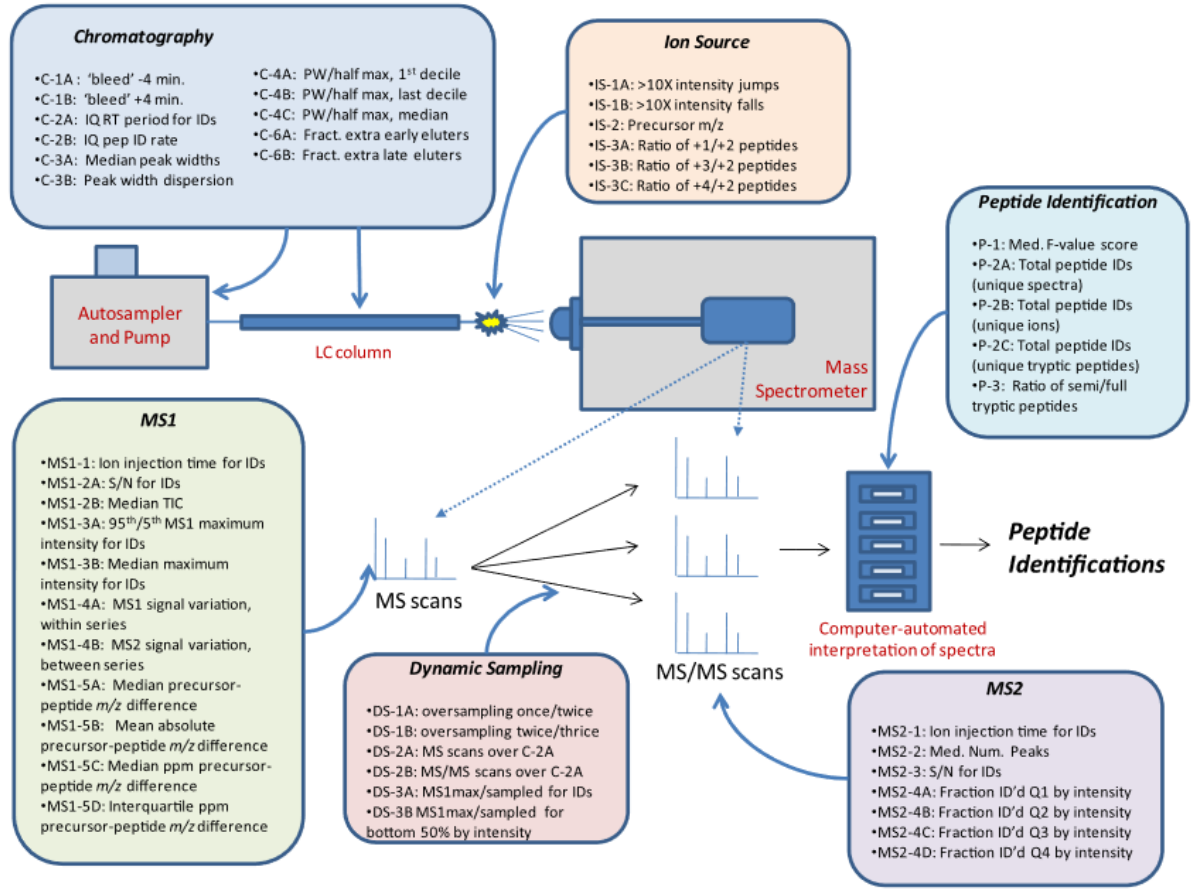
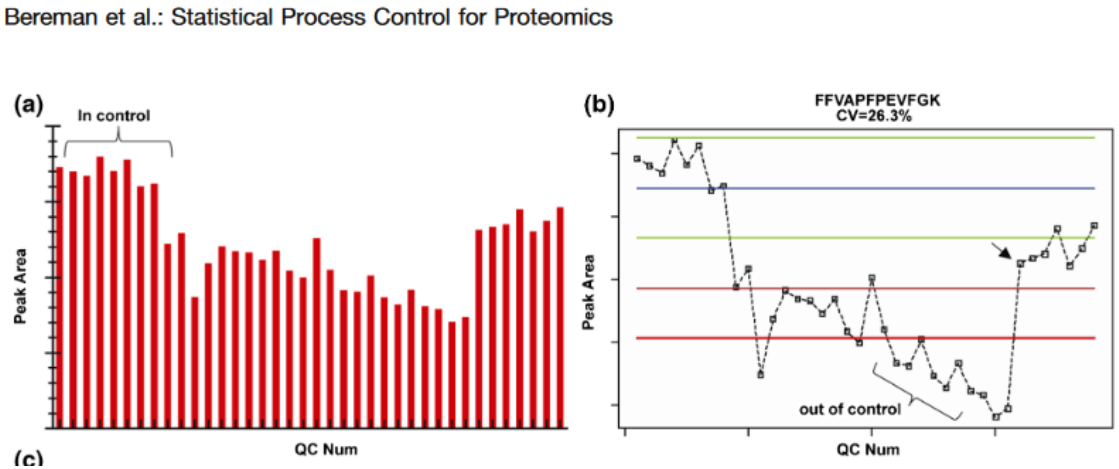


FIG. 1. **Schematic representation of performance metrics mapped to LC-MS/MS system elements.** *PW*, peak width; *IQ*, interquartile; *pep*, peptide; *ID*, identification; *Med.*, median; *ID'd*, identified; *Fract.*, fraction; *Num.*, number.

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



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

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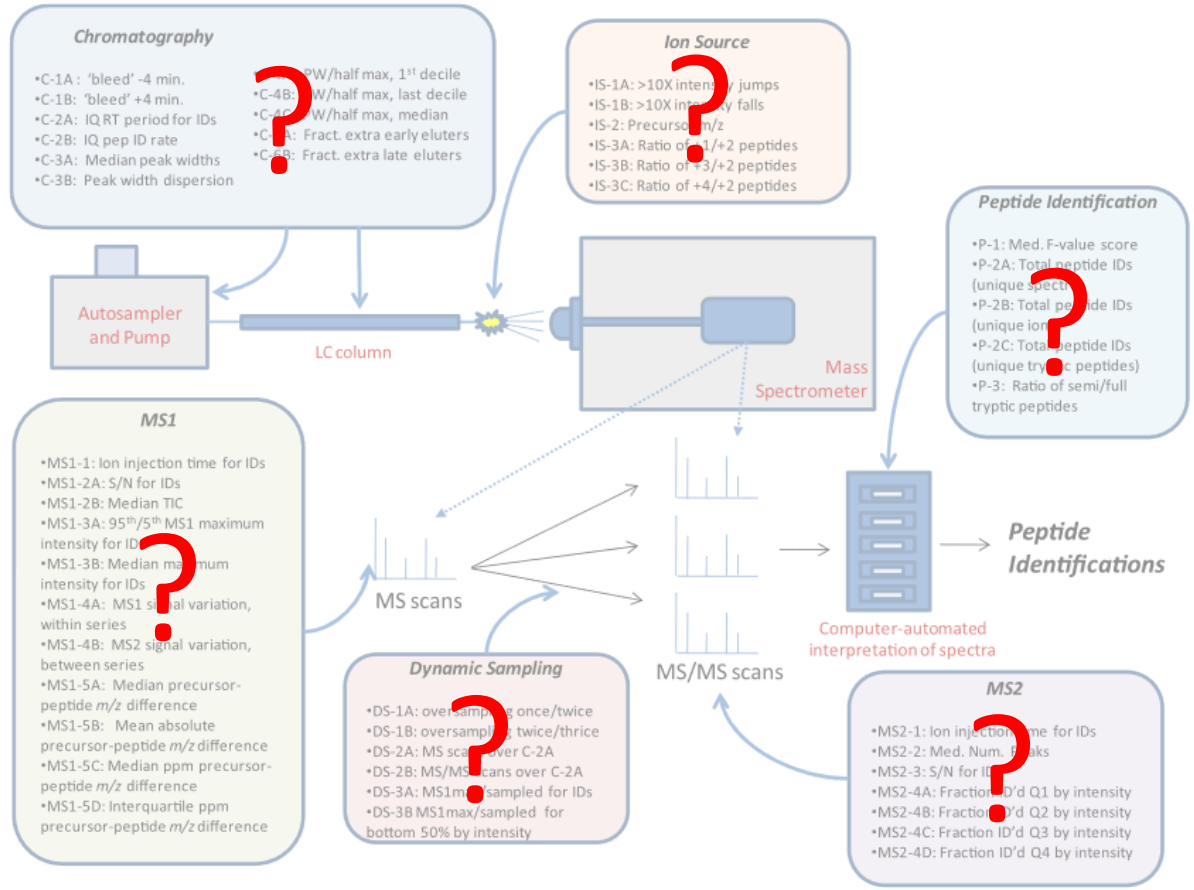
System Suitability in Mass Spectrometry 'Omics



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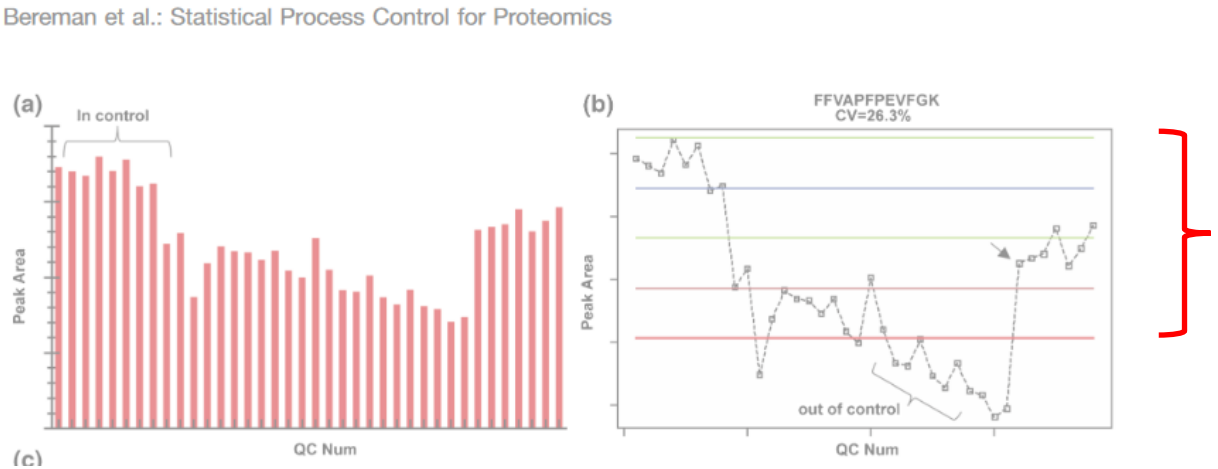

Bereman et al. *J Am Soc Mass Spectrom*. **2014** Apr;25(4):581-7.

