ALTHEA | THE POWER TO MAKE™

SPEEDING UP REPRODUCIBILITY WITH SMART DIGEST AND THE VANQUISH UHPLC

MAY 2017

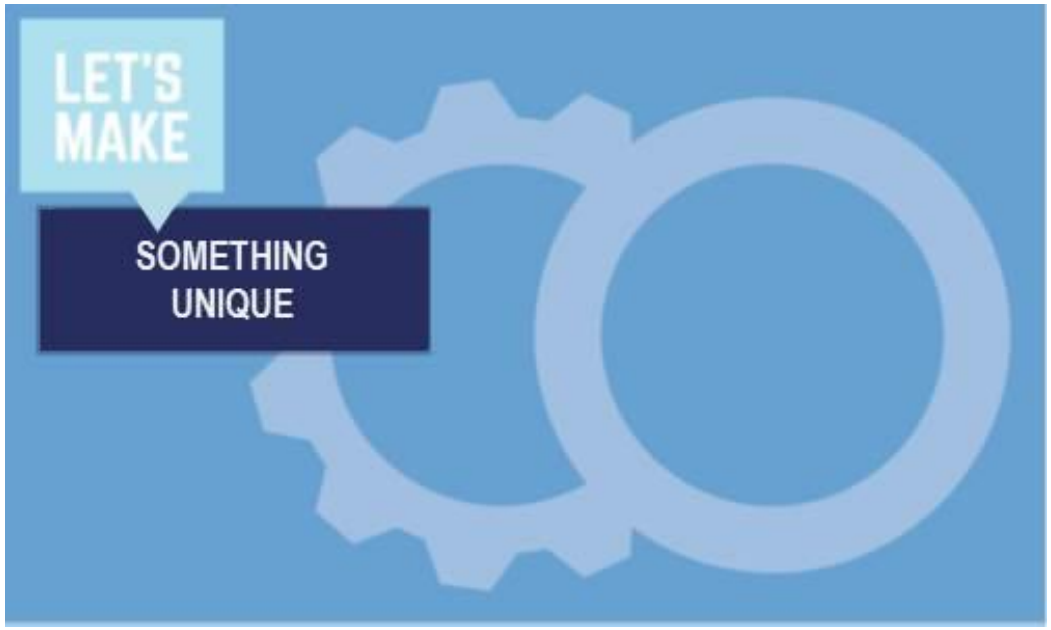
ANALYTICAL DEVELOPMENT TEAM

OVERVIEW

- Short introduction to Althea
- Background in peptide mapping
- Moving up to SMARTer digests
- Vanquish UHPLC

ALTHEA OVERVIEW

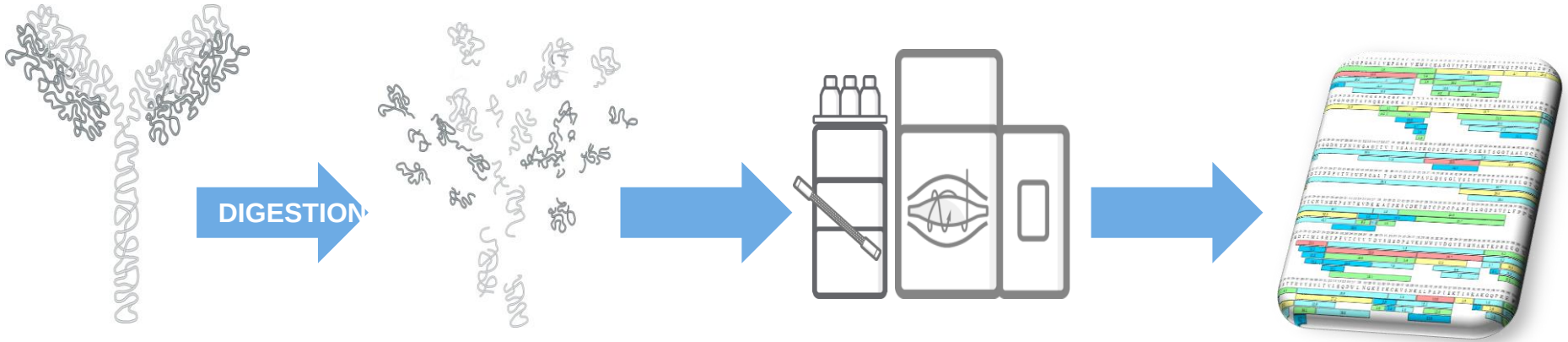
- Full service contract manufacturing organization (CMO)
- Analytical development (early phase-commercial manufacturing support)
- Formulations (proprietary Crystalomics technology)
- State of the art Antibody Drug Conjugate facility (ADC)

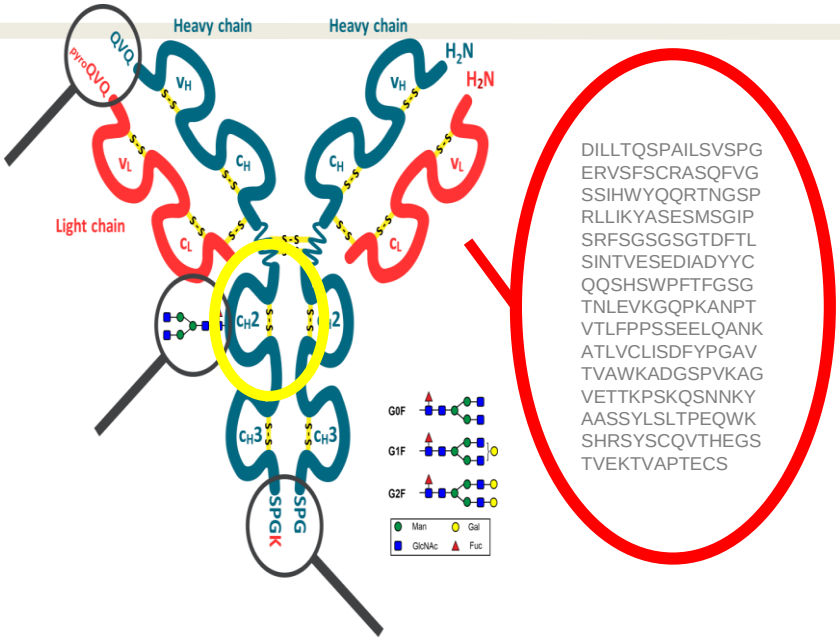


ALTHEA ANALYTICAL DEVELOPMENT

- Supports external and internal clients (manufacturing, process development, Formulations/Crystalomics)
- Work with development partners in early phase development through commercial manufacturing
- Antibody drug conjugate testing and development

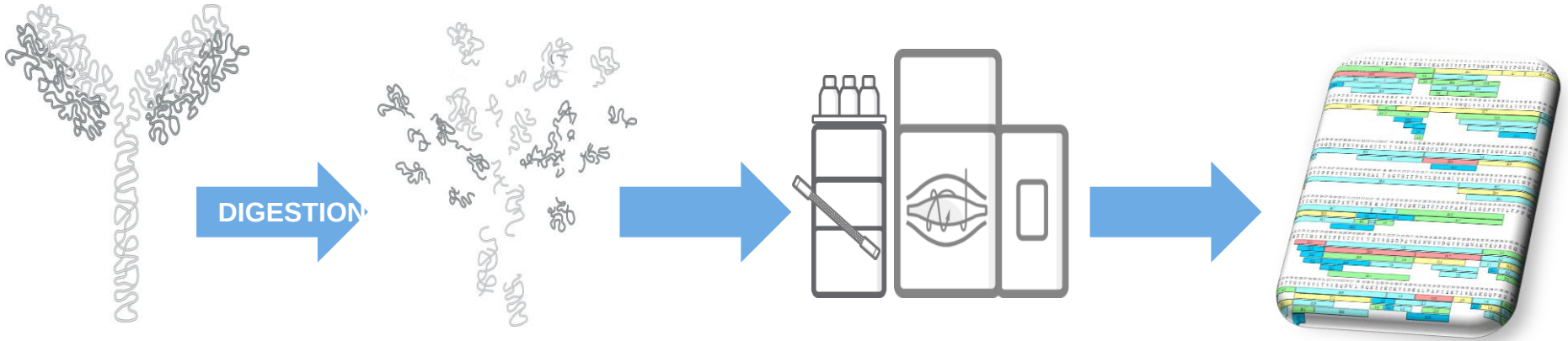
Why Peptide Mapping?





