



ThermoFisher
SCIENTIFIC

Peptide separations with pinpoint precision

Mauro De Pra, PhD

Identity confirmation or purity analysis



(See Webinar 1)



Thermo Scientific™
SMART Digest Kits™



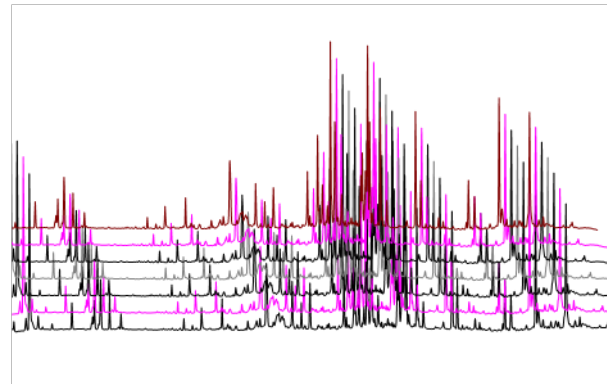
Trypsin digestion of mAbs

- LC-MS → 2000 components
- LC-UV → 100 peaks

Method requirements

High resolving power in reasonable time

- **30-45 minutes run time**
- Theoretically capable of **resolving > 300 peaks**



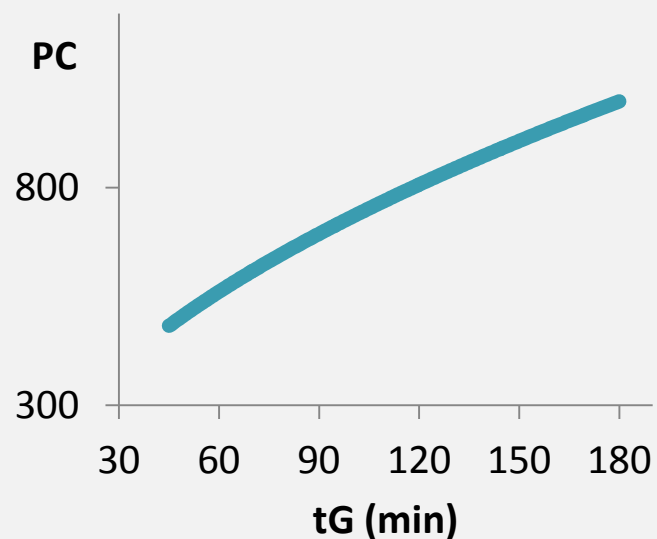
Comparison against reference

- **Highly reproducible method**

How do we achieve high resolving power?

Gradient Time

$$\text{Peak Capacity} = f\left(\frac{t_G}{t_0}\right)$$



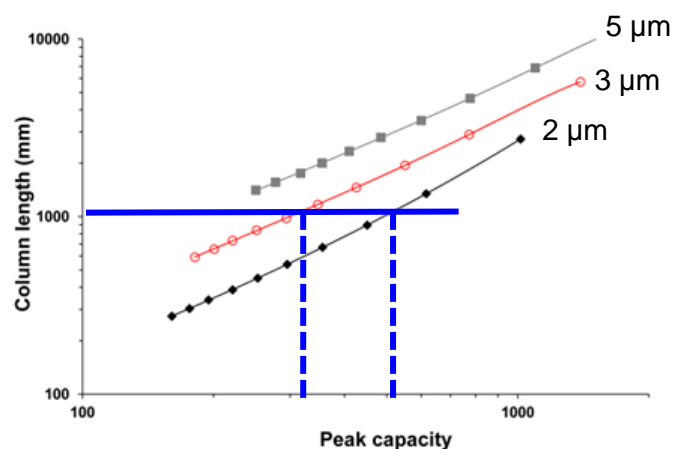
Column Efficiency

$$\text{Peak Capacity} = f(\sqrt{N})$$

N proportional to:

1. Particle size
2. Column length

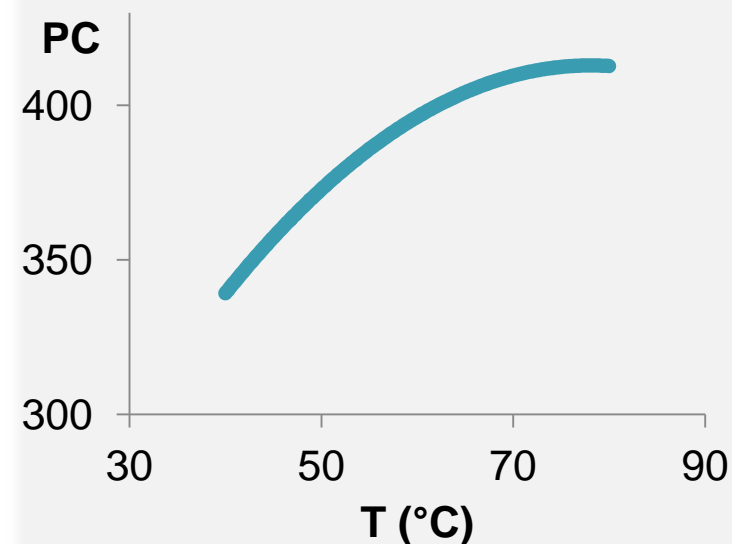
Example: performance limits at 800 bar



Nevakova, Vaast, Stassen, Broeckhoven, Desmet, Eeltink, et al. J. Sep. Sci. 2013, 36, 1192

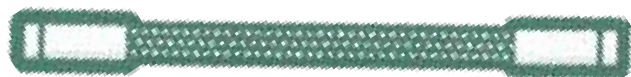
Column Temperature

Column temperature strongly affect peak capacity (faster molecular diffusion)



High resolving power without compromising on time

Thermo Scientific™ Acclaim™ VANQUISH™ C18



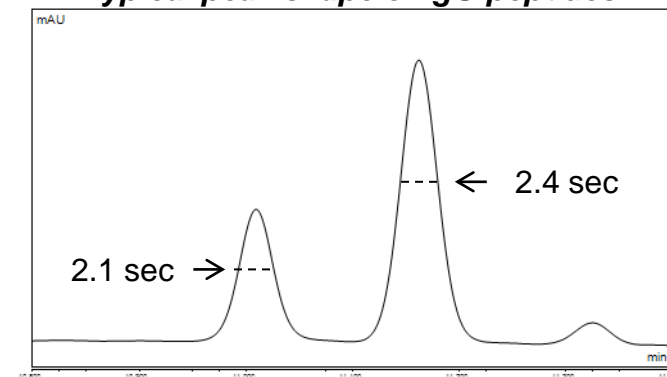
- 2.2 µm particles
- 120 Å
- 250 mm length
- 2.1 mm ID
- 60 °C
- Excellent peak shape with FA and TFA

Thermo Scientific™ Vanquish™ Flex UHPLC Quaternary System



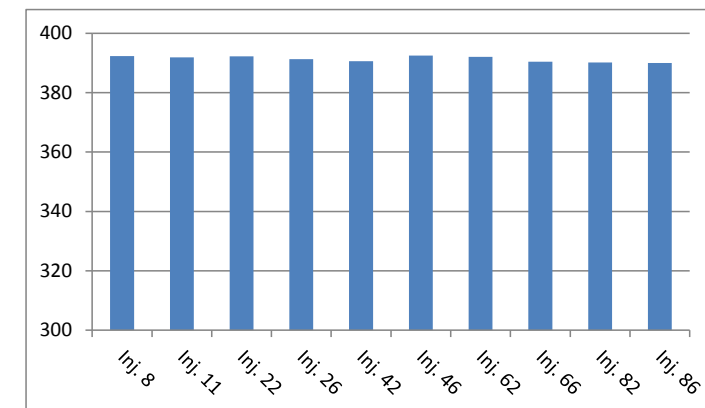
- 1000 bar (15,000 psi)
- Quaternary pump
- Advanced temperature control
- Low dispersion fluidics
- SmartInject
- Seamless integration with HRAM MS

Typical peak shape of IgG peptides



2x Acclaim VANQUISH C18 25 cm (50 cm total)
30 minutes gradient @ 400 µL/min
0.05% TFA