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

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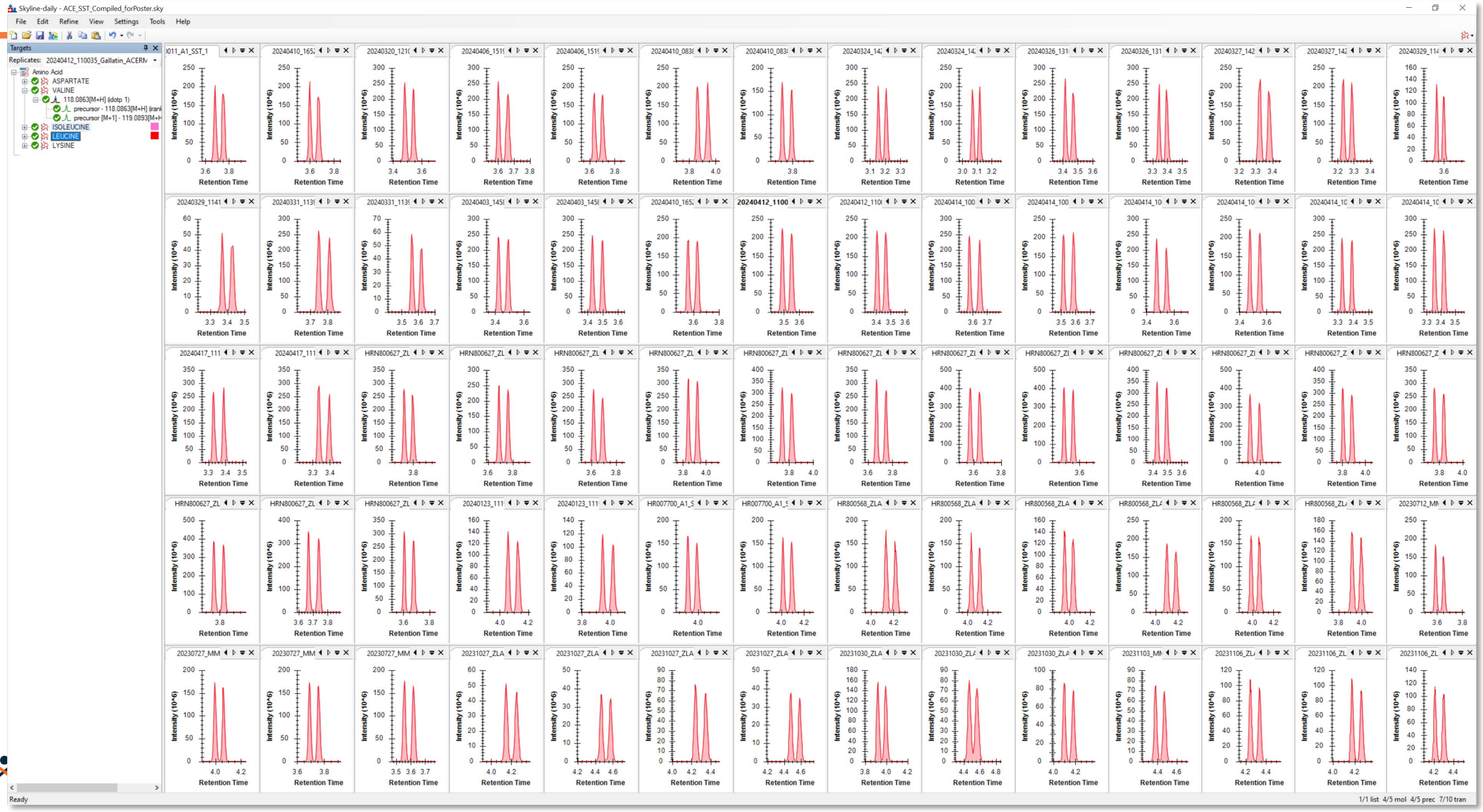
System Suitability Testing – Importance of Automation



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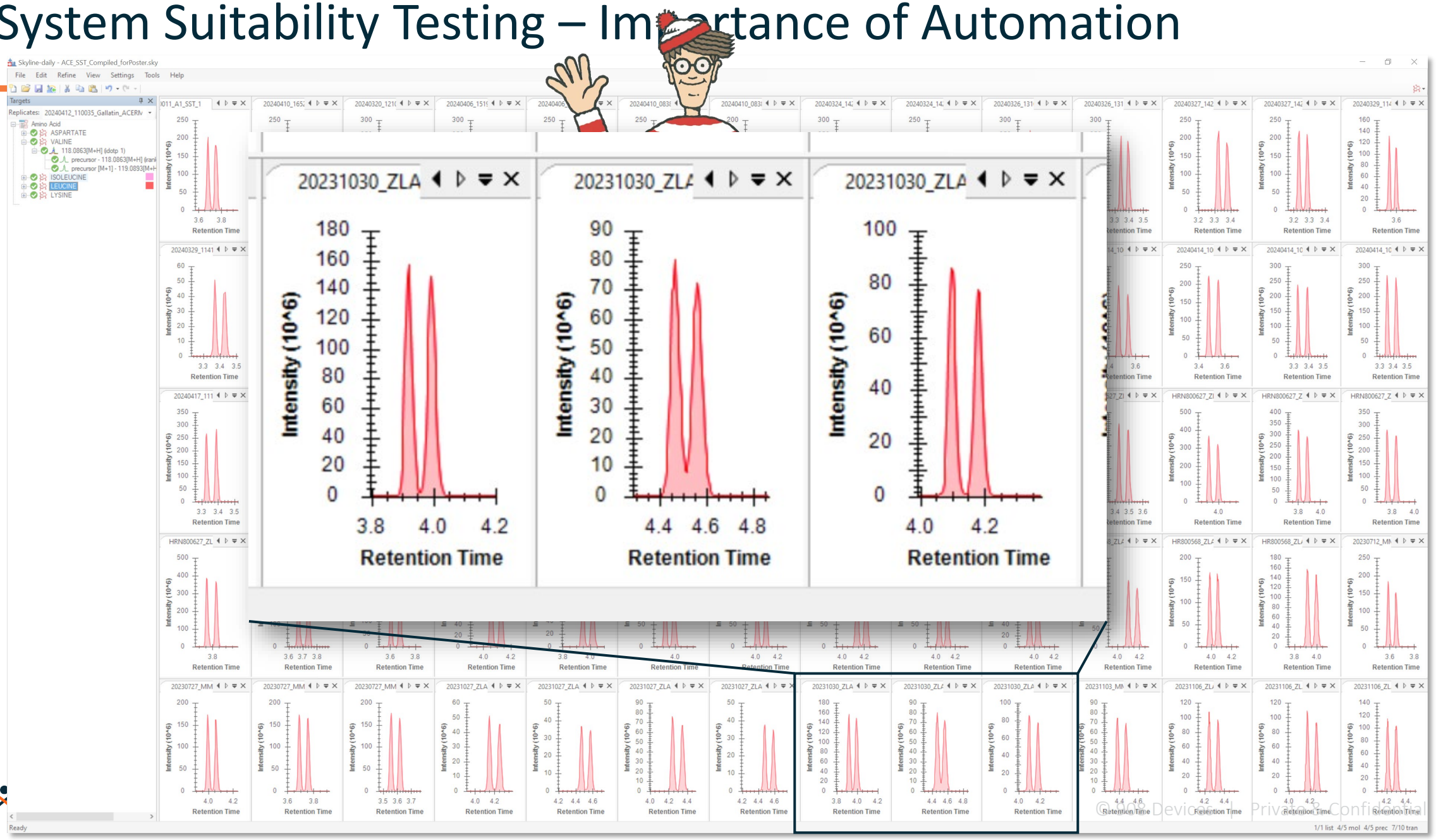
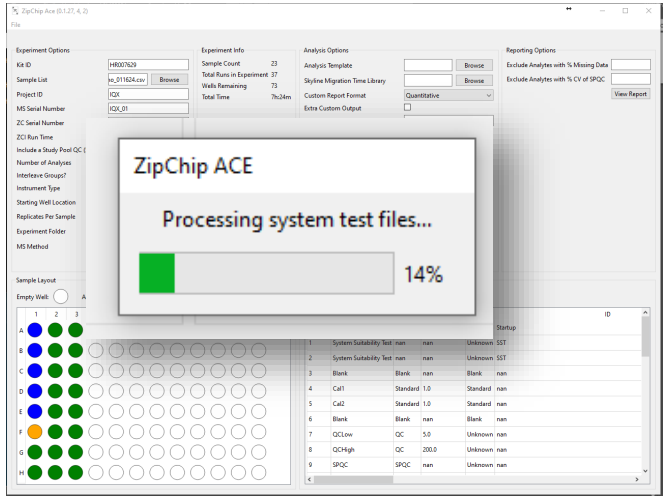
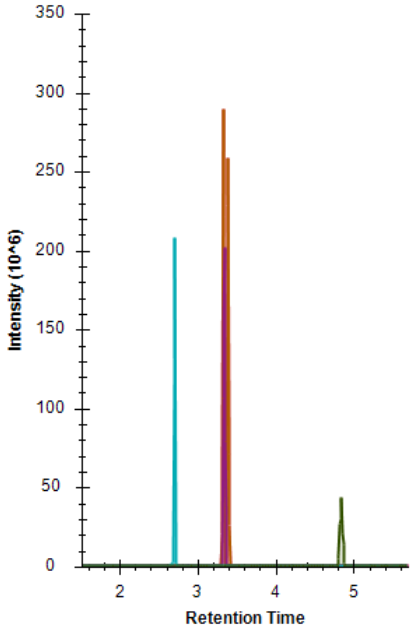


mCZE System Suitability Testing – Integration with Skyline

Single “Touchpoint” in ZipChip ACE App

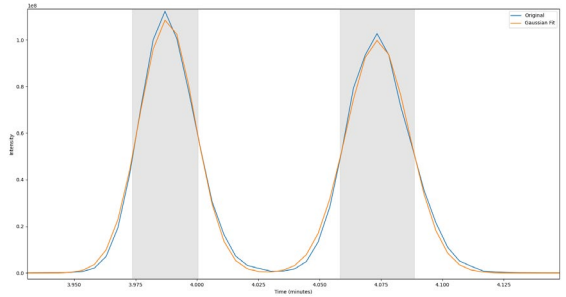


Skyline Template for Extracting Chromatograms

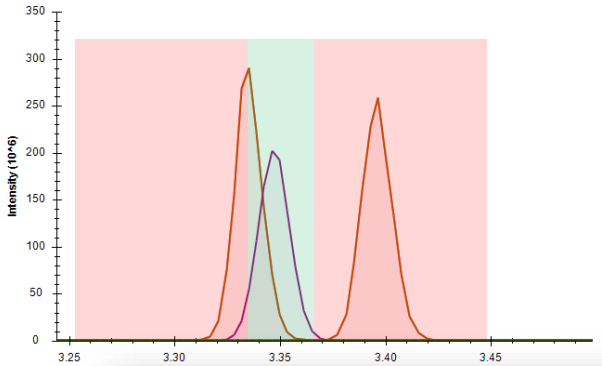


5 analytes, 4 m/z values
ZipChip HR, Peptides BGE
Exploris 240 MS
10 uM Promega AA Mix

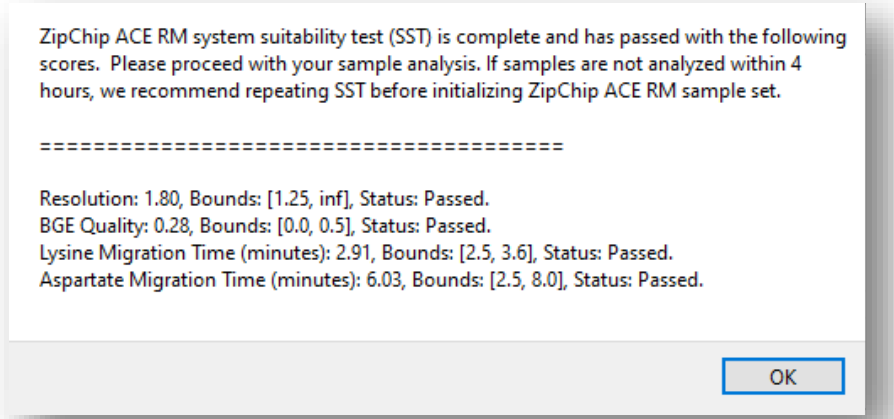
Calculate and Report Metrics



Resolution



BGE “Quality” (effectively, pH)



