



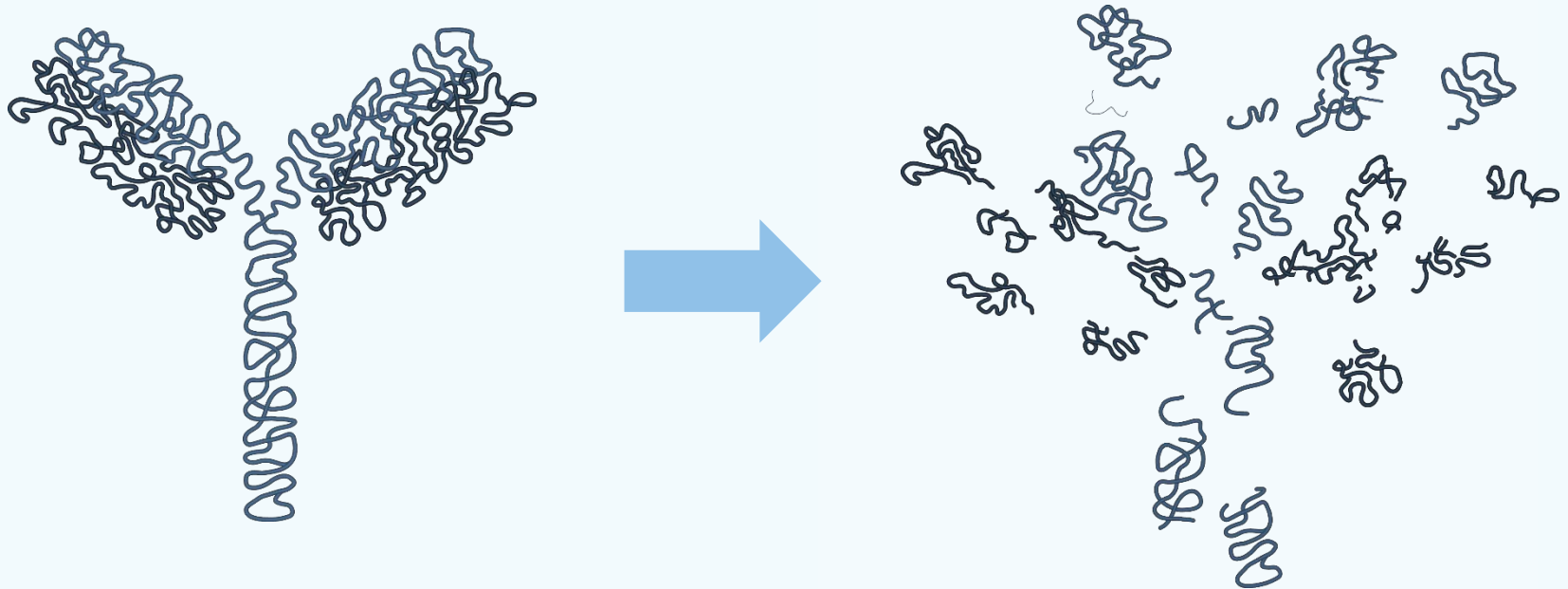
ThermoFisher
SCIENTIFIC

New, rapid & reproducible peptide mapping workflows

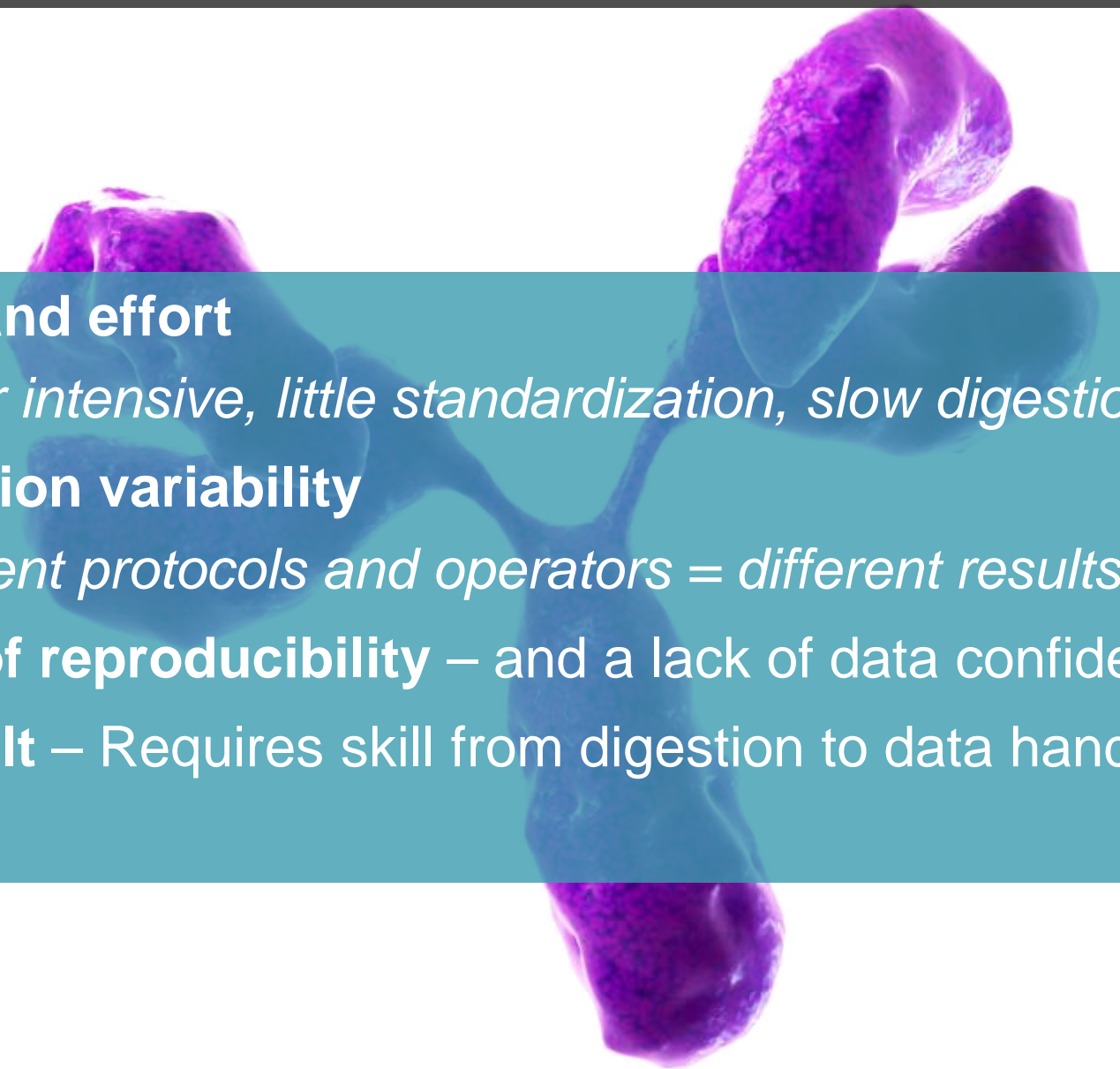
Pharma & Biopharma Tours | 2016

Why Peptide Mapping?

- Essential step in biopharmaceutical characterization
- Used from discovery to QC
- Routine... but slow and painstaking



What Are The Pain Points?

- 
- **Time and effort**
labour intensive, little standardization, slow digestion
 - **Digestion variability**
different protocols and operators = different results
 - **Lack of reproducibility** – and a lack of data confidence
 - **Difficult** – Requires skill from digestion to data handling

We Have Called Our Solution: Upgrade Your Maps!



- Full workflow solution
- Robust and reliable
- Fast and reproducible
- Easy and convenient
- Gives you:
 - *Sequence verification*
 - *Modifications analysis*
 - *Sequence variants*
 - *Relative abundance quantification*

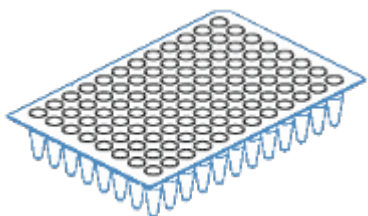
What's The Goal of Upgrade Your Maps?

Sample preparation, separation, MS detection & data analysis of proteins.

Confirm amino acid sequence with 100% coverage, identify and quantify modifications, identify variants.

Most Important: Make it simpler! little training, results within a few hours.

Upgrade Your Maps: Our Workflow Solution



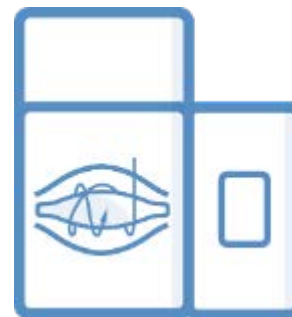
Thermo Scientific™ **SMART Digest™** kits offer extremely reproducible and rapid protein digestion



Thermo Scientific™ **Vanquish™ Flex UHPLC** is engineered for high resolution, reproducible peptide separations



Thermo Scientific™ **Acclaim™ 120 C18 column** is the perfect column choice to ensure sharp peaks during peptide mapping



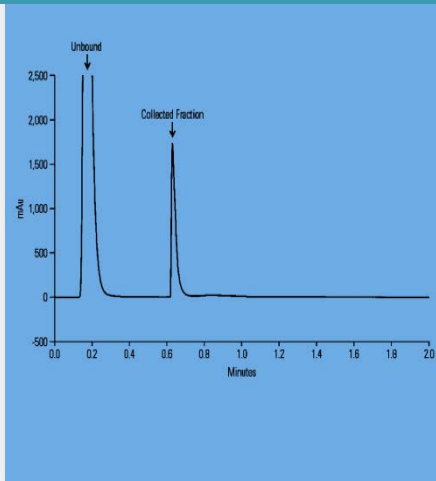
Thermo Scientific™ **Q Exactive™ Hybrid Quadrupole-Orbitrap™ mass spectrometers** are the gold standard for accurate mass measurement



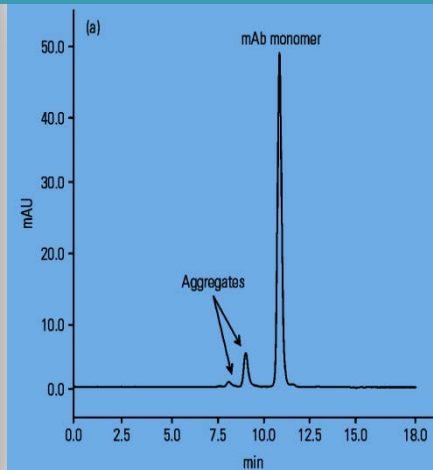
Thermo Scientific™ **BioPharma Finder™** software is the perfect software tool for peptide identification and sequence mapping