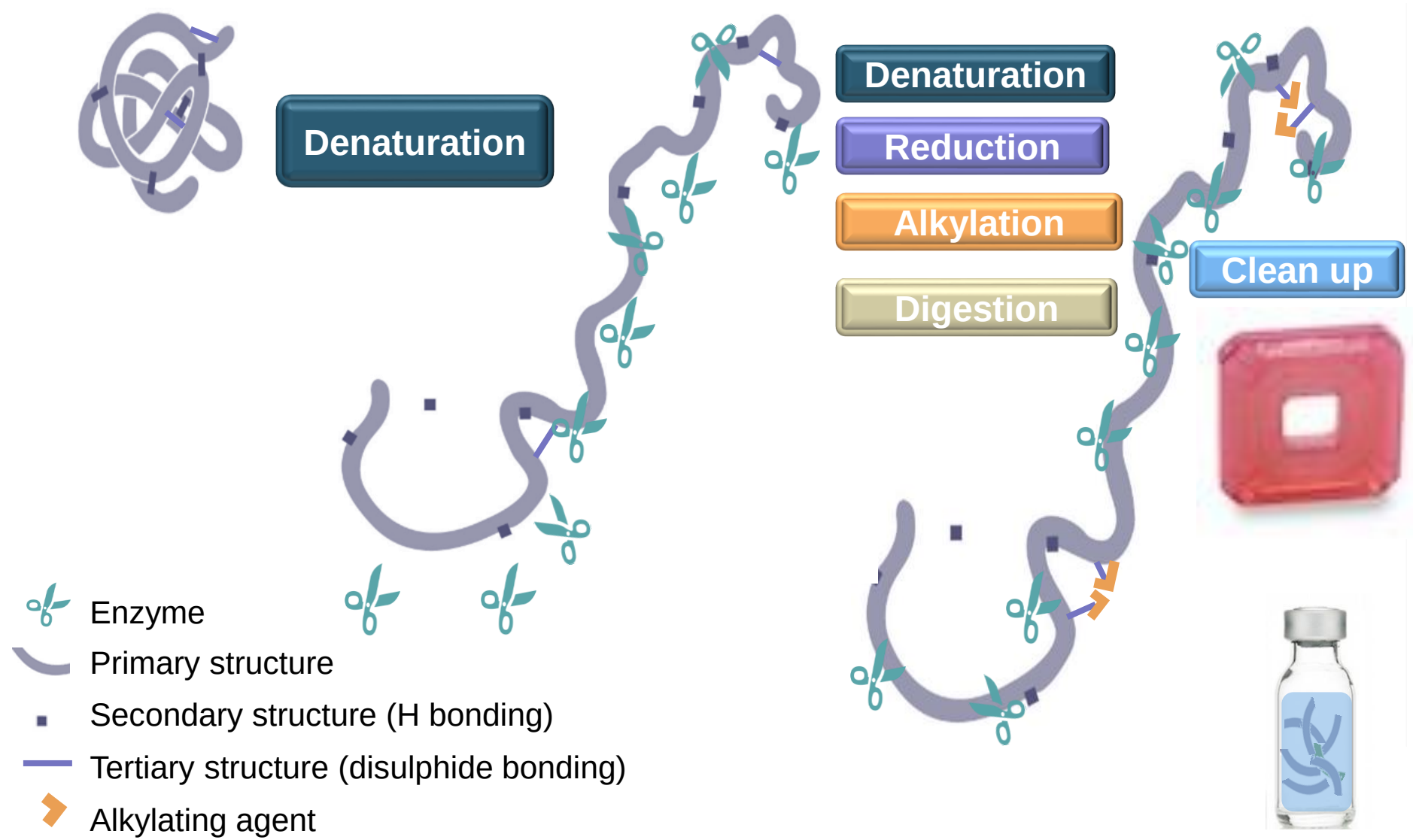
DILLTQSPAILSVSPG
ERVSFSCRASQFVG
SSIHWYQQRTNGSP
RLLIKYASESMSGIP
SRFSGSGSGTDFTL
SINTVESEDIADYYC
QQSHSWPFTFGSG
TNLEVKGQPKANPT
VTLFPPSSEELQANK
ATLVCLISDFYPGAV
TVAWKADGSPVKAG
VETTKPSKQSNNKY
AASSYLSLTPEQWK
SHRSYSCQVTHEGS
TVEKTVAPTECS

Why Peptide Mapping?