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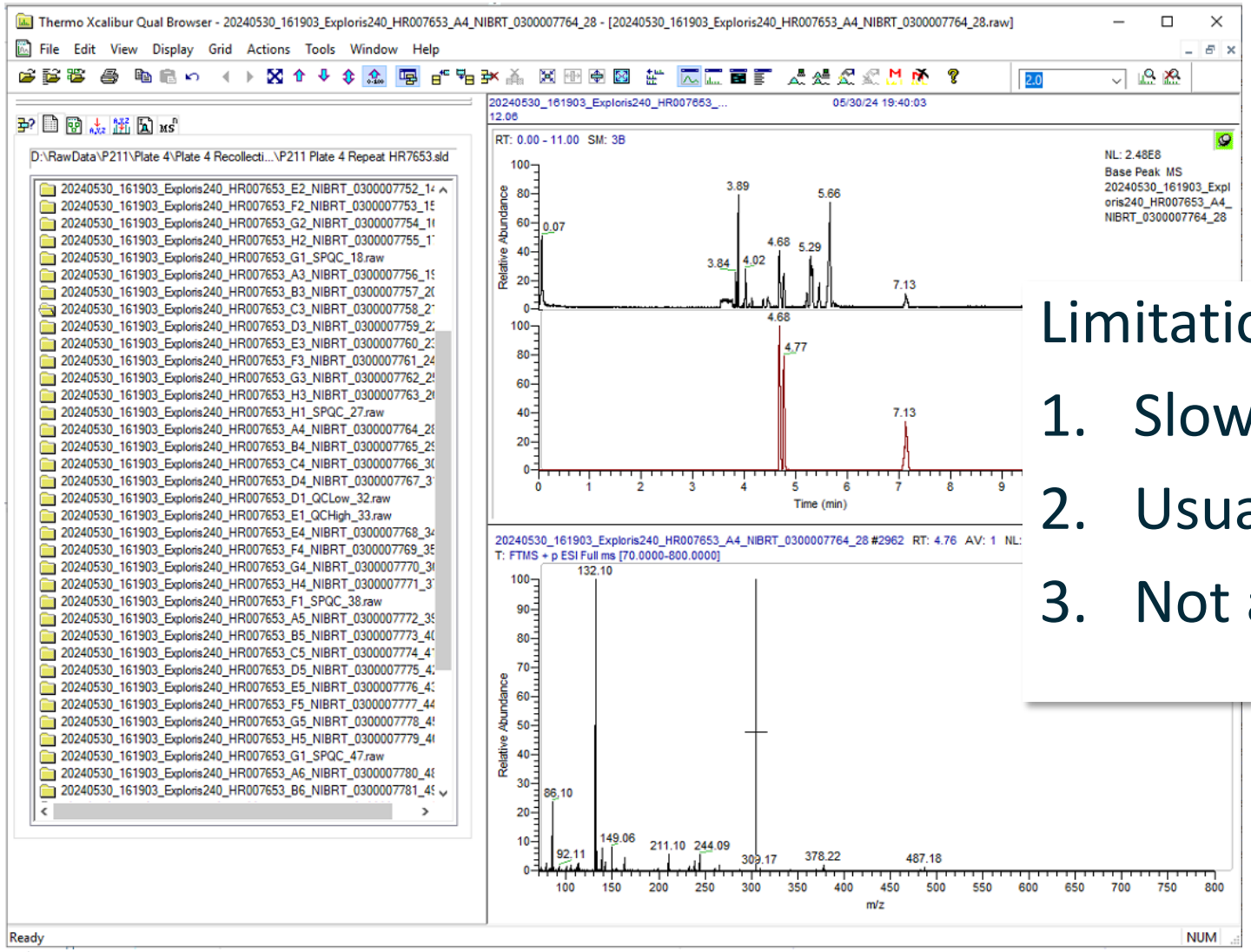
Data Check Standard Practice: Manual Data “Check”

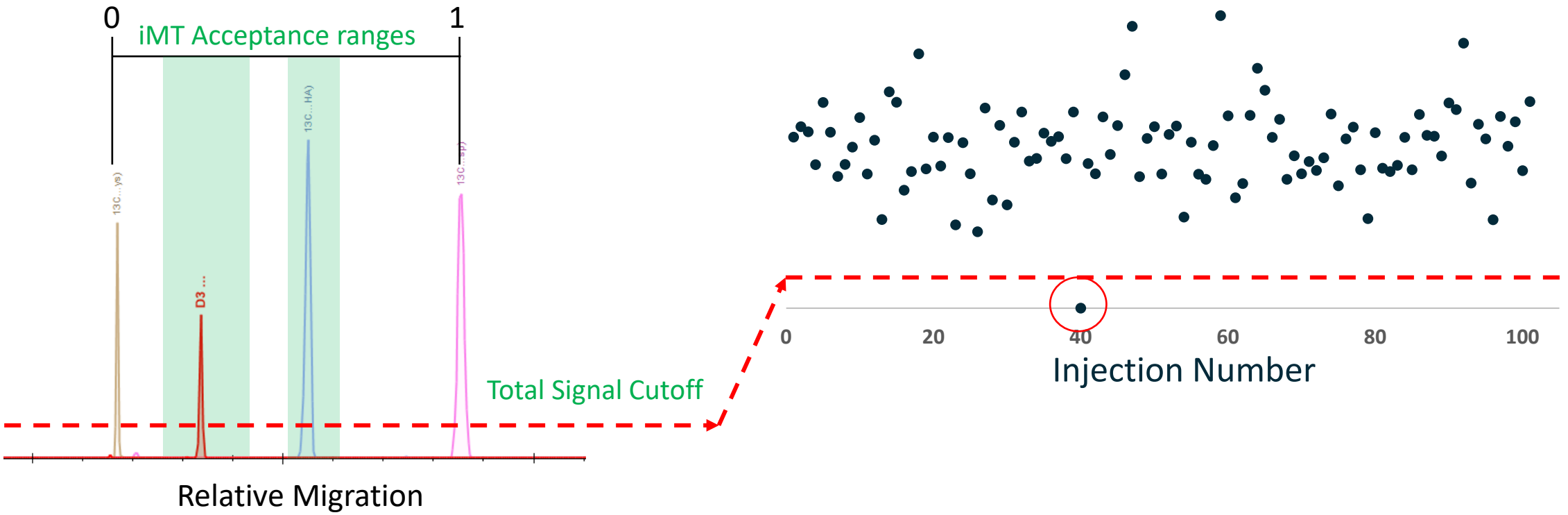


Limitations to Manual Data Check

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

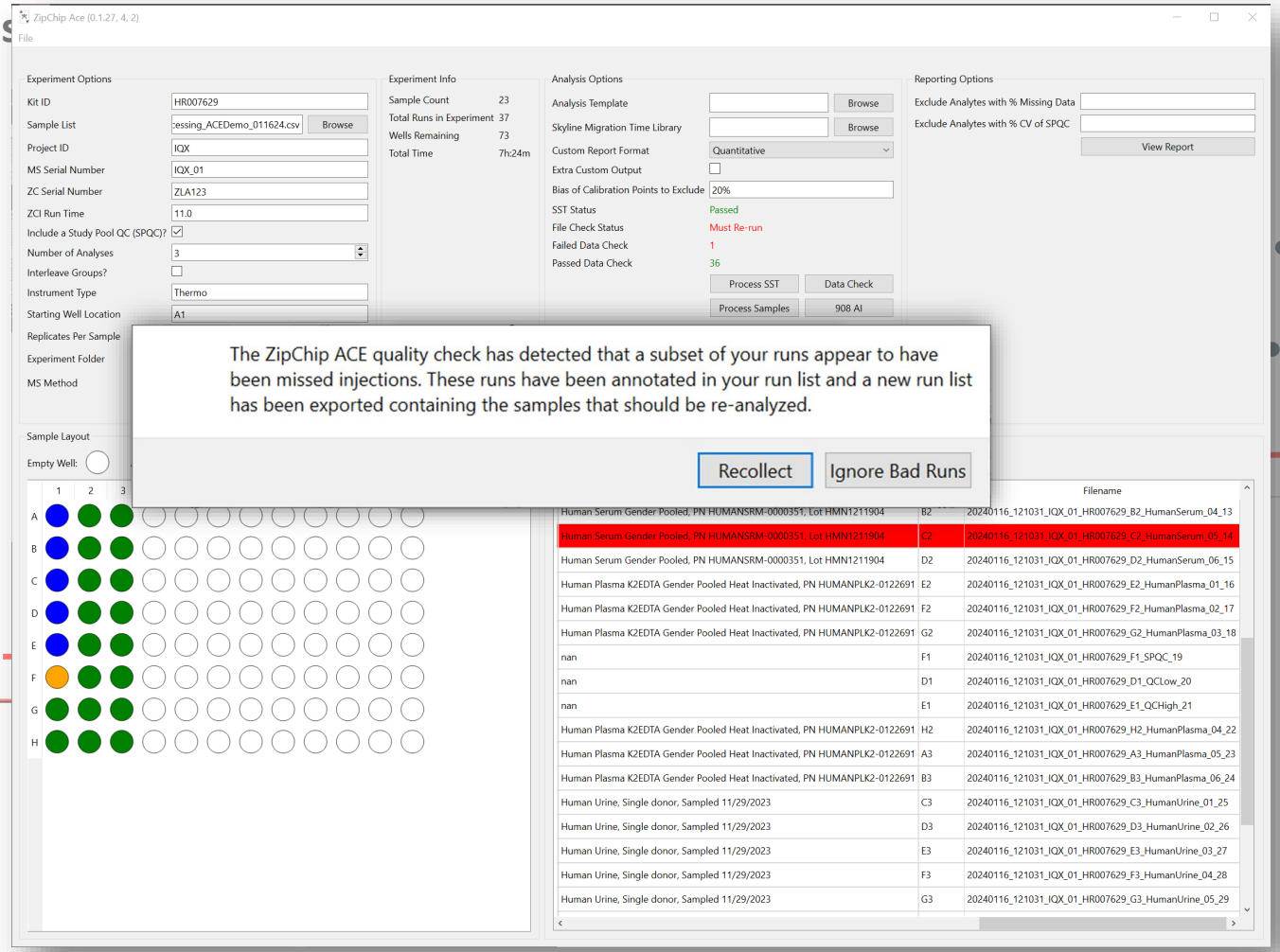
“Data Check” Acceptance Criteria

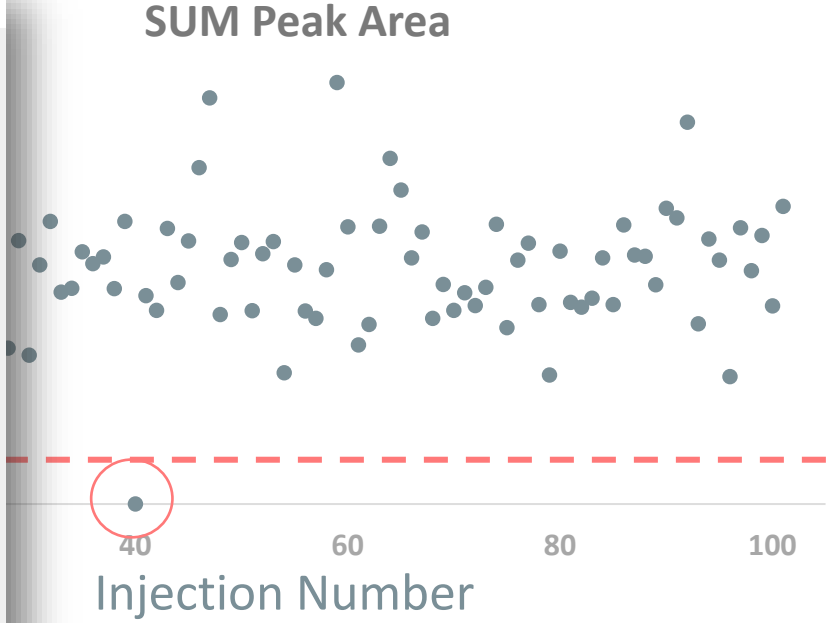
Uses 4 Method-Specific Internal Standards