Wide Range of Bioanalytical Characterization Methods

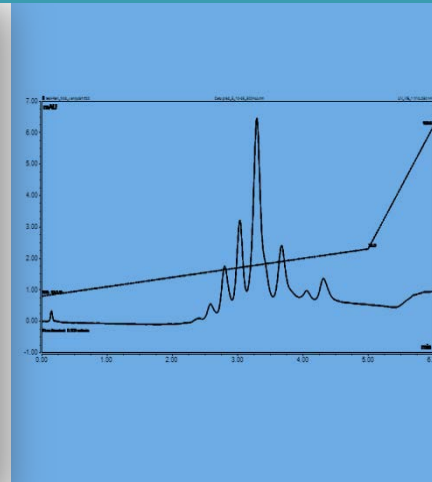
Protein A
capture of IgG



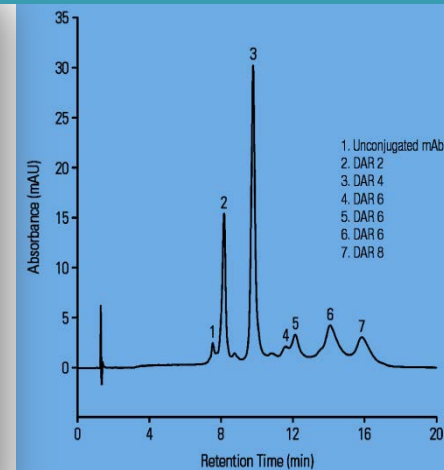
SEC aggregation
screening



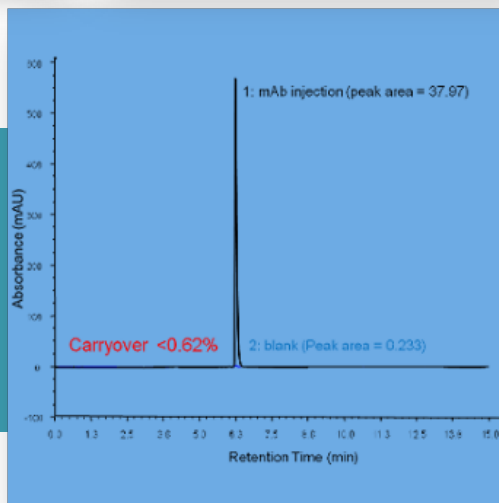
IEC pH-gradient charge
variant analysis



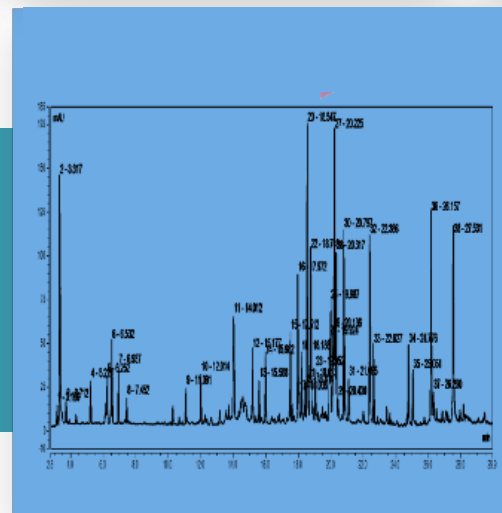
ADC DAR
determination



Intact MAb RP
separation

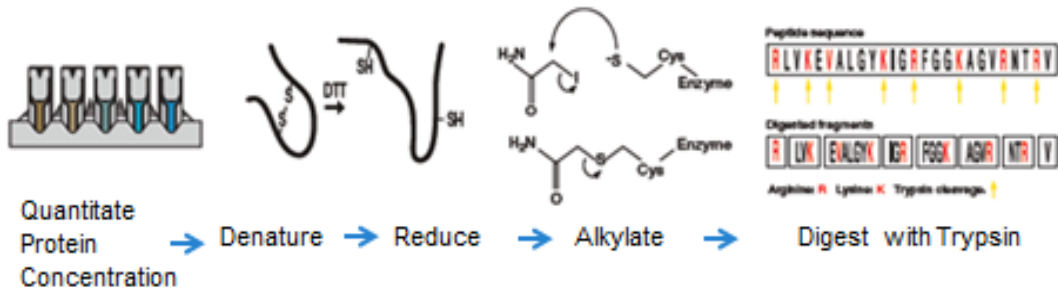


Digestion and
peptide
mapping

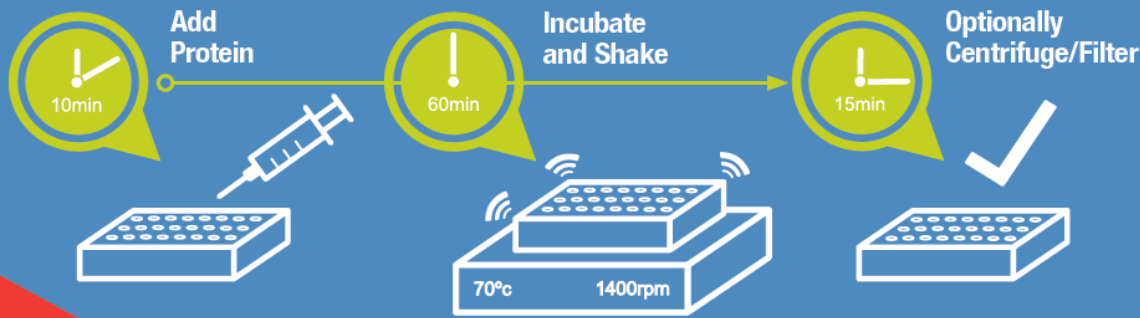


SMART Digest Kits

From this...



To this...!



- Simple, three step protocol
- Quicker than in-solution digestion
- Reduces operator variability
- Increases reproducibility
- Higher throughput
- Ability to automate

SMART Digest Kits: What Is The Benefit?

What it is?

Highly stable, immobilized trypsin digestion

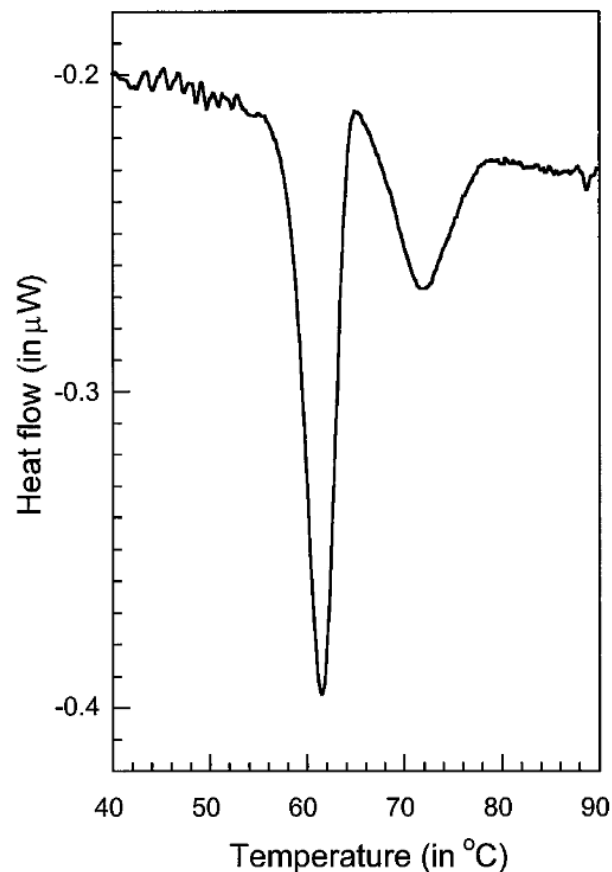
What is the benefit?

- Proteins are heat-denatured for digestion
 - - No additional denaturing agents
 - - No reduction / alkylation required
 - - No handling of mutagenic substances
 - - Minimized trypsin autolysis
 - - Enzyme can be use in excess



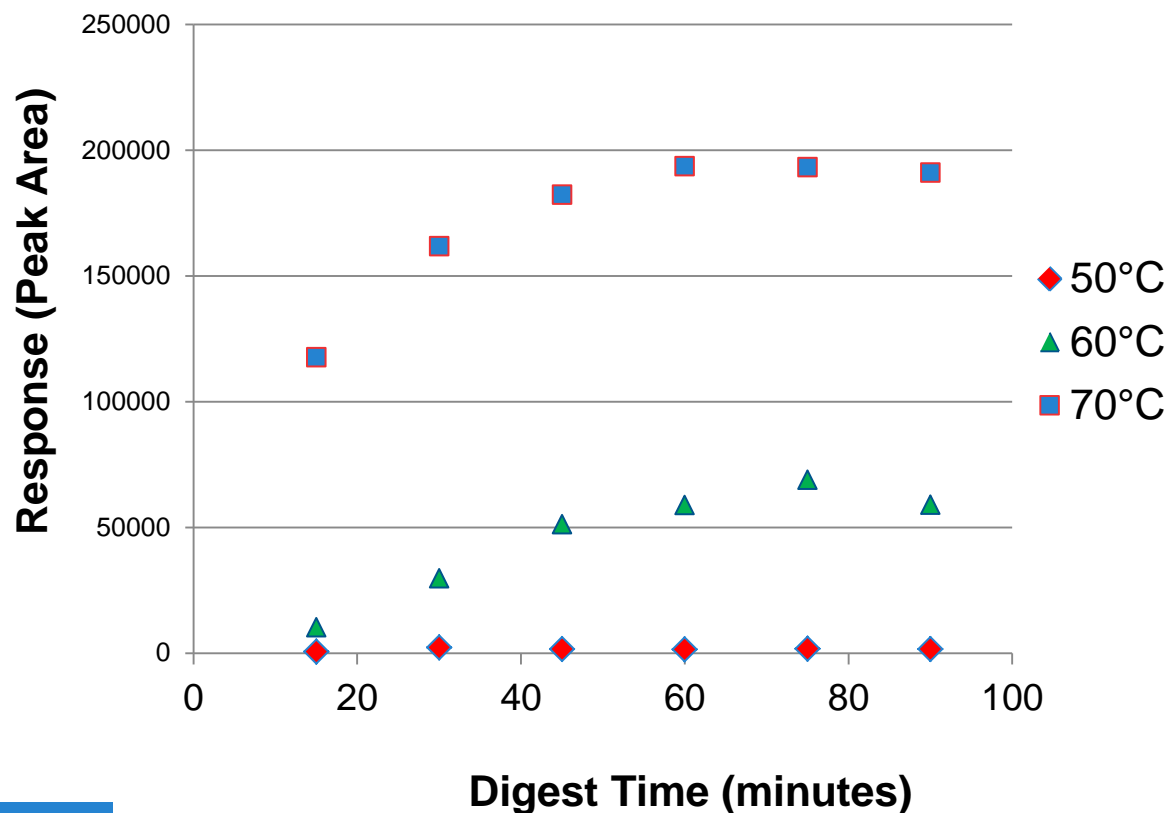
SMART Digest Kits: IgG digestion in 45 min

