thermo scientific

# Analytical solutions for biopharmaceutical **characterization** and **control**

Your partner on every step of your journey

ThermoFisher SCIENTIFIC

# Together we can address the challenges of biotherapeutic drug development

## Your molecules are complex

Biotherapeutics such as monoclonal antibodies, biosimilars, antibody-drug conjugates, and nucleotide-based therapies are increasingly important drug product classes. Most biotherapeutic drug products are fundamentally more complex than traditional chemically synthesized products, and are subject to modification events, throughout development, manufacturing and storage.

## You need fit-for-purpose tools at each biotherapeutic development stage

Well-designed characterization technologies provide unique insights which advance the development of biotherapeutics. Comprehensive characterization, allowing for full product understanding can save significant time and development costs. Throughout process development and manufacturing, tools are required for reliable monitoring of product microheterogeneity to ensure drug safety and efficacy.

## Technology innovations to help you on your drug development journey

Our technology innovations have been specifically designed with the rapid pace and complexity challenges of biotherapeutic development in mind. From early discovery through to delivery of a commercial product, we provide technologies to confidently answer your analytical questions, bringing life-changing biotherapeutics to market faster than ever before.

# Enabling analytical tools for each stage of biopharmaceutical development

**Characterization**
Companies that develop biotherapeutics need versatile instrumentation capable of answering any characterization question.

**Discovery**
Early development phases identify potential molecules to develop as drug candidates. Discovery includes the search for pipeline candidates.

**Requires**
high-throughput capabilities and versatile, advanced tools.

**Product characterization**
At this stage, the analytical focus shifts to gaining a deep product understanding.

**Requires**
advanced tools to answer in-depth molecular questions with full confidence.

**Process development**
In process development, an in-depth understanding of process effects on biomolecules allows for quicker progression through the development pipeline.

**Requires**
robust and reproducible analyses to monitor molecular attribute changes with speed and confidence.

**Quality control and monitoring**
Small changes in manufacturing conditions can have a significant impact on product quality. To ensure the release of safe and effective products, advanced analytical methods are implemented to closely monitor CQAs for each batch.

**Requires**
reproducible, robust, GMP compliance-ready methods.

**Control**
Many modifications that cause structural heterogeneity of biotherapeutic products have profound impact on function, efficacy, and safety. These modifications, known as critical quality attributes (CQAs), must be defined and analytical methods implemented to monitor them.

## Analytical workflows

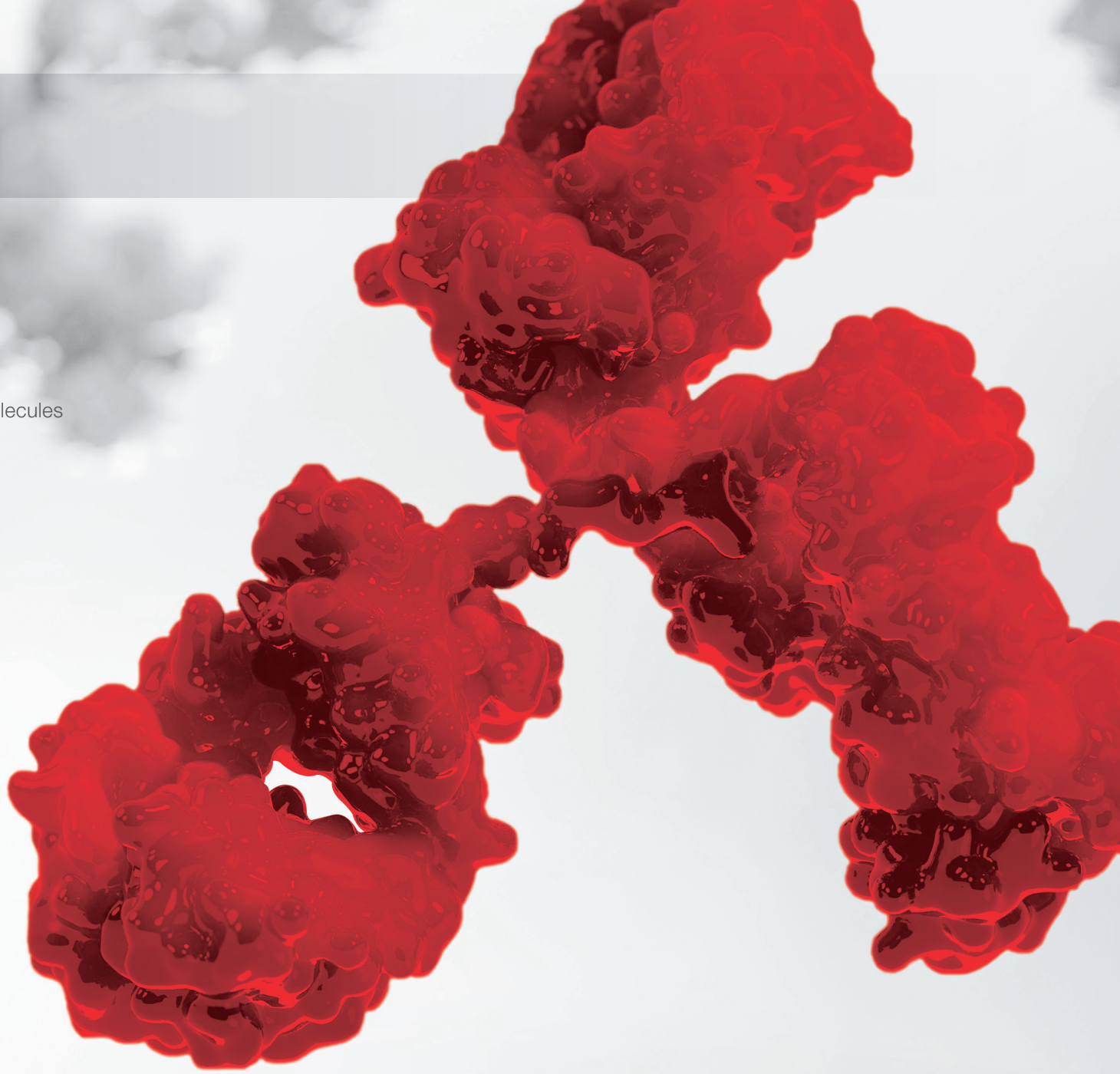
# Discovery

Early development phases identify potential molecules to develop as drug candidates.

**Requires**
Advanced tools and high-throughput screening capabilities to elucidate structure.

## Enabling technologies for drug discovery

- Advanced mass spectrometry capabilities
- Exceptional separations
- Flexible software tools



# Enabling discoveries in BioPharma

## Go beyond what is possible

### See more than ever before

Unprecedented insights into the most advanced biotherapeutics are revealed by utilizing the pioneering technology and software developments incorporated into the Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer.

BioPharma discovery and development scientists can now achieve new levels of structural insight into therapeutic proteins; revealing ultra-low-level modifications and allowing site-specific critical quality attribute (CQA) determination—delivering more confidence when progressing candidates throughout the development pipeline, whilst ensuring drug efficacy and patient safety.

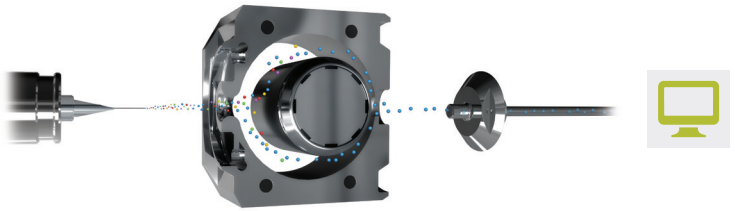
## Orbitrap Eclipse Tribrid MS with HMR$^{n}$ option

- Mass range *m/z* 50–8,000 with HMR Option
- Max. resolution up to 1 M at *m/z* 200
- Proton Transfer Charge Reduction (PTCR)
- Multiple fragmentation techniques: CID, HCD, ETD, EThcD, ETciD, UVPD, available at any stage of MS$^{n}$

> The Orbitrap Eclipse Tribrid mass spectrometer brings a new dimension to native MS, enabling us to discover and chemically define the ligands, lipids, and drugs that regulate the function of membrane protein assemblies.
> **Dr. Joseph Gault, University of Oxford, the UK**

WP Go beyond in biopharmaceutical characterization

## Thermo Scientific FAIMS Pro interface



### Orthogonal selectivity expands confidence in characterization

Add a new dimension of selectivity with high-field asymmetric waveform ion mobility spectrometry (FAIMS).

Enhance peptide level and intact protein characterization by reducing interferences and enhancing sensitivity, whilst reducing time-consuming sample preparation.

> FAIMS Pro™ enhances the deep characterization of proteoforms. Our data has generated a lot of excitement, in part due to robustness of the ion source over time.
> **Prof. Neil Kelleher, Northwestern University**

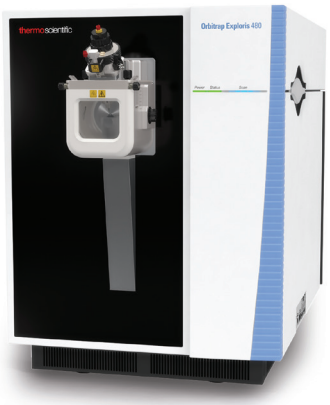
# Enabling discoveries in BioPharma

## Research-grade Mass Spectrometry

### Thermo Scientific Orbitrap Exploris 480 MS with BioPharma option

**Extraordinary simplified – maximized throughput**

The Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer goes beyond routine analytical needs with advanced capabilities and intelligence driven data acquisition strategies for rigorous, high throughput identification, quantitation, and structural characterization of biotherapeutics. Along with the superior resolving power of up to 480,000 at *m/z* 200, adding the BioPharma option enables analysis of intact biotherapeutics under native-like conditions with an ion transmission and mass detection up to *m/z* 8,000.