Peak capacity for BSA digest



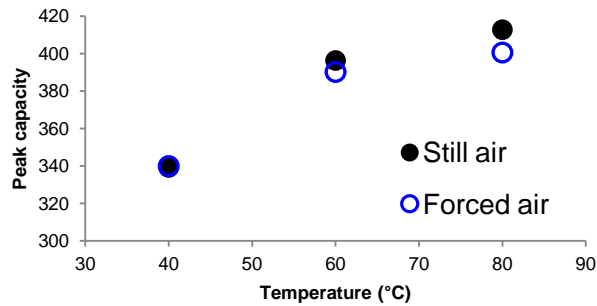
Acclaim VANQUISH long lasting performances

Column thermostating

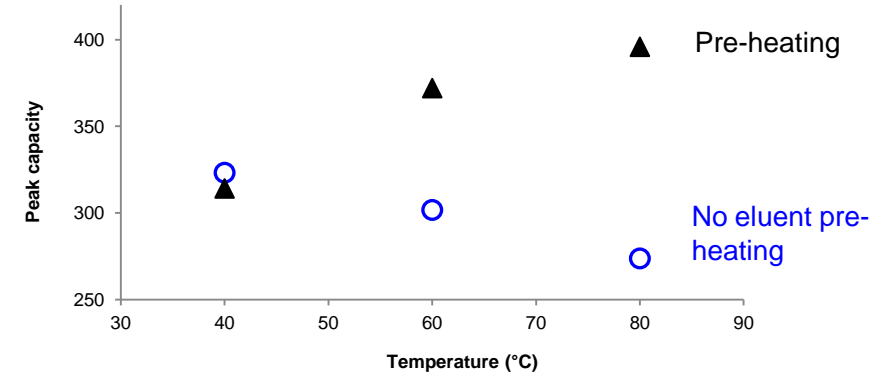
Column thermostating

Forced air

Still air



- *Slight capacity increase with still-air*
- *Dual heating mode facilitates method transfer from wide range of instruments*
- *Forced-air heating beneficial for fast system preparation*



IgG peptides: peak capacity and mobile phase pre-heating

Pre-column heater

Active

Low dispersion

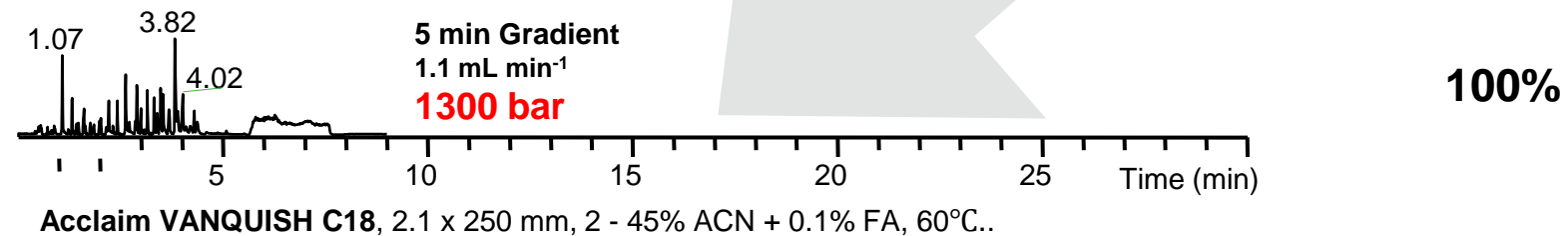
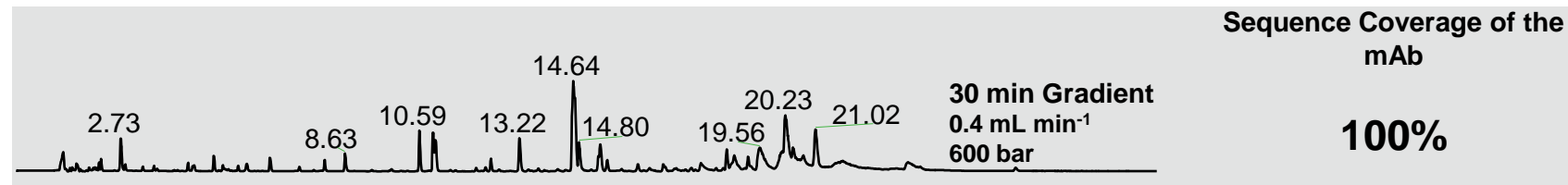
Accurate

Vanquish Horizon UHPLC System – Ultra High Throughput

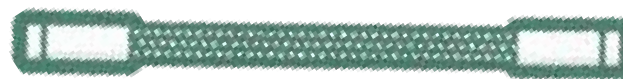
Thermo Scientific™ Vanquish™ Horizon UHPLC



- **1500 bar (22,000 psi)**
- **Binary pump**
- **Advanced temperature control**
- **Low dispersion fluidics**
- **Sample pre-compression**
- **Seamless integration with HRAM MS**



Acclaim VANQUISH C18



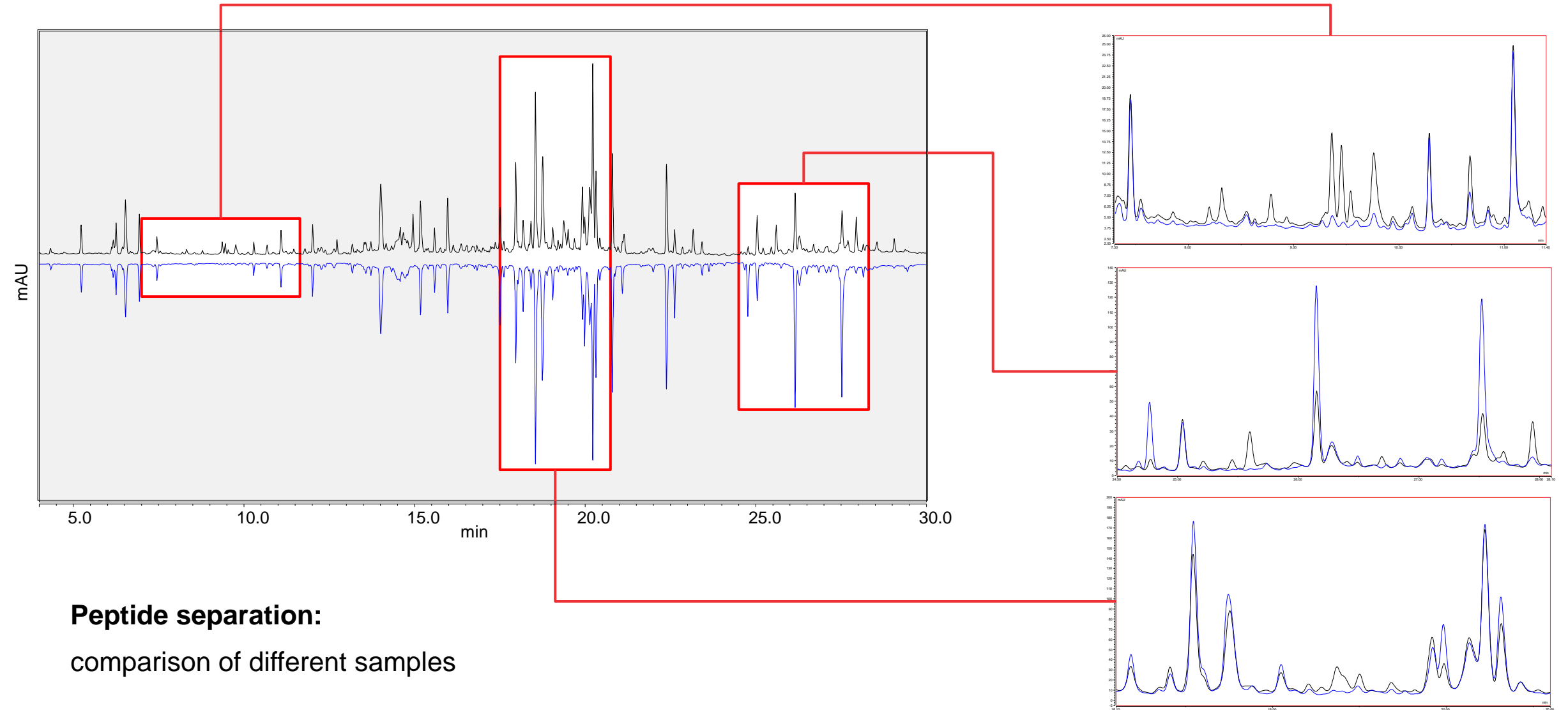
1500 bar (22,000 psi)