“Data Check” Acceptance Criteria

Uses





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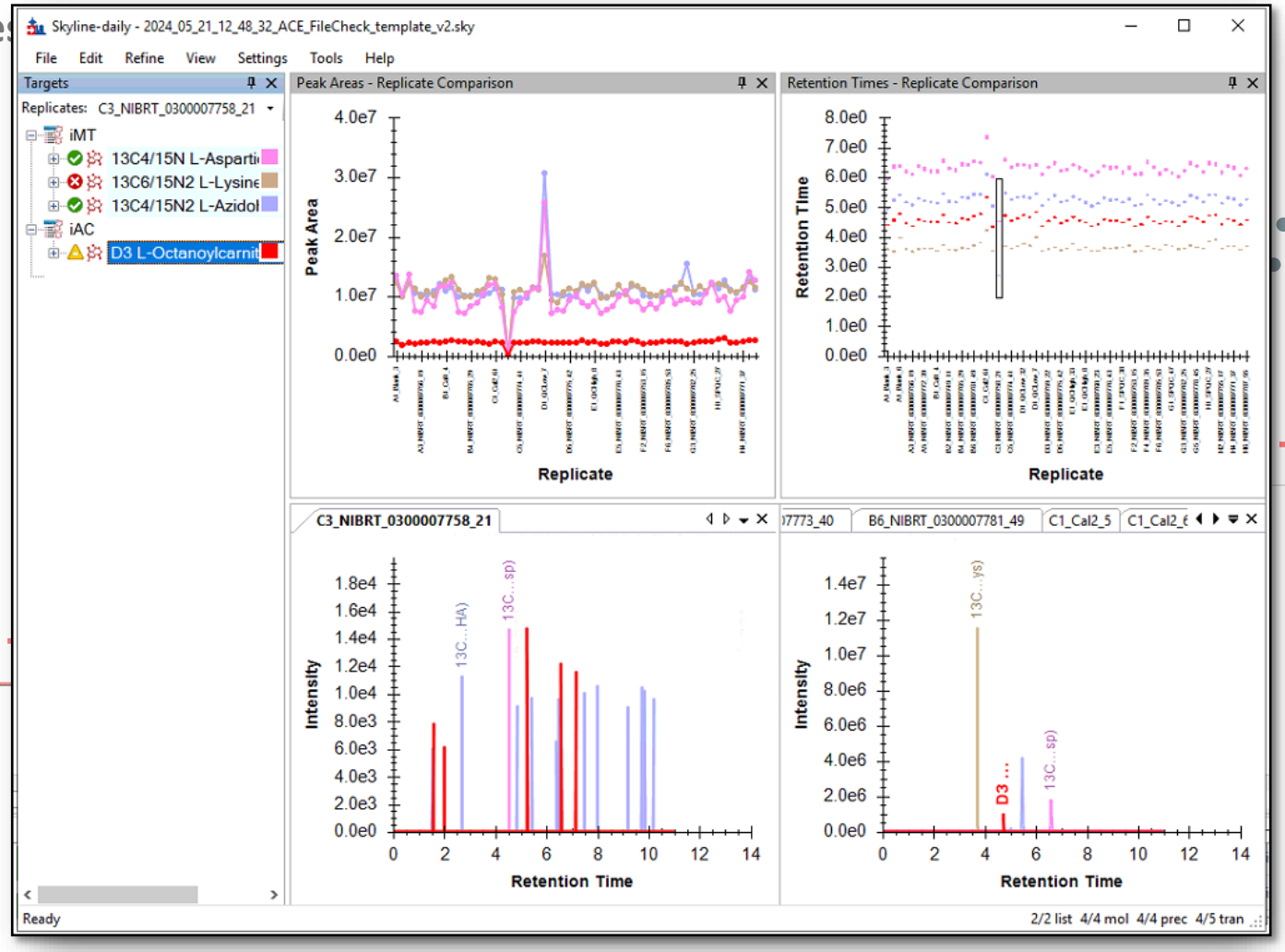

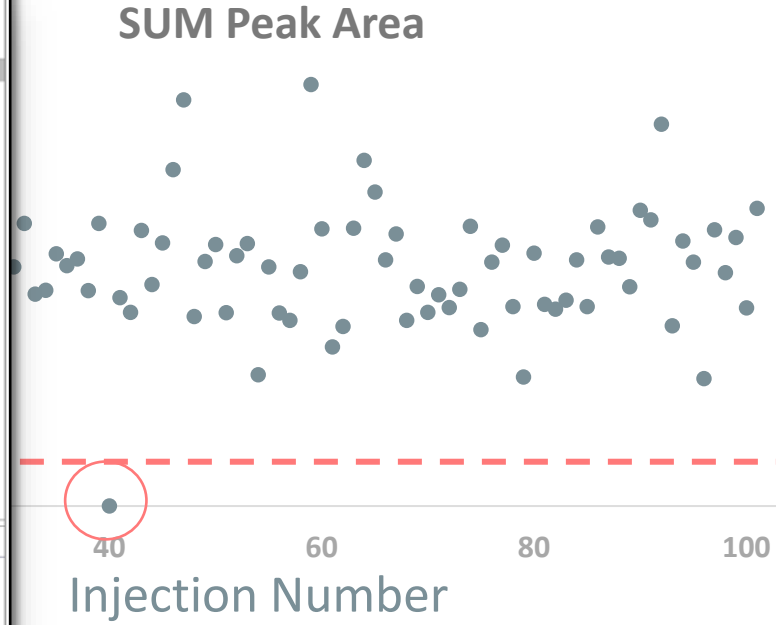

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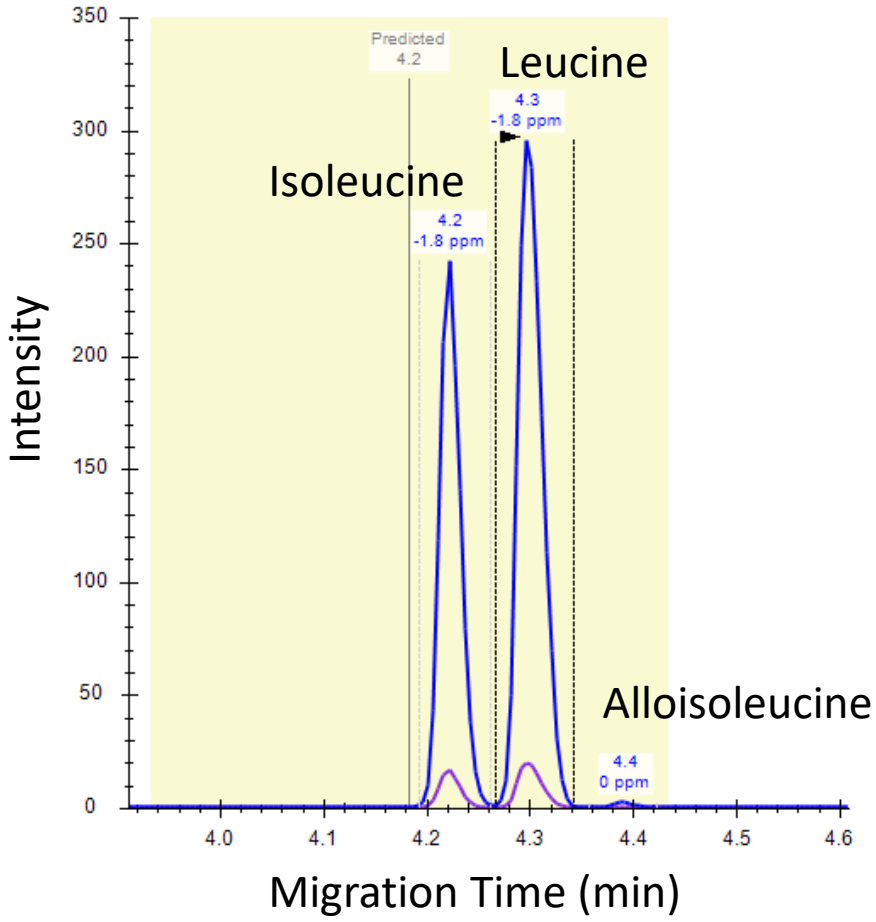
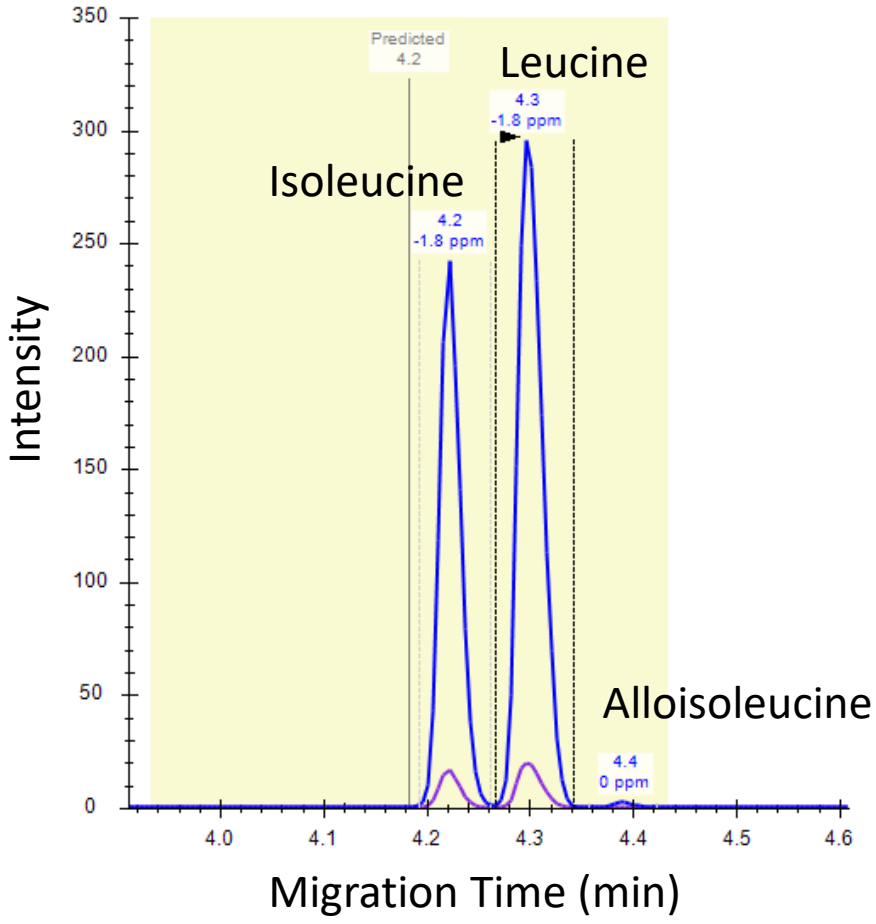
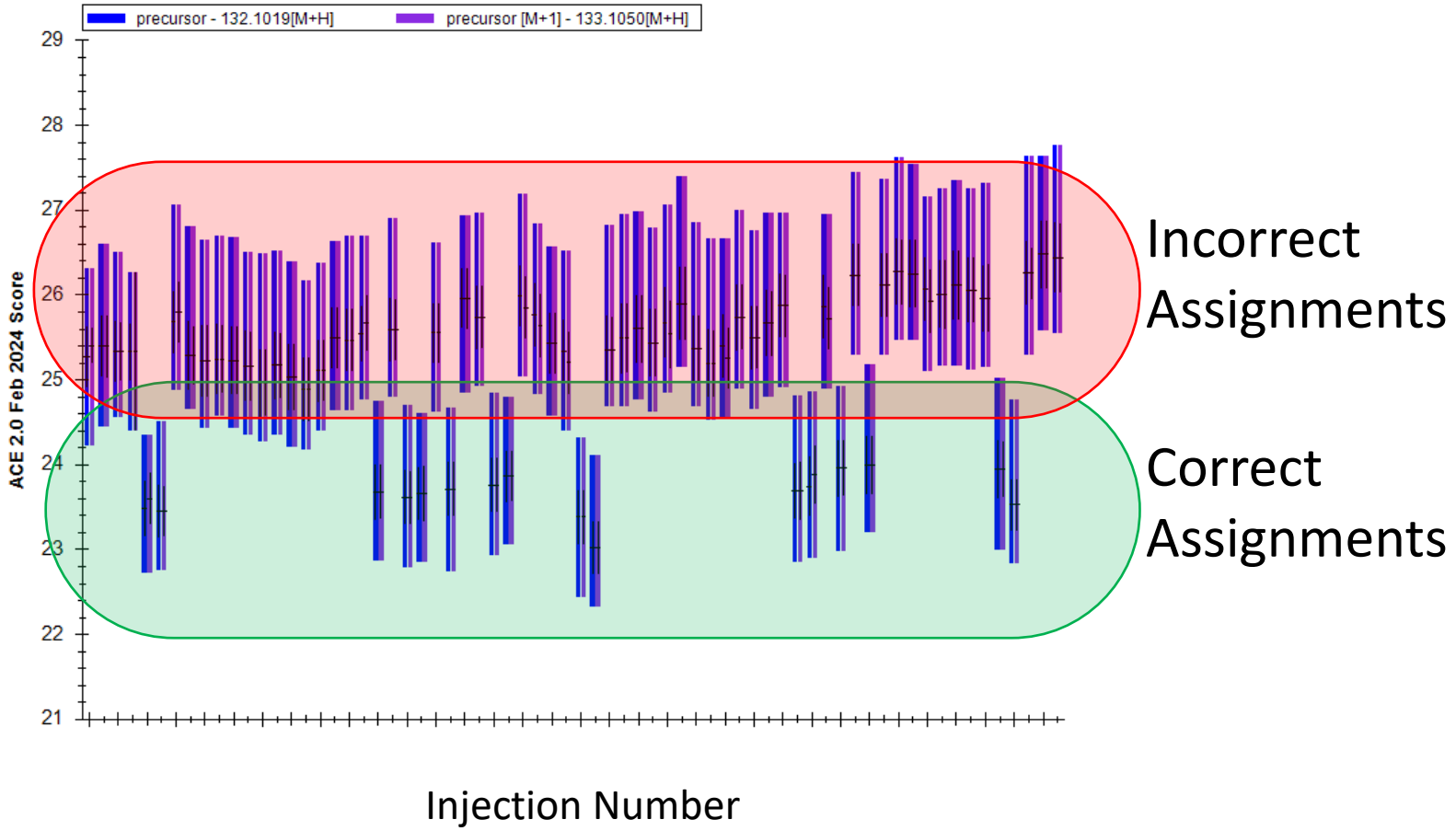
The Most Common and Tedious Problem In Targeted Metabolomics is Peak Assignment for Closely-Eluting, Isobaric Compounds