- *m/z* 40–8,000 with BioPharma option
- Resolution up to 480,000 at *m/z* 200
- Scan modes: Full MS, dd MS/MS, TopN, TopSpeed, targeted MS/MS, MSX, tSIM, tSIM MSX, DIA, BoxCar DIA, Acquire X

> With the Orbitrap Exploris 480 we can leverage optimal combination of signal to noise ratio and spectral resolution for our native SEC-MS lead selection studies with exceptional robustness demonstrated through the analysis of hundreds of samples.
> **Dr. Dan Bach Kristensen, Principal Scientist, Symphogen**

### Thermo Scientific Q Exactive UHMR Hybrid-Quadrupole Orbitrap MS

**Delivering unmatched performance for ultra-high mass analysis**

Designed for scientists needing higher resolution and sensitivity in the ultra-high mass range (up to *m/z* 80,000) and ability to perform highest quality native MS and experiments run under native condition. The unique combination of high resolution, high sensitivity and $MS^2$/pseudo-$MS^3$ capabilities makes the Thermo Scientific™ Q Exactive™ UHMR Hybrid-Quadrupole Orbitrap mass spectrometer ideal to further analyze complex macromolecules and aggregate forms of your biotherapeutic.

- *m/z* 350 – 80,000
- Resolution up to 200,000 @ *m/z* 400
- In-source trapping capability that enables improved transmission and efficient desolvation and fragmentation.
- Pseudo-$MS^3$ capability
- Quadrupole isolation up to *m/z* 25,000

> The Q Exactive UHMR mass spectrometer has been a game changer in native mass spectrometry. Due to the very high resolution, we can now measure small molecules bound to large proteins, which is an important step forward for fragment-based drug discovery.
> **Dr. Idlir Liko, CTO, OMass Therapeutics**

# Enabling discoveries

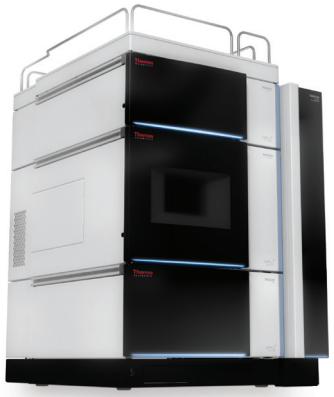
## Unrivaled separation capabilities

### Thermo Scientific Vanquish Horizon UHPLC System

**Deliver results without compromise**

The Thermo Scientific™ Vanquish™ Horizon UHPLC system delivers confidence with a fully biocompatible flow path and proven compliance. With it, today's biopharmaceutical scientists can enjoy unprecedented performance in retention time stability, sensitivity and separation efficiency with the widest range of column chemistries for biotherapeutic proteins. You can expect operational simplicity with easy, freely available, one-click workflows and tool-free Viper fittings.

The system features high sample capacity for high-throughput workflows and versatility through seamless integration with market-leading mass spectrometry, ultraviolet, fluorescence and charged aerosol detection, allowing you to do more with less.



- 1,500 bar
- Ultra-low gradient delay volume
- Fully biocompatible

> One of the key features for us is really the simplicity of use.
> **Prof. Jonathan Bones, National Institute for Bioprocessing Research and Training**

### The 908 Devices ZipChip® platform

**Seamless workflow for BioPharma applications**

The Thermo Scientific™ compatible 908 Devices™ ZipChip™ platform Integrates capillary electrophoresis (CE) and electrospray ionization (ESI) into a single microfluidic device to rapidly prepare, separate, and electrospray biological samples directly into a mass spectrometer. The portable size ZipChip interface directly mounts onto select models of Thermo Scientific Orbitrap-based mass spectrometers, and creates a seamless CE/ESI-MS workflow that offers fast CE separation, nano-spray level sensitivity, and HRAM mass spectrometry for the characterization of intact proteins, antibody-drug conjugates, antibody subunits, peptides, and metabolites.

Unrivalled separations,
faster results,
and easier operation

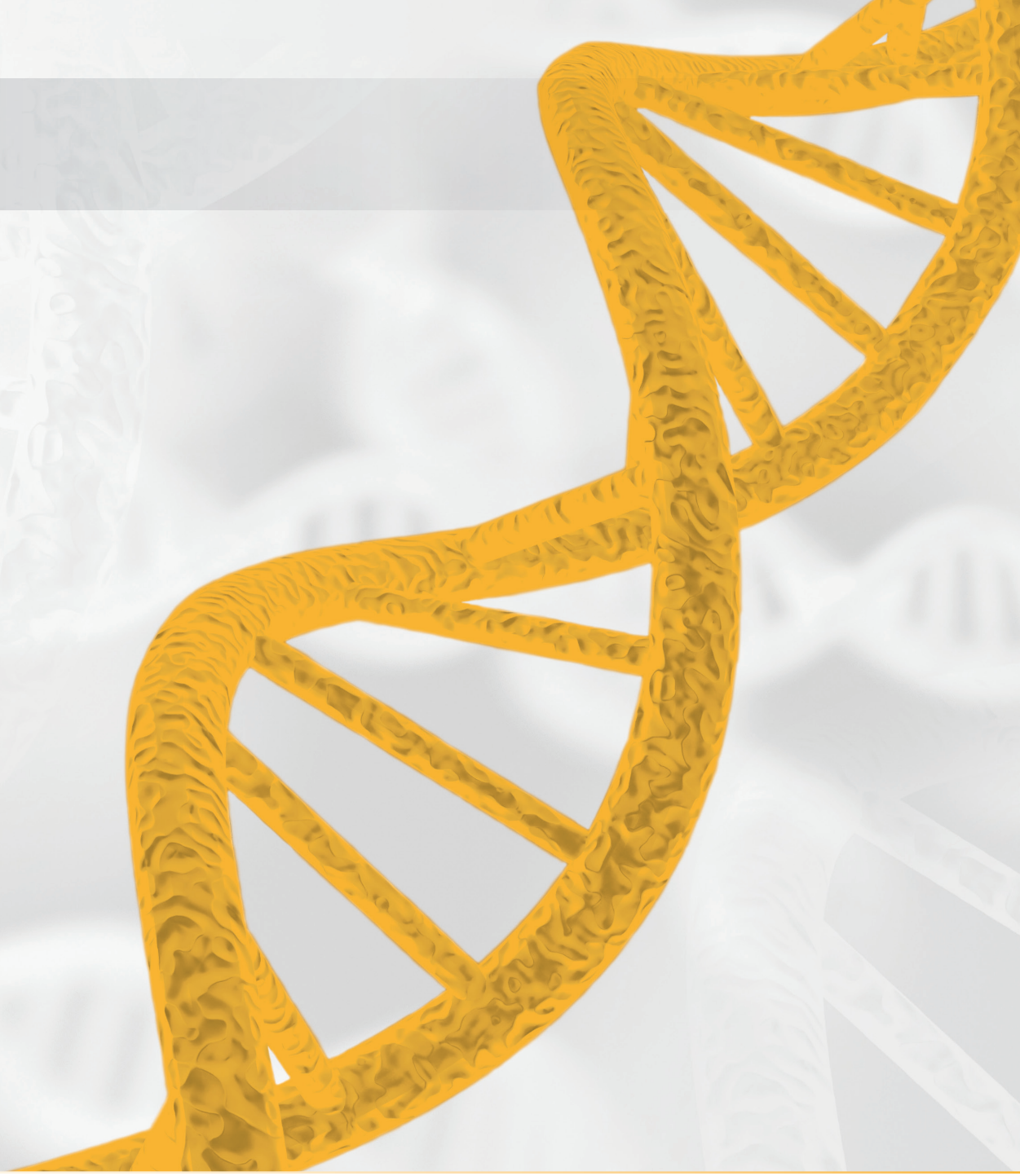
# Product characterization

Here analytical innovations are applied to gaining a deep product and impurity understanding.

**Requires**
advanced tools to answer in-depth molecular questions with full confidence.

## Featured technologies for confident characterization

- Unique purpose-built chemistries
- High productivity separations
- Workhorse mass spectrometry
- Enabling software



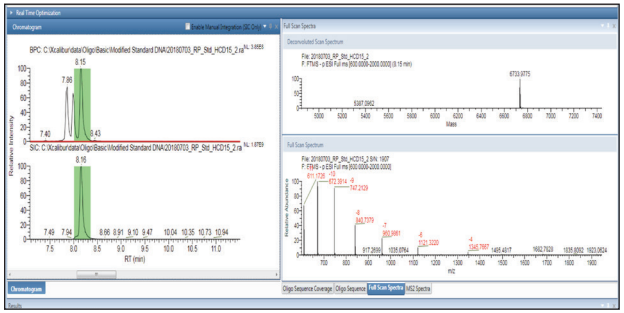
# Confident characterization

## Easy to use, flexible software

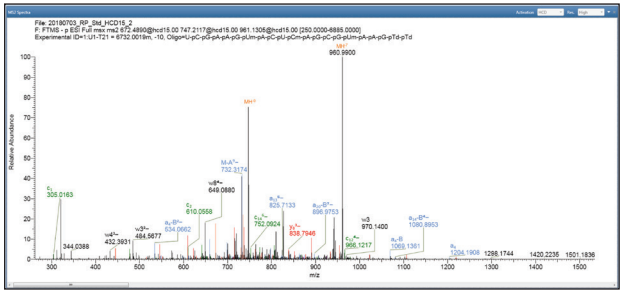
**Thermo Scientific™ BioPharma Finder™ software** answers your biotherapeutic characterization needs.