Thermo Scientific™ Q Exactive™ HF
LC-MS/MS System



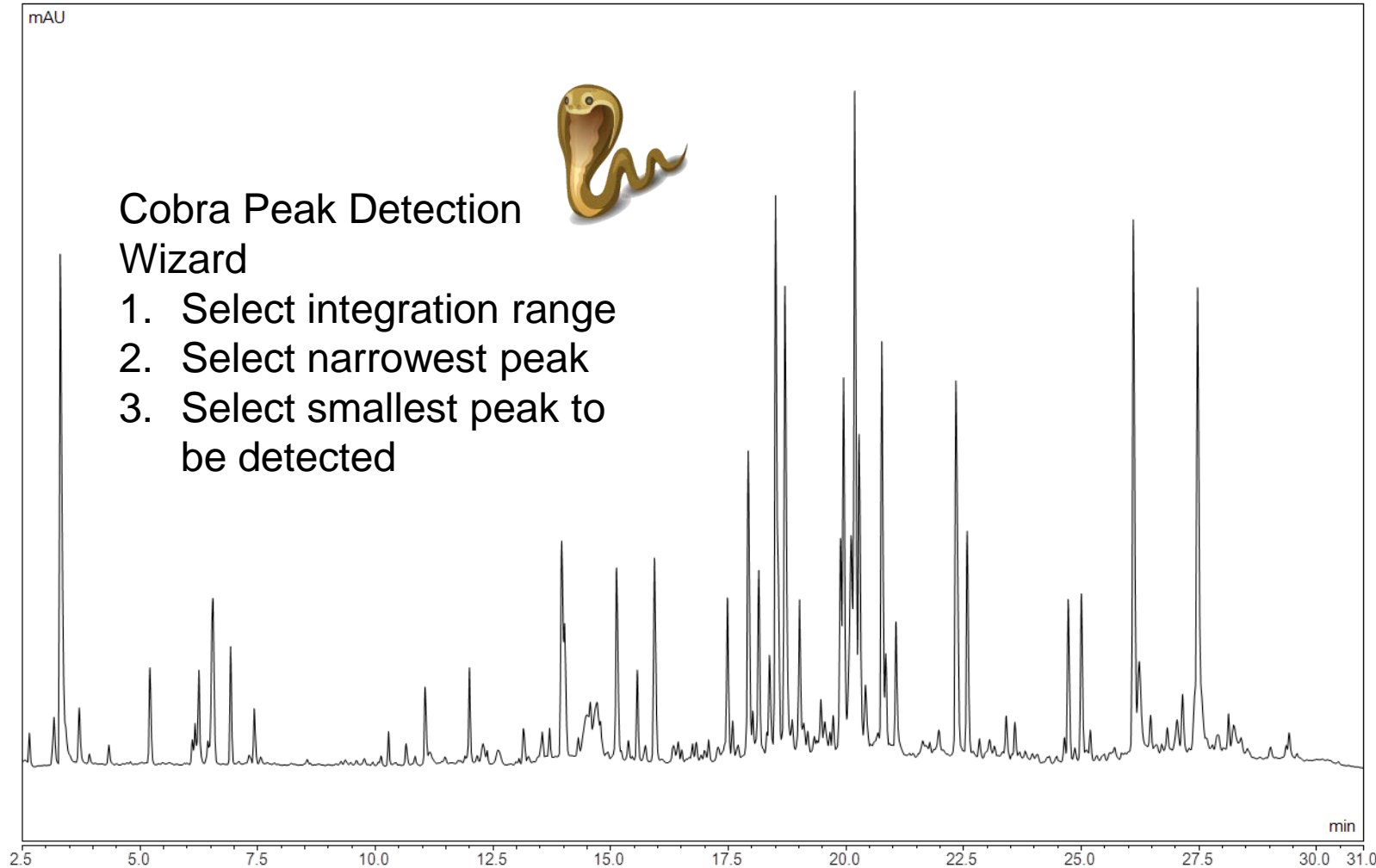
Ultra-fast data acquisition

Visual comparison reference & unknown



Processing of peptide LC data – Comparison reference unknown samples

Reference → Raw data



Identity confirmation

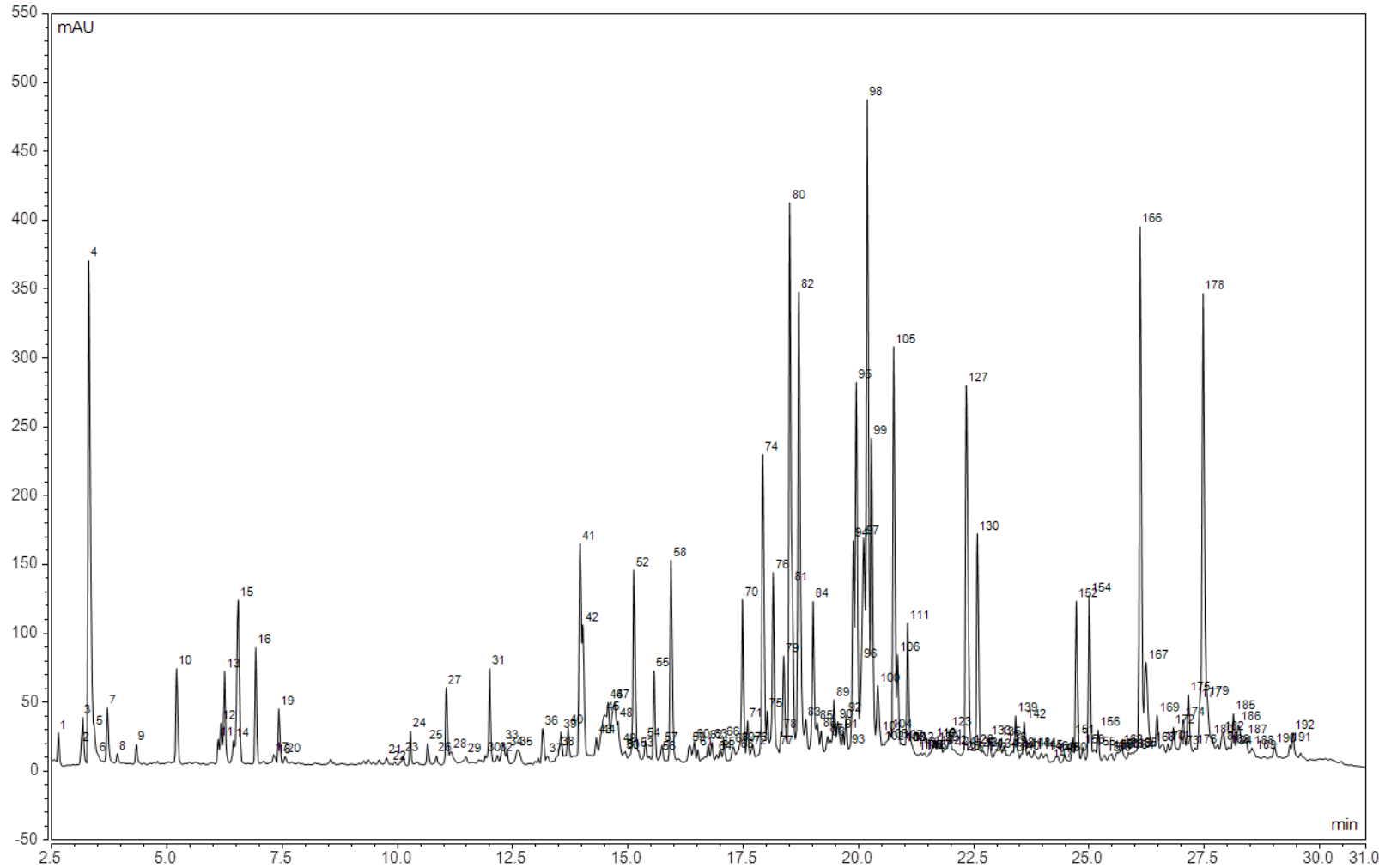
- Each component represented with same relative abundance
- No additional peaks in the unknown