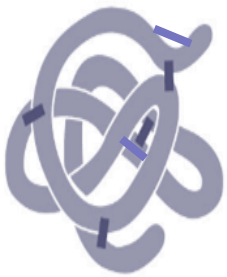


Identity & Purity | Comparability | Quantitation | Lot Release | Regulatory

The Fundamental Steps In a Typical Protein Digest



FIVE TYPICAL TIME CONSUMING STEPS

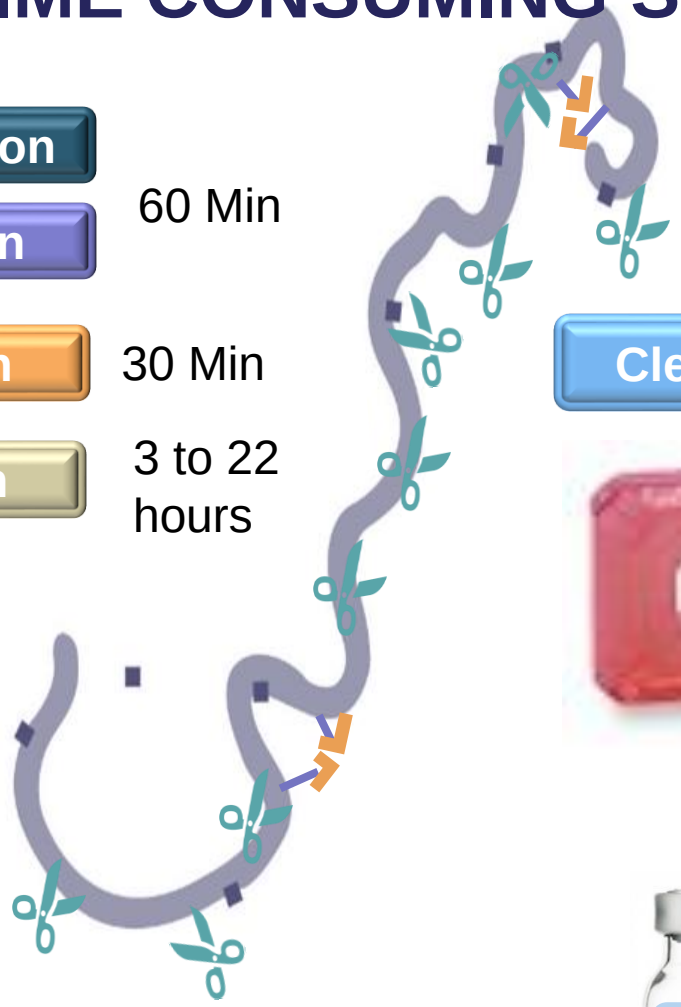


Denaturation

Reduction 60 Min

Alkylation 30 Min

Digestion 3 to 22 hours

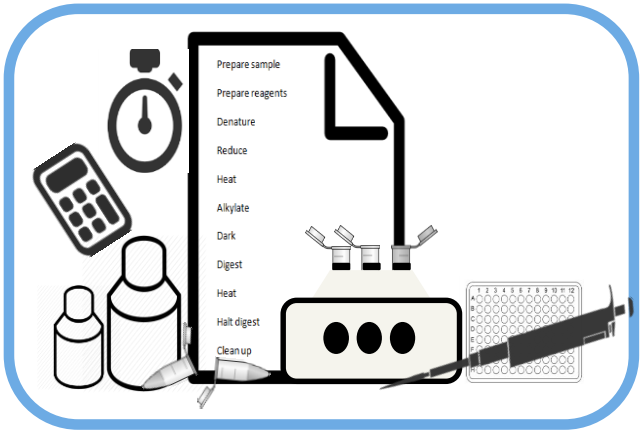


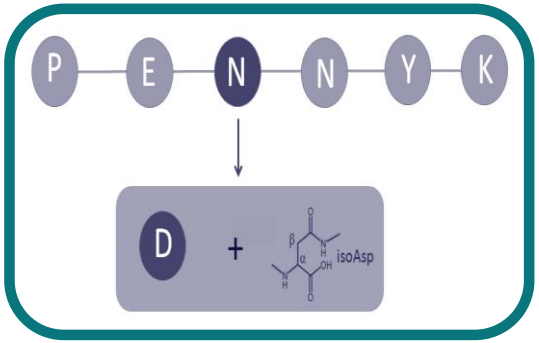
Clean up 3 to 12 hours

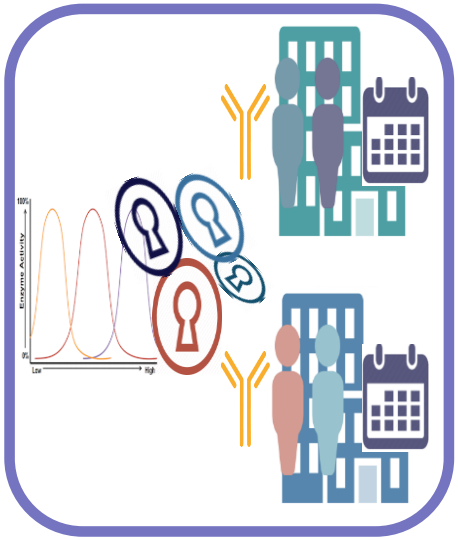
Total Time to Injection: 8 to 36 hours

Improvement Opportunities

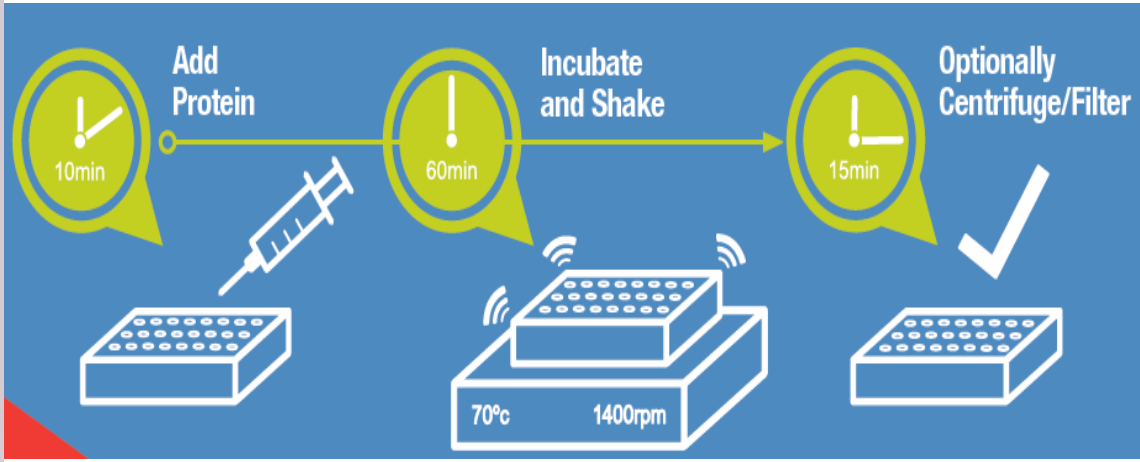
- Lengthy multi-step protocols
- Process-induced PTMs
- Reproducibility
- Throughput/speed
- Method development ease







Thermo Scientific™ SMART Digest™ Kits



Denaturation

Digestion

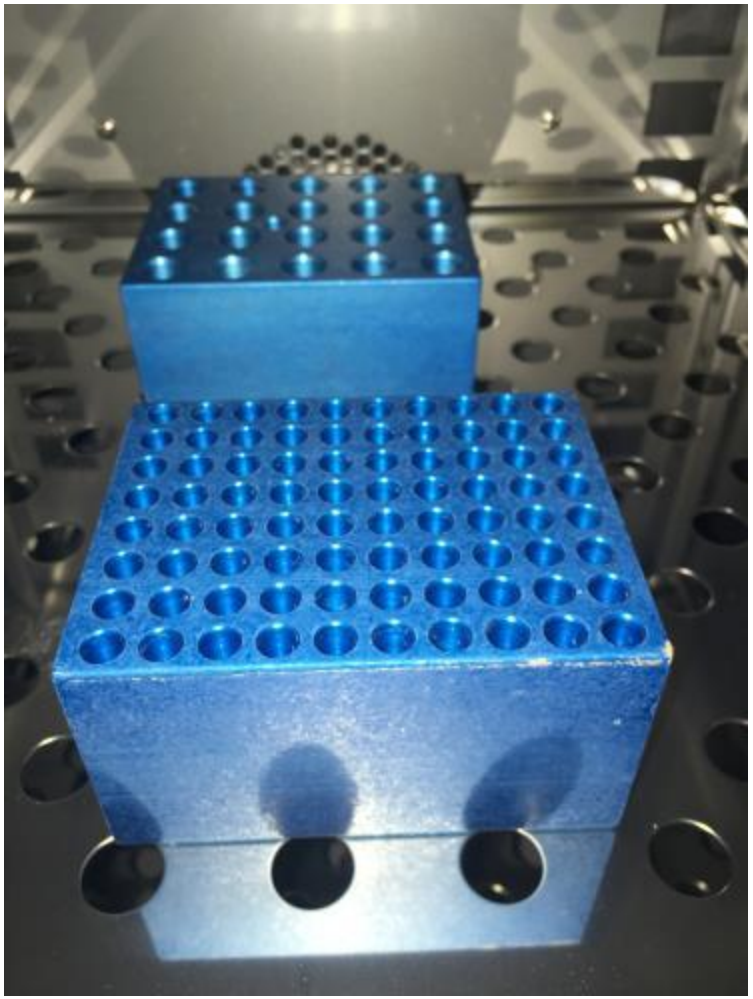
Clean up

5 steps simplified

ALTHEA ANALYTICAL DEVELOPMENT CASE STUDY

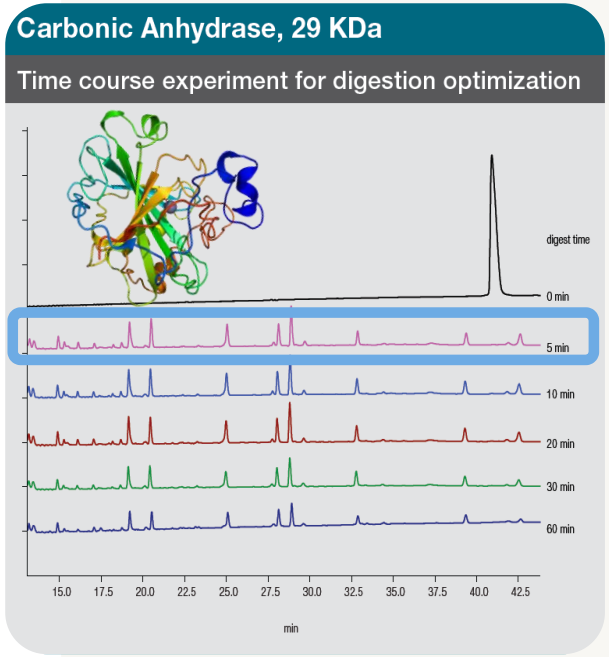
- How much can we decrease analysis time/increase throughput by incorporating SMART digest and using the Vanquish UHPLC
- User to user reproducibility
- Can the technology easily be transferred to a QC setting

INITIAL SET UP



DEVELOPMENT OF DIGEST METHOD

- Time-course to determine optimum digestion time
- Determine if reduction or alkylation are necessary for your application
  - I am worried I may see scrambled disulphides/ my protein has free cysteines – **alkylate before**
  - I want to know where my disulphides are! – **yes & no**
  - My mass spectrometer won't acquire/is not optimized for higher mass peptides - **maybe**

