

Leveraging LC-MS peptide mapping in protein raw material identity confirmation

CASSS – Next Generation Investigator

Alexzandrea Woudenberg

Scientist I R&D

Analytical Method Development

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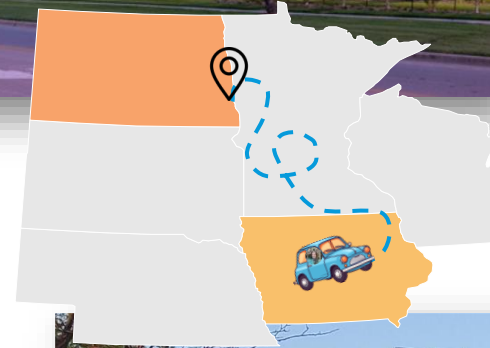
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Introduction – Alexzandra Woudenberg

- PhD in Biochemistry from Iowa State University
 - Protein signaling
 - Kinase activity and regulation
 - Protein complex formation
 - Conformational dynamics/HDX-MS
- Joined Aldevron in August 2025
- Transitioning to industry



Aldevron
Breakthrough Campus

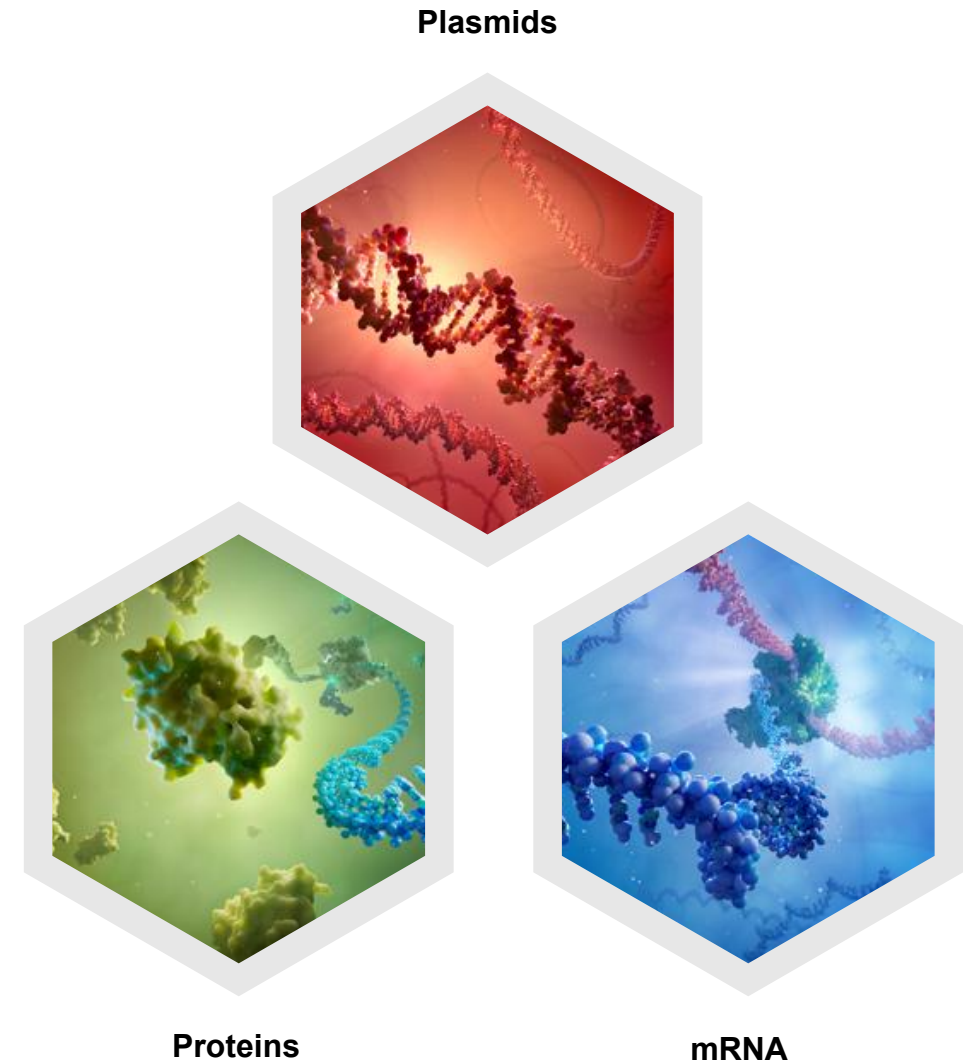


Roy J. Carver
Department of
Biochemistry,
Biophysics, and
Molecular Biology



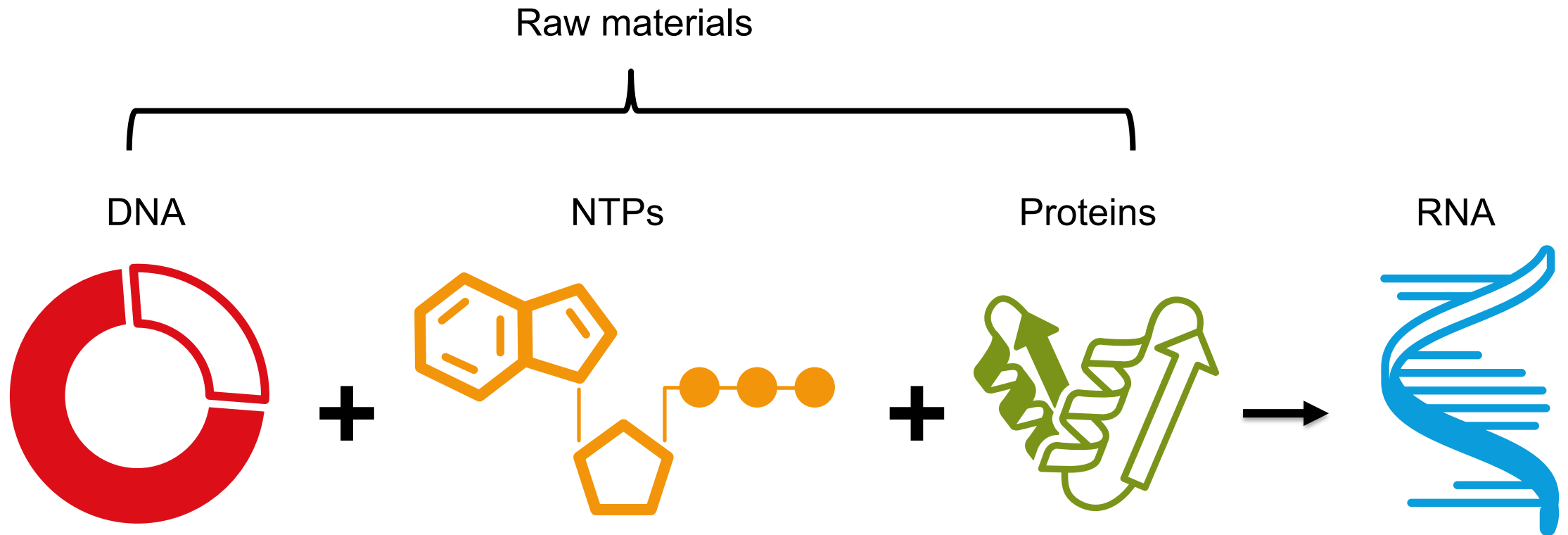
What is Aldevron?