- Novel deconvolution algorithms ensure determination of the correct molecular weight, confident identification of glycoforms and accurate measurement of drug-to-antibody ratio (DAR).
- Combine in-depth knowledge of post-translational modifications (PTMs), disulfide bonds, and low level impurities with unique data visualization tools.
- Simple software workflows guide along the characterization pathway, all centrally managed within an integrated library for sequences.

**Multiple interactive plot display options for flexibility in visualization – Oligonucleotide component detection and deconvolution**



**Automatic annotation of MS²**

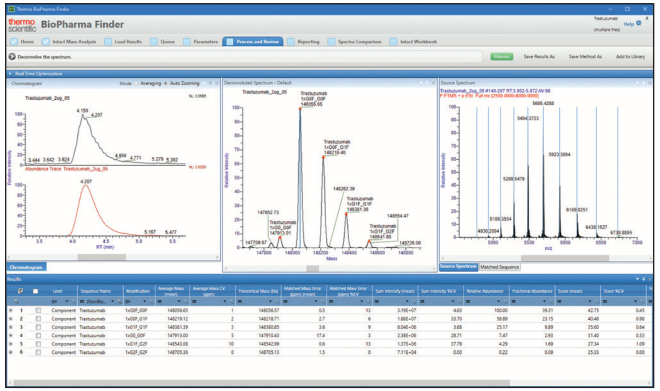


### Simple, confident characterization software:

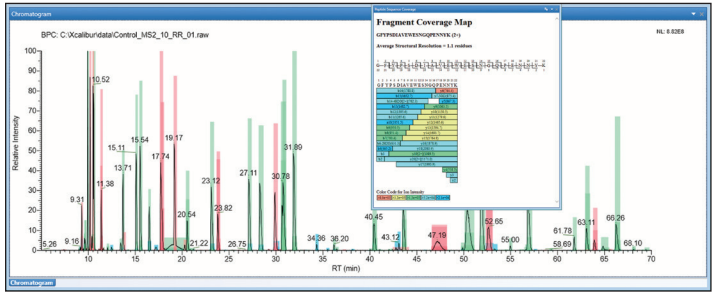
- Peptide mapping
- Intact mass analysis
- Fragment mass analysis
- Oligonucleotide mapping and impurity analysis
- Multi-attribute method development
- Top-down sequencing
- DAR calculations
- PTM site location
- Host cell protein analysis

BioPharma Finder Software

**Superior deconvolution algorithms**



**Informative and interactive sequence coverage map, colored by intensity**



**Automatically annotate peaks in the chromatogram**

**Chromatographic shading, predicted vs. experimental fragment ion spectra and sequence variant searching allows visualization of every peptide, modified or not.**

# Confident characterization

## Dependable, flexible, productive separations

Thermo Scientific™ Vanquish™ HPLC and UHPLC systems are the most advanced liquid chromatography instruments available. The Vanquish LC platform improves performance, repeatability, and dependability with no trade-offs in quality or ease-of-use.

### Thermo Scientific™ Vanquish™ Duo HPLC and UHPLC systems

- Double the throughput of your LC-MS application without the need for additional bench space
- Improve return on investment by reducing cost per sample
- Reduce column carry over through extended column washing without sacrificing throughput
- Run parallel experiments on the same sample simultaneously
- Full biocompatibility to address the most challenging biotherapeutic applications

BR LC that takes your productivity to new heights

AN Tandem UHPLC operation for high-throughput LC-MS peptide mapping analyses

### Four workflows. Two flow paths. Maximized productivity.

Expand the benefits of our award-winning Vanquish platform with four workflows (Dual LC, Dual LC-MS, Tandem LC or LC-MS, and Inverse Gradient). Save time and cost per sample without adding bench space, and without compromising performance, robustness, or ease-of-use.

### Thermo Scientific™ Vanquish™ Flex UHPLC systems

- Seamless integration with the broad Thermo Scientific LC-MS portfolio
- Fast, automated, and comprehensive method development
- Full biocompatibility to address the most challenging biotherapeutic applications
- Easy instrument familiarization with intuitive system operation and simplified user maintenance

**Easier method development and routine analysis**

Whether you need an LC or LC-MS solution, the flexibility of the Vanquish Flex systems with binary or quaternary solvent blending provides the most advanced performance through better separations, more results and easier interaction.

> At Symphogen the implementation of Vanquish Duo UHPLC system has radically reduced analysis time during early lead selection studies.
> **Dr. Dan Bach Kristensen, Principal Scientist, Symphogen**

# Confident characterization

## Workhorse mass spectrometry solutions

### Thermo Scientific Orbitrap Exploris 240 MS with BioPharma option

- *m/z* 40– 8,000 with BioPharma option
- Resolution up to 240,000 at *m/z* 200

**Confidently achieve greater insights with even more productivity**

With superb spectral clarity, the Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer delivers the high-confidence characterization required to reliably quantitate and identify biotherapeutics in both research and routine laboratory environments. With new-generation system architecture and instrument control software, the system provides easy to use and powerful data acquisition capabilities, addressing the most demanding analytical challenges for intact mass analysis, subunit analysis, and peptide or disulfide bond mapping.

> The Orbitrap Exploris 240 mass spectrometer is a reliable and flexible solution for high performance biopharmaceutical characterization. I am really impressed by the sensitivity achieved for native MS analyses. The system is a must-have instrument for analytical and characterization labs supporting biopharmaceutical development and manufacture.
> **Dr. Jonathan Bones, Principal Investigator, NIBRT**

### Thermo Scientific Q Exactive Plus Hybrid Quadrupole-Orbitrap MS with BioPharma option

- *m/z* 50–8,000 with BioPharma option
- Resolution up to 140,000 at *m/z* 200

**Everyday performance and flexibility to see more and quantify more**

The Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer delivers everything you need for demanding routine and QC environments where the right results matter. It facilitates a wide range of protein analysis applications, from top- and middle-down antibody characterization and peptide mapping applications to data-independent acquision (DIA)-based peptide and protein quantitation.

> The system gets tuned and calibrated once a week. It's incredibly stable and easy to set-up.
> **Dr. David Tickle, Senior Principal Scientist, LifeArc**

# Confident characterization

## Complete suite of advanced chromatography chemistries

Our unique sample preparation and column chemistries for biomolecule workflows have been specifically designed for the separation and analysis of proteins, peptides, monoclonal antibodies, glycans, oligonucleotides, viral vectors and more.

### Oligonucleotide separation

**Thermo Scientific**™ DNAPac™ RP columns
**Thermo Scientific**™ DNAPac™ PA200 columns
**Thermo Scientific**™ DNAPac™ PA200RS columns
**Thermo Scientific**™ DNASwift™ SAX-1S Monolith columns

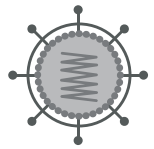
Oligonucleotide HPLC Columns

> In my work, I have found the DNAPac PA200 is quite versatile for oligonucleotide analysis, with excellent resolution for both DNA and RNA oligonucleotides of a size of up to about 100 nucleotides as well as profiling of mRNA.
> **Anastassia Kanavarioti, Founder and Director, Yenos Analytical LLC**

### Gene therapy analytics

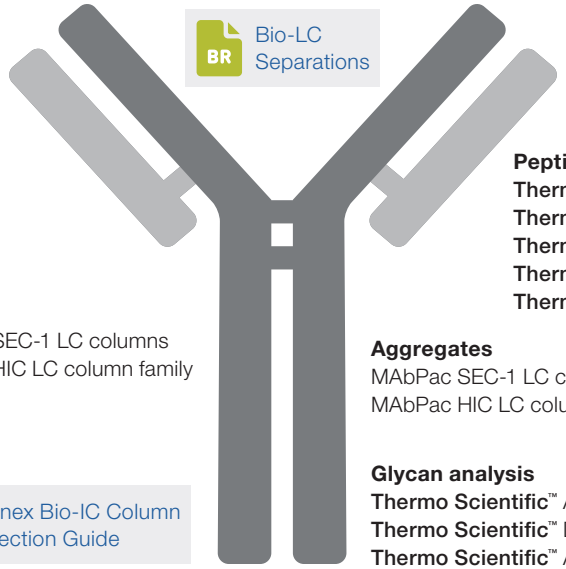
**Thermo Scientific**™ EASY-Spray™ PepMap™ RSLC columns
**Thermo Scientific**™ ProPac™ SAX-10 HPLC columns
**Thermo Scientific**™ SMART Digest™ kits
**Thermo Scientific**™ MAbPac™ RP columns
**Thermo Scientific**™ Hypersil GOLD™ C4 LC columns

BR EASY-Spray Columns



## Protein separation

BR Bio-LC Separations



**Charge variant**
**Thermo Scientific**™ CX-1 pH Gradient Buffers
**Thermo Scientific**™ MAbPac™ SCX-10 LC columns
**Thermo Scientific**™ ProPac™ Elite WCX LC columns

**Intact mass**
MAbPac RP LC columns
**Thermo Scientific**™ MAbPac™ SEC-1 LC columns
**Thermo Scientific**™ MAbPac™ HIC LC column family
MAbPac SCX-10 LC columns
ProPac Elite WCX LC columns

ST Dionex Bio-IC Column Selection Guide

**Antibody – drug conjugates**
MAbPac HIC LC column family
MAbPac RP LC columns

**Peptide mapping**
**Thermo Scientific**™ SMART™ Digest Kits
**Thermo Scientific**™ SOLAμ™ Plates and Cartridges
**Thermo Scientific**™ Accucore™ Vanquish™ C18+ LC columns
**Thermo Scientific**™ Hypersil GOLD™ C18 LC columns
**Thermo Scientific**™ Acclaim™ VANQUISH™ C18 columns

**Aggregates**
MAbPac SEC-1 LC columns
MAbPac HIC LC column family

**Glycan analysis**
**Thermo Scientific**™ Accucore™ 150-Amide-HILIC LC columns
**Thermo Scientific**™ Dionex™ BioIC columns
**Thermo Scientific**™ Acclaim™ 120 C18 columns

> The CX-1 pH Buffer Kits are really popular with the team in the lab because they are easy to prepare. In a few minutes you are ready to go. Plus we have full traceability from the certified kits.
> **Dr. Martin De Cecco, Biochemistry R&D, Sartorius Stedim BioOutsource**

# Process development

During process development, an in-depth understanding of process effects on biomolecules allows for quicker progression through the development pipeline.