Thermal denaturation of IgG



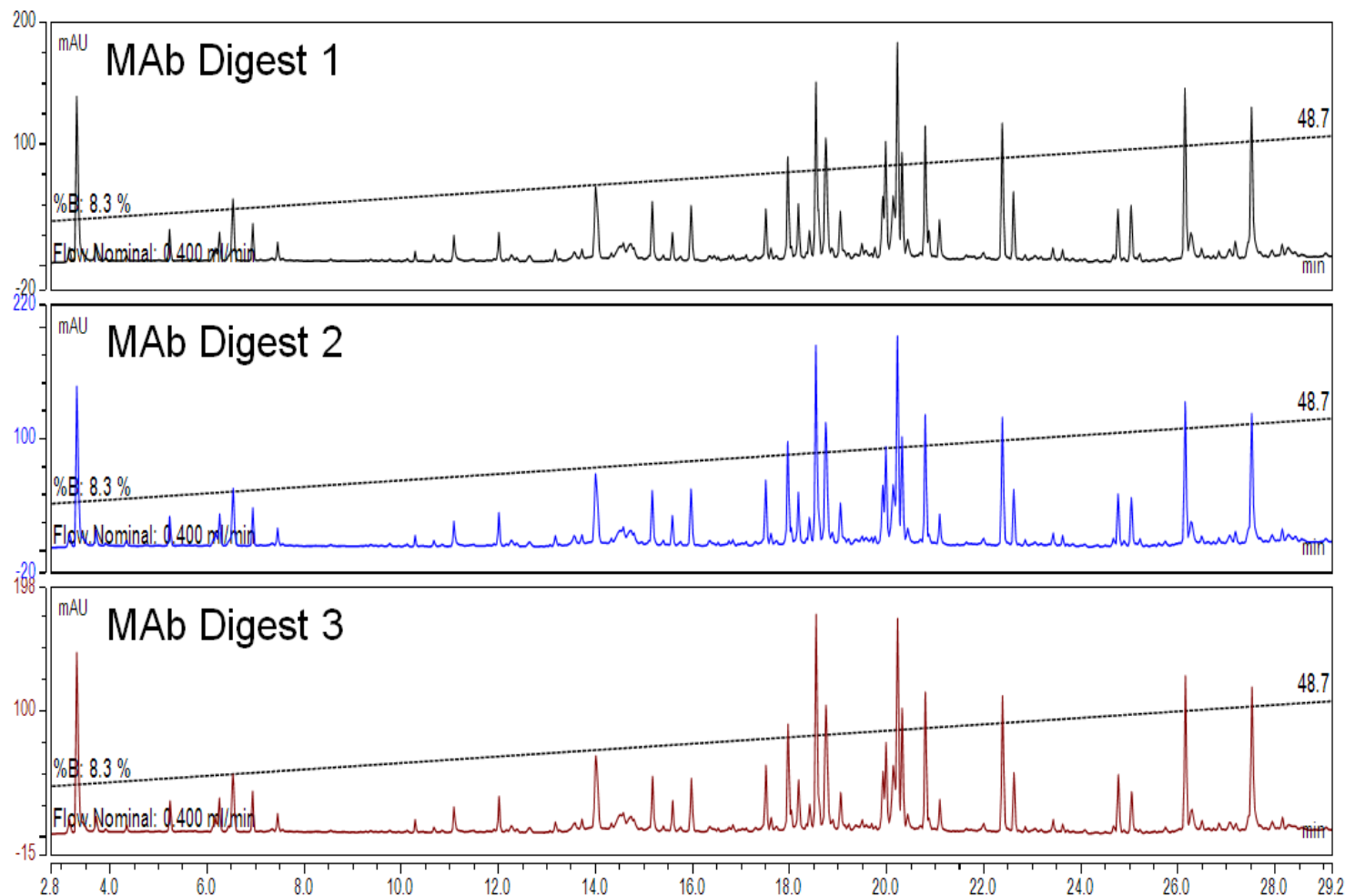
DSC thermogram of IgG (6 mg/ml; mouse IgG2b)
in a 10 mM phosphate buffer pH 8.1; 0.5 $^{\circ}C$ /min