Purity assessment

- Unidentified peaks in unknown sample above threshold need to be reported

Processing of peptide LC data – Comparison reference unknown samples

Reference → Integrated



Identity confirmation

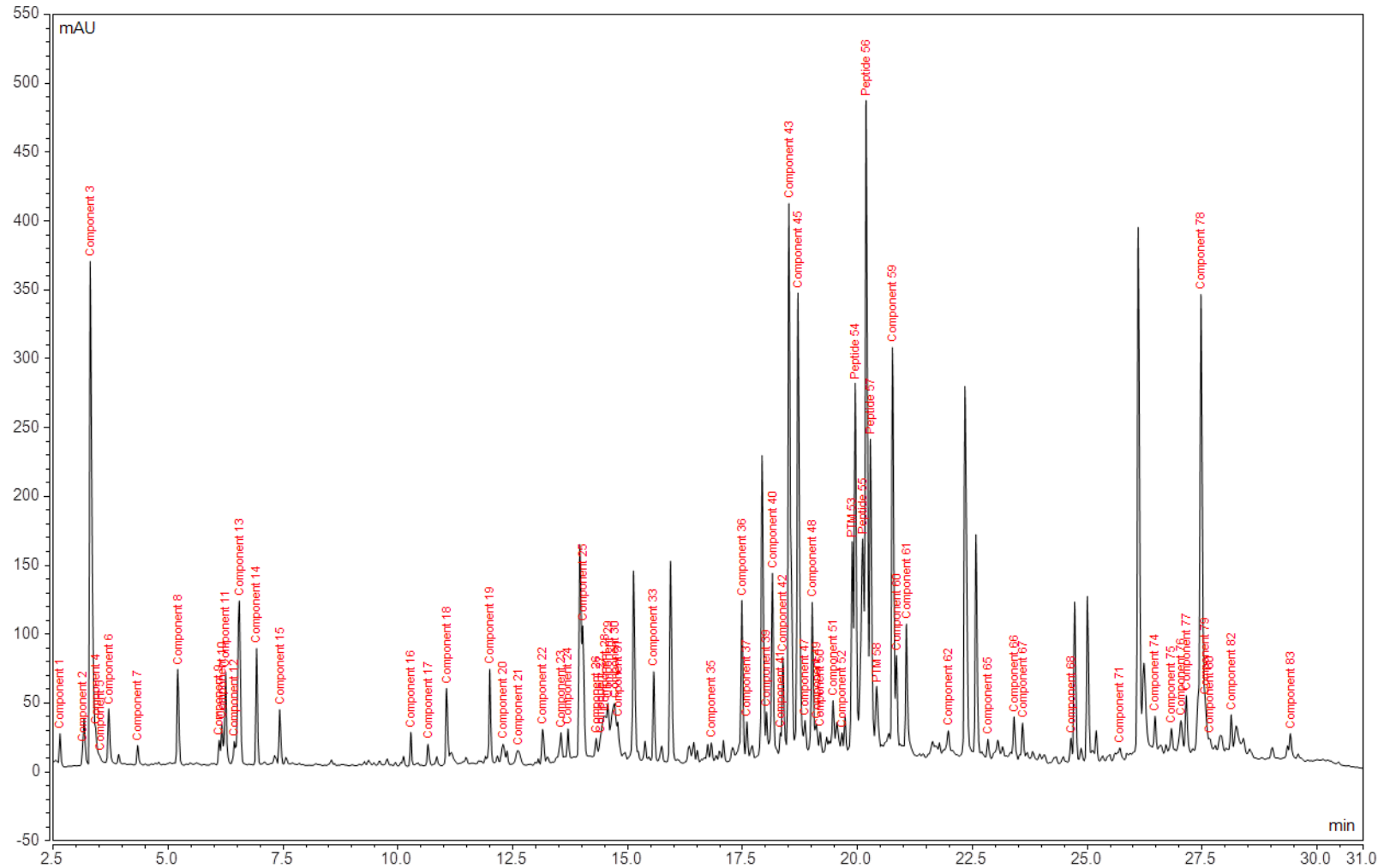
- Each component represented with same relative abundance
- No additional peaks in the unknown

Purity assessment

- Unidentified peaks in unknown sample above threshold need to be reported

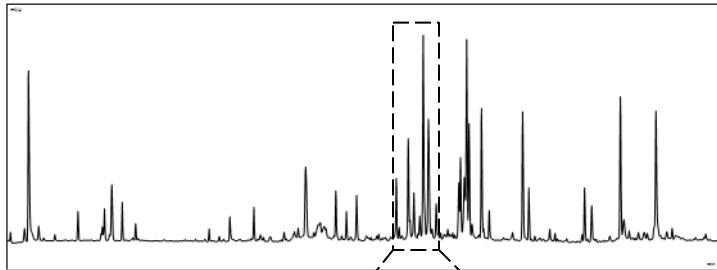
Processing of peptide LC data – Comparison reference unknown samples

Reference → Peak assigned

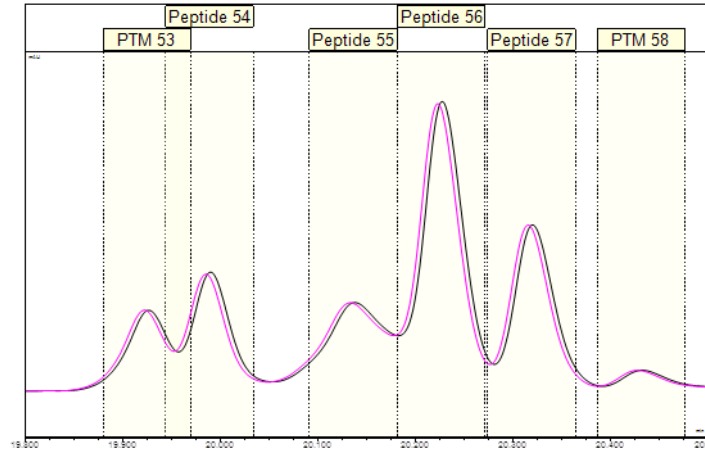
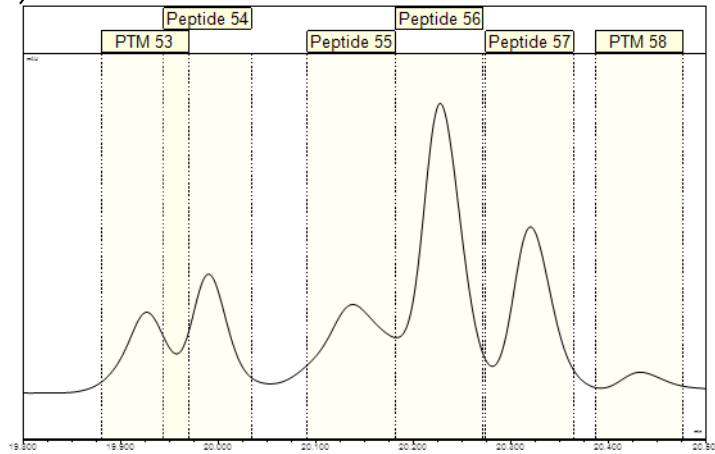


- Generic assignment or identity based on MS data
- Peak assignment saved as Processing Method
- Check if all assigned peaks are present in the unknown sample
- Check if non-assigned peaks are present in the unknown sample