- Manufacturing
 - cGMP & RG products
 - End-to-end DS and DP
- Cell & gene therapy CDMO



Raw material testing for cGMP manufacturing

- Raw material testing is required by the FDA



Protein raw materials provide unique challenges

- 23 protein raw materials
 - 7 enzymes
 - 4 vendors

	Aldevron	Vendor 2	Vendor 3	Vendor 4
DNase I				
Inorganic Pyrophosphatase				
RNase Inhibitor				
T7 RNA Polymerase				
Poly(A) Polymerase				
Guanylyltransferase				
2'-O-Methyltransferase				

FDA guidance for raw material testing

- Identity testing is required for all raw materials
- Accurate and reliable characterization

21 CFR part 211

“At least one test shall be conducted to verify the identity of each component of a drug product. Specific identity tests, if they exist, shall be used.”

Incoming materials must undergo identity testing as part of Phase 1 cGMP requirements. The results should be evaluated together with the vendor's Certificate of Analysis (CoA) to support material acceptance and release. Vendor is qualified as part of supplier quality assurance.

Raw material testing – Phase I vs. Phase III

- Phase-appropriate testing

Phase I

✓ **Identity**

Phase III

✓ **Identity**

✓ Purity

✓ Concentration

✓ Activity

✓ Endotoxin

✓ Bioburden

How is identity determined?

ICH Q2(R2) - Identity

“For identification tests, a critical aspect is to demonstrate the capability to identify the analyte of interest based on unique aspects of its molecular structure and/or other specific properties.”

Table 1: Typical performance characteristics and related validation tests for measured product attributes

Type of measured product attribute Analytical Procedure Performance Characteristics to be demonstrated (2)	IDENTITY	IMPURITY (PURITY) Other quantitative measurements (1)		ASSAY content/potency Other quantitative measurements (1)
		Quantitative	Limit	
Specificity (3) Specificity Test	+	+	+	+
Working Range	-	+	-	+
Suitability of Calibration model	-	QL (DL)	DL	-
Lower Range Limit verification	-			
Accuracy (4) Accuracy Test	-	+	-	+
Precision (4) Repeatability Test	-	+	-	+
Intermediate Precision Test	-	+ (5)	-	+ (5)

- signifies that this test is not normally evaluated

+ signifies that this test is normally evaluated

ICH Q2(R2)

Utilizing a phase-appropriate approach for ID

ICH Q2(R2) - Identity

“For identification tests, a critical aspect is to demonstrate the capability to identify the analyte of interest based on unique aspects of its molecular structure and/or other specific properties.”

How can we use mass spectrometry to confirm identity?

Phase I

- RP-HPLC with intact protein

Phase III

- Peptide mapping
- LC-UV characterization

Challenges for protein raw materials

What did we learn from Phase I raw material testing?

- Unique sequences → same enzyme/functionality
- PTMs – impact RT for RP-HPLC
- Protein tags

Phase I

- RP-HPLC with intact protein

Phase III

- Peptide mapping
- LC-UV characterization

HPLC vs. LC-MS for routine use

Why choose LC-UV over LC-MS?

- Analyst training requirements
- Equipment cost
- Instrument availability



LC-UV (HPLC)