Native IgG Digest Profile monitoring VVSVLTVLHQDWLNGK

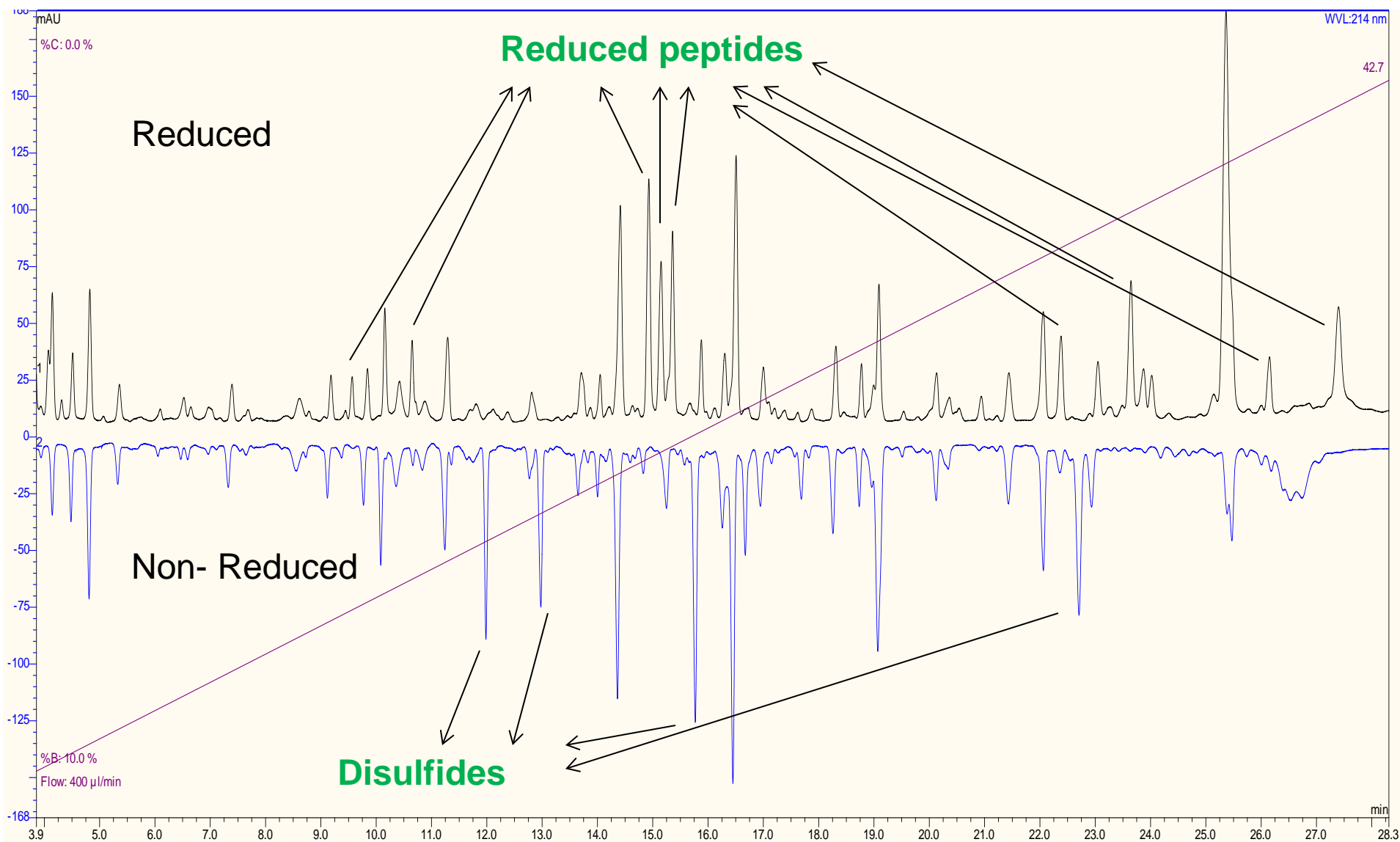


SMART Digest Kits: Outstanding Digestion Reproducibility

Monoclonal antibody SMART digestion by three different analysts



Rituximab Peptides for Disulfide Mapping



Acclaim 120 C18 Column

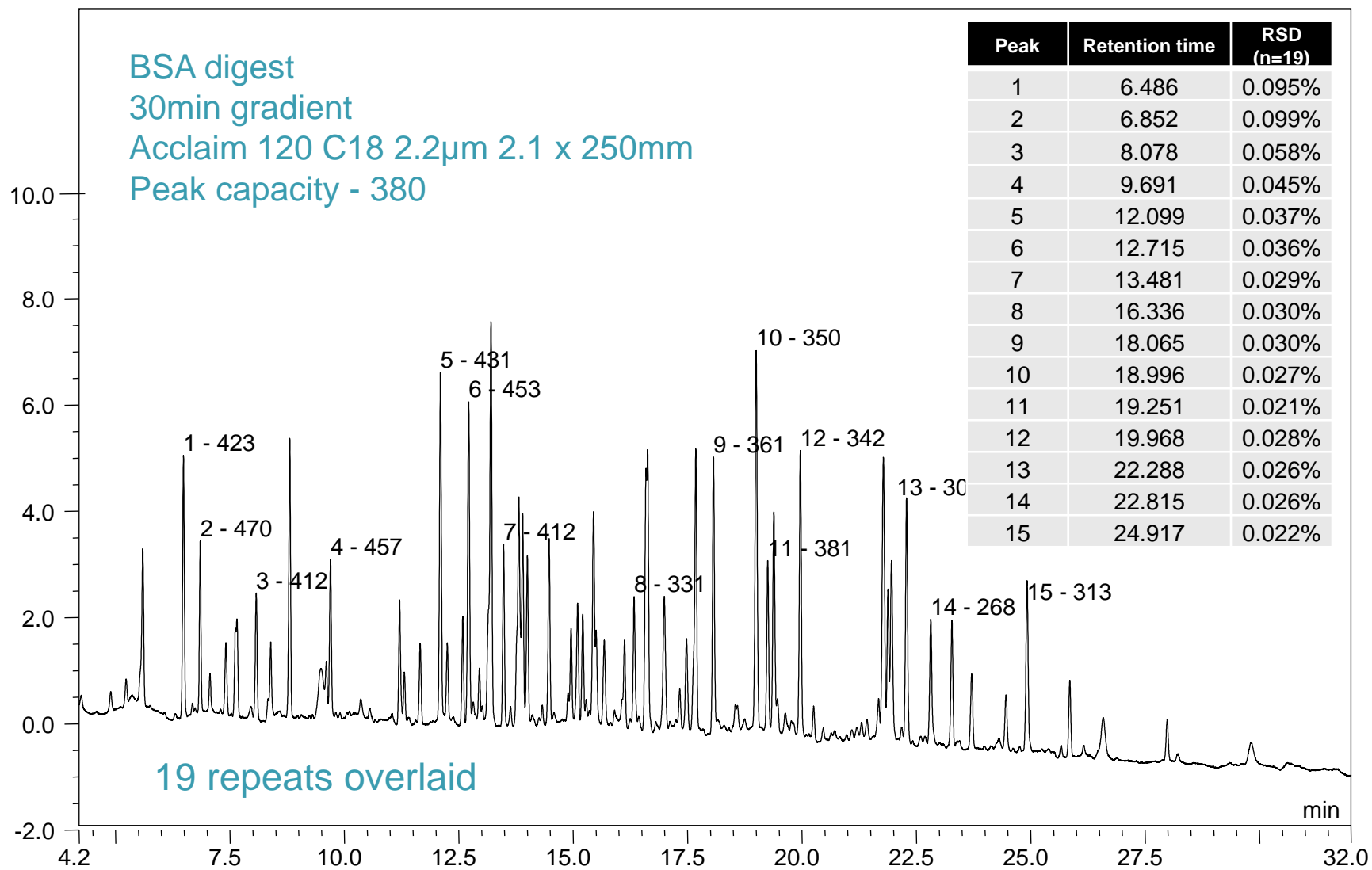


- High resolution - better protein identification
- 250mm in length
- High loadability - high sensitivity LC/MS
- High column-to-column reproducibility
- 1500 bar Vanquish UHPLC compatible
- Viper™ fittings: robust & easy to install

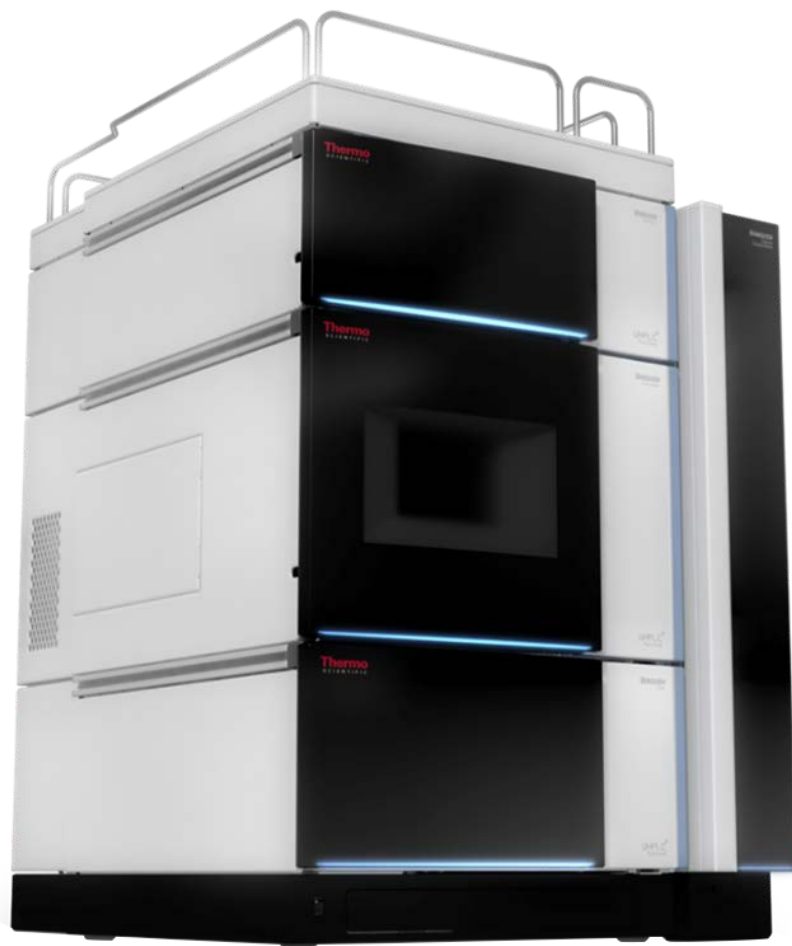


Viper™ finger tight fitting

Acclaim 120 C18 Column: High Peak Capacity



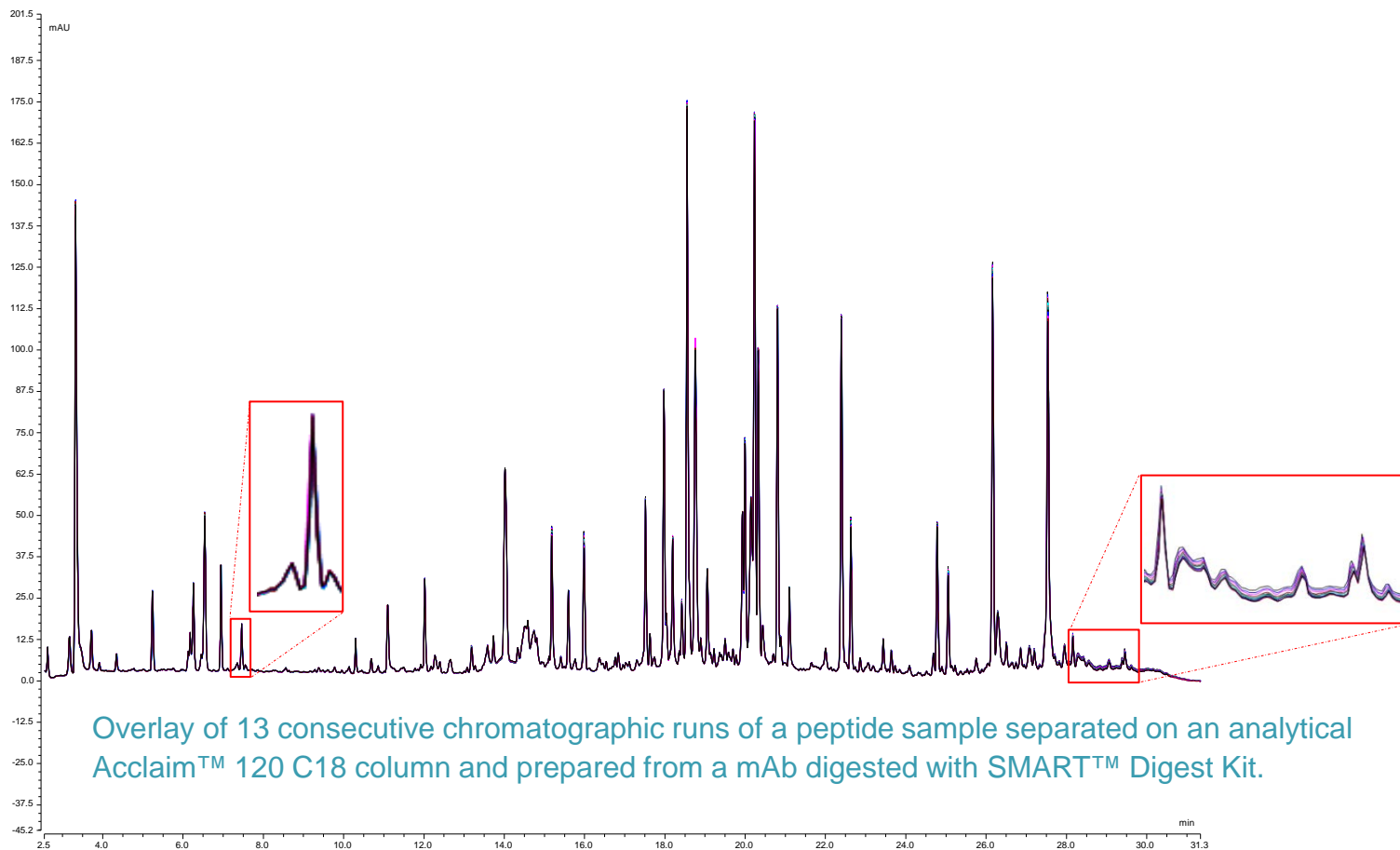
Vanquish Flex UHPLC System



- Developed for biopharma
- Fully biocompatible flow path
- Powerful detectors – all workflows
- Sample pre-compression for maximum peak retention time reproducibility and column lifetime
- Ceramic valve technology
- Column thermostating technology with two temperature control modes
- Supports latest column technology