No Targeted Analysis Software is Immune, Including Skyline





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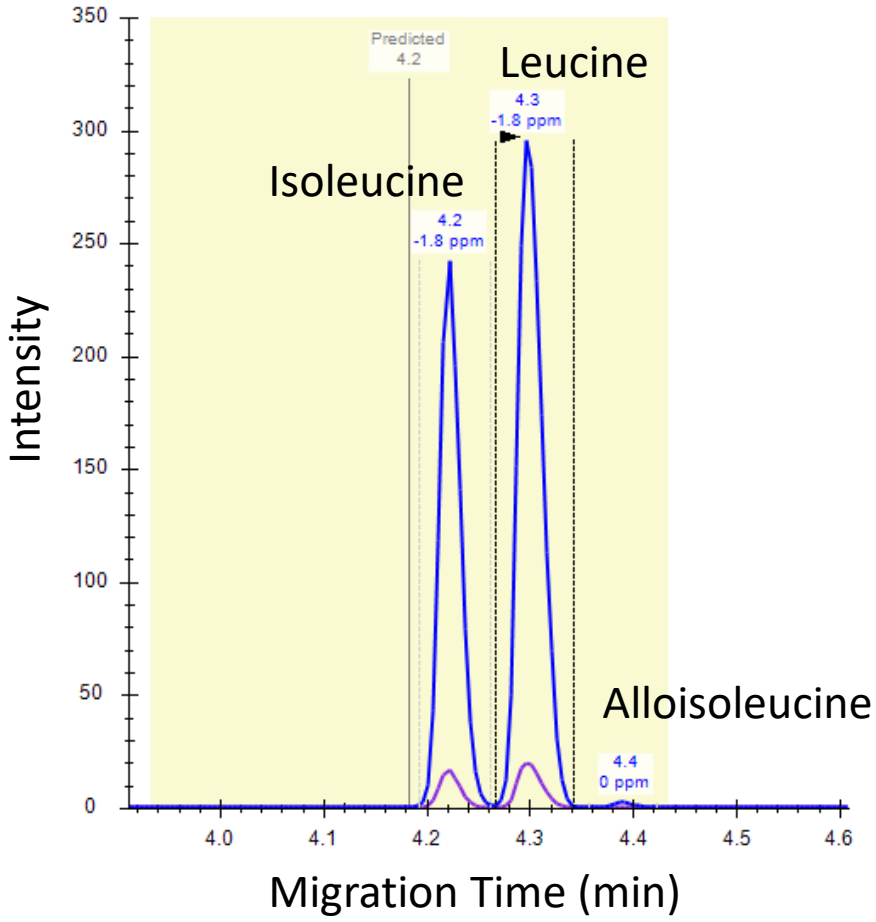
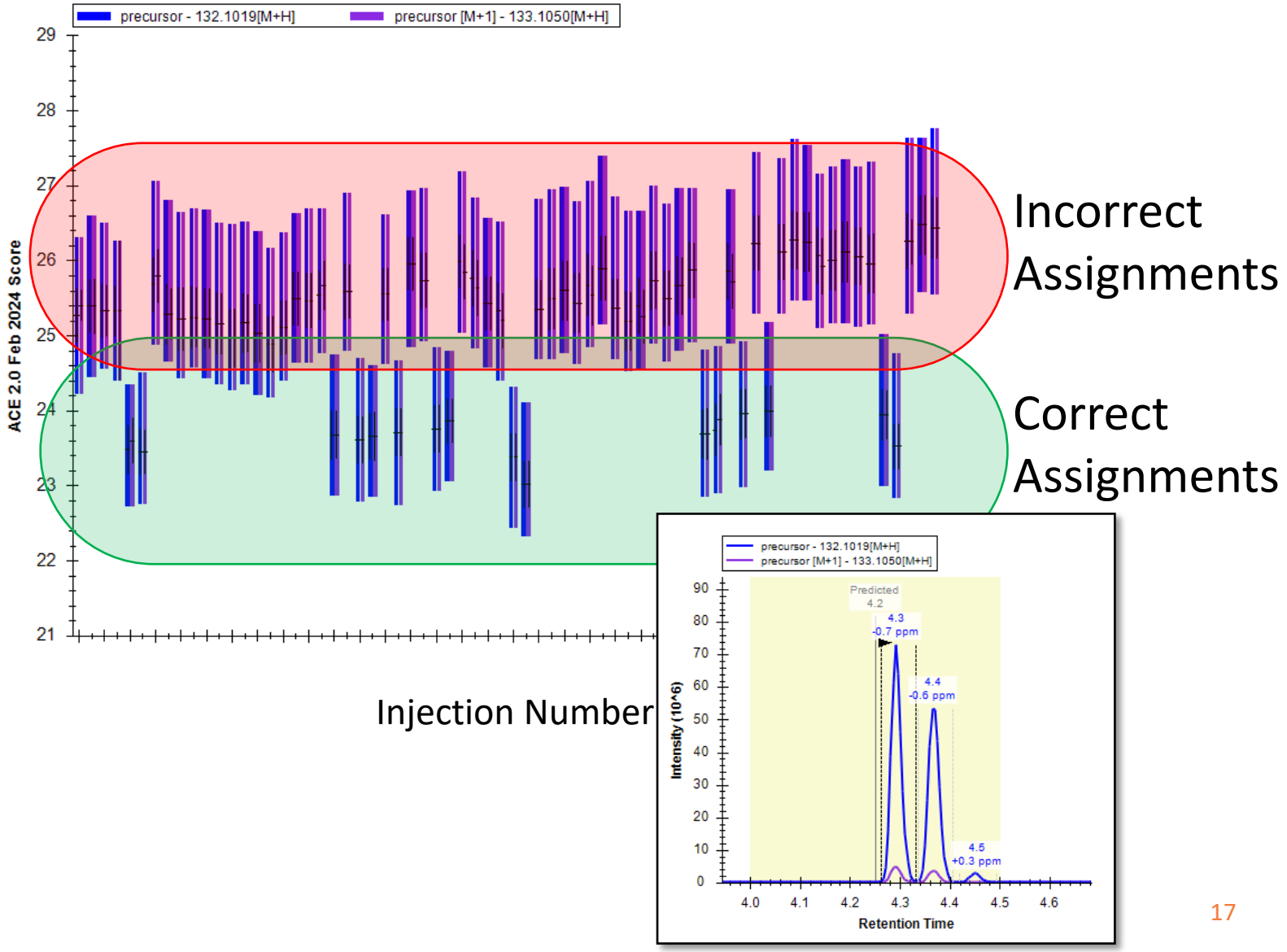
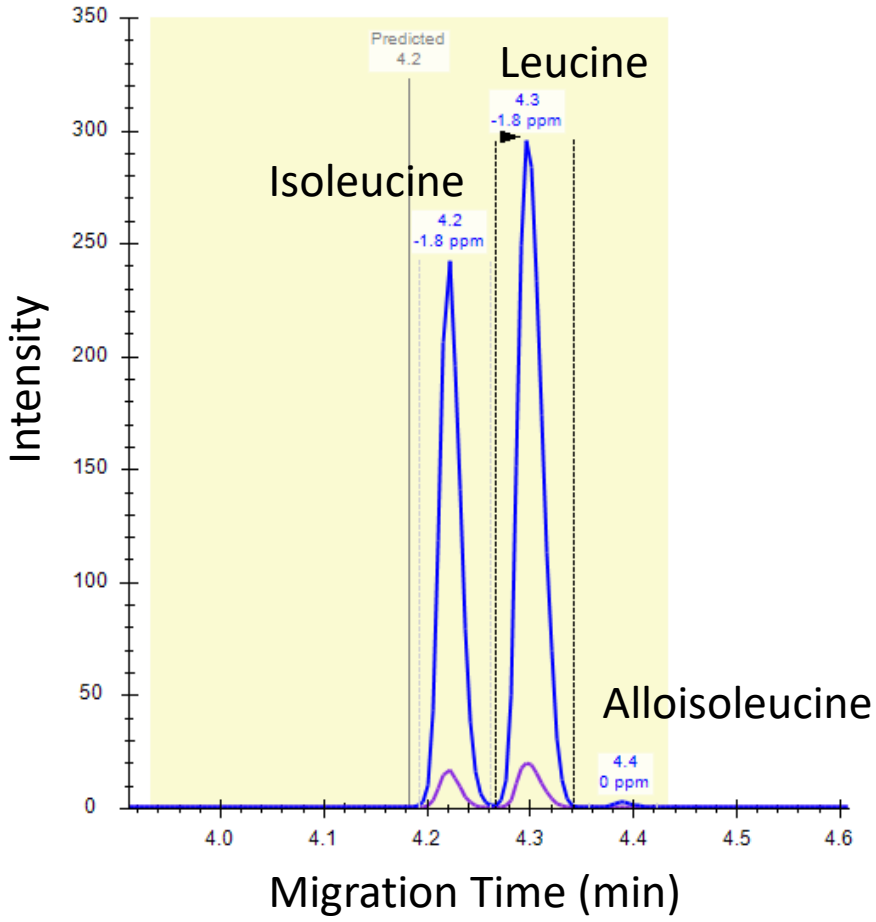