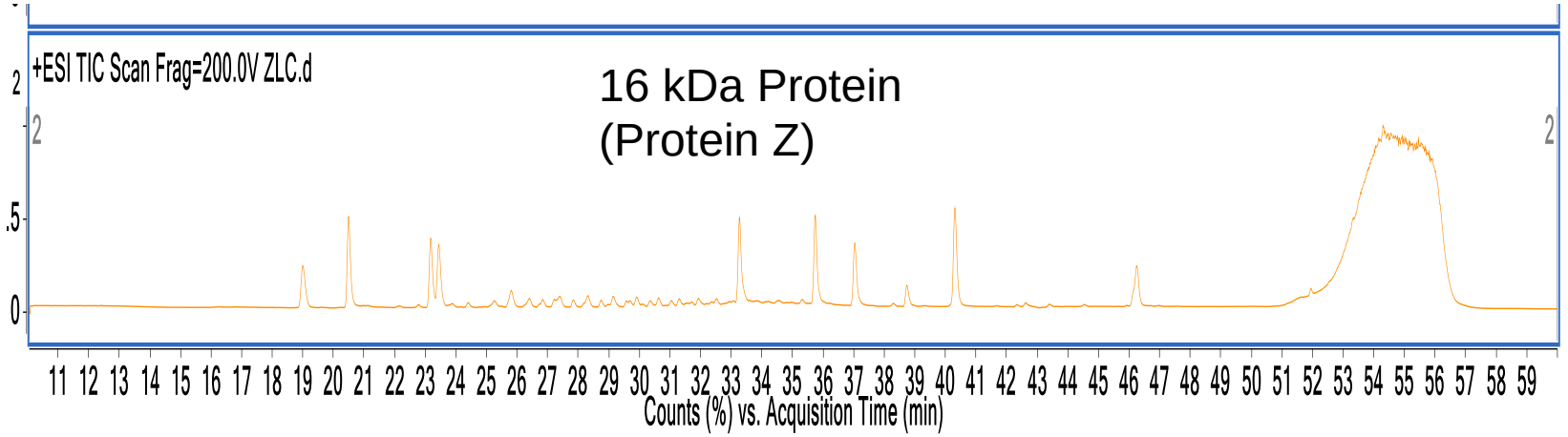


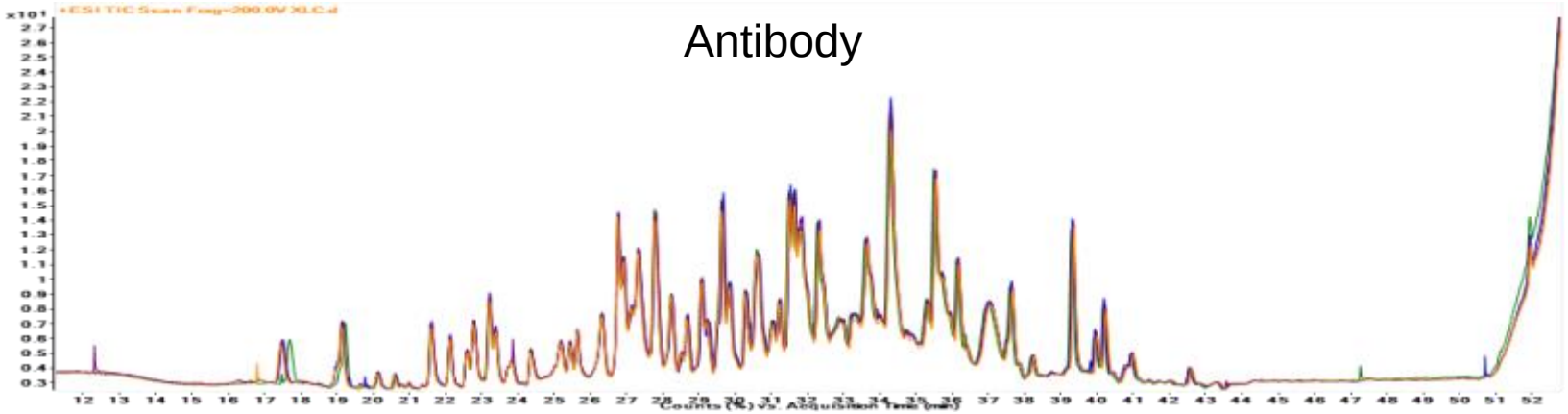
Total Time To Develop 4 Protein Methods: 8 hours