VS



LC-MS

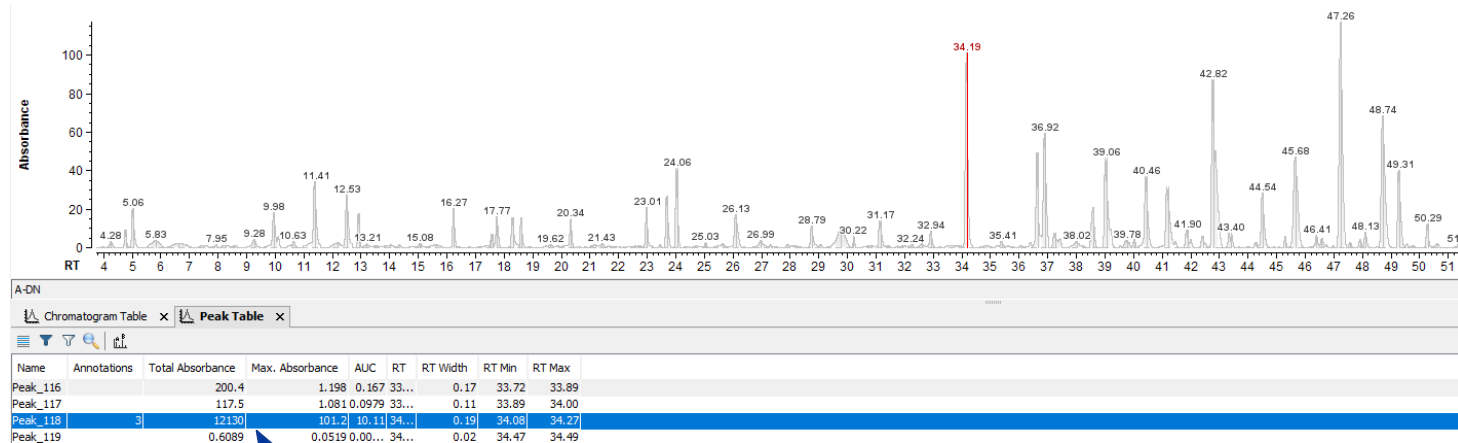
Peptide mapping on Sciex ZenoTOF 7600

- Waters™ ACQUITY Premier UPLC Front-End
- Sciex ZenoTOF 7600
- Generate a gradient that can be reproduced on the QC Agilent instruments.