**Requires**
robust and reproducible analyses to monitor molecular attribute changes with speed and confidence.

# Quality control and monitoring

Small changes in manufacturing conditions can have a significant impact on the quality and consistency of a biotherapeutic. To ensure the release of safe and effective products, advanced analytical methods are implemented to closely monitor critical quality attributes for each batch.

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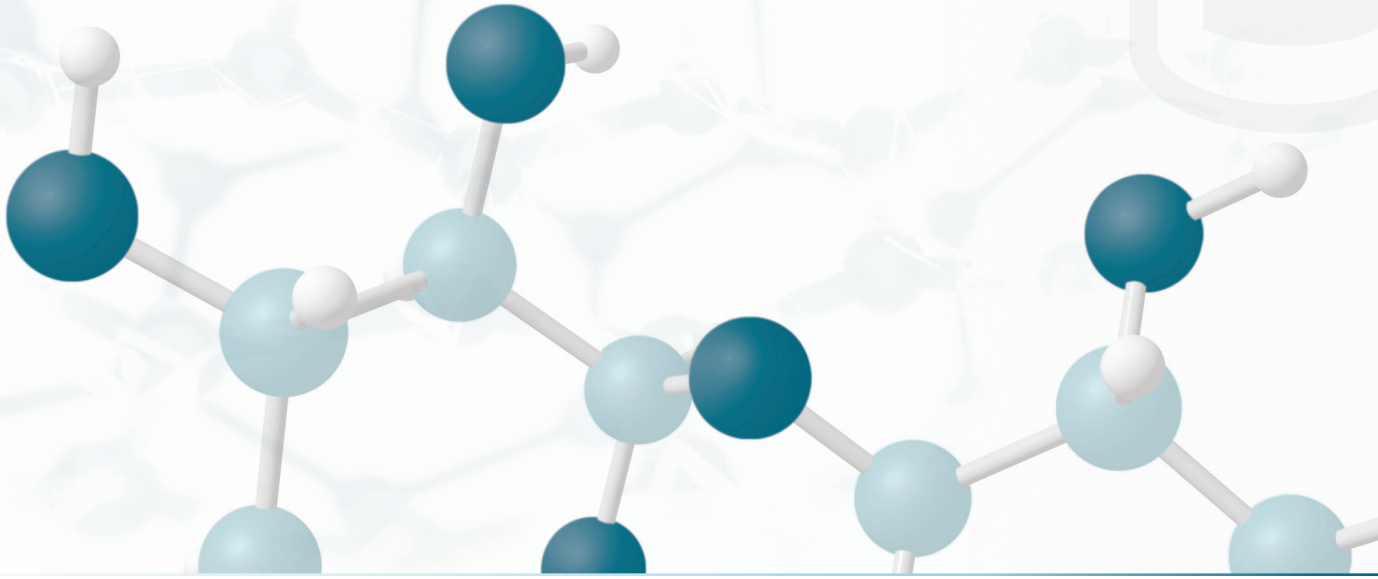
reproducible, robust, GMP compliance-ready methods.



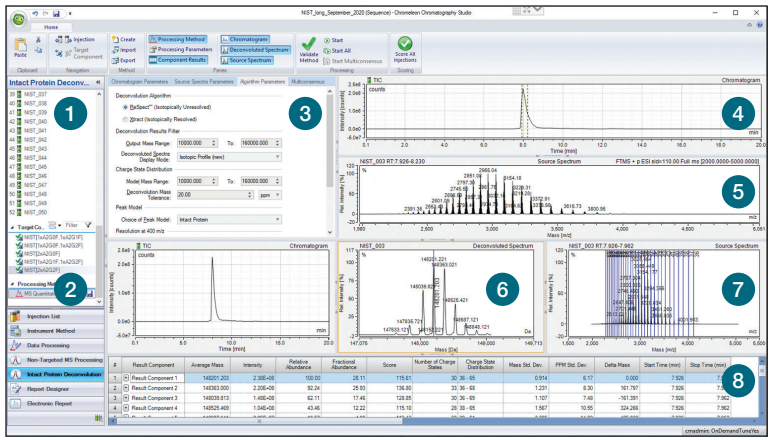
## Dependable technologies for monitoring and control

- Simplified sample preparation and automation
- Multi attribute method capabilities proven in GMP
- Compliance-ready software

# GMP proven, compliance-ready enterprise chromatography and mass spectrometry software

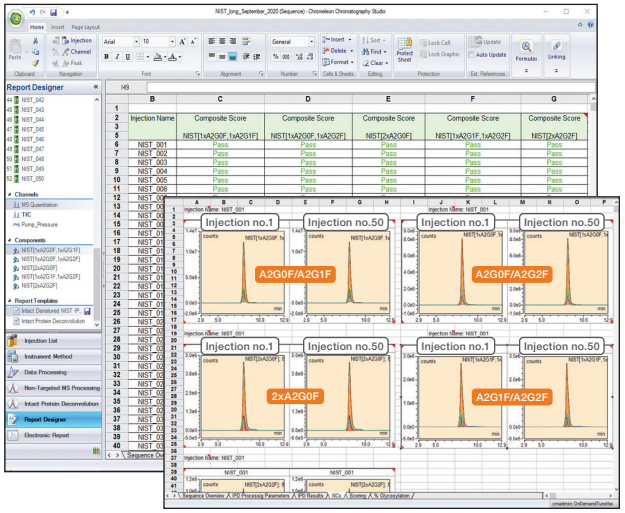
**Streamlined chromatographic and MS workflows with a compliance-ready single software platform – Thermo Scientific™ Chromeleon™ Chromatography Data System**

Chromeleon CDS delivers comprehensive compliance tools, full networking capabilities, instrument and data acquisition control, automation, compliance-ready data processing and easy reporting, making the software an ideal platform for routine monitoring at any phase of drug development. Compliant instrument control is afforded for HRAM-MS or MS/MS, as well as front end chromatography systems, including many third-party LC systems.




## Customizable Chromeleon Studio

1. Sequence
2. Target components
3. Deconvolution parameters
4. Chromatogram view
5. Full MS source spectrum
6. Deconvoluted spectrum
7. Source spectrum with indication of a specific charge state series
8. Result component table




**An example report for intact protein deconvolution displaying sequence analysis results, generated by Chromeleon CDS. The panel in the back shows the injection list with the five target components with pass/fail results for each injection. The panel in the front represents XICs for the target components for each injection.**

> Chromeleon CDS is a very efficient platform for acquiring, processing and evaluating data in a single, seamless platform.
> **Trine Meiborg Sloth,**
> **Senior Technician, Symphogen**

AN Monoclonal antibody mass confirmation using intact protein deconvolution for a compliance-ready manufacturing environment

# Simple and automated sample preparation tools

## For manufacturing control

### Fast, simple, reproducible protein digestion in an hour or less

SMART Digest kits use optimized heat-stable enzymes on magnetic or non-magnetic beads. This innovation generates high confidence in your LC and LC/MS results. Digestion procedures can be performed manually or automated with the Thermo Scientific™ KingFisher™ Duo Prime purification system.


SMART Digest protein digestion kits

AN Investigating process-related post-translational modifications

### Easy and simple

- Significantly increased speed of sample processing
- Simple process
- Easily automatable protocol for high-throughput processing

### Reliable results

- Higher sensitivity
- Increased reproducibility over existing protocols
- Higher data confidence
- Low levels of sample preparation-induced post-translational modifications

### Your samples, fast, consistent, and simple

KingFisher Duo Prime purification system improves your digestion by removing manual steps and reducing overall processing time, reducing user processing errors while increasing the reproducibility of your results, and uses optimized, easy-to-follow protocols for nearly every downstream application or sample type.