-4 hours for set up and perform initial digest studies

-4 hours to analyze the digests

TWO EXAMPLES



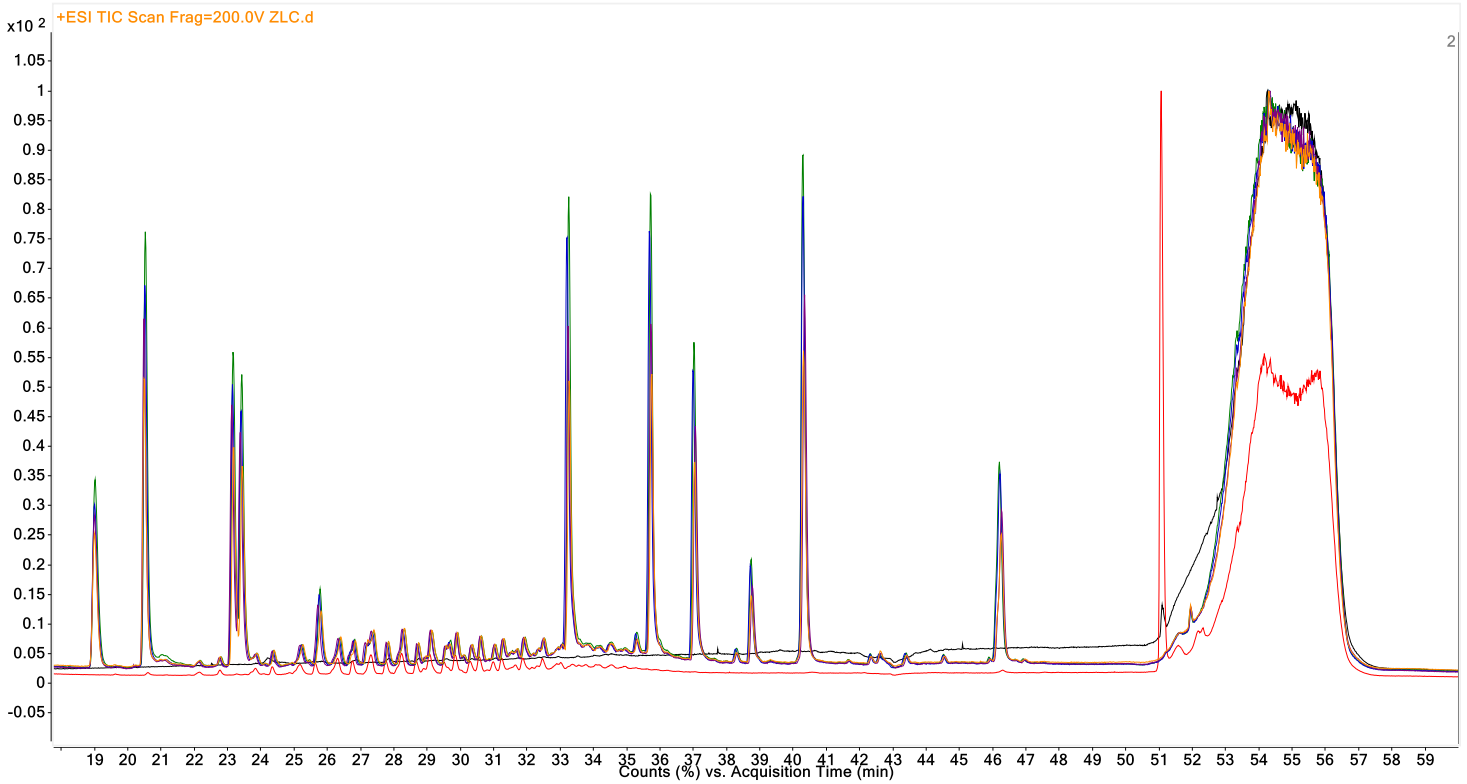


REPRODUCIBILITY

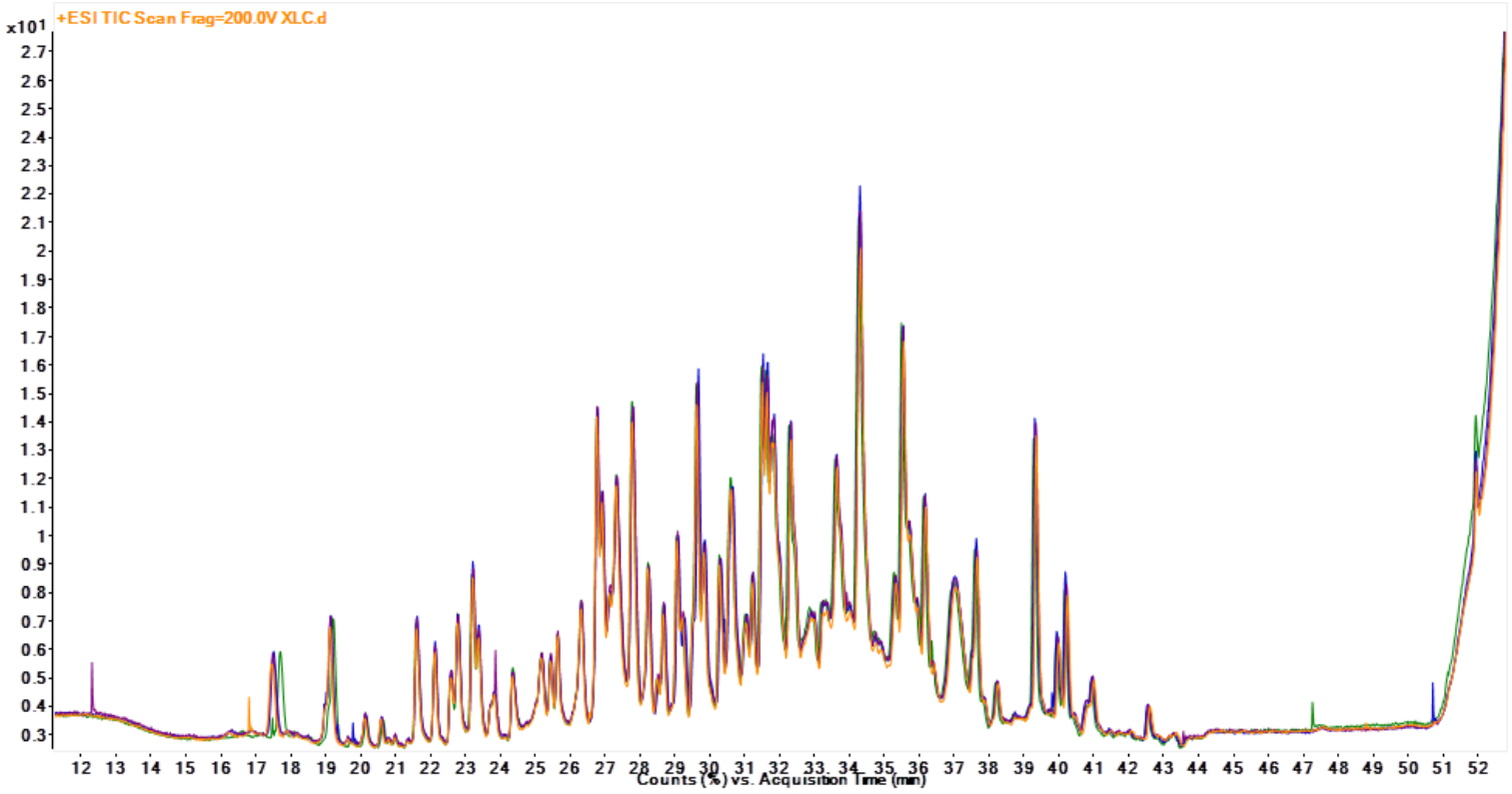
Four Individual Analysts

- Two experienced in mass spectrometry and enzyme digestions
- One lab analyst inexperienced in enzyme digestions
- One sales rep

REPRODUCIBILITY (PROTEIN Z)

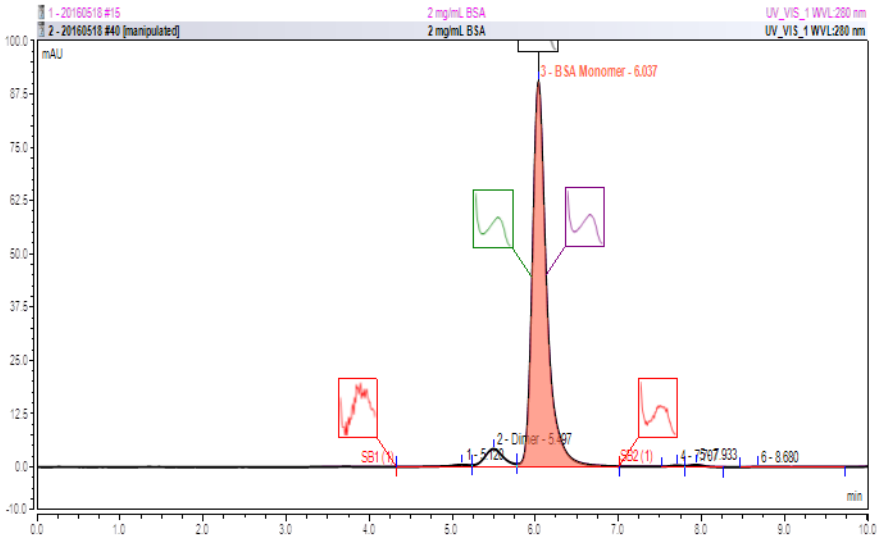


REPRODUCIBILITY (ANTIBODY)