Vanquish Flex UHPLC System: Retention Time Reproducibility

Retention time reproducibility of a peptide separation

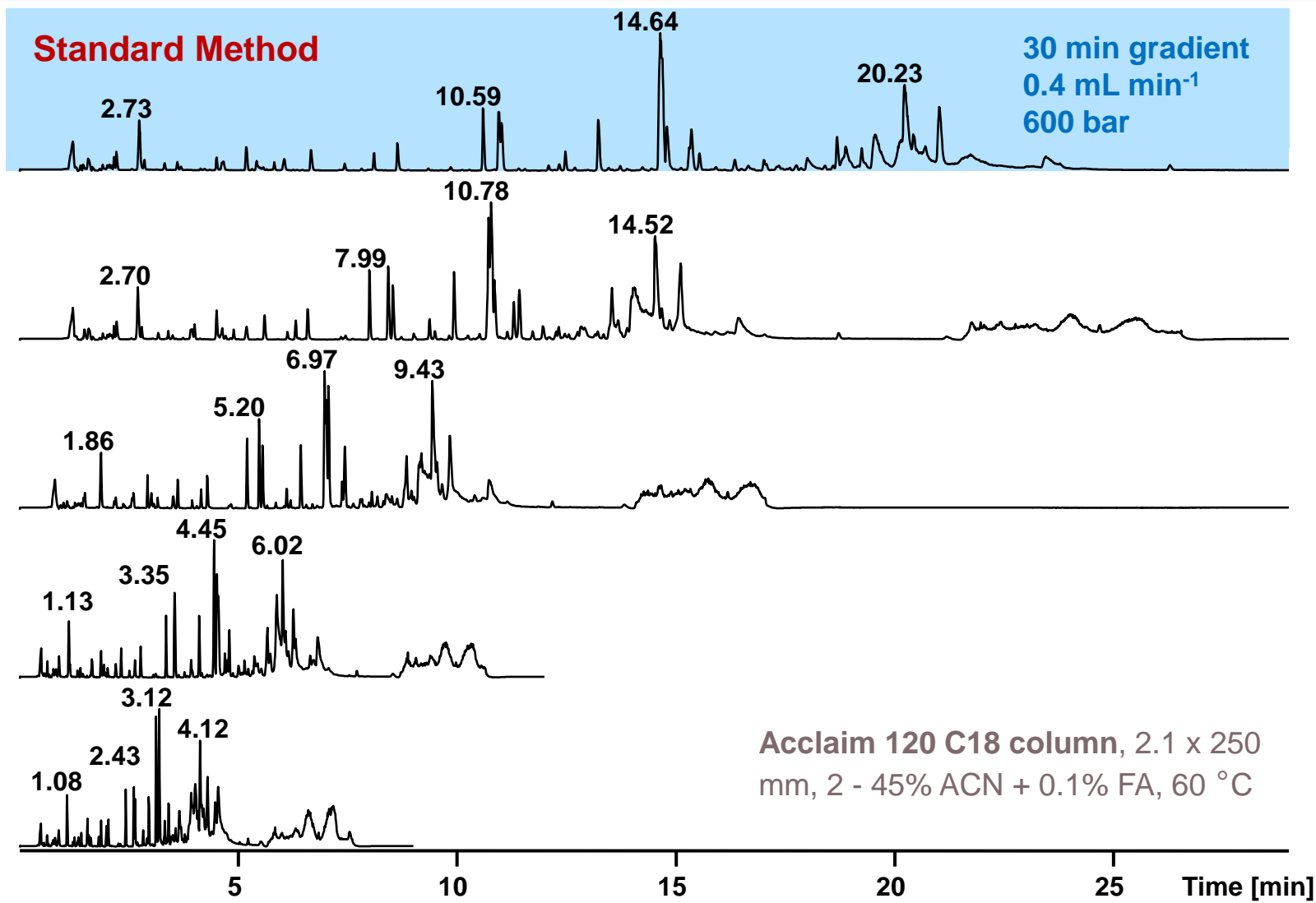


Overlay of 13 consecutive chromatographic runs of a peptide sample separated on an analytical Acclaim™ 120 C18 column and prepared from a mAb digested with SMART™ Digest Kit.

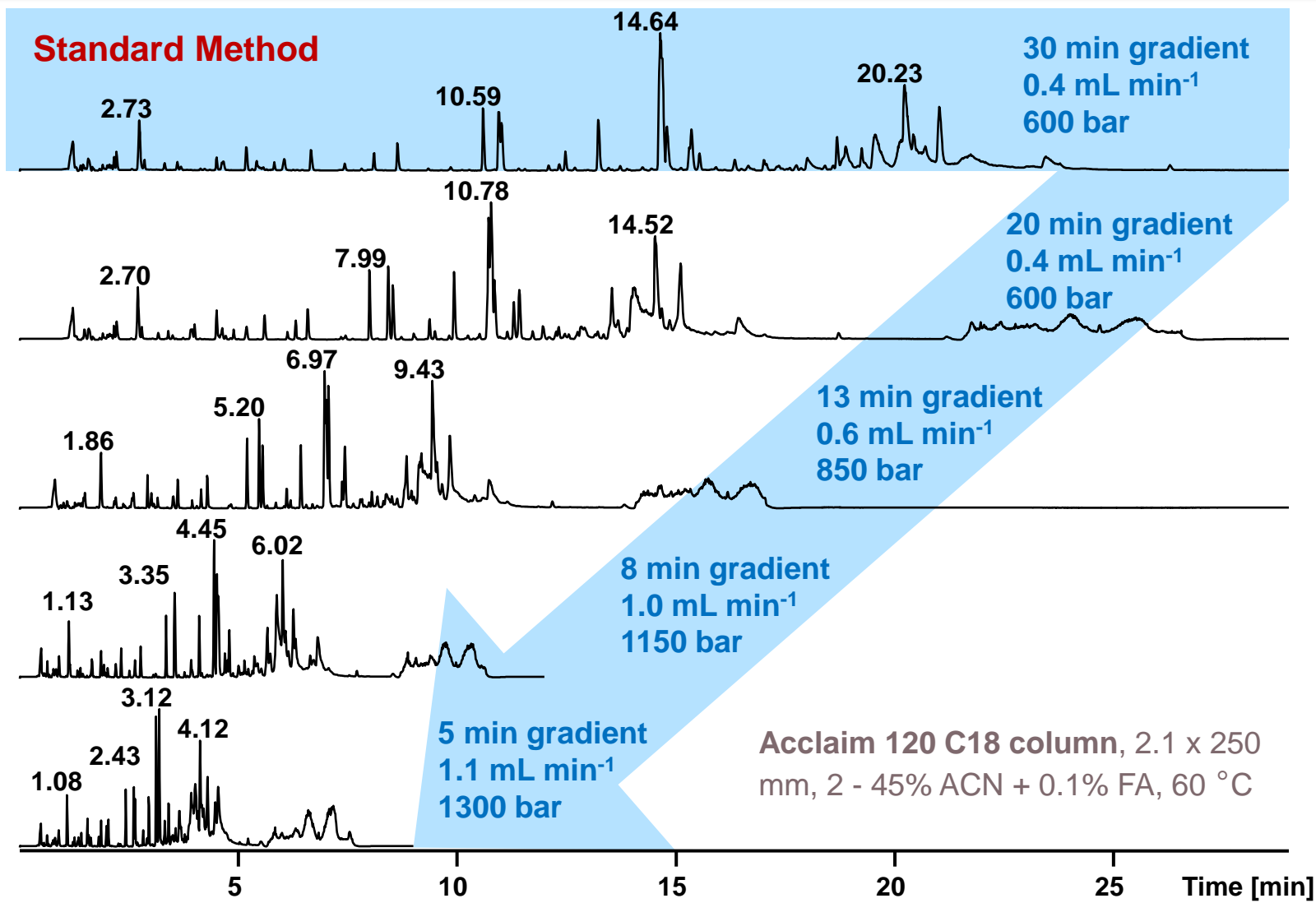
Retention time Repeatability

peak #	RT (min)	RSD (%)
3	3.315	0.082
9	5.231	0.065
14	6.532	0.017
15	6.937	0.023
19	10.290	0.021
23	12.013	0.012
31	14.011	0.013
39	15.177	0.012
42	15.589	0.010
51	17.511	0.007
55	17.969	0.011
61	18.546	0.010
83	20.798	0.010
85	21.095	0.012
87	22.386	0.009
96	24.774	0.012
103	26.155	0.009
106	26.155	0.009
109	27.529	0.010

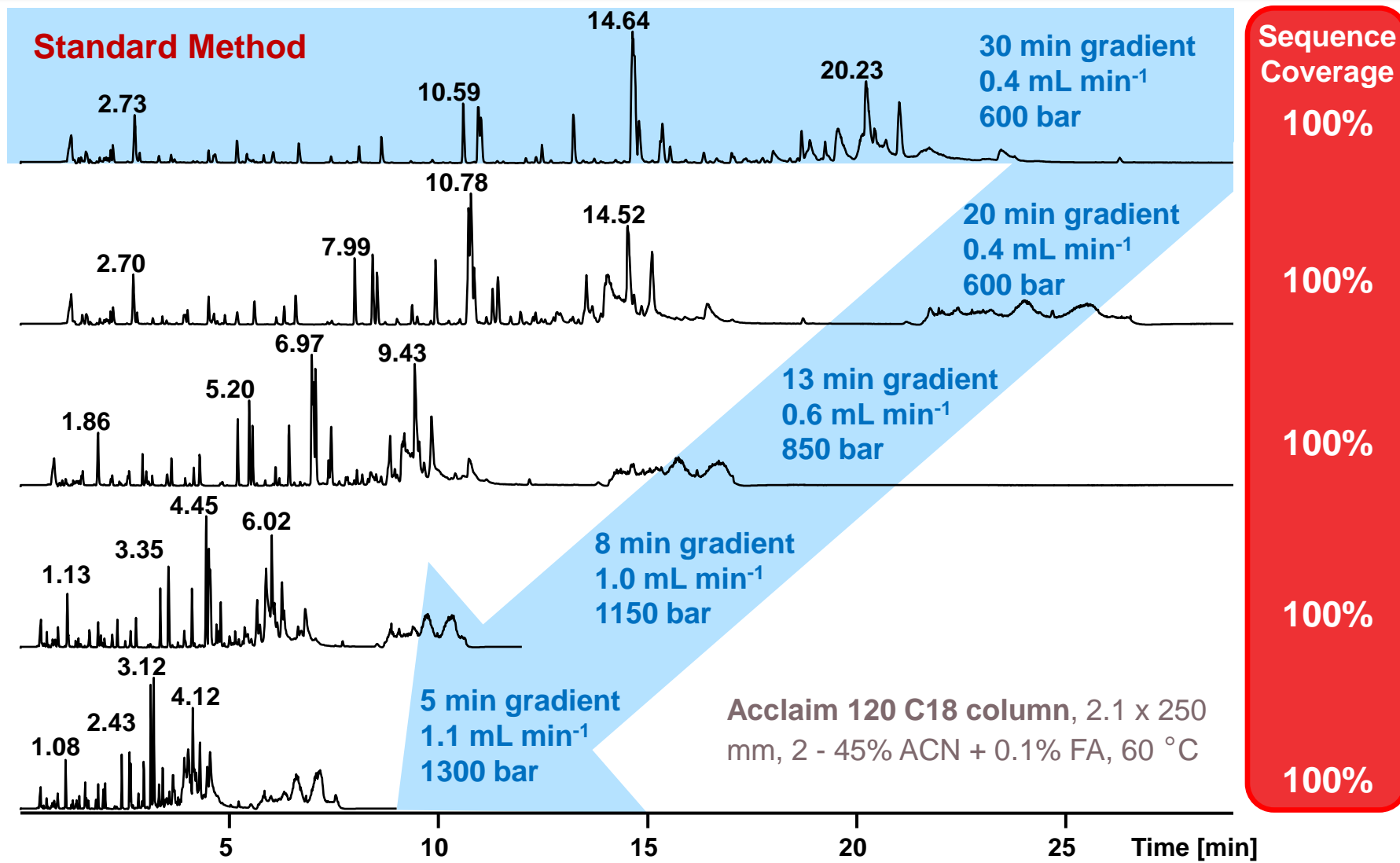
Vanquish Flex: Rituximab Analysis With Reducing Gradient