KingFisher Duo Prime System

AN High-precision, automated peptide mapping of proteins

> Our bottleneck lies in the sample preparation where we have taken a process that takes days down to minutes. With the easy to use, simple kit the data quality is absolutely excellent with the SMART Digest kit.
> **Charles Olea Ph.D, Analytical Development, Ajinomoto Althea**

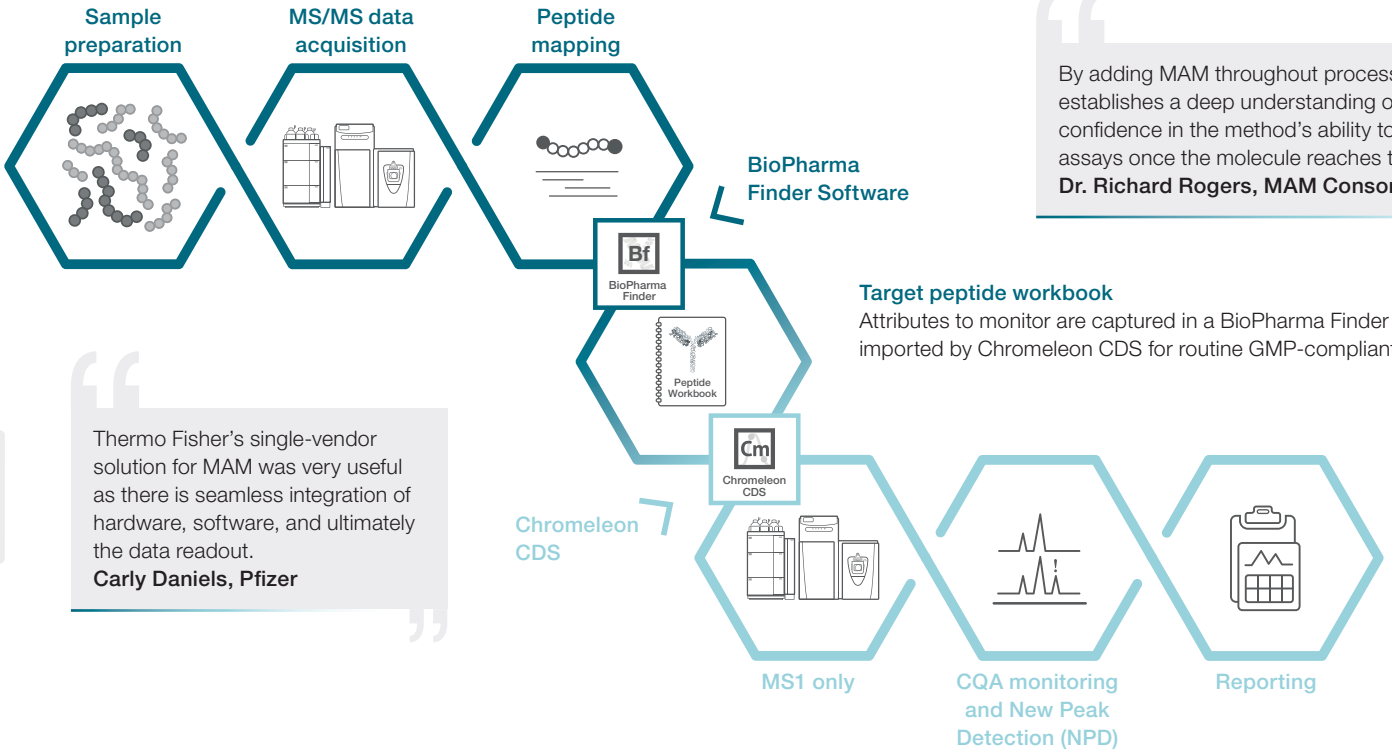
# High resolution Multi-Attribute Method

## Because resolution matters from research to routine

A primary goal of the biopharmaceutical industry is to deliver the highest quality product faster and at the lowest cost possible. HR MAM workflow can replace numerous conventional lot release assays, offering potential for significant cost and time-to-market savings.

Benefits of the HR MAM full workflow solution include:

- Verified single-vendor solution containing all necessary components of a MAM workflow
- Standardized solution allows for easy lab-to-lab method transfer, for consistency from characterization through manufacturing.
- Standardization offers peace of mind when preparing regulatory filing.




**Attribute characterization**
Peptide mapping with HRAM LC-MS/MS data is processed in Thermo Scientific BioPharma Finder software.

**BioPharma Finder Software**

**Target peptide workbook**
Attributes to monitor are captured in a BioPharma Finder workbook, which is imported by Chromeleon CDS for routine GMP-compliant monitoring.

**Chromeleon CDS**

**Attribute monitoring and new peak detection**
Targeted quantitation of the CQAs using high resolution MS1 only measurements, along with detection of new peaks when compared to a reference standard.

> By adding MAM throughout process development, one establishes a deep understanding of the method, leading to higher confidence in the method's ability to replace traditional release assays once the molecule reaches the quality control laboratory.
> **Dr. Richard Rogers, MAM Consortium**

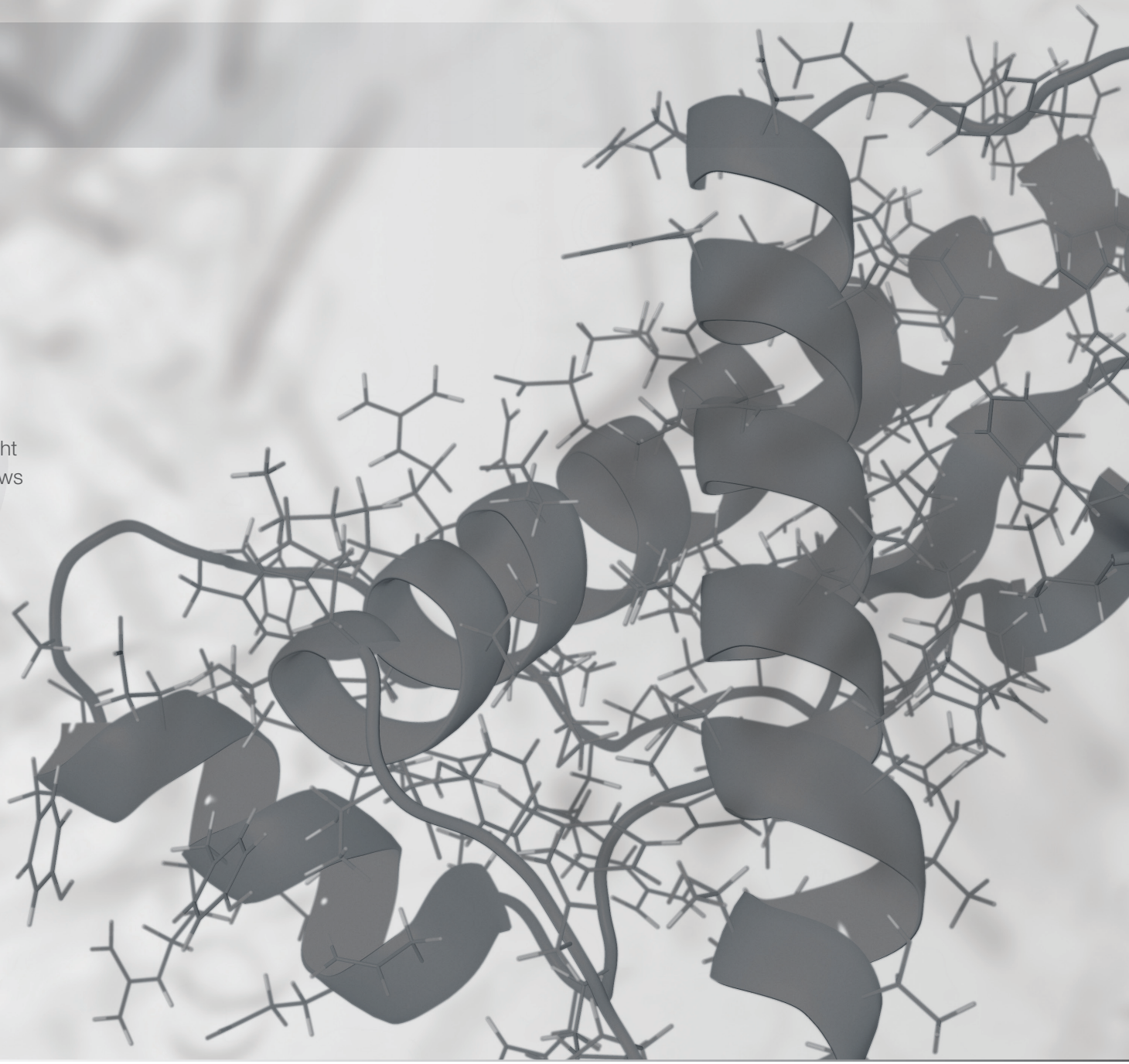
> Thermo Fisher's single-vendor solution for MAM was very useful as there is seamless integration of hardware, software, and ultimately the data readout.
> **Carly Daniels, Pfizer**