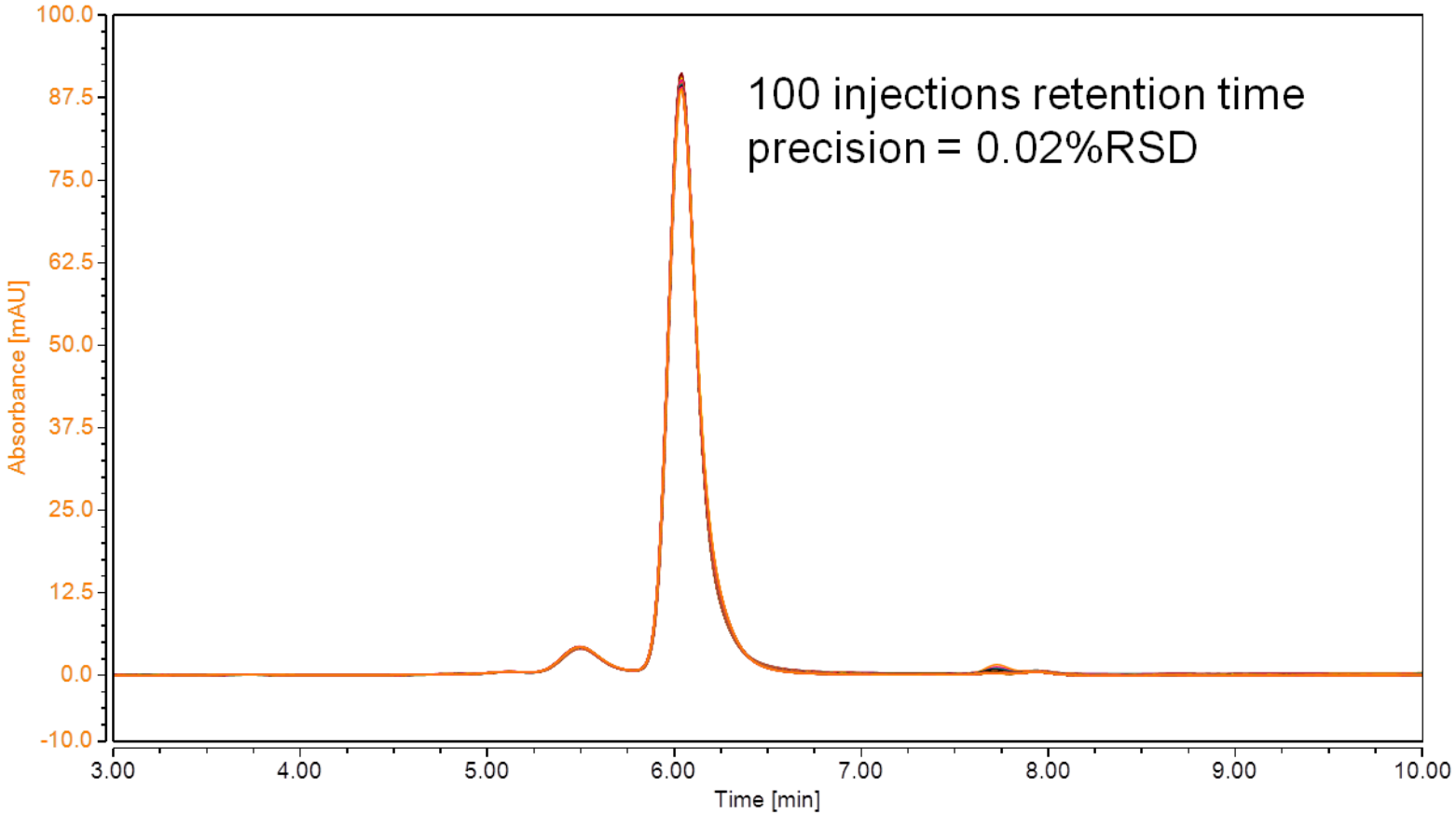


INSTRUMENTATION OVERVIEW

UHPLC:Thermo Scientific™ Vanquish™ Horizon UHPLC System
Detectors: DAD

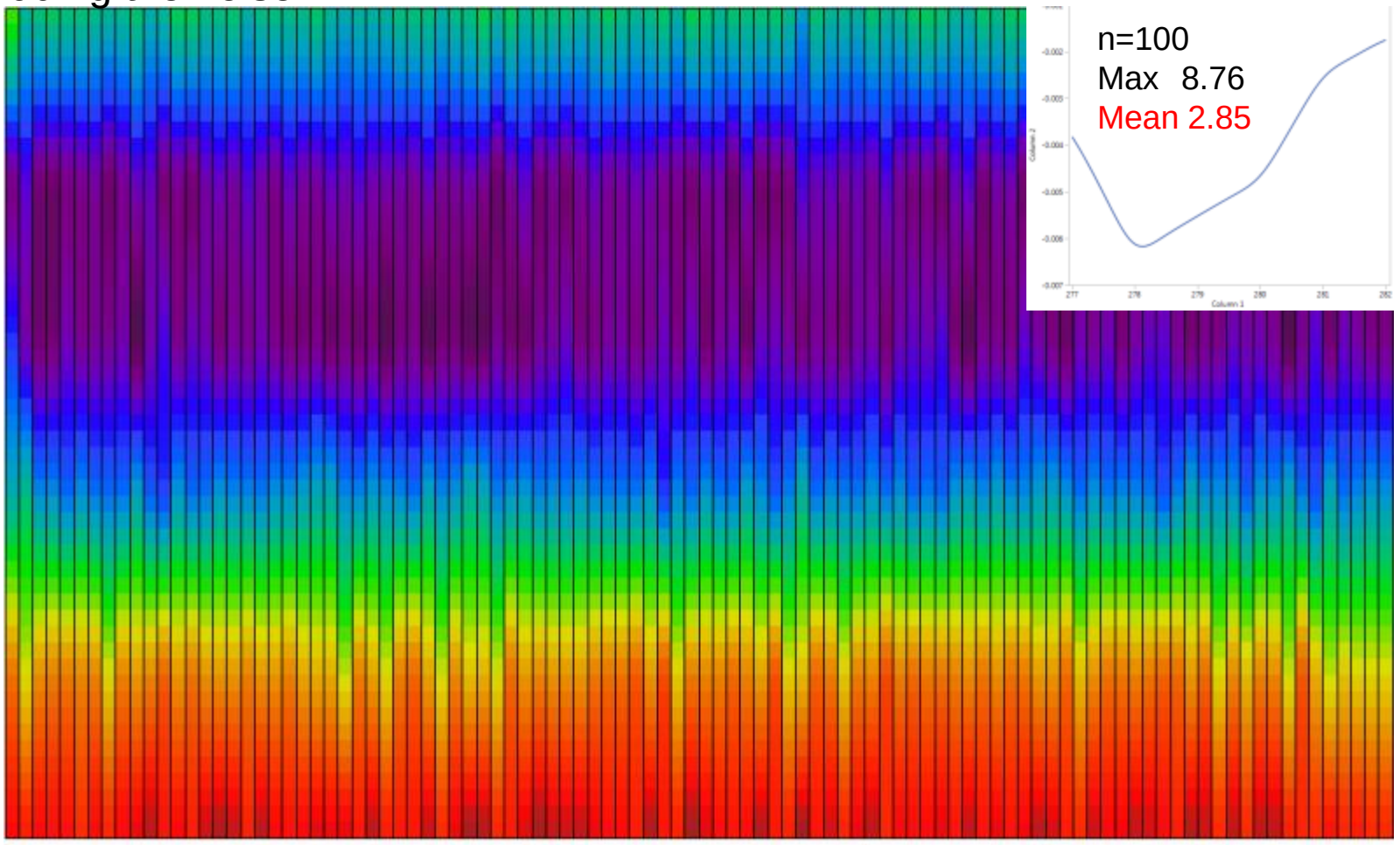


RETENTION TIME PRECISION

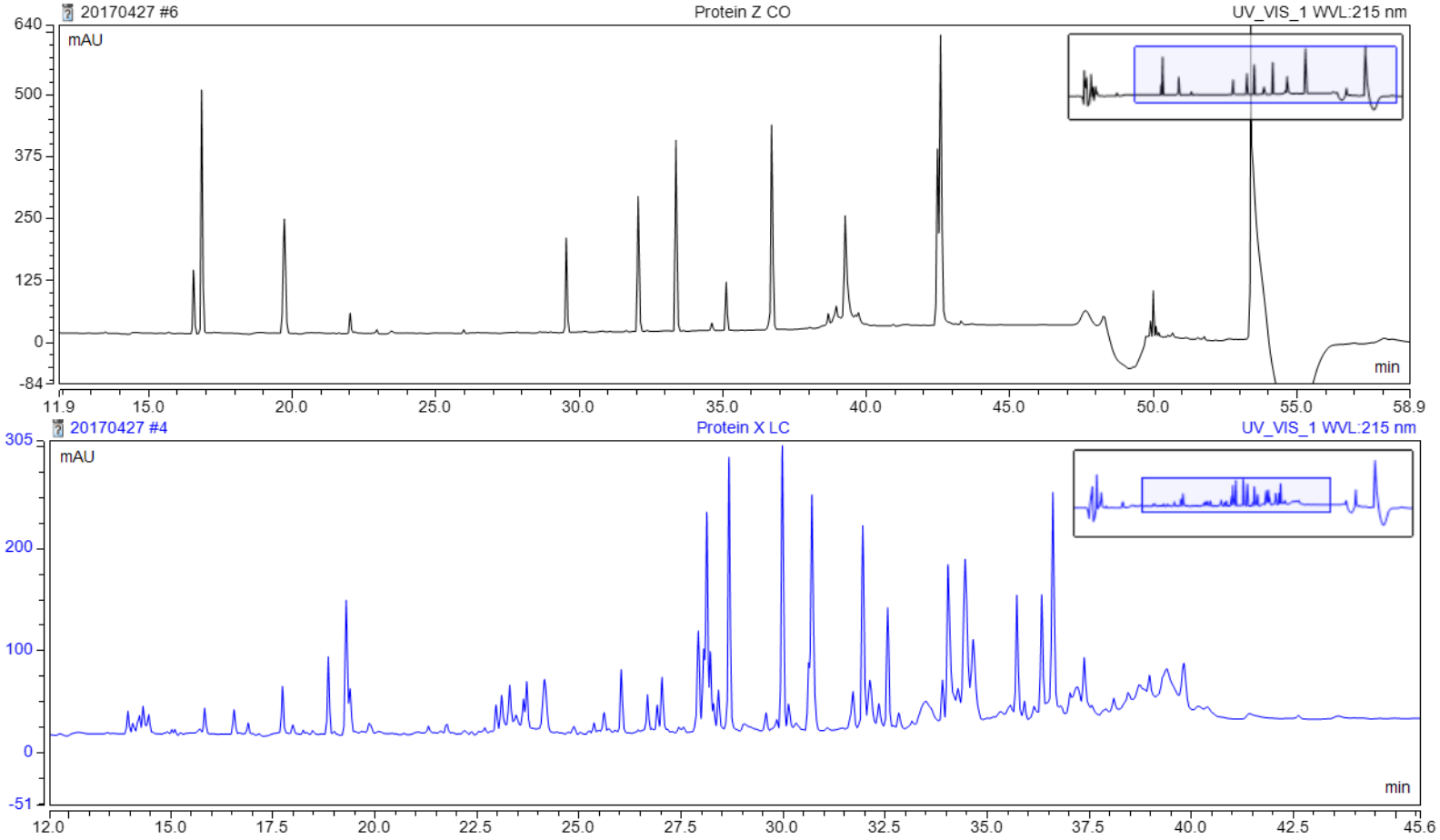


SIGNAL REPRODUCIBILITY OF THE VANQUISH DAD

100 injections X 600 data points with the average RSD less than 3%, including the noise