Vanquish Flex: Rituximab Analysis With Reducing Gradient

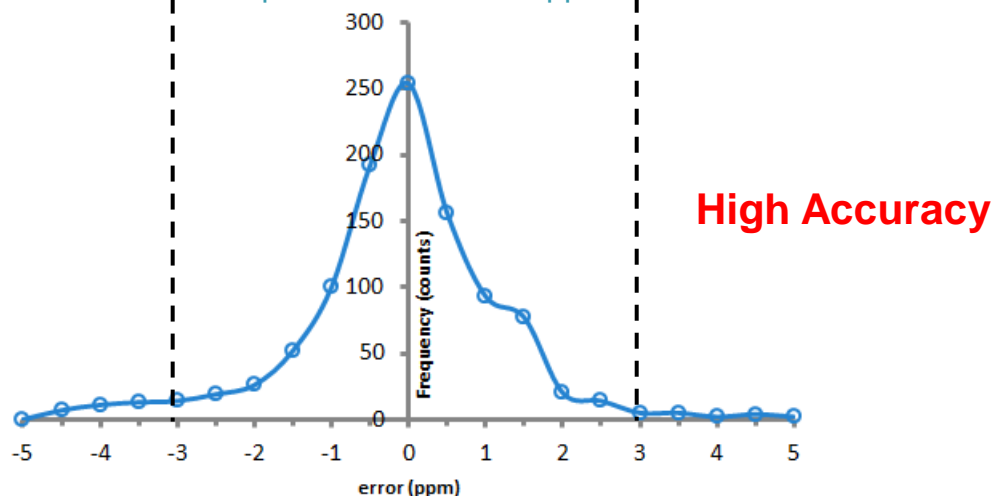


Vanquish Flex: Rituximab Analysis With Reducing Gradient

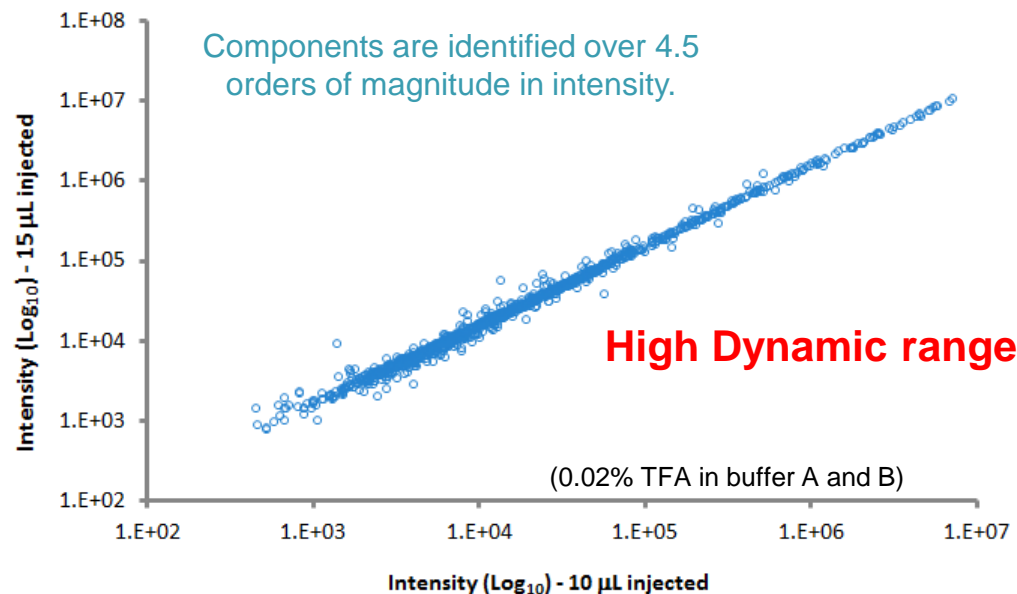


Q Exactive Plus Mass Spectrometer

95% of the 1067 identified components are in ± 3 ppm



Components are identified over 4.5 orders of magnitude in intensity.



- Ideal for intact proteins and peptides
- Very high accuracy
- High dynamic range
- Gold standard Orbitrap™ detector
- Optional intact protein mode.



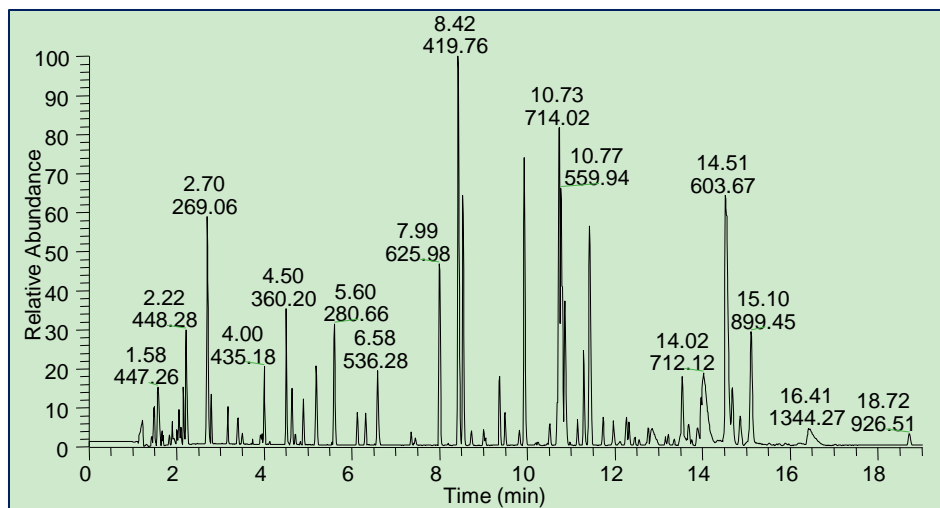
Sample Analysis By Mass Spectrometry

Q Exactive Plus

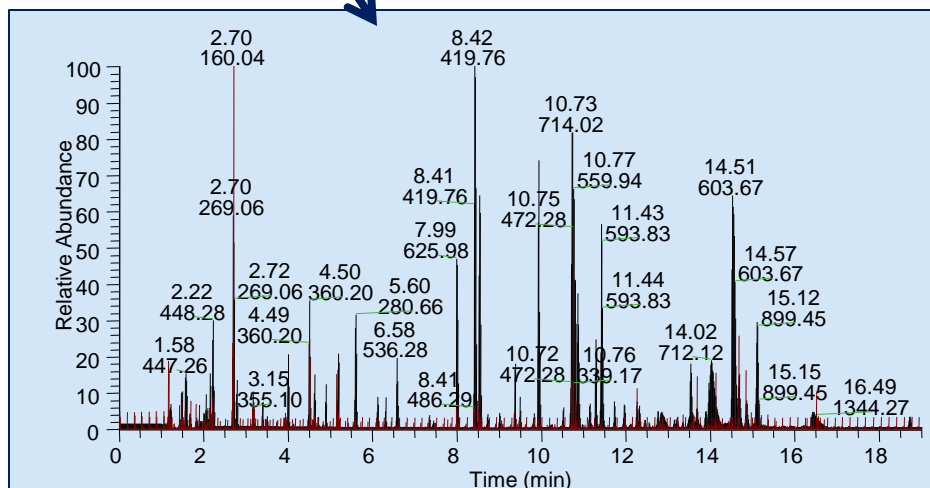


**Full MS scan
method
m/z 200-2000**

**ddTopN Method:
Full MS with
dd MS2 scans**



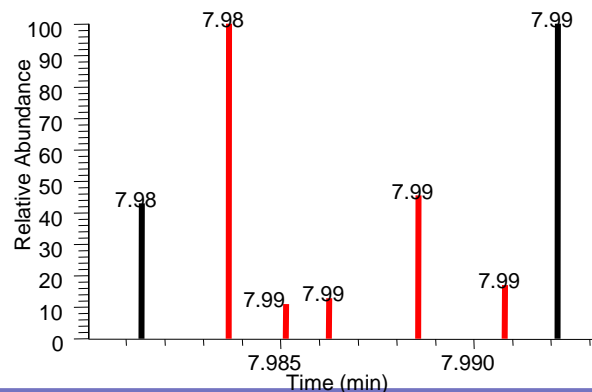
Base Peak Chromatograms



ddTopN Method with N=5:

MS/MS data-dependent analysis of 5 most abundant peptide ions (red) based on precursors obtained in a full scan (black)

zoom



BioPharma Finder

- Intact protein analysis and peptide mapping in one package
- Peptide mapping of biotherapeutics and other recombinant proteins
- Supports all Orbitrap™ & ion-trap-based instruments



NEW!

BioPharma Finder™ 1.0
Mass Informatics Platform for Protein Characterization

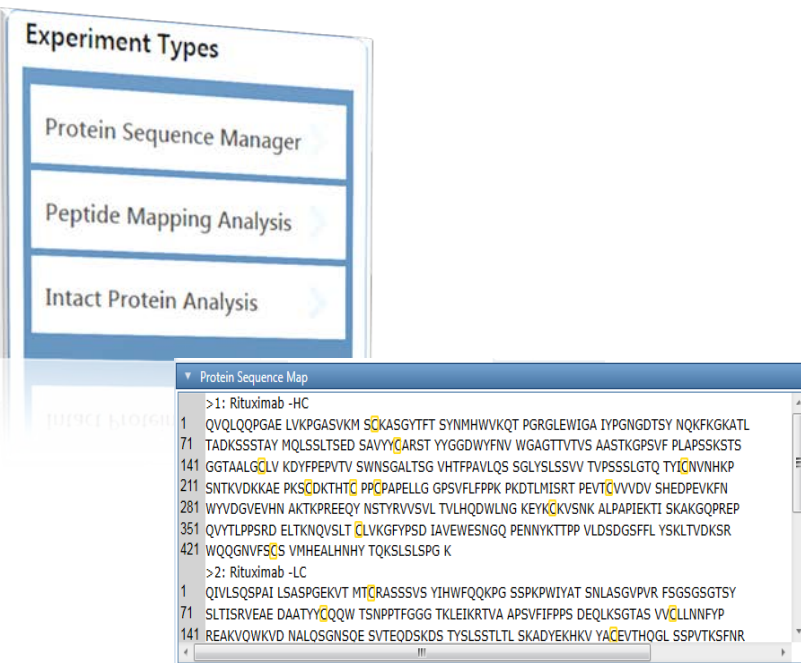
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Thermo
SCIENTIFIC

**More confidence, more
modifications, less time**

Data Analysis With BioPharma Finder: Peptide Mapping

- Protein sequence manager stores sequence information for quick use



Data Analysis With BioPharma Finder: Peptide Mapping

- Protein sequence manager stores sequence information for quick use
- Maximum throughput through simple method editor, allowing batch analyses

The screenshot displays the BioPharma Finder software interface, specifically the Peptide Mapping Analysis section. On the left, a sidebar titled 'Experiment Types' lists 'Protein Sequence Manager', 'Peptide Mapping Analysis', and 'Intact Protein Analysis'. The main window is divided into two panes. The top pane, titled 'Protein Sequence Map', shows a list of protein sequences with their corresponding peptide mappings. The bottom pane, titled 'Peptide Mapping Analysis Definition', contains a 'Load Raw Data File' section with a 'Raw Data Directory' field and a 'Load Raw Data File' button. Below this is a table with columns 'Raw file name' and 'Condition', listing files like 'Herceptin_Q2Plus_10ug.raw' and 'Herceptin_Q2Plus_15ug.raw'. To the right, the 'Protein Sequence' section displays a table with columns: 'Select', 'Name', 'Category', 'Last Modified Time', 'Manufacture/MS', 'Num. of Chains', 'Min. Num. of Modifications', and 'Total Number of Amino Acids'. The table lists sequences such as 'Example mAb', 'Example mAb var', 'Herceptin', and 'Herceptin, full'. Below this is the 'Processing Method' section, which includes a table with columns 'Name' and 'Description', listing methods like 'Basic Default Method' and 'Disulfide Bond Default Method'. At the bottom of the interface are two buttons: 'Start Processing' and 'Edit Method'.