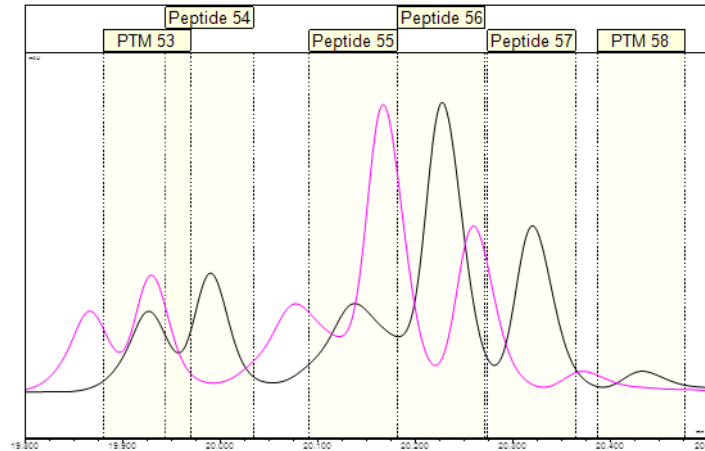
Processing of peptide LC data – Comparison reference unknown samples



reference



$RSD (n=2) = 0.018\% \Delta=0.3 \text{ sec}$

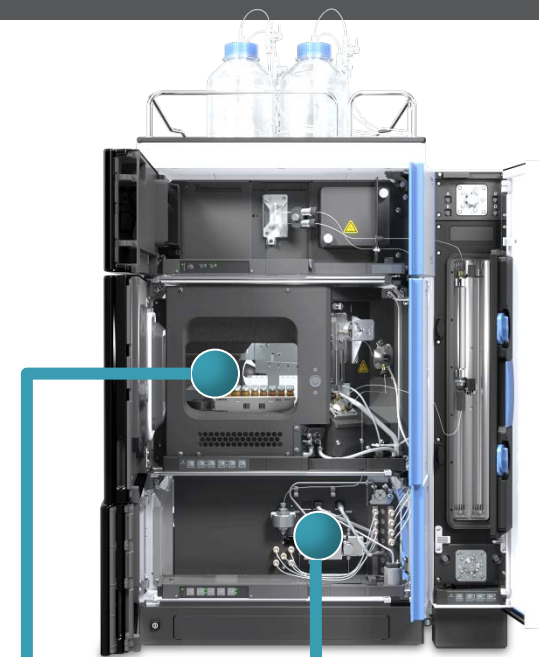
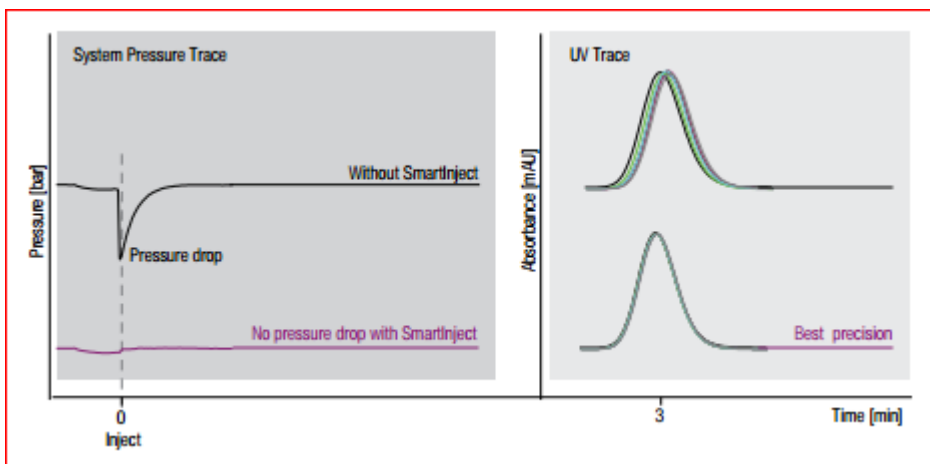
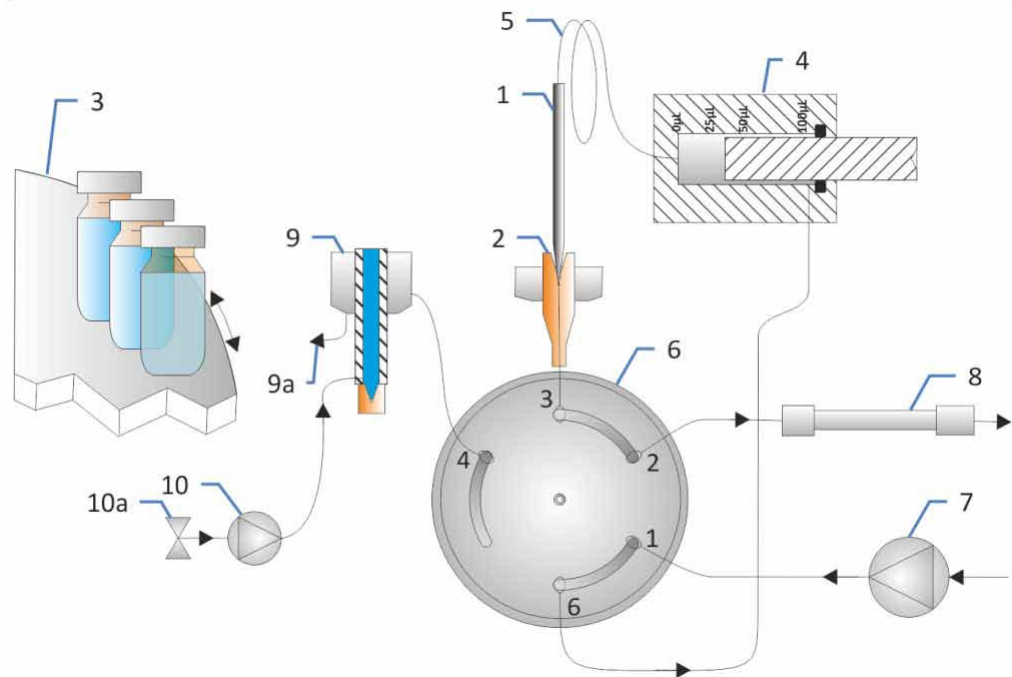


$RSD (n=2) = 0.160\% \Delta= -3.8\text{sec}$

Component	Reference Rel. Area (%)	Unknown Rel. Area (%)
PTM 53	1.9	1.8
Peptide 54	2.6	2.4
Peptide 55	3.1	2.9
Peptide 56	6.7	6.3
Peptide 57	3.8	3.5
PTM 58	0.7	0.6

Component	Reference Rel. Area (%)	Unknown Rel. Area (%)
PTM 53	1.9	2.4
Peptide 54	2.6	-
Peptide 55	3.1	6.3
Peptide 56	6.7	3.5
Peptide 57	3.8	-
PTM 58	0.7	-