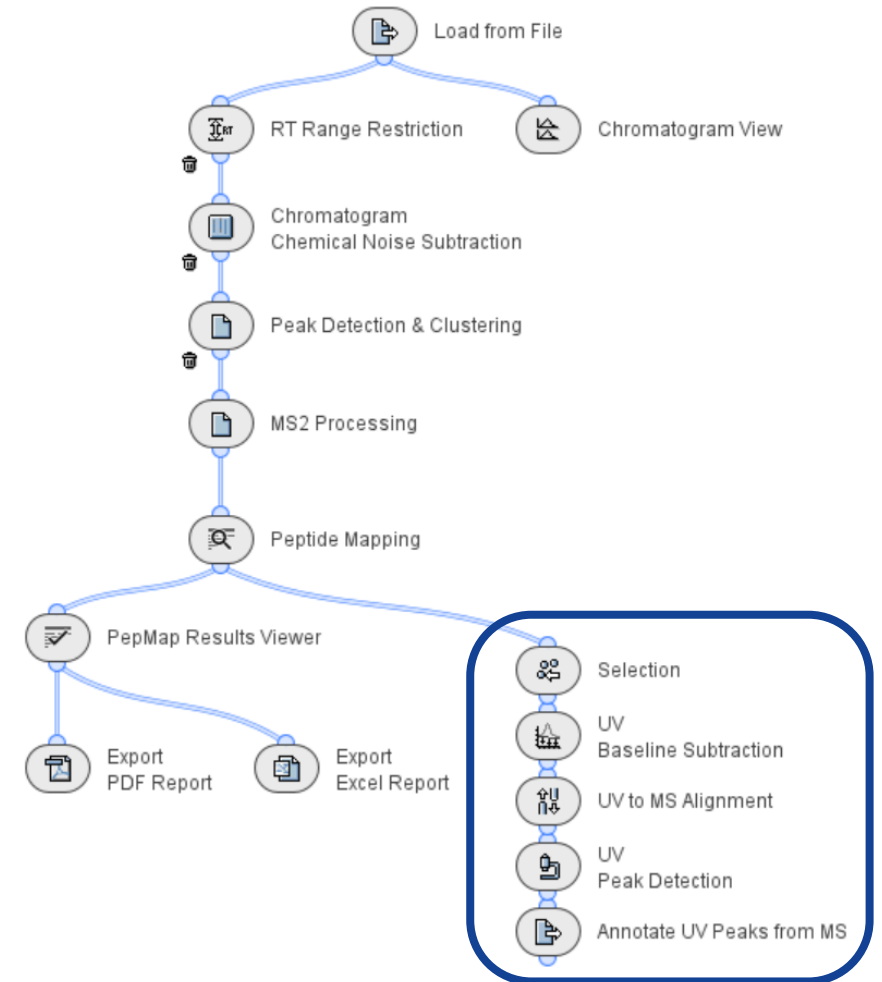


Mapping peptides to retention time with Genedata Expressionist

1. ID peptides using Genedata Expressionist
2. Annotate UV peaks for peptides with MS/MS confirmation
3. Transfer LC-UV mapping to QC LC-UV instruments