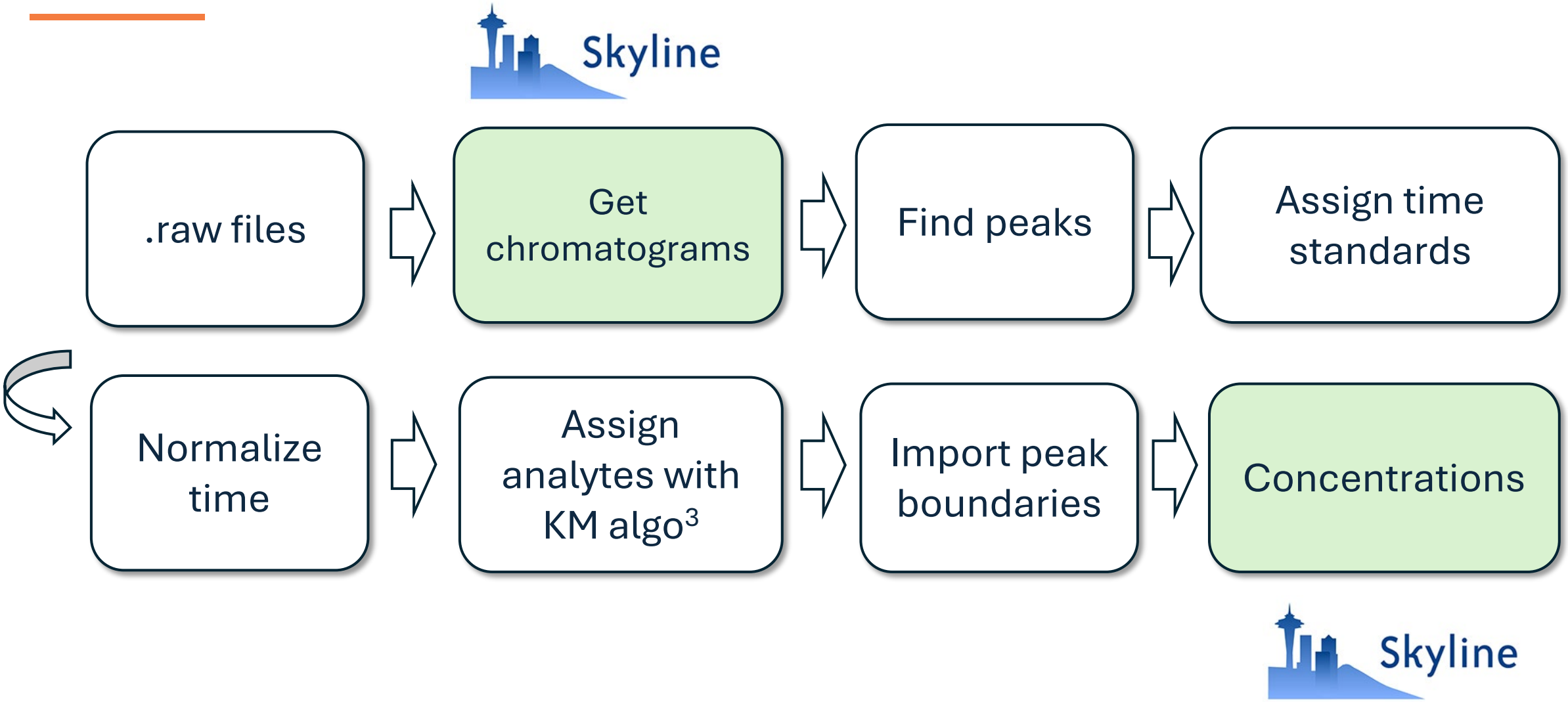
The Mis-assignments Are Made Largely for Two Simple Reasons



Skyline has No “Knowledge” of:

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

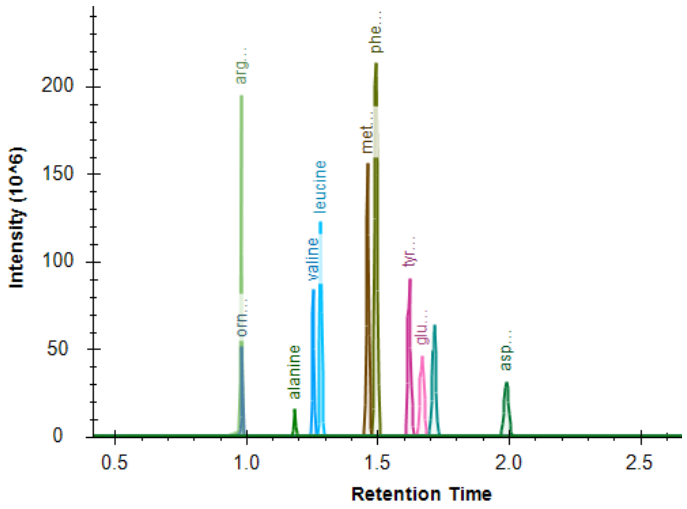
How we interact with Skyline (Metabolite Quantification)

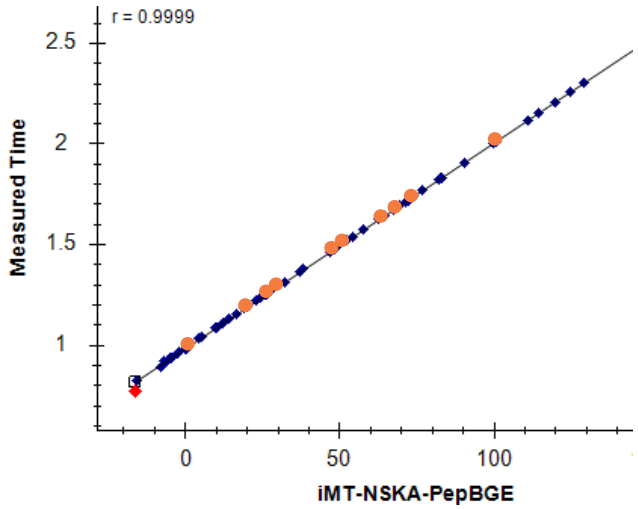


[3]Munkres, J. *Journal of the Soc. for Ind. and Appl. Math.* **1957** 5(1):32–38

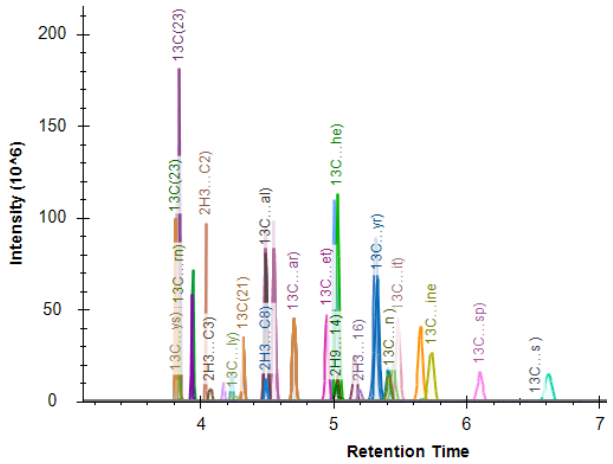
Start by adopting the iRT Approach for microchip CZE

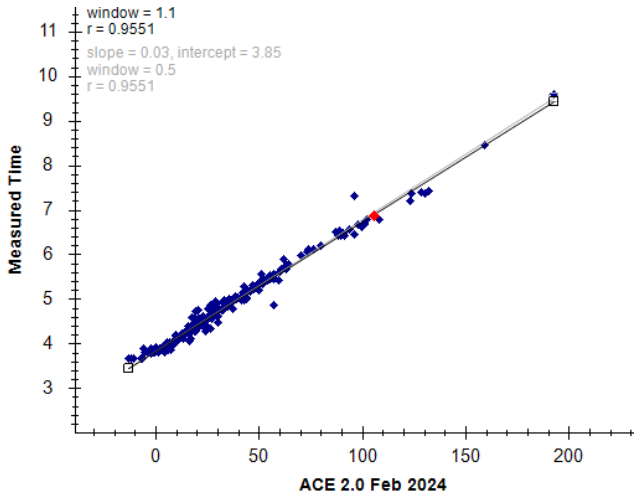
CIL “NSK-A” as iMT Standards (2021) - ~60 library compounds



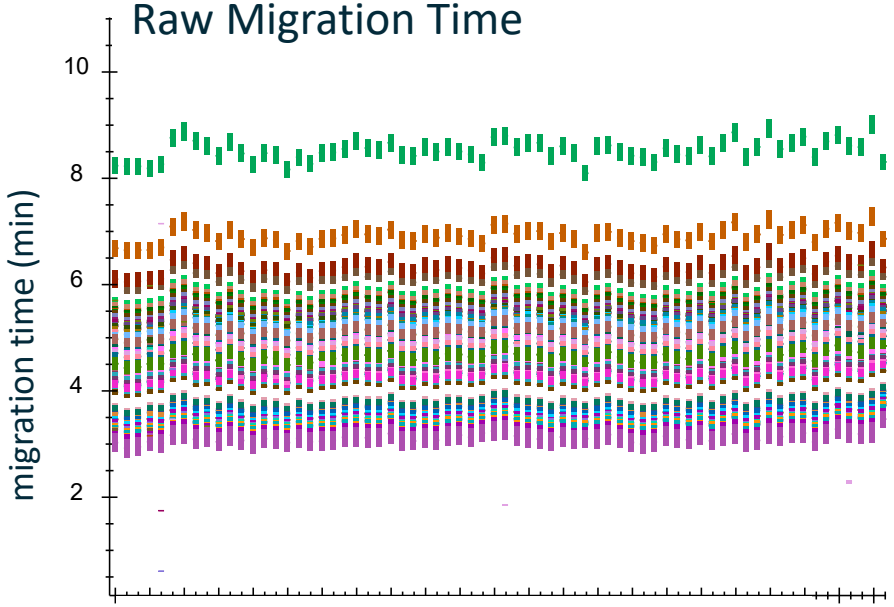


ZipChip ACE iMT Standards (2023) - ~325 library compounds

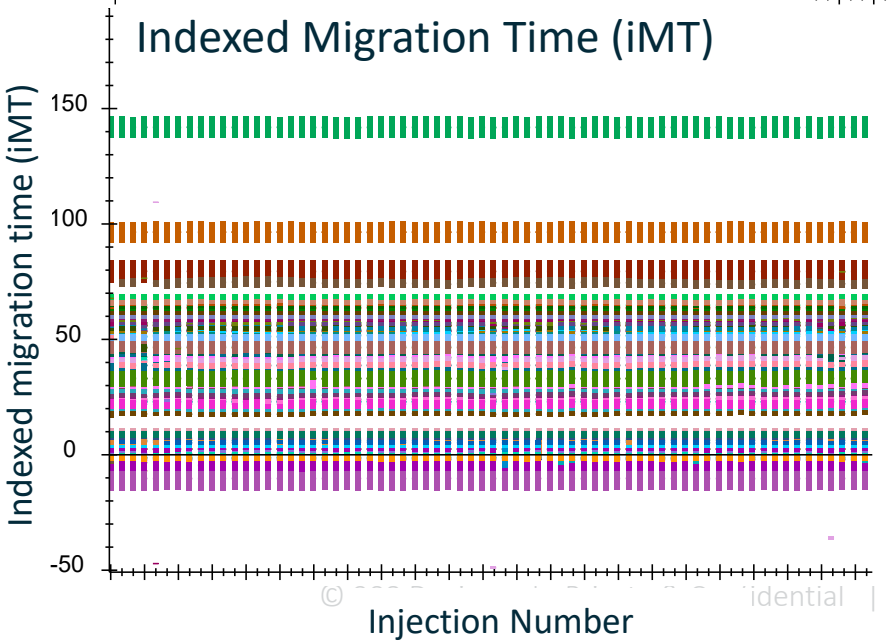




Raw Migration Time



Indexed Migration Time (iMT)