Retention time precision Vanquish Flex Quaternary UHPLC System

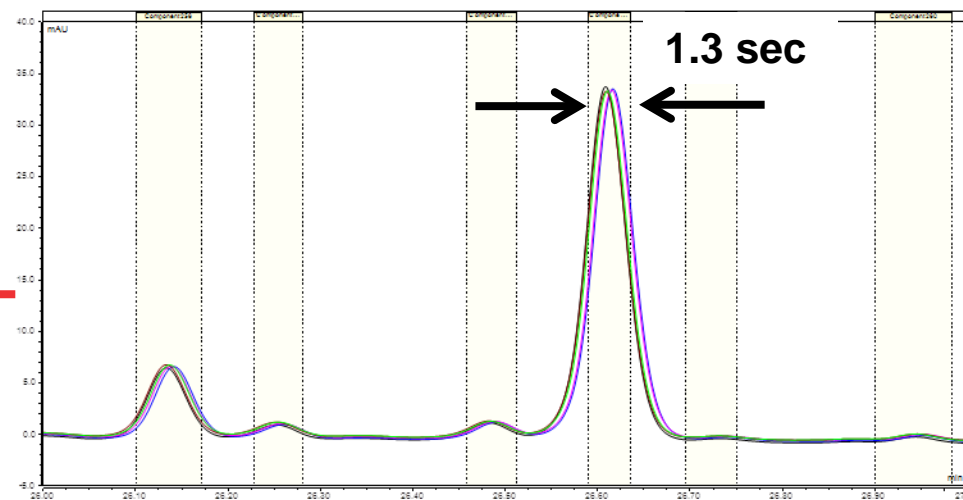
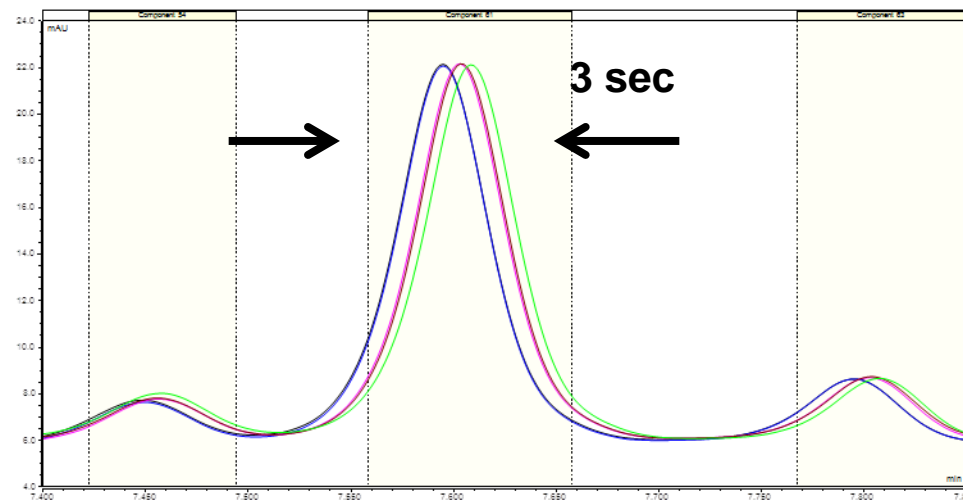
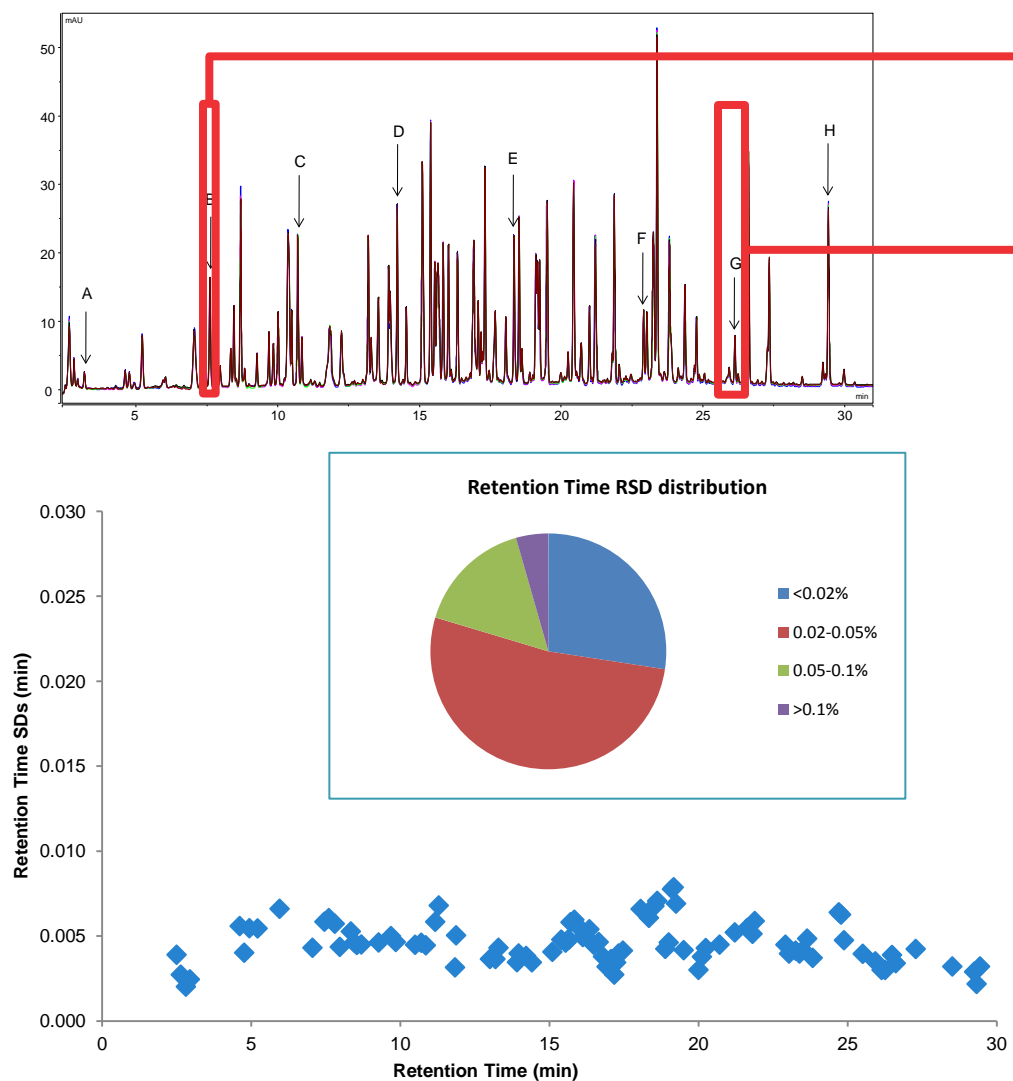


SmartInject
Sample pre-compression

SmartFlow
Reproducible flow

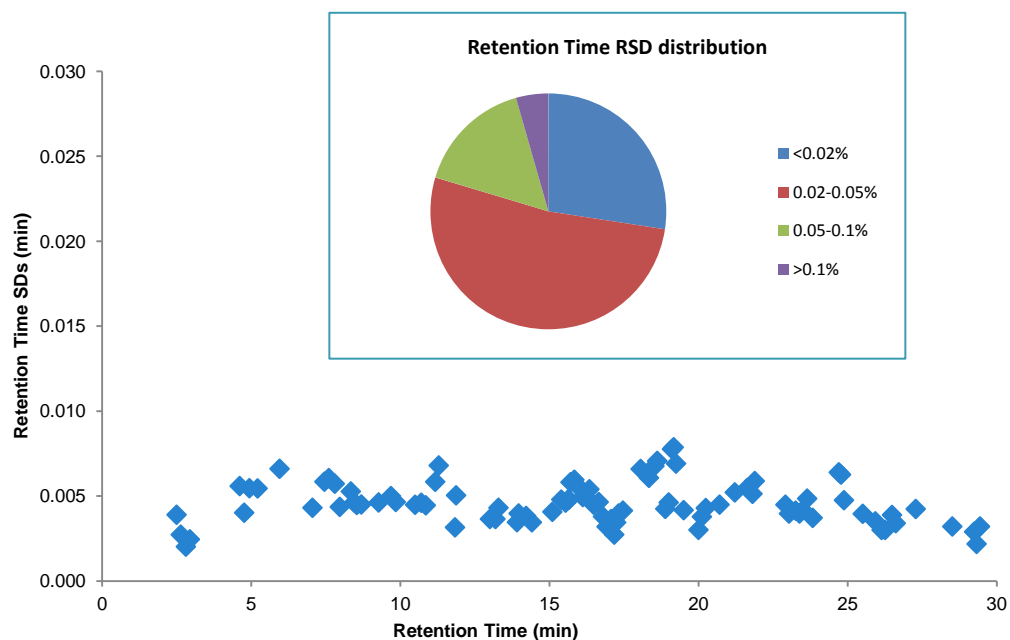
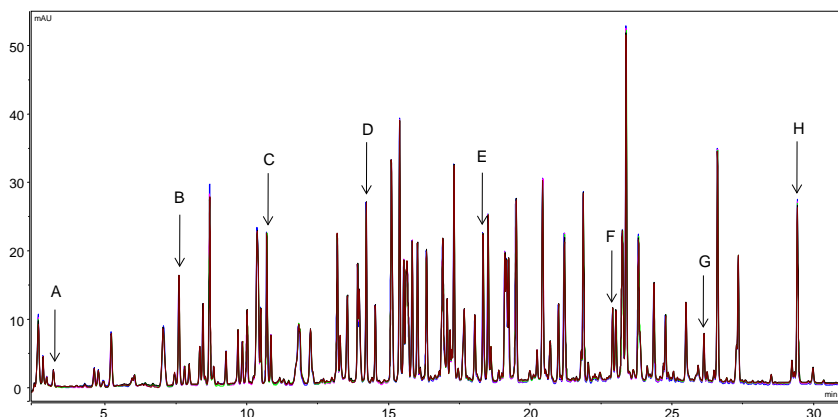
What are the precision we expect from Vanquish Flex Quaternary?