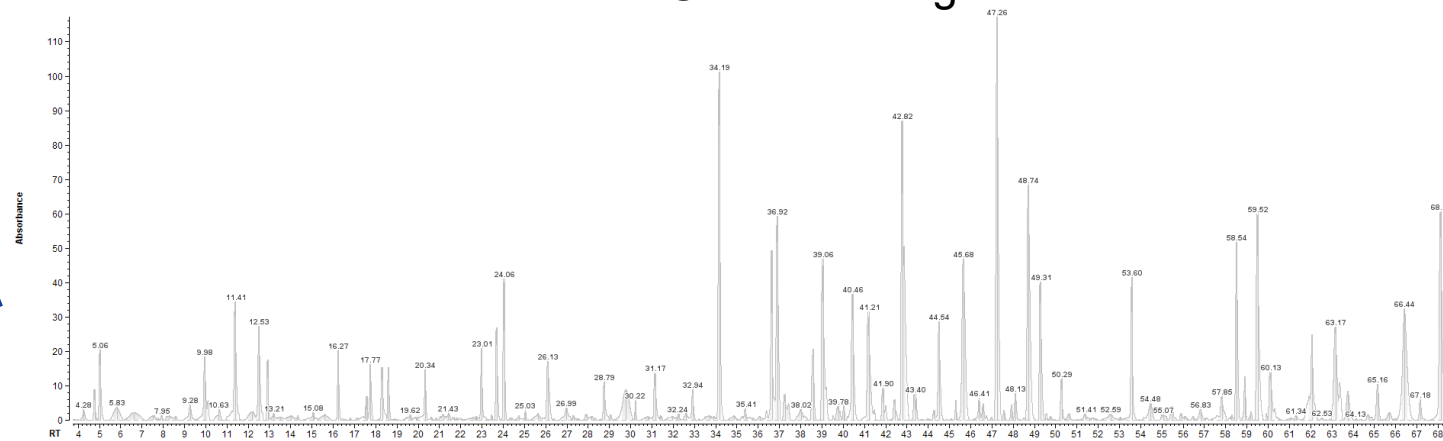


Annotate UV peaks with MS data



Using Genedata – mapping peptides to retention time

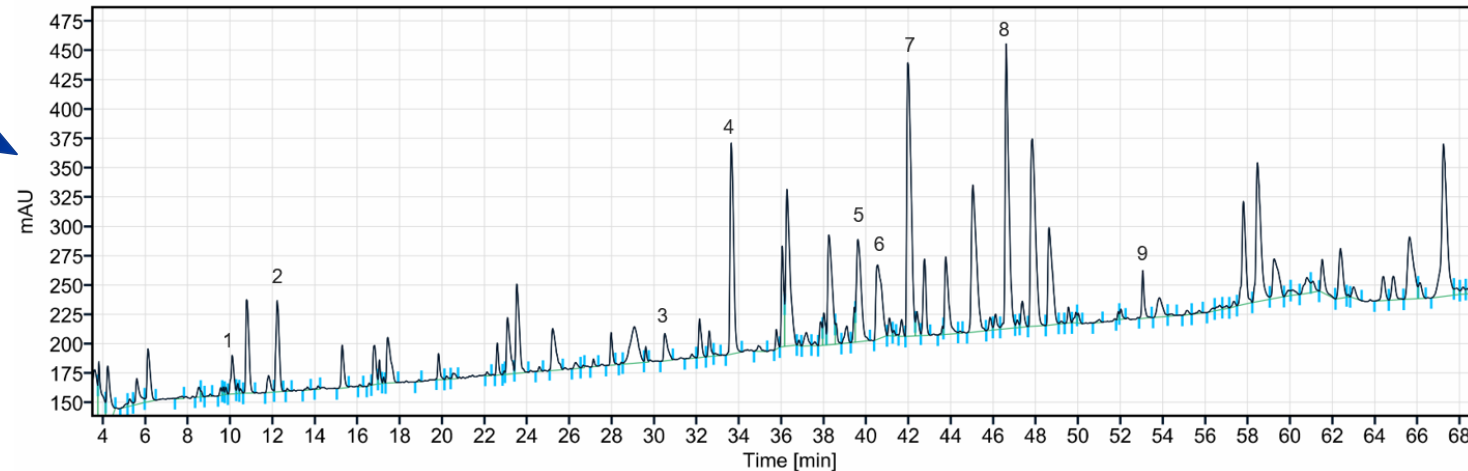
DNase I UV chromatograms



UV annotations with
MS/MS data



Sample chromatogram on
QC HPLCs



Benefits of raw material testing

- Raw material testing helped learn about the products we're using
 - Multiple unique sequences
 - Product variants
 - Sample mix – which enzymes are in the sample?

Selecting peaks for LC-UV analysis

- 5 peptides were selected for each sequence
- Peaks were chosen based on:
 - Complete digestion (no missed cleavages)
 - Potential modifications
 - Signal in UV
 - Consistent digests (reproducible peaks and RT)
 - Pattern (easy visualization)

Specificity

All 5 peaks from one sequence cannot overlap with all 5 peaks from another sequence.

Selecting peaks for LC-UV analysis

- 5 peptides were selected for each sequence
- Peaks were chosen based on:
 - Complete digestion
 - Potential modifications
 - Signal in UV
 - Consistent digests
 - Pattern



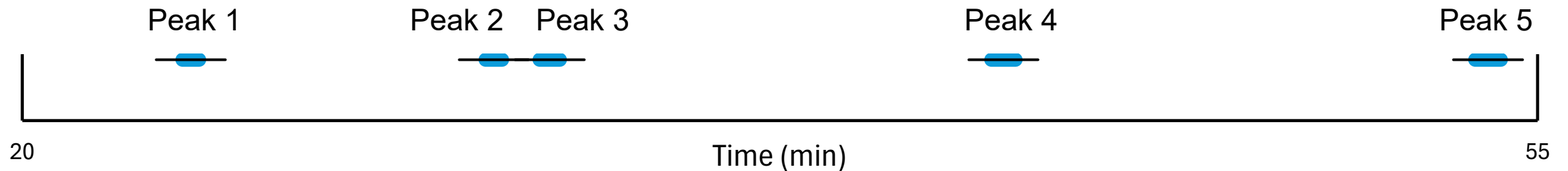
Assessing retention time repeatability

- 2 instruments, 3 columns, 12+ analytical runs
- Plotted the range of retention times