Experiment Types

- Protein Sequence Manager
- Peptide Mapping Analysis
- Intact Protein Analysis

Protein Sequence Map

>1: Rituximab -HC

1 QVQLQQPGAE LVKPGASVKIM SCKASGYFT SYNMHWVKQT PGRGLEWIGA IYPNGDTSY NQKFKGKATL

71 TADKSSSTAY MQLSSLTSED SAVYCARST YYGGDWYFNW WGAGTTVTVS AASTKGPSVF PLAPSSKSTS

141 GGTAALGLV KDYPEPVTY SWNSGALTSG VHTFPAVLQS SGLYSLSSV TVPSSSLGTQ TYQNVNHKP

211 SNTKYDKKAE PKSCDKHTHTGPPCPAPELLG GPSVLFPPK PKDTLMISRT PEVTGVVDV SHEDPEVKFN

281 WYVDGVEVHN AKTKPREEQY NSTYRVVSVL TVLHQDWLNG KEYCKVSNK ALPAIEKTI SKAKGQPREP

351 QVYTLPPSRD ELTKNQVSLT CLVKGYPSD IAVEWESNGQ PENNYKTPPP VLDSGGSFFL YSKLTVDKSR

421 WQQGNVFSQS VMHEALHNHY TQKSLSLSPG K

>2: Rituximab -LC

1 QIVLSQSPAI LSASGEEKVT MTCRASSSVS YHWFQKPG SSKPWIVAT SNLASGVPVR FSGSGSGTSY

71 SLTISRVEAE DAATYCCQW TSNPPTFGG TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VQCLLNFPY

141 REAKVQWKVD NALQSGNSOE SVTEQDSKDS TYSLSSTLT SKADYEKHKV YACEVTHOGL SSPVTKSFNR

Peptide Mapping Analysis Definition

Name (Experiment): Herceptin

Load Raw Data File

Raw Data Directory: C:\lab\ur\data\Herceptin\Peptide Mapping

Load Raw Data File

Condition: 10ug, 15ug

Raw file name	Condition
Herceptin_Q2Plus_10ug.raw	10ug
Herceptin_Q2Plus_15ug.raw	15ug

Protein Sequence

Select	Name	Category	Last Modified Time	Manufacture/MS	Num. of Chains	Min. Num. of Modifications	Total Number of Amino Acids
<input type="checkbox"/>	Example mAb	Unknown	04/04/2015	75,623.20	2	2	660
<input type="checkbox"/>	Example mAb var	Unknown	04/04/2015	72,033.83	2	1	660
<input checked="" type="checkbox"/>	Herceptin	Unknown	04/03/2015	72,982.06	2	2	654
<input type="checkbox"/>	Herceptin, full	Unknown	04/03/2015	72,667.95	2	2	654

Processing Method

Name	Description
Basic Default Method	Default Method for Basic Peptide Mapping
Disulfide Bond Default Method	Default Method for Disulfide Bond

Start Processing Edit Method

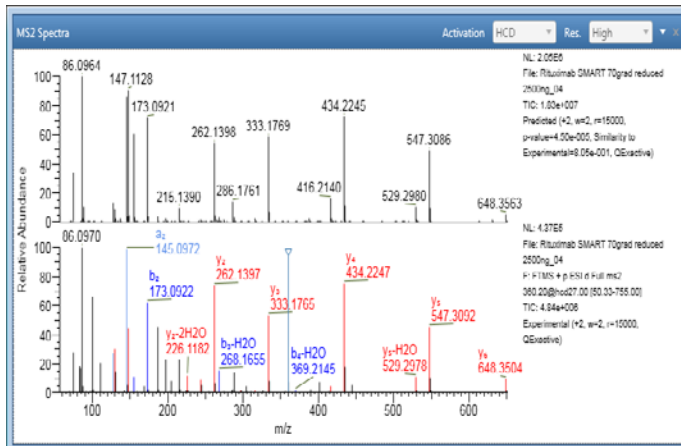
Data Analysis With BioPharma Finder: Peptide Mapping



- Protein sequence manager stores sequence information for quick use
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- Interactive results display allows you to review data how you want

Data Analysis With BioPharma Finder: Peptide Mapping

- Protein sequence manager stores sequence information for quick use
- Maximum throughput through simple method editor, allowing batch analyses
- Interactive results display allows you to review data how you want
 - Sequence/fragment coverage maps
 - Compare real and predicted spectra



Data Analysis With BioPharma Finder: Peptide Mapping



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- Maximum throughput through simple method editor, allowing batch analyses
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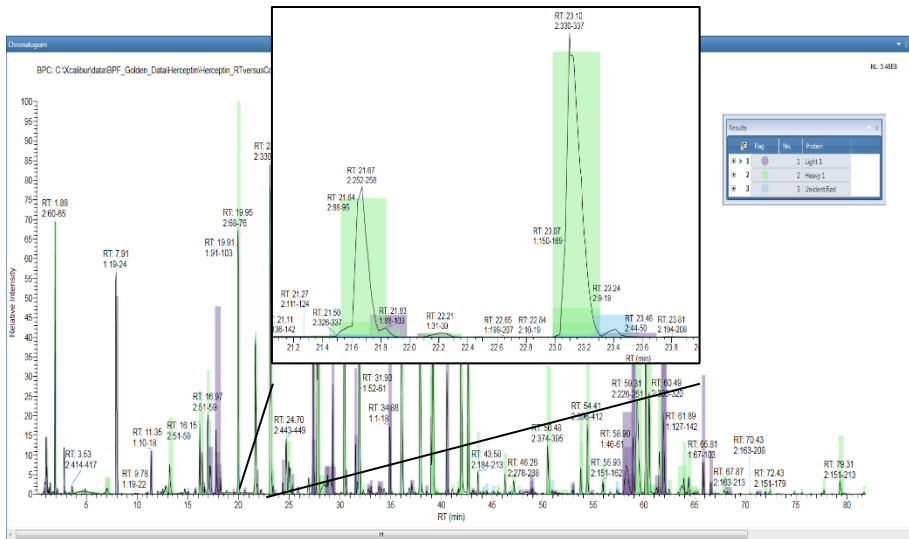
#	Level	No.	Identification	Peptide Sequence	Modification	Site	Delta (ppm)	Confidence Score	ID Type	RT (min)
1	Component	1101	1A343-R348 = 655.3765m	AKGQIR	None		0.09	100.0%	MS2	2.17
2	Component	1105	1A343-R348 = 655.3765m	AKGQPR	None		0.75	100.0%	MS2	2.17
3	Component	1371	1-E325-K330 = 677.35304m	CKVSNK	None		0.54	100.0%	MS2	2.67
4	Component	1427	1-F64-K67 = 478.29037m	FKGK	None		-2.55	99.9%	MS2	2.82
5	Component	1434	2-F29-K44 = 571.29658m	PGSSPK	None		0.53	100.0%	MS2	2.83
6	Component	1463	1-E322-K324 = 438.21145m	EYK	None		-0.28	100.0%	MS2	2.84
7	Component	1467	1-F327-K324 = 438.21145m(F327-18.0104)	EYK	-18.0104	F322	-0.36	91.2%	MS2	2.84
8	Component	1475	2-S12-K18 = 674.32352m(nonspecific)	SASPGEK	nonspecific		-0.36	100.0%	MS2	2.84

Modification Results				
Created		Data Folder		
Protein	Residue #	Modification	Category	
Aa		Aa	Aa	
51	Rituximab -HC	289 ~H289+135.9642	Unknown Modifica	
52	Rituximab -HC	289 ~H289-17.0251	Unknown Modifica	
53	Rituximab -HC	290 ~N290+0.9840	Unknown Modifica	
54	Rituximab -HC	292 ~K292-18.0096	Unknown Modifica	
55	Rituximab -HC	301 N301+A1G0	Glycoform	
56	Rituximab -HC	301 N301+A1G0F	Glycoform	
57	Rituximab -HC	301 N301+A1G1F	Glycoform	