HR Multi-Attribute Method

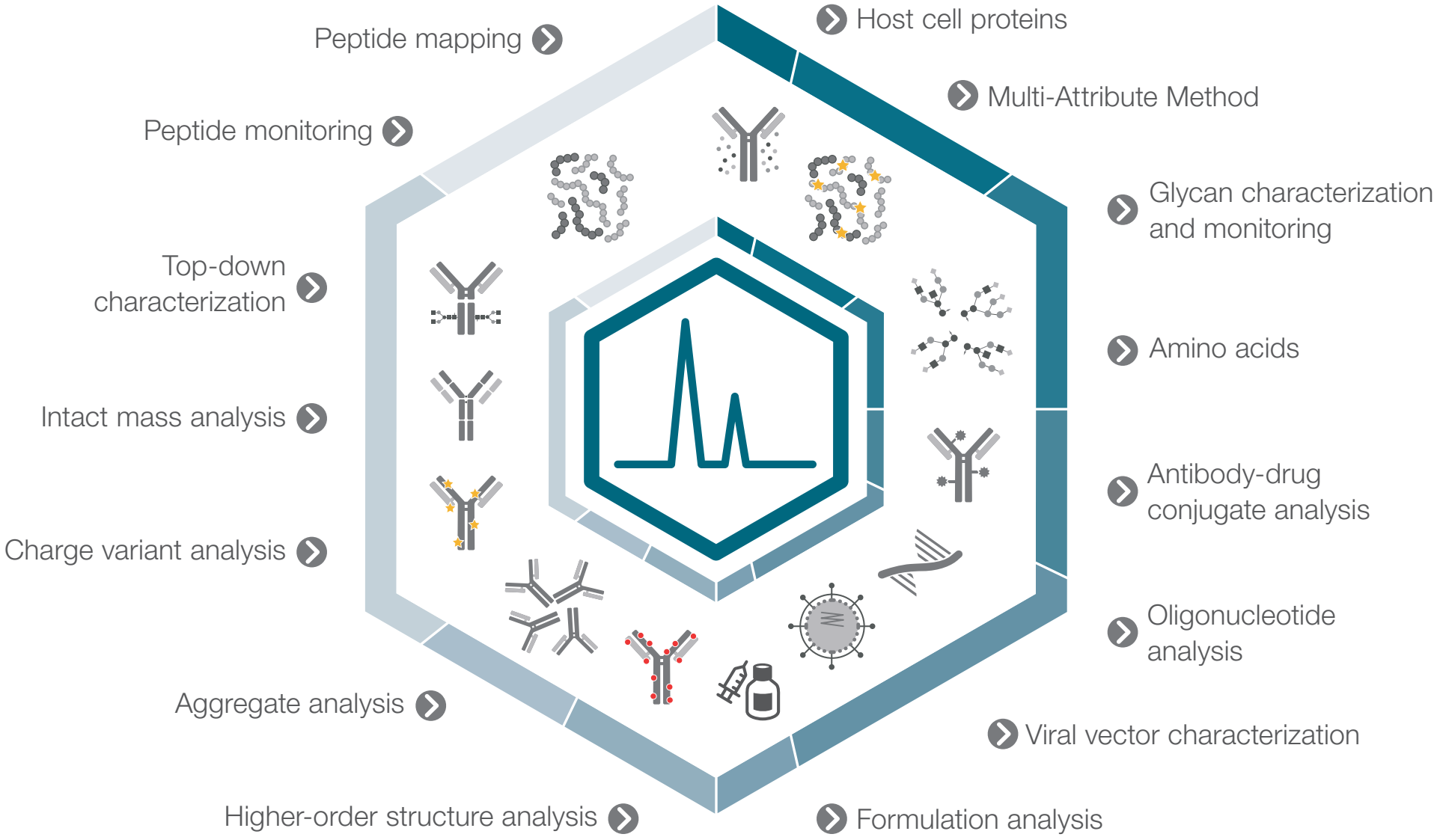
# Analytical workflows

Powerful, efficient and accurate tools are brought together to provide seamless analytical workflows purpose built to address the challenges of biotherapeutic drug development.

- Unrivalled technologies
- Rapid, high throughput capabilities
- Robust and reproducible methods
- Unique structural insights
- Data confidence
- Industry endorsed



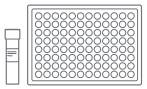
# Analytical workflows



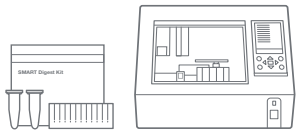
# Fast, reproducible and accurate peptide mapping

Peptide mapping is a critical workflow for product characterization and is essential for elucidating the primary amino acid structure of proteins.

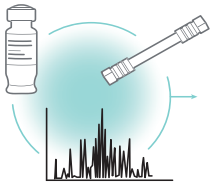
## Peptide mapping workflow for product characterization



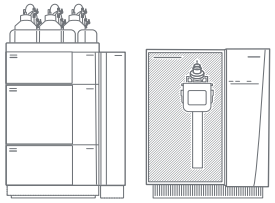
**Sample handling**
Thermo Scientific™ WebSeal Plate + glass coated well plates Thermo Scientific™ National 9 mm Total Recovery Vials



**Protein digestion**
KingFisher Duo Prime purification system and SMART Digest Kit



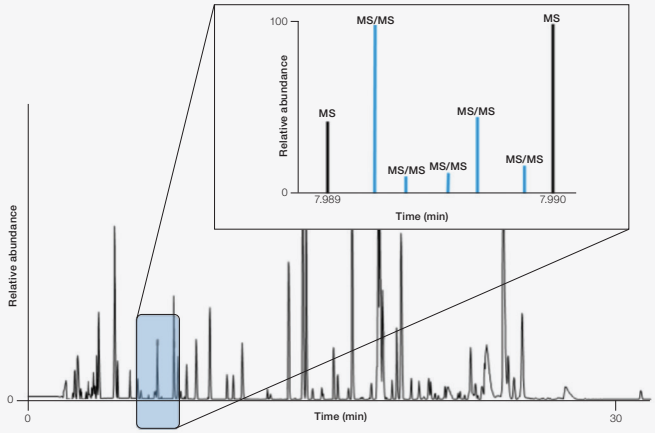
**Peptide separation**
Acclaim VANQUISH C18 column



**Resolution and confidence**
Vanquish UHPLC systems and Orbitrap-based mass spectrometers

## End-to-end solutions for complete product characterization

- Sequence coverage
- PTMs (identification and quantitation)
- Disulfide bonds
- Sequence variants
- *De novo* sequencing




### Characterize all peptides and modifications with a wide dynamic range

Class leading accurate mass and resolving power provide the most effective way to accurately and confidently identify peptides. Working together with the Acclaim VANQUISH C18 column and Vanquish UHPLC system, our Orbitrap-based mass spectrometers give you the confidence to perform peptide mapping where full characterization is required and complete sequence information needed.

**Sequence coverage**
Confident and complete sequence coverage with versatile BioPharma Finder software

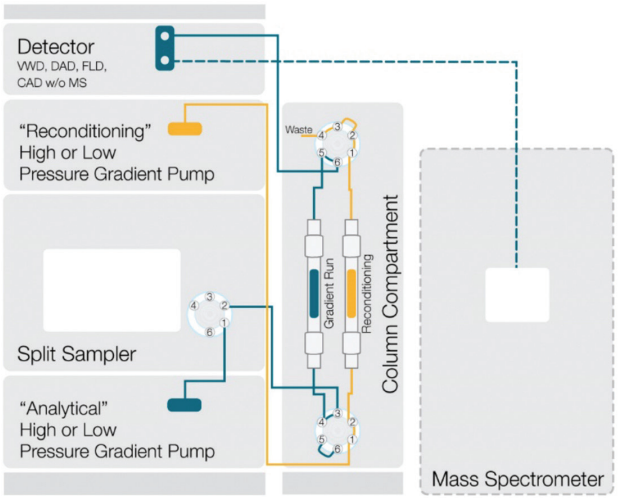

**BioPharma Finder software is purpose-built for biotherapeutic analyses, with efficient workflows that ensure you get the right answers first time. Novel data visualization tools, from chromatographic shading and in-depth coverage maps mean that you won´t miss a thing.**

# High throughput peptide mapping with Vanquish Duo UHPLC system

We understand product monitoring requires the analysis of many samples and results are needed as soon as possible. Our Vanquish UHPLC systems coupled to a wide variety of fit-for-purpose Thermo Scientific columns can help to achieve the productivity and resolution needed.

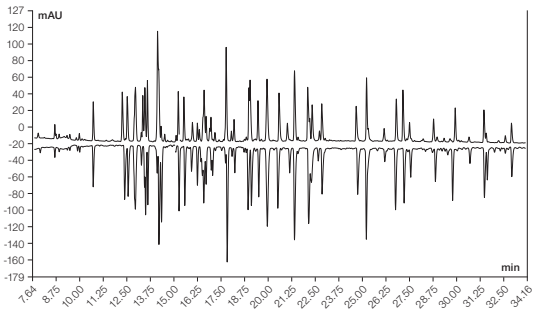
## Increase productivity with a dual UHPLC system

### Vanquish Duo UHPLC system




AN High-throughput peptide mapping of trastuzumab using a tandem LC-MS workflow

### Tandem UHPLC for high-throughput peptide mapping

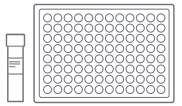



BSA digest analyzed using diode-array detection with first lot of Hypersil GOLD VANQUISH C18 UHPLC column.

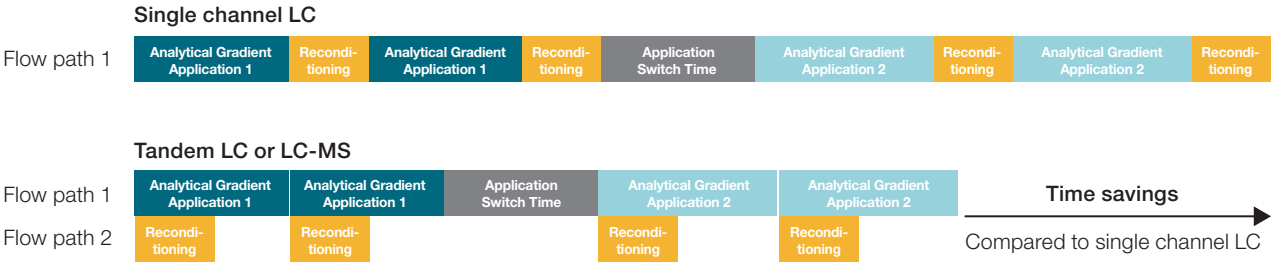
BSA digest analyzed using variable wavelength detection with second lot of Hypersil GOLD VANQUISH C18 UHPLC column.