	Instrument A	Instrument B
Column A		
Column B		
Column C		

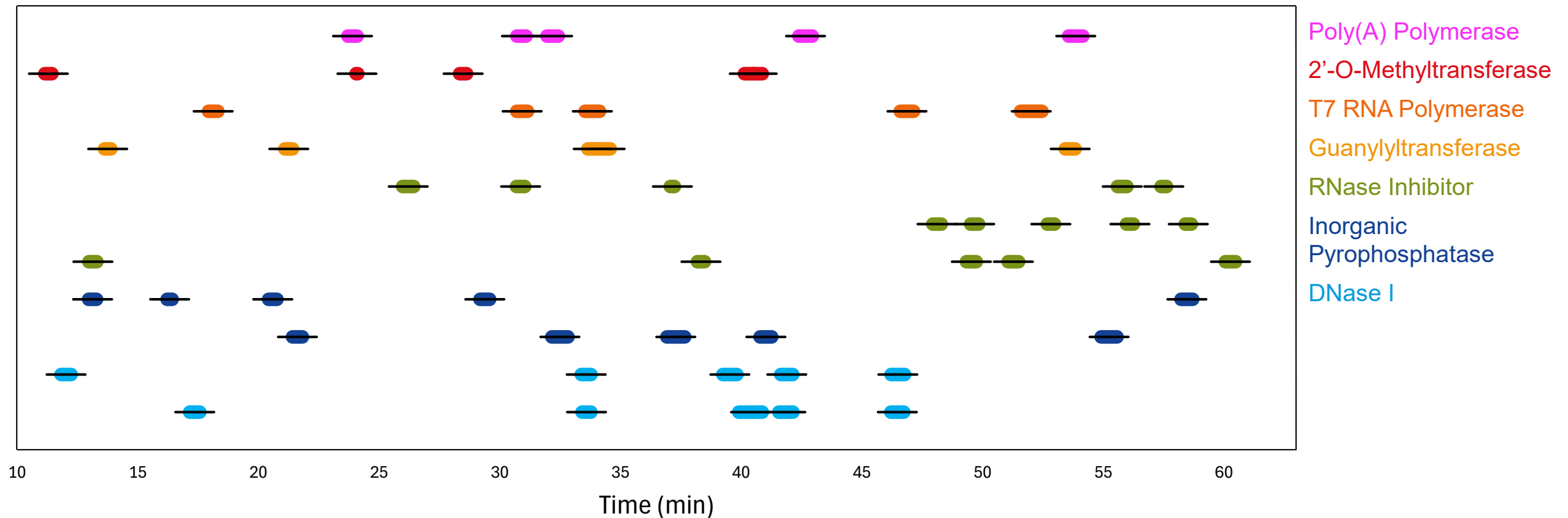
 Observed retention times

 Retention time range



Assessing retention time repeatability

- 2 instruments, 3 columns, 12+ analytical runs

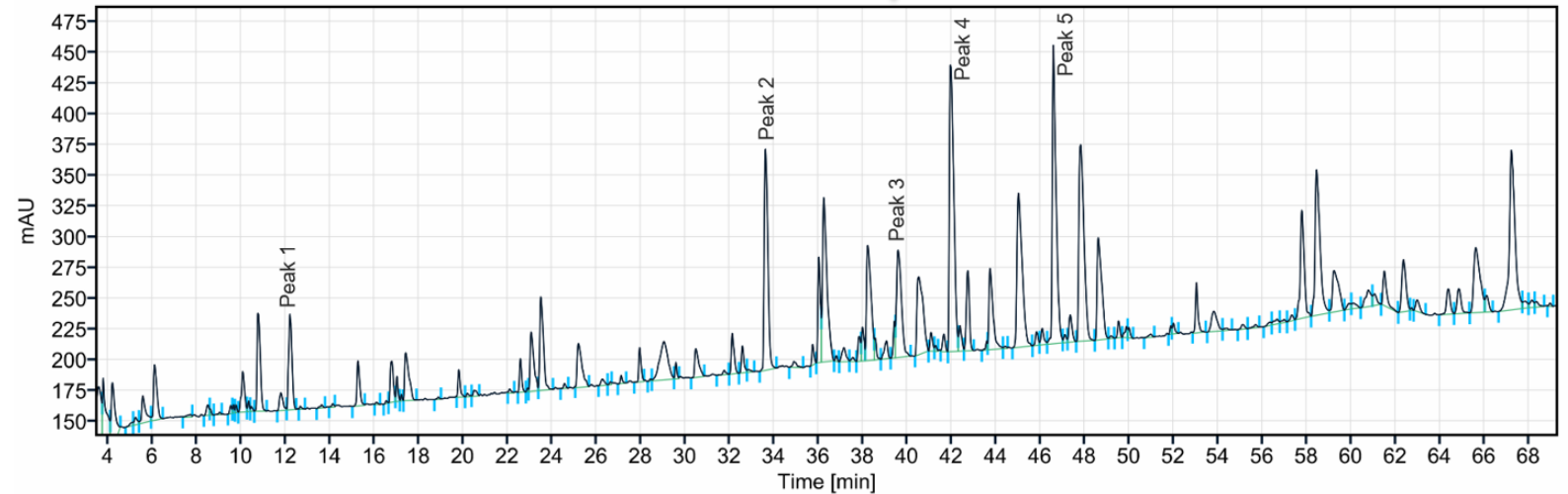
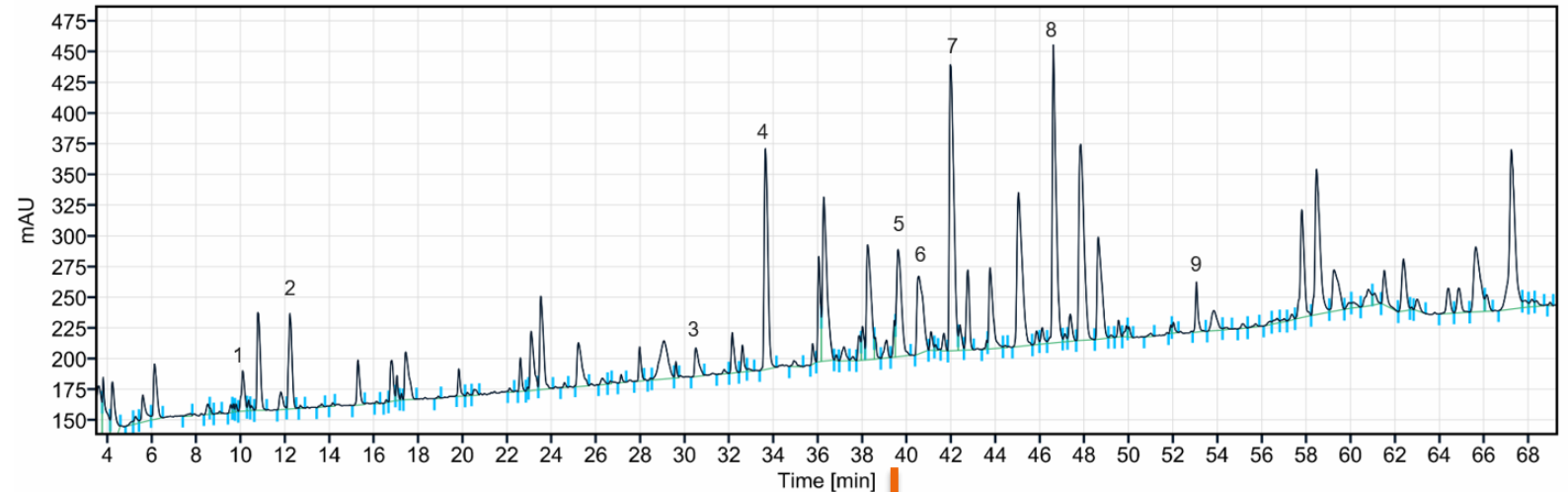


Selecting unique peptides for all samples

DNase I UV chromatograms

Routine use in QC:

1. Does the pattern match?
2. Are peptides within the retention time range?



Next steps

- ID methods for Phase I and Phase III methods have been qualified
- Projects utilizing LC-MS in development
 - Protein contamination of products in Alchemy™ Cell-free DNA
 - Supporting protein R&D
 - Expanding ID assays

Acknowledgements

Analytical Method Development

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 danaHER.



Aldevron is proud to be part of DanaHER.

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