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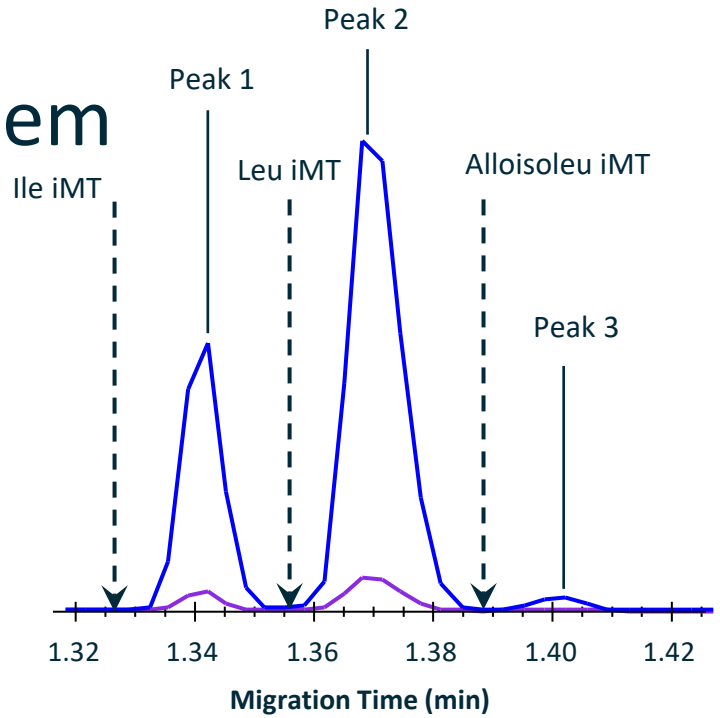
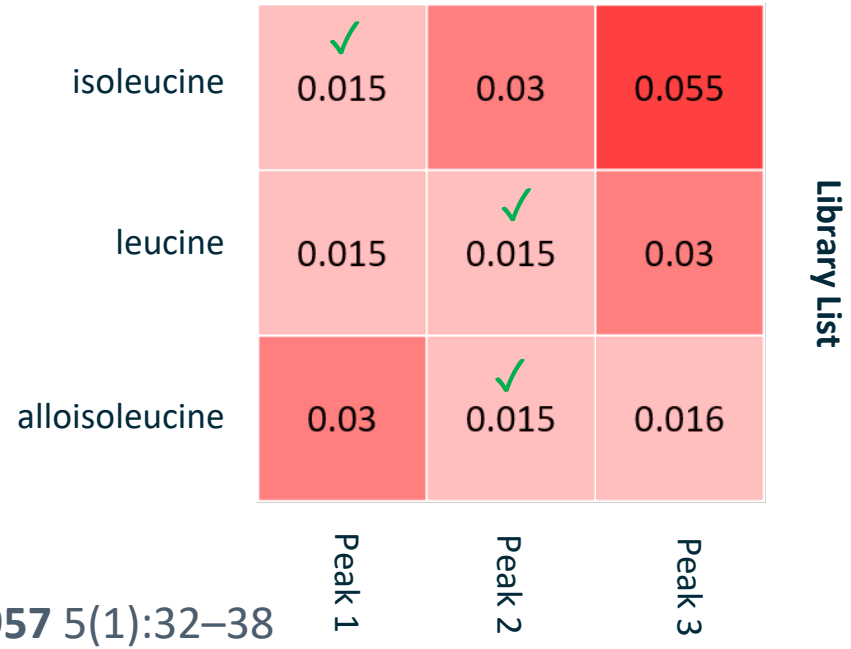
KM Algorithm[3] for Linear Sum Assignment Problem

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





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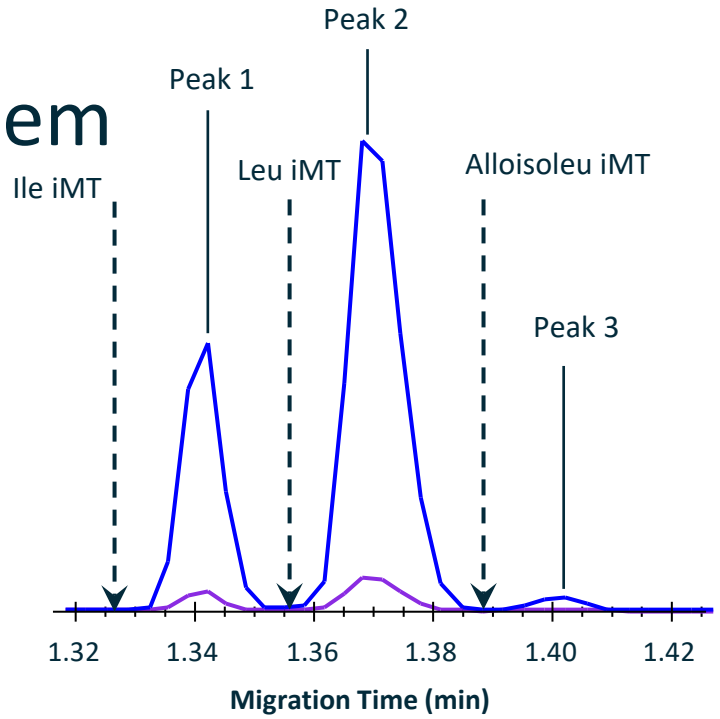
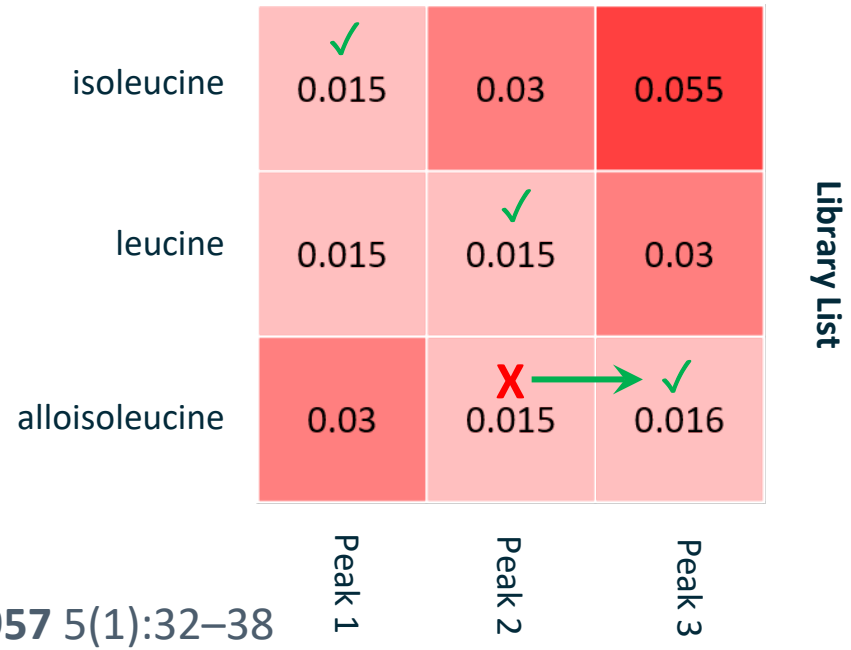




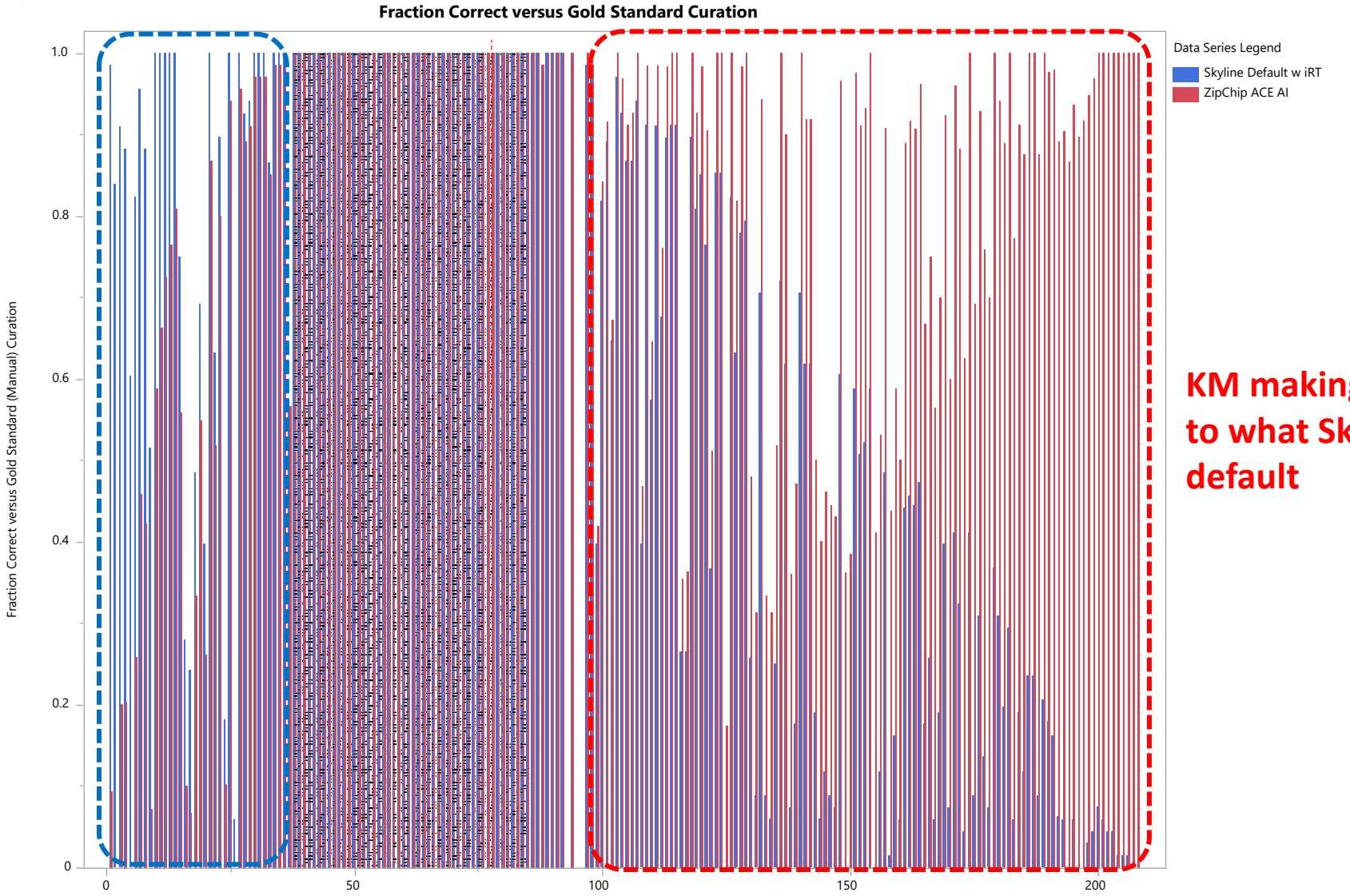
[3]Munkres, J. *Journal of the Soc. for Ind. and Appl. Math.* **1957** 5(1):32–38