**The figure shows well-aligned chromatograms produced using the Vanquish Duo UHPLC system, run in tandem mode with 2 different lots of the Hypersil Gold VANQUISH column.**


**Hypersil GOLD VANQUISH C18 UHPLC columns**



**Thermo Scientific™ barcoded WebSeal™ Well Plates**

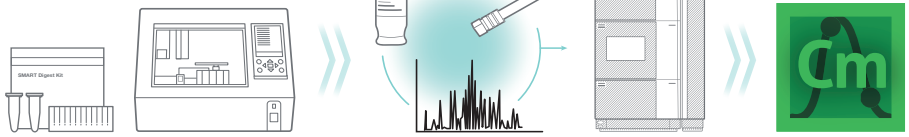



**Real productivity savings can be achieved by converting single channel LC methods to tandem, enabling full utilization of detector time.**

> Since installing the Tandem LC-MS system we have doubled our throughput and increased the precision of our data!
> **Dr. Richard Rogers, Just Biotherapeutics, Inc.**

# Confident peptide monitoring

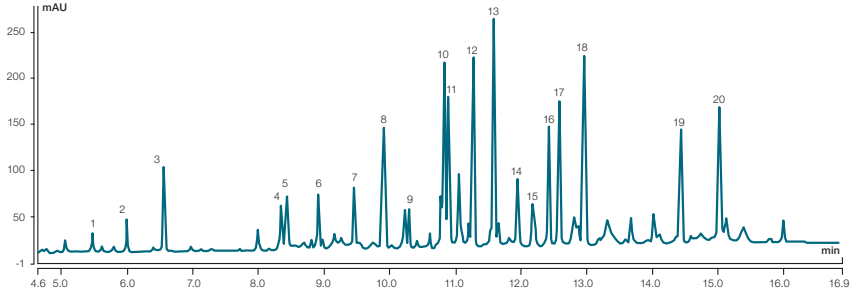
Peptide monitoring can confirm the primary structure of a product compared to a reference standard on a lot-to-lot basis for determining identity, stability, lot release, and monitoring manufacturing changes. Sample preparation and non-reproducible chromatography are common issues for reproducibility in peptide monitoring. Using an end-to-end workflow for automated peptide monitoring, your lab can achieve the robustness and reproducibility needed every time.



**Protein digestion**
KingFisher Duo Prime purification system and SMART Digest Kit

**Peptide separation**
Acclaim VANQUISH C18 column
Vanquish UHPLC system

**Data acquisition and processing**
Chromeleon CDS
- Compliance-ready
- Intelligent run control
- Enterprise capabilities, single software platform



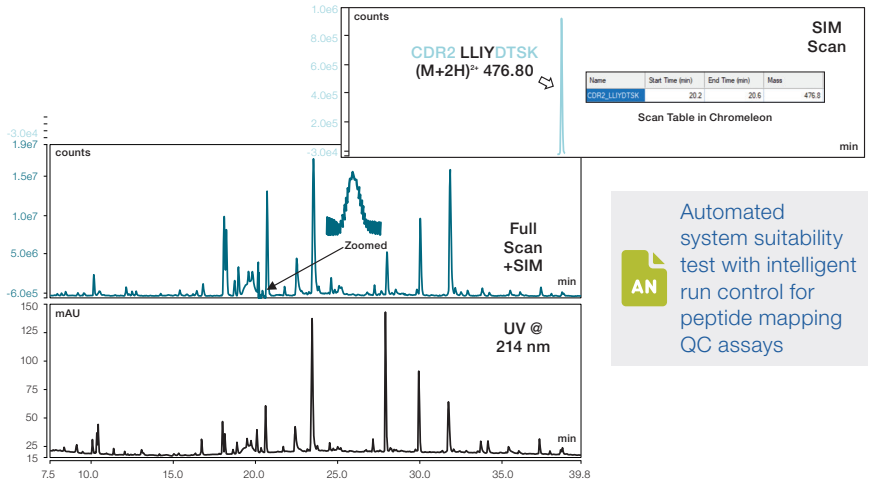

**An overlay of UV-chromatograms from 5 separate protein digests with**
- **exceptionally low RSD (average RSD 2.82%)**
- **excellent digestion and separation reproducibility**

## Thermo Scientific ISQ EM Single Quadrupole MS

### Flexible and routine detection for additional confidence

- Mass range *m/z* 10–2,000 with unit mass resolution
- Mass accuracy ≤0.1 Da

The Thermo Scientific™ ISQ™ EM single quadrupole mass detector can be coupled to UV to aid in method optimization and add confidence in peak identification within the compliance-ready environment of Chromeleon CDS. Peptide confirmation is demonstrated to the right.




Automated system suitability test with intelligent run control for peptide mapping QC assays

# Top-down structural characterization of complex biologics under native conditions

Complex biologics demand in-depth characterization employing multiple orthogonal analytical workflows to confidently determine protein structure ideally in its unmodified therapeutic form.

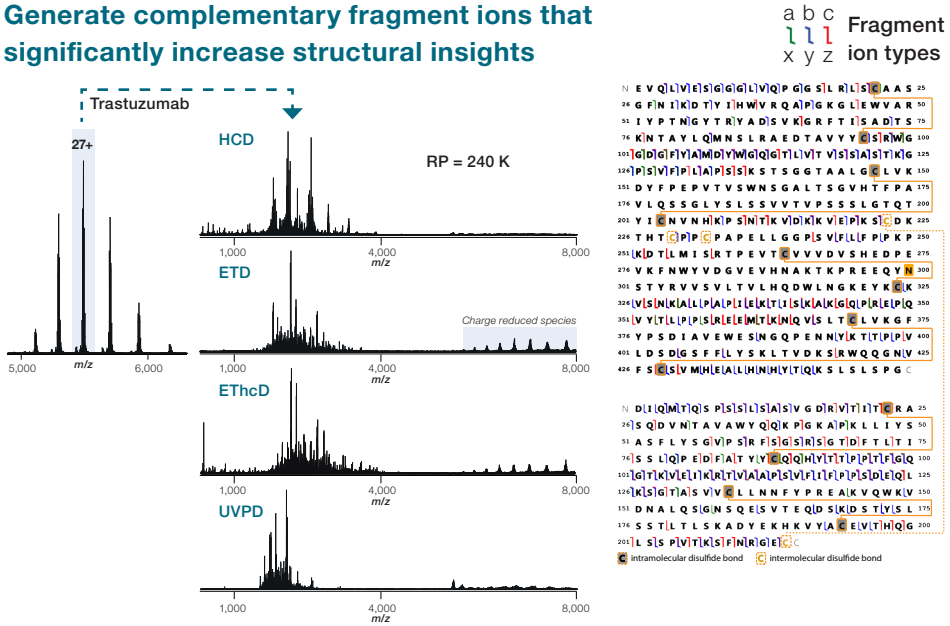
Characterization of proteins in their native state can now be achieved on the Orbitrap Eclipse Tribrid mass spectrometer with its enhanced vacuum technology and high mass range accessible in the HMR$^n$ mode. The instrument provides the ability to analyze native molecules at the MS/MS level for detailed structural insights without sample preparation.

## Deeper structural understanding, earlier in drug development, to make confident decisions faster.

Specific benefits of top-down analysis approaches:

- Fewer sample preparation steps
- Avoids preparation-induced chemical modification thereby reducing the number of experimental artefacts.
- Detect modifications that are removed or scrambled during peptide sample preparation.
- Elucidate functional relationships (for example, cross-talk) between PTMs on the same protein molecule.
- Characterize drug–target interactions
- Identify and quantify proteoforms that would have been convoluted by endoproteinase digestion.
- Achieve more in-depth structural heterogeneity characterization during biopharmaceutical development and control.

## Generate complementary fragment ions that significantly increase structural insights




**Top-down analysis of intact trastuzumab (148 kDa) supplied by direct infusion. A single charge state was isolated and fragmented using ETD, EThcD, UVPD and HCD. Fragment ion spectra were acquired with resolution of 240,000 @ *m/z* 200.**

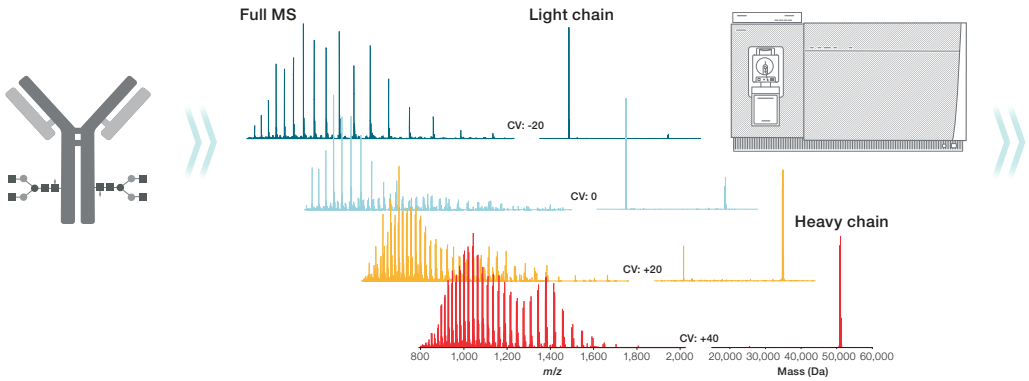
**The advantage of this approach is that the IgG molecule can be efficiently probed in its native state, with covalent (S-S) and non-covalent bonds still intact. The resulting sequence coverage for intact IgG was 43% with 3.7 ppm RMS.**

# Structural insights in minutes with FAIMS and top-down

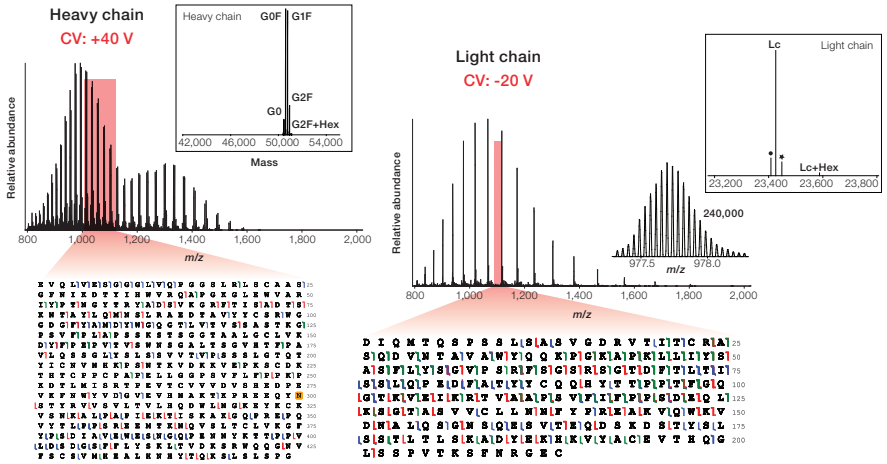
## Incorporation of the FAIMS Pro interface for enhanced top-down characterization

The addition of the FAIMS Pro interface to the Orbitrap Eclipse Tribrid mass spectrometer can enhance gas-phase selectivity of complex protein mixtures, enabling high-quality intact mass analysis, as well as top-down or middle-down sequencing using the multitude of dissociation options in conjunction with PTCR for unmatched characterization capabilities.