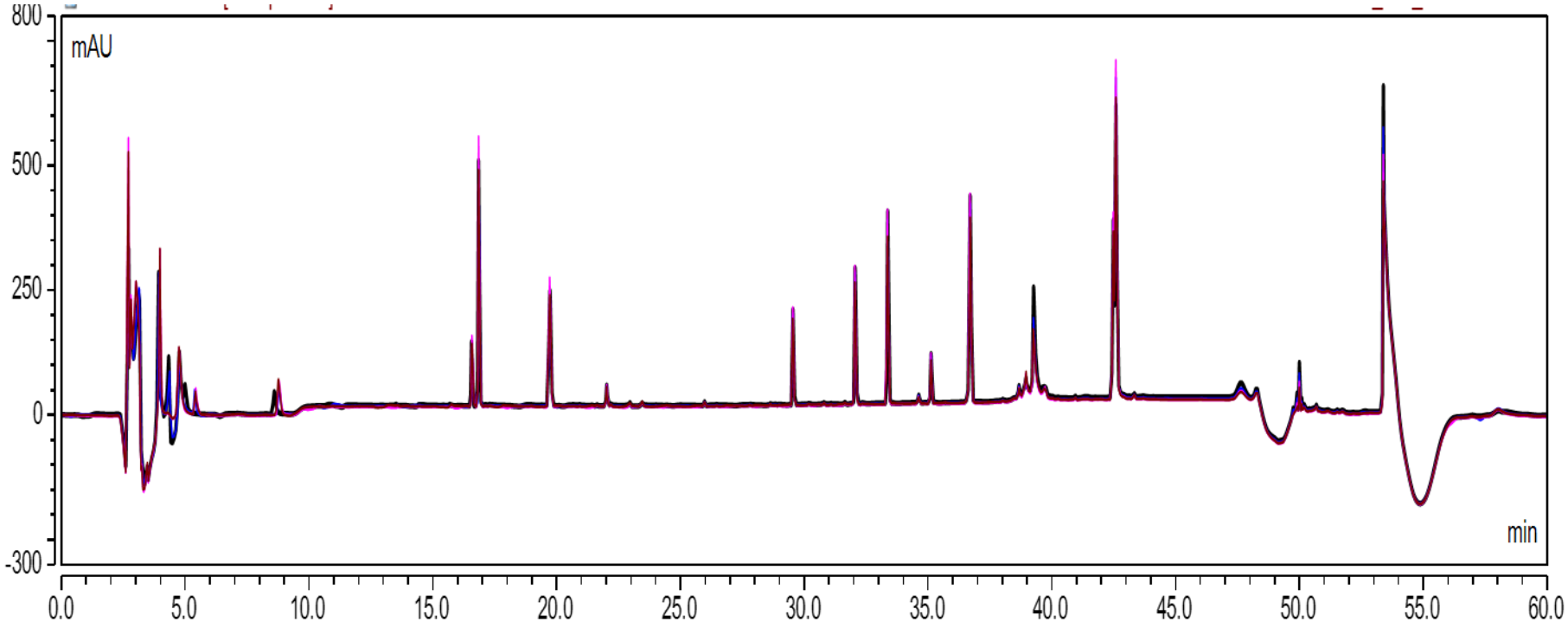


INITIAL TRANSFER OF THE METHOD TO THE VANQUISH

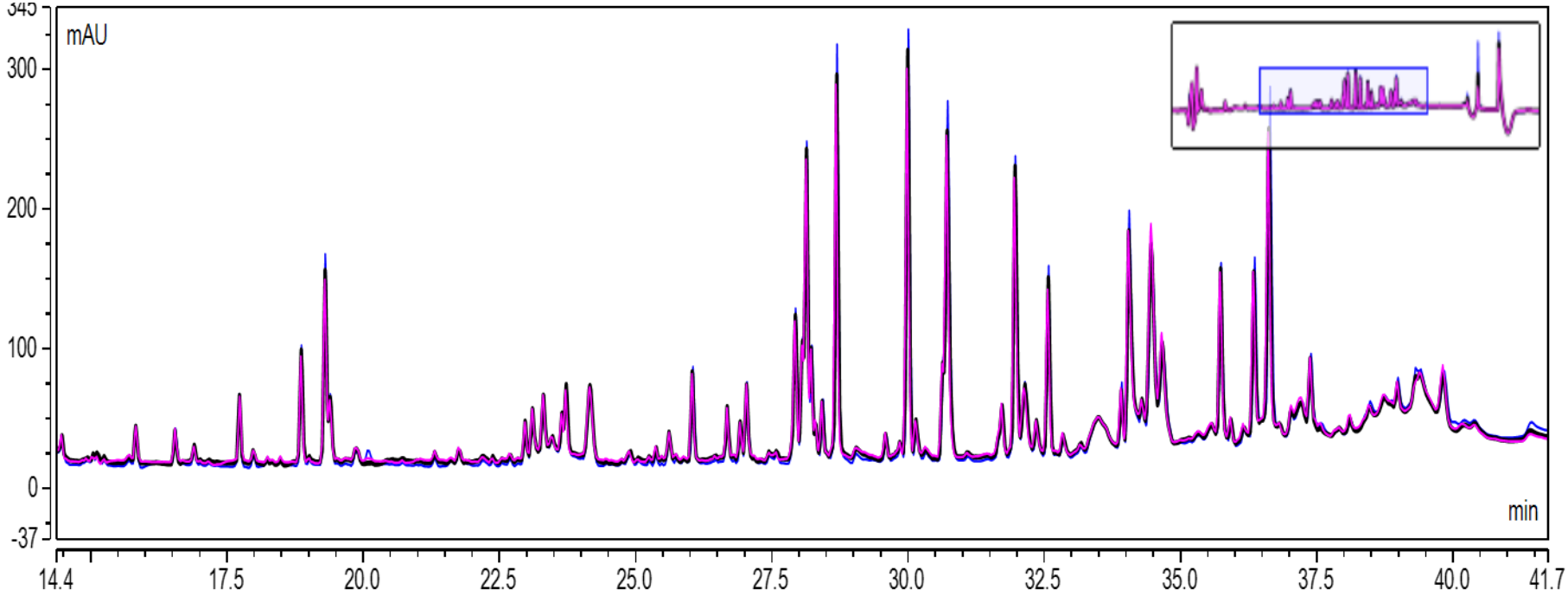


Same Flow Rate and Gradient
Forced Air Column Heating

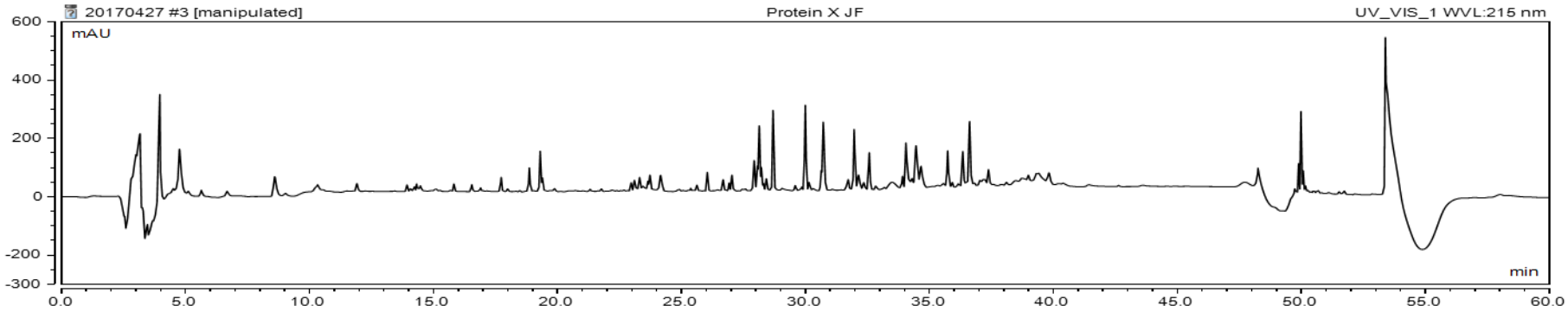
4 ANALYSTS PROTEIN Z

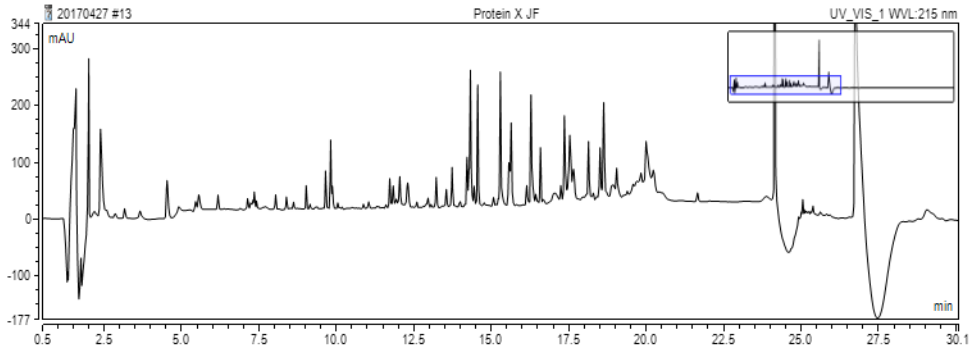


FOUR ANALYSTS (ANTIBODY)