BSA tryptic digest (n=5) with Vanquish Flex Quaternary



What are the precision we expect from Vanquish Flex Quaternary?

BSA tryptic digest (n=5) with Vanquish Flex Quaternary



Peak ID	Retention Time (min)	Retention Time RSD (%)	RSD Area (%)
A	3.171	0.077	0.61
B	7.601	0.077	0.31
C	10.702	0.042	0.22
D	14.217	0.028	0.24
E	18.345	0.036	0.77
F	22.912	0.018	0.79
G	26.137	0.013	0.20
H	29.438	0.007	2.10

1. Optimize resolving power of the peptide separation
2. Streamline peak assignment for efficient comparisons of different chromatograms
3. Importance of retention time precision on peak assignment
4. Ultra fast separation of peptides with great reproducibility



Biopharmaceutical Peptide Mapping –A User Perspective

Amy Farrell, PhD





- **World-class facility dedicated to address the training and research needs of the global biopharmaceutical industry.**
- **Unique competency based training experience in an environment that replicates the most modern industrial bioprocessing facilities.**
- **Research with impact – developing solutions to address real challenges faced within the biopharmaceutical industry.**
- **CCL - complete characterisation of bioprocesses and expressed therapeutic glycoproteins**

Benefits of Thermo Scientific Platform for Peptide Mapping Analysis



UHPLC System Set-Up

- Smart start-up, standby and shut-down
- eWorkflows - <https://appslab.thermofisher.com>



SMART Digest

- Time-Saving – 30 minute digestion for broad sequence coverage
- User friendly – reduction in sample preparation steps compared to traditional digest methods



Vanquish Flex Quarternary UHPLC – Acclaim 120 C18 5 μm 2.1 x 250 mm analytical column

- Reproducibility
- Separation Efficiency

SMART Digest Time Course Study – Light Chain Sequence Coverage



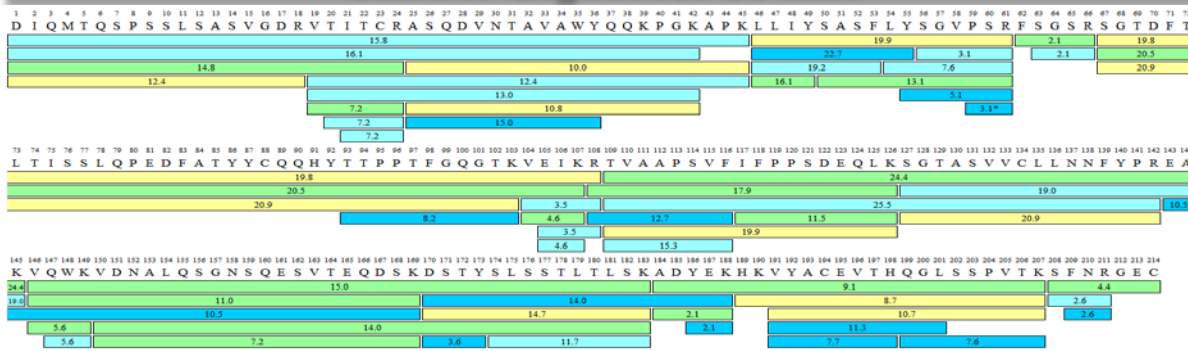
Sample Preparation:

- 50 µL of 2 mg.mL⁻¹ IgG1 sample digested.
- Reduction with 50 mM TCEP preformed after digestion with Thermo Scientific SMART Digest Kits.

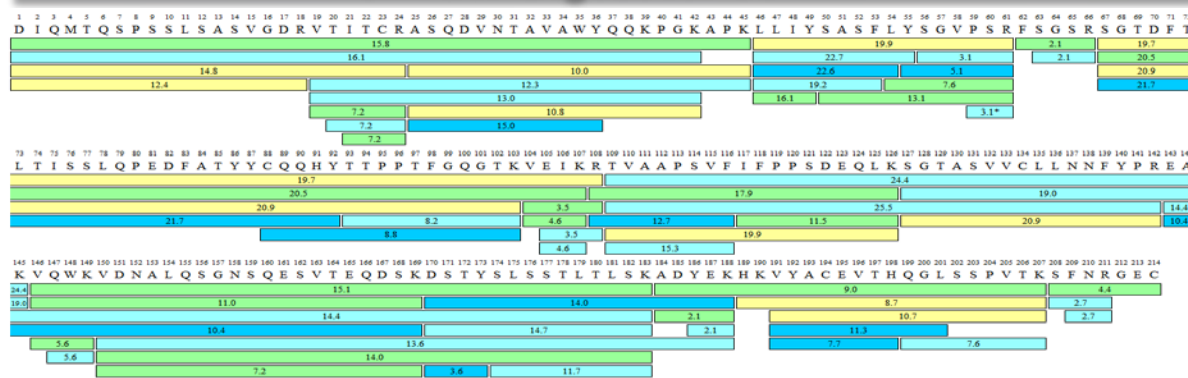
Sample Analysis:

- Acclaim 120 C18 2.1 x 250 mm.
- Thermo Scientific™ Q Exactive™ BioPharma platform
- Thermo Scientific™ BioPharma Finder™