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

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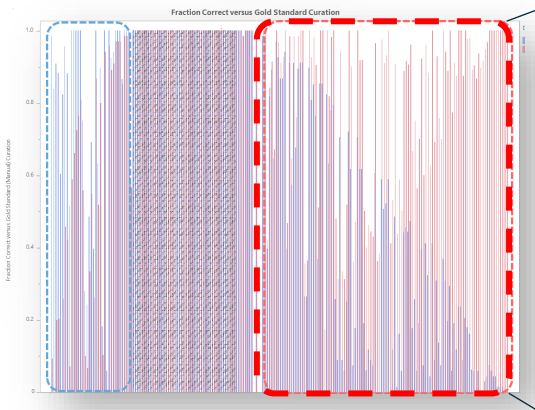
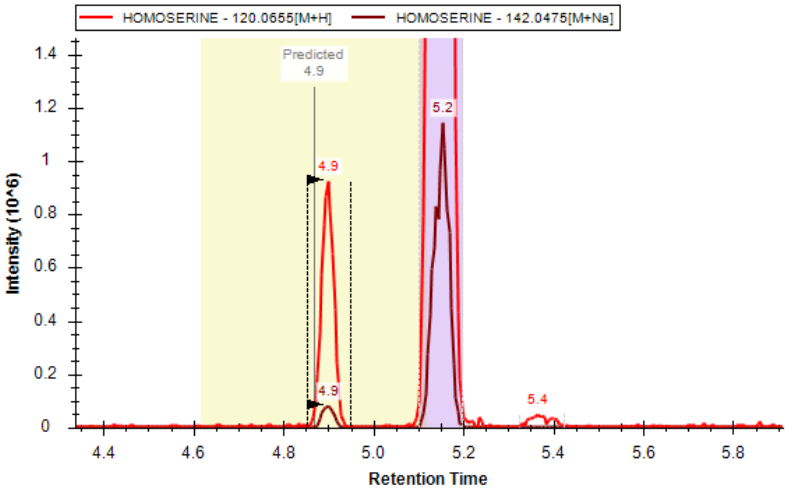
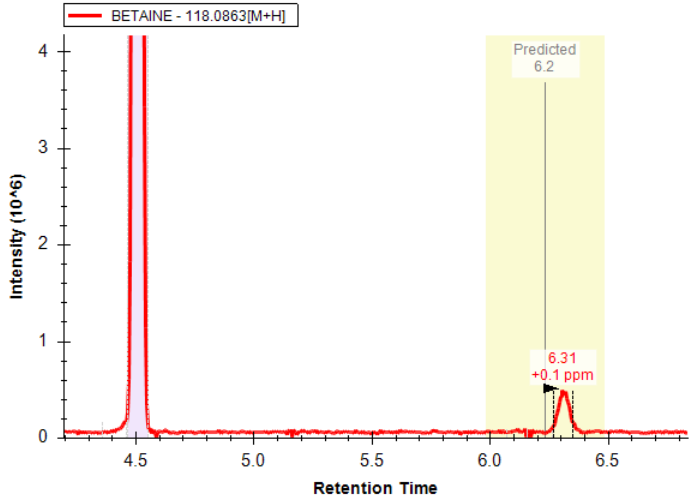
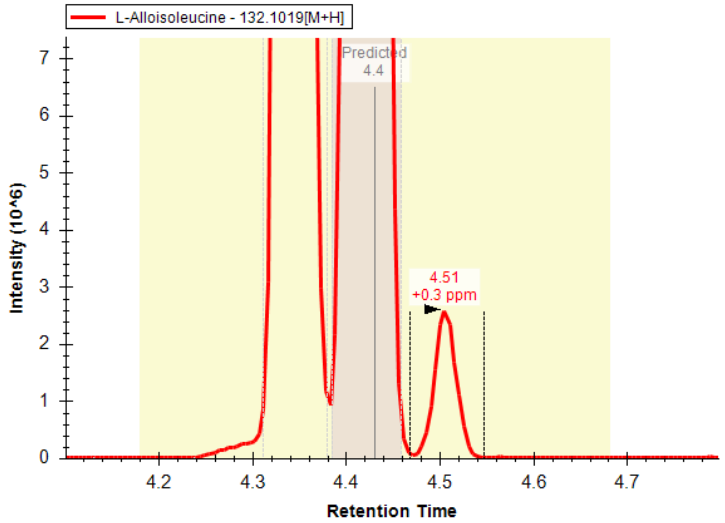
KM Algorithm is solving some time-intensive assignment problems



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%







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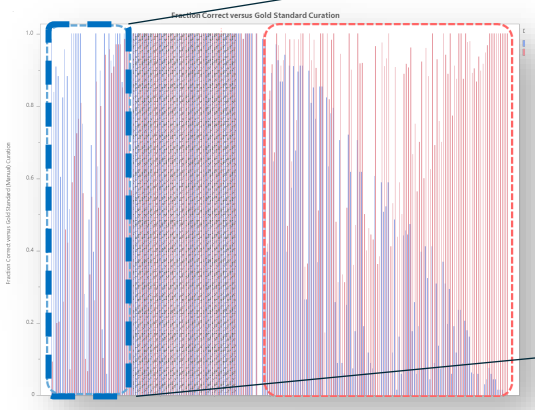
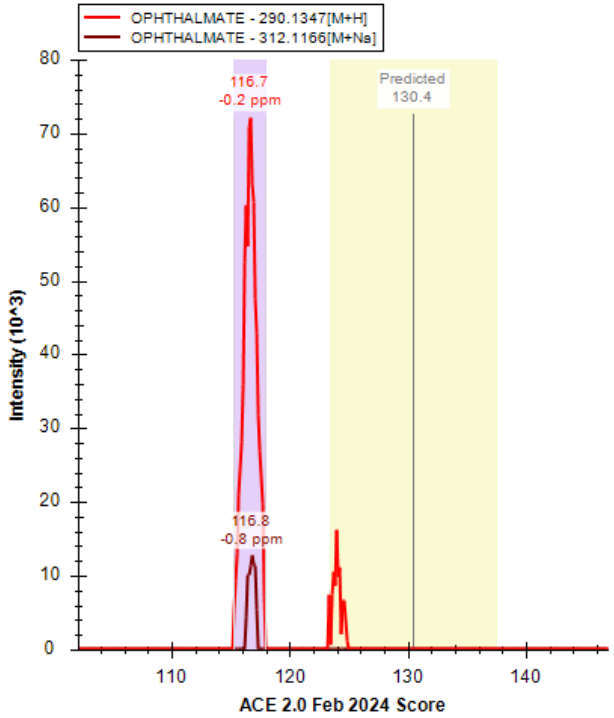
KM Algorithm needs ‘tuning’ for some cases

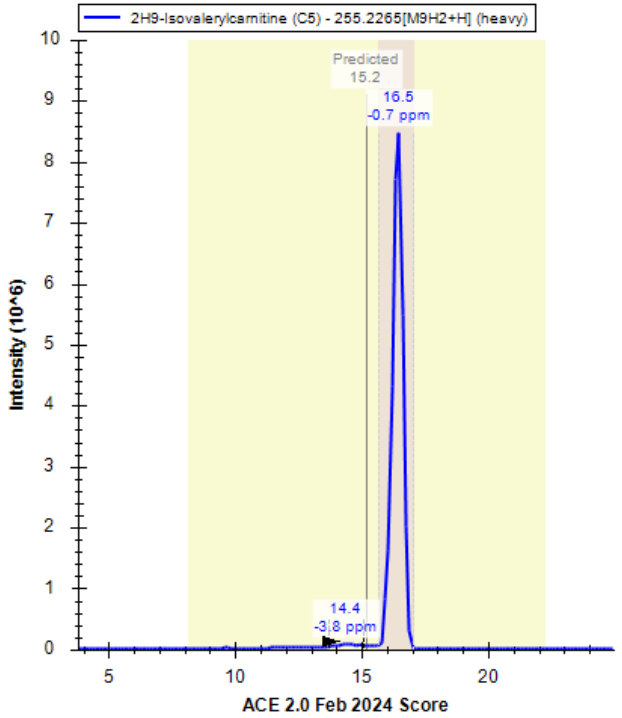


Molecule List Name	Molecule Name	%Correct Auto	%Correct 908AI	Improvement %
Dipeptide	epsilon-(gamma-Glutamyl)lysine	98.5%	9.3%	-89.2%
Dipeptide	N2-gamma-Glutamylglutamine	83.8%	0.0%	-83.8%
Vitamin	NICOTINAMIDE	90.9%	20.0%	-70.9%
Sugar	GLUCOSAMINE	88.2%	20.3%	-67.9%
Other	OPHTHALMATE	60.3%	0.0%	-60.3%
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/Modulat	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



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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

Extra slides

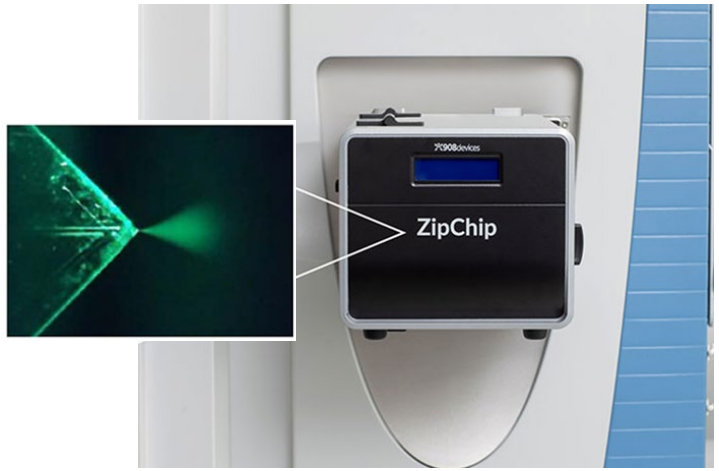
Metabolomics with ZipChip (microchip CZE-MS)

KIT

Reagents/Consumables

+

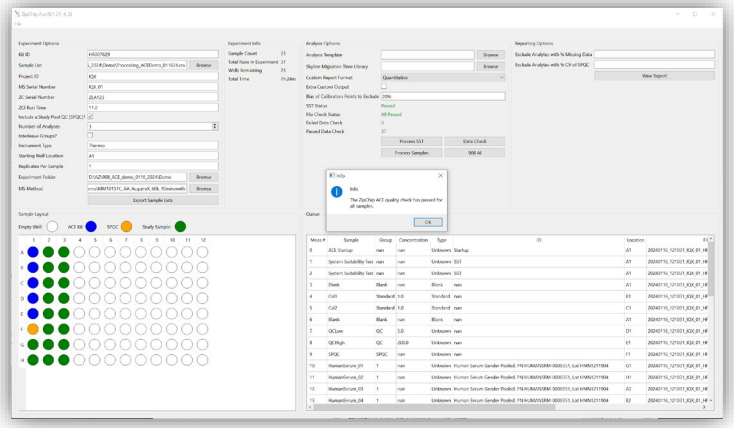
HARDWARE



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

+

SOFTWARE



ZipChip ACE App