## Rapid characterization by gas-phase separation of mAb light and heavy chains




**Rapid characterization of monoclonal antibody chains: FAIMS Pro interface denaturing top-down MS on IgG 1 mAb by changing the compensation voltage and recording the subsequent full scan MS.**




**Trastuzumab middle-down sequencing using multiple fragmentation modes.**

Light and heavy chains can be analyzed simultaneously or separated in the gas phase and introduced sequentially into the MS. Top-down or middle-down sequencing can then be performed utilizing multiple fragmentation modes including ETD, EThcD, HCD and UVPD. The figure shows rapid characterization of monoclonal antibody chains, with resulting data processing showing 65% sequence coverage for the light chain and 45% for the heavy chain which was achieved in less than five minutes. In addition, the full scan mass spectra acquired at each CV setting were deconvoluted prior to top-down analysis resulting in the molecular weight determination for the various glycoforms.

Fast high quality intact mass and sequence information with automated sample introduction, in just 5 minutes

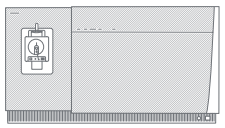
# Intact mass analysis

## Exceptional spectral clarity, from discovery to QC

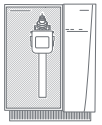
**Extended mass range** on Orbitrap-based instruments with BioPharma option and operational modes for mass determinations up to *m/z* 8,000, opening the door for **simple, robust, and high spectral quality intact mass analysis** of large proteins like mAbs under denaturing and native-like conditions.

> Orbitrap capabilities have been expanding enormously over these years in multiple dimensions: from detecting elements and isotope ratio analysis to protein complexes and intact viruses; from disease pathway elucidation to biopharmaceutical quality control.
> **Alexander Makarov, the inventor of the Orbitrap mass analyzer**

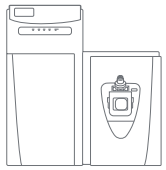
Unparalleled **sensitivity, resolution, and accuracy, effortlessly** at any stage of the drug development.



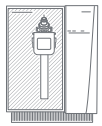
**Orbitrap Eclipse Tribrid MS with HMR$^n$ Option**
- **Up to *m/z* 8,000 with HMR$^n$ option**
- **Resolution up to 1 M at *m/z* 200**
- **Proton Transfer Charge Reduction (PTCR), (ETD)/PTCR ion source**



**Orbitrap Exploris 480 MS**
- Up to ***m/z* 8,000 with BioPharma option**
- **Resolution up to 480,000 at *m/z* 200**



**Q Exactive UHMR Hybrid Quadrupole-Orbitrap MS**
- **Up to *m/z* 80,000**
- Resolution 200,000 at *m/z* 400
- **Quadrupole isolation up to *m/z* 25,000**



**Orbitrap Exploris 240 MS**
- Up to *m/z* 8,000 with BioPharma option
- Resolution up to 240,000 at *m/z* 200

**Q Exactive Plus MS**
- Up to ***m/z* 8,000 with BioPharma option**
- **BioPharma workhorse**
- Dedicated protein analytical workflow modes

Orbitrap LC-MS

SA Fundamentals and Advances of Orbitrap Mass Spectrometry

# Intact mass analysis

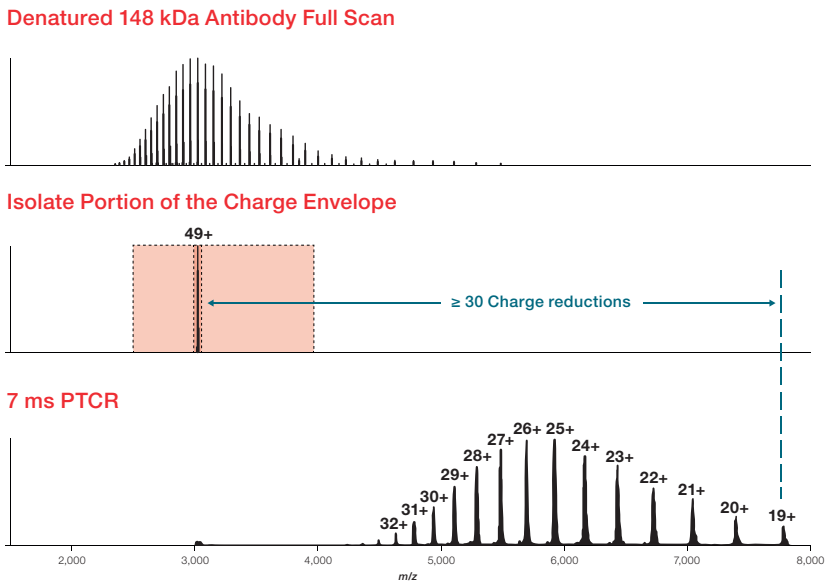
## Delivering structural insights for complex proteins

### Simplify complex spectra by decreasing the charge state of ions with PTCR

As the biopharmaceutical industry works toward a deeper product understanding, the need for innovative approaches to facilitate deeper protein characterization grows. The pioneering technology and software developments incorporated into the Orbitrap Eclipse Tribrid mass spectrometer enable superior analytical performance across all biopharmaceutical characterization workflows. Take advantage of high mass range $MS^n$ ($HMR^n$) on a Thermo Scientific Tribrid Orbitrap mass spectrometer.

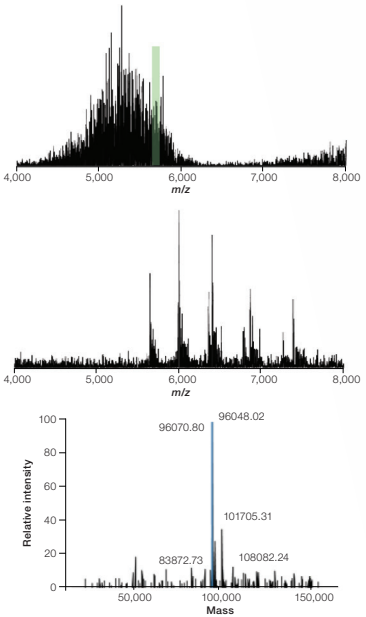
WP Go beyond in biopharmaceutical characterization

### PTCR of denatured monoclonal antibody to reduce spectral complexity




PTCR of an isolated portion of the denatured mAb charge envelope (49+) reveals a charge-reduced spectra, facilitating more accurate data interpretation.

### Analysis of cytokine-Fc fusion protein under native conditions with PTCR MS




MS spectrum of native desialyated Cytokine-Fc

PTCR $MS^2$: PTCR of 80 amu window *m/z* 5,677-5,757

Deconvolution result

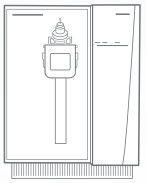
Proton Transfer Charge Reduction (PTCR) makes it possible to deconvolve the mass spectrum obtained from a desialylated Cytokine-Fc fusion protein acquired under native conditions. The charge reduced product ions are detected in the Orbitrap mass analyzer taking advantage of the extended mass range.

# Intact mass analysis

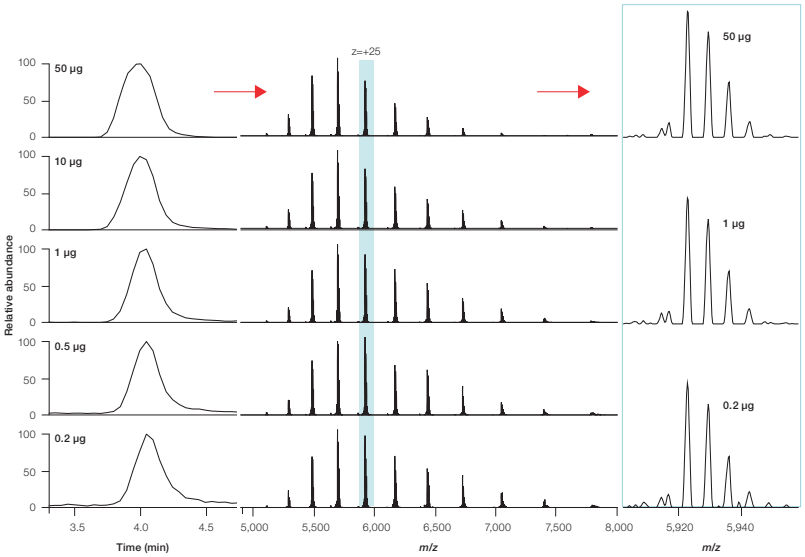
## Exceptional data from low sample concentrations

### Instruments made for intact mass analysis

- Fast, sample prep-free, robust method to determine molecular weight, post-translational modifications, and impurities of biotherapeutics
- Intact Protein Mode available on several Orbitrap-based mass spectrometers for mass detection up to *m/z* 8,000, supports intact mass analysis performed under both denaturing and native conditions
- Assign more spectral features and maximize your productivity through improved flexibility, speed, dependability, and operational simplicity