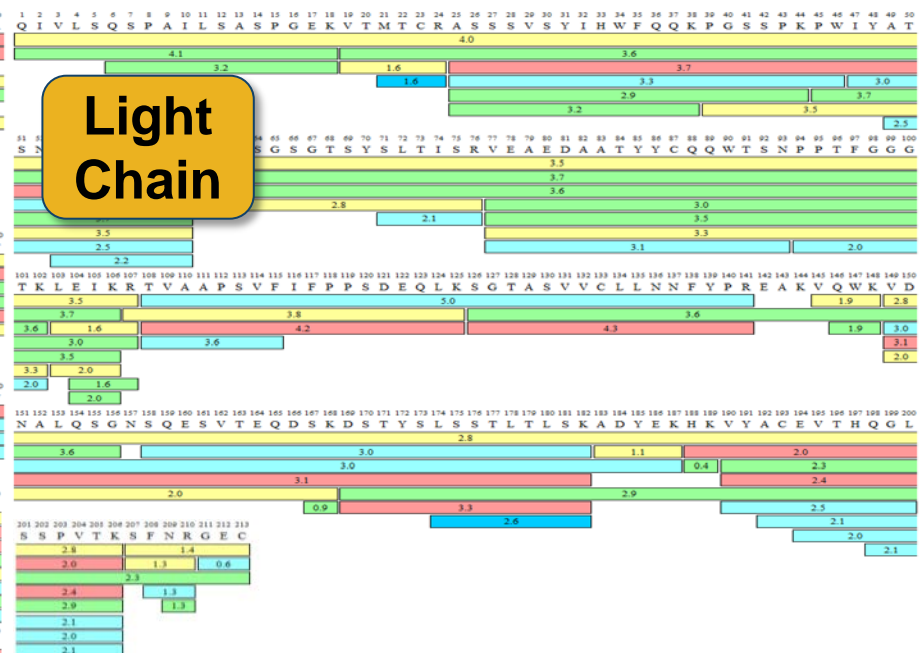
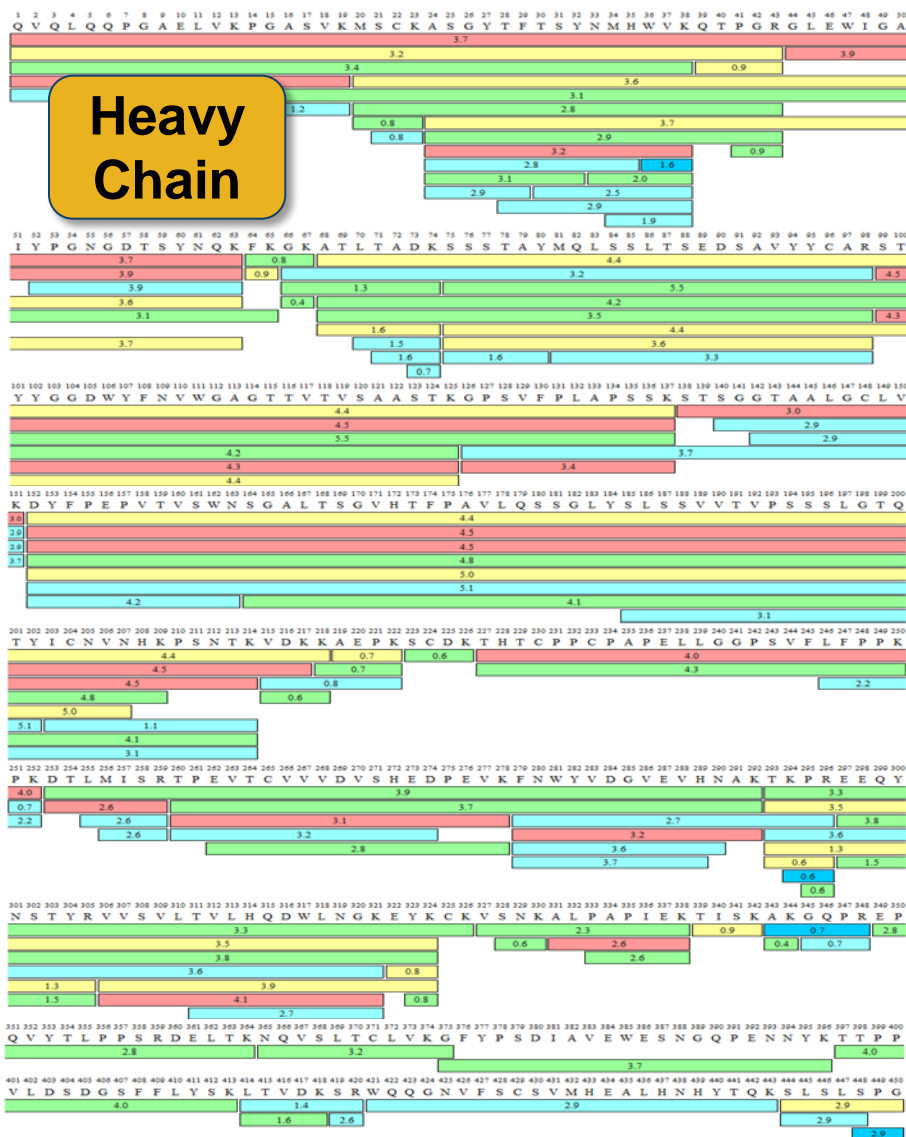
- Sequence/fragment coverage maps
- Compare real and predicted spectra
- Powerful modification and results summary

Data Analysis With BioPharma Finder: Peptide Mapping

- Protein sequence manager stores sequence information for quick use
- Maximum throughput through simple method editor, allowing batch analyses
- Interactive results display allows you to review data how you want
 - Sequence/fragment coverage maps
 - Compare real and predicted spectra
 - Powerful modification and results summary
 - Chromatographic shading – never miss a thing

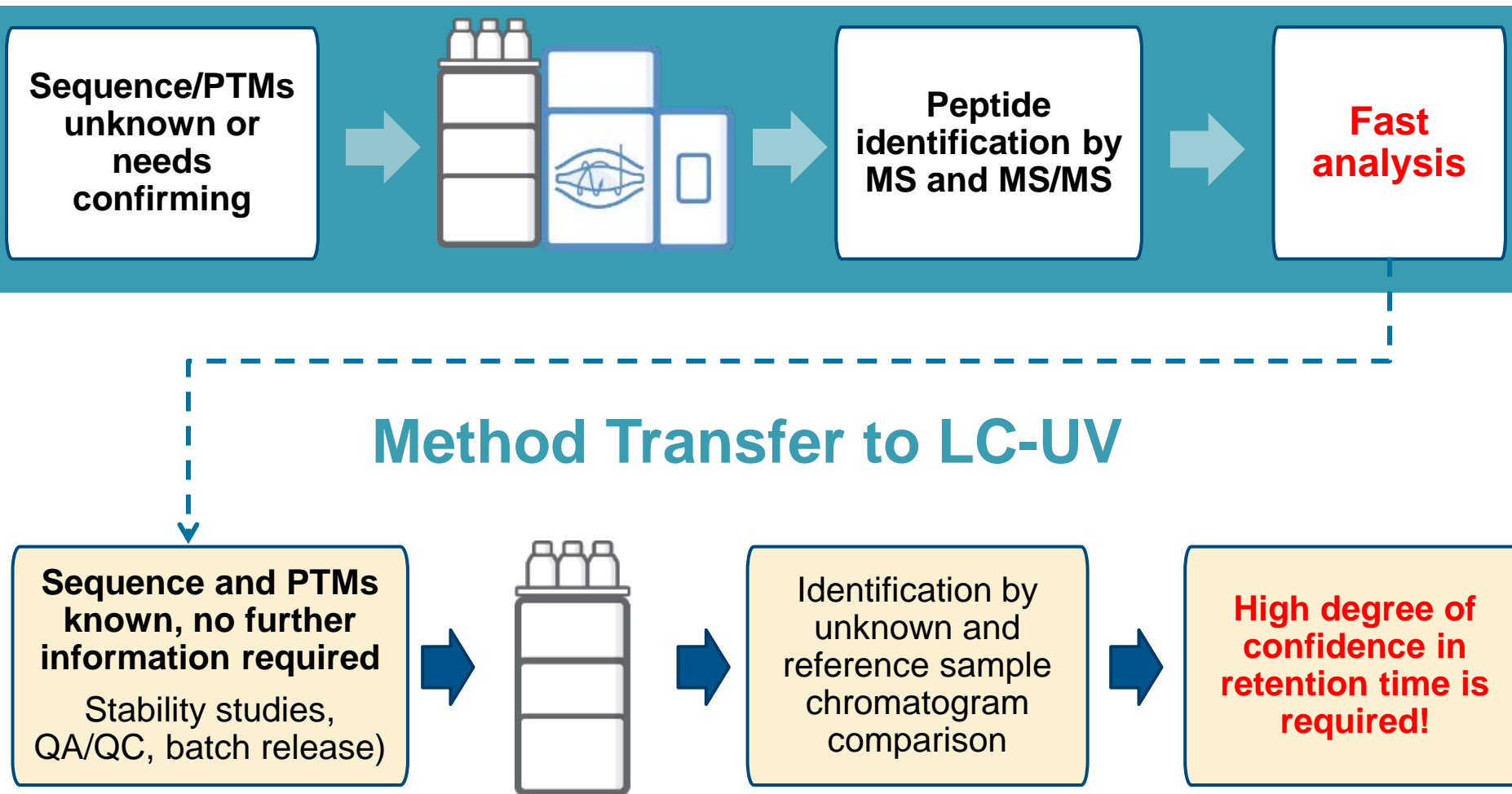


Sequence Coverage Obtained For 5 Minute Gradient

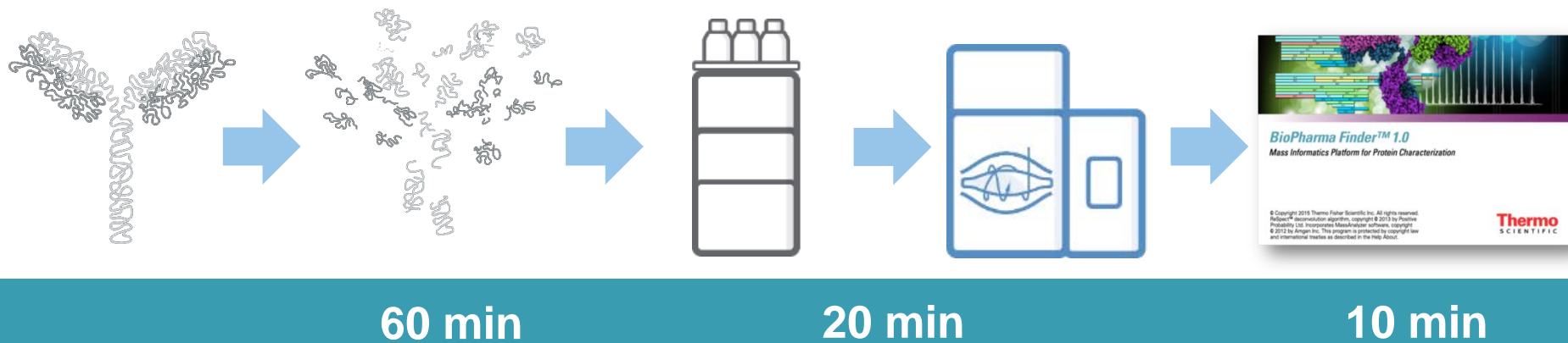


100% Sequence coverage
obtained for light and heavy
chain of Rituximab

Peptide Mapping: Method Transfer From LC-MS to LC-UV



Peptide Mapping Workflow: Time Saving



- Complete analysis can be done in less than 90 minutes
- MS analysis is rapid, stand-alone LC-UV even quicker
- Inexperienced analyst can obtain reproducible results from LC-UV

Thank You – Any Questions?