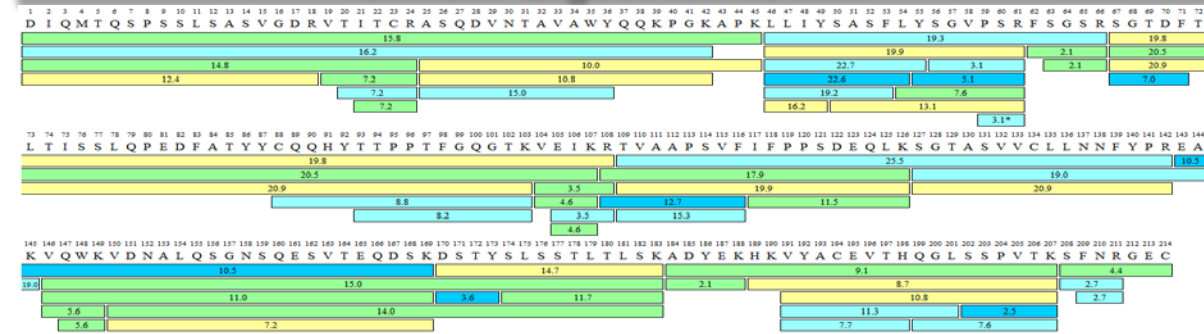
SMART Digest for 30 min



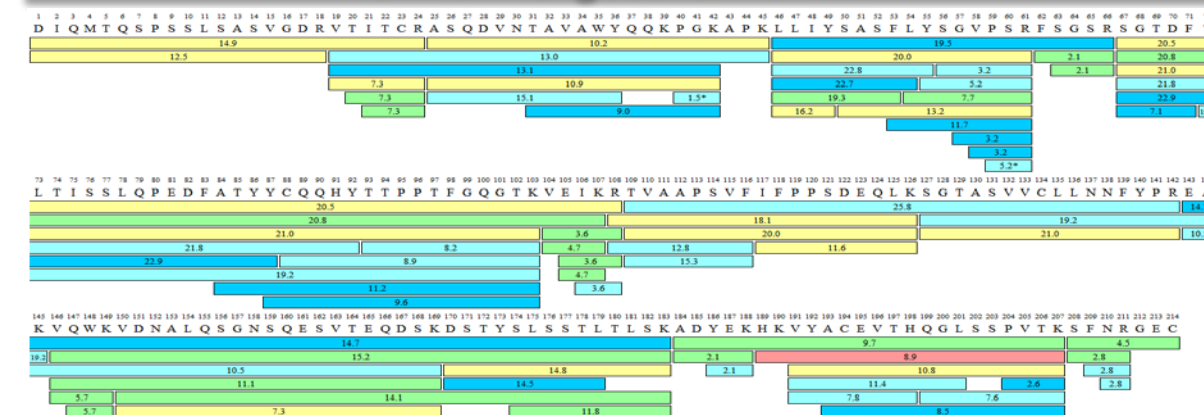
SMART Digest for 60 min



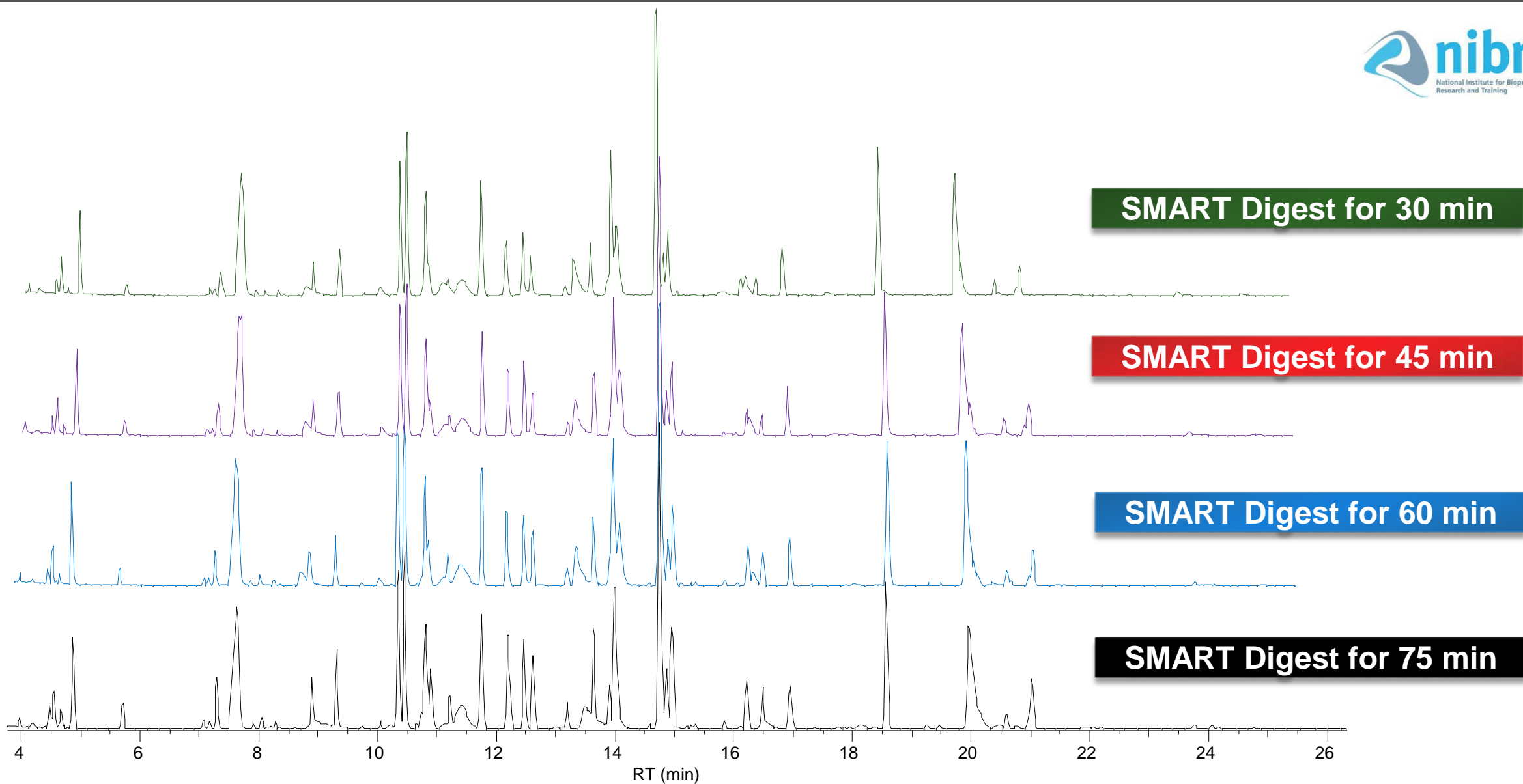
SMART Digest for 45 min



SMART Digest for 75 min

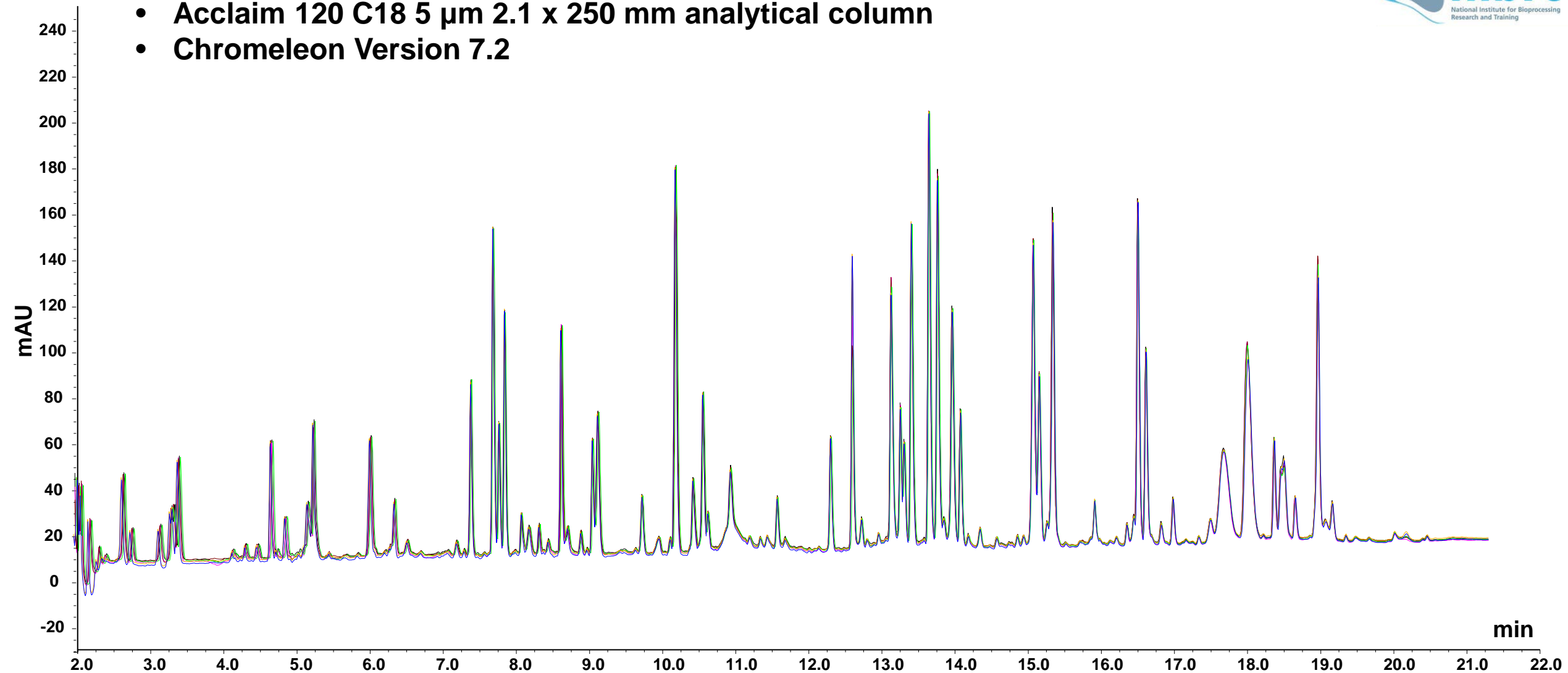


SMART Digest Kits - Time Course Study – Total Ion Chromatograms



Vanquish UHPLC - Reproducible Sample Separation

- Vanquish UHPLC – DAD detector at 214 nm
- Acclaim 120 C18 5 μ m 2.1 x 250 mm analytical column
- Chromeleon Version 7.2



Identified beneficial attributes of the Chromeleon 7.2:

- ✓ Minimal hands-on time for system set-up
- ✓ Ability to download developed methods from AppsLab

Identified beneficial attributes of the Vanquish:

- ✓ Retention time precision across multiple analyses

Identified beneficial attributes of SMART digest:

- ✓ Efficient Monoclonal Antibody digestion in 30 minutes
- ✓ Full sequence coverage