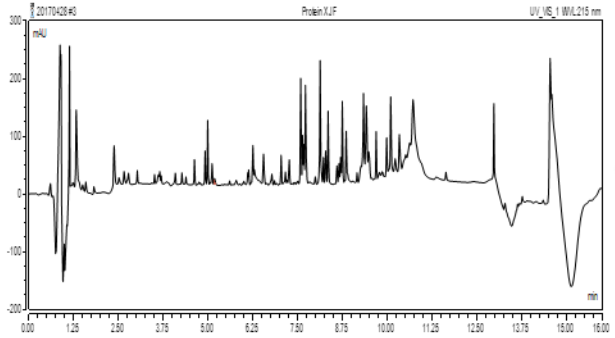


HOW FAST CAN IT GO



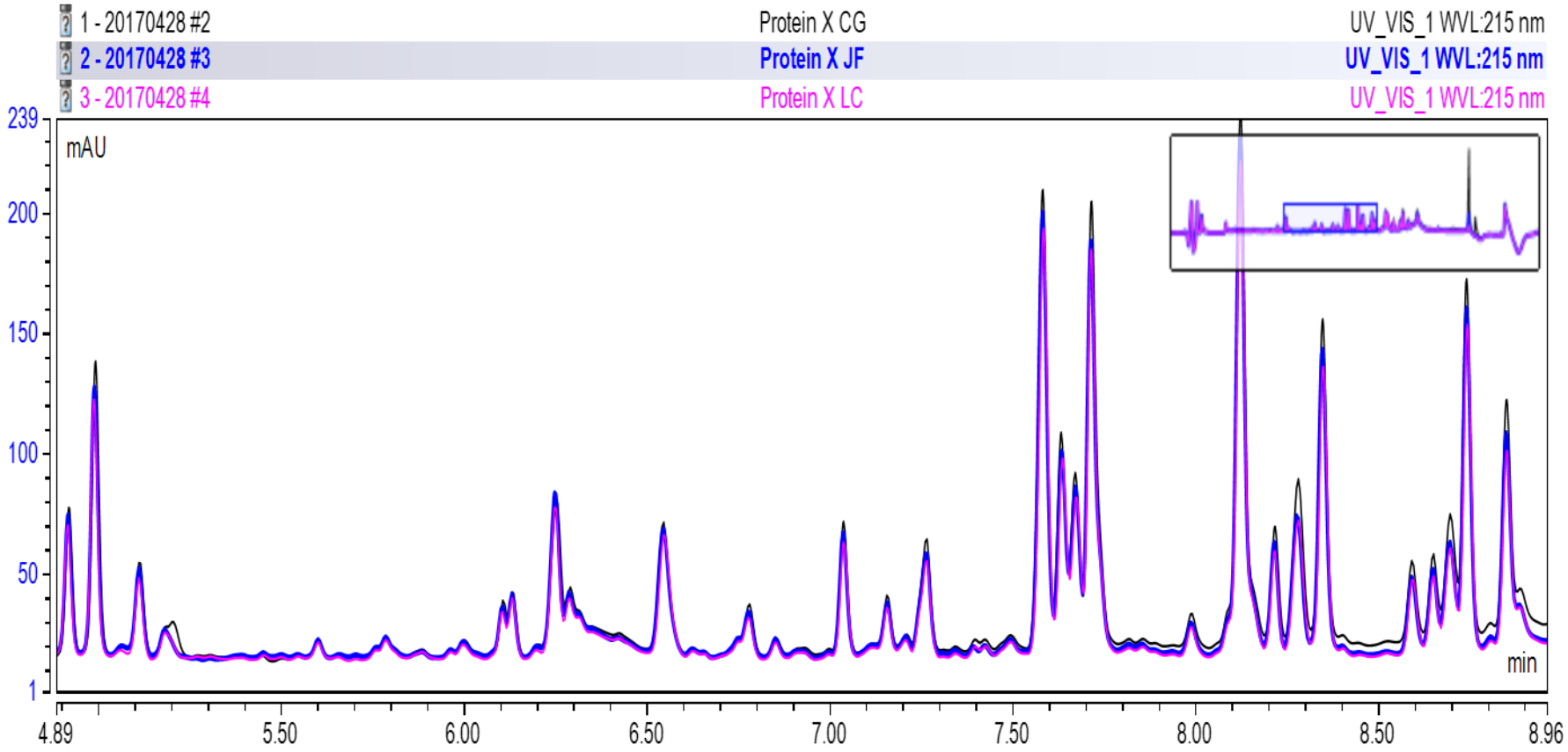


30 Minutes



15 Minutes

REPRODUCIBILITY (ANTIBODY)



PRECISION

N=4

Protein Z Peak Area Precision									
	Component 1	Component 2	Component 3	Component 4	Component 5	Component 6	Component 7	Component 8	Component 9
Protein Z-1	8.31	31.05	23.06	12.05	19.08	26.21	6.05	33.17	77.76
Protein Z-2	8.26	28.10	20.95	11.78	18.41	25.18	6.01	31.81	82.93
Protein Z-3	9.47	34.04	25.67	12.60	19.55	26.87	6.24	34.24	87.32
Protein Z-4	8.08	28.72	22.27	10.75	16.72	22.93	5.22	29.10	77.63
RSD	7.43	8.84	8.65	6.56	6.71	6.81	7.70	6.93	5.71

Protein Z Retention Time Precision									
	Component 1	Component 2	Component 3	Component 4	Component 5	Component 6	Component 7	Component 8	Component 9
Protein Z-1	16.56	16.84	19.73	29.54	32.05	33.37	35.12	36.70	42.59
Protein Z-2	16.58	16.86	19.73	29.53	32.04	33.36	35.12	36.70	42.58
Protein Z-3	16.58	16.84	19.72	29.54	32.04	33.36	35.11	36.69	42.58
Protein Z-4	16.58	16.85	19.72	29.54	32.04	33.36	35.12	36.70	42.58
RSD	0.07	0.04	0.02	0.01	0.01	0.01	0.01	0.01	0.01

GOALS OF THE STUDY

1. Determine if SMART digest could be easily implemented in the lab

2. Determine how reproducible the results are

3. Determine how experienced the analysts need to be to perform a digest from start to finish (i.e. can a sales rep perform a SMART digest with confidence)

4. How much throughput can be gained by combining SMART with Vanquish UHPLC

GOALS OF THE STUDY

1. Determine if SMART digest could be easily implemented in the lab
   A: Yes, even if a time study was needed to determine digestion times a SMART digest can be developed in a single day in most cases

2. Determine how reproducible the results are
   A: results amongst four individual analysts are highly reproducible, even considering analyst experience

3. Determine how experienced the analysts need to be to perform a digest from start to finish (i.e. can a sales rep perform a SMART digest with confidence)
   A: I could probably perform a SMART digest . . . Maybe!

4. How much throughput can be gained using the Vanquish UHPLC
   A: At least a 6x increase is achievable, depending on the complexity of the peptide map 12X or more increase in efficiency seems achievable

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ACKNOWLEDGEMENTS

Ajinomoto Althea

- Charlie Olea
- Lena Ceballos

Thermo Scientific

- Jon Ferguson
- Christina Guintu
- Jeanine Pippitt
- Zee Nadjie
- Rowan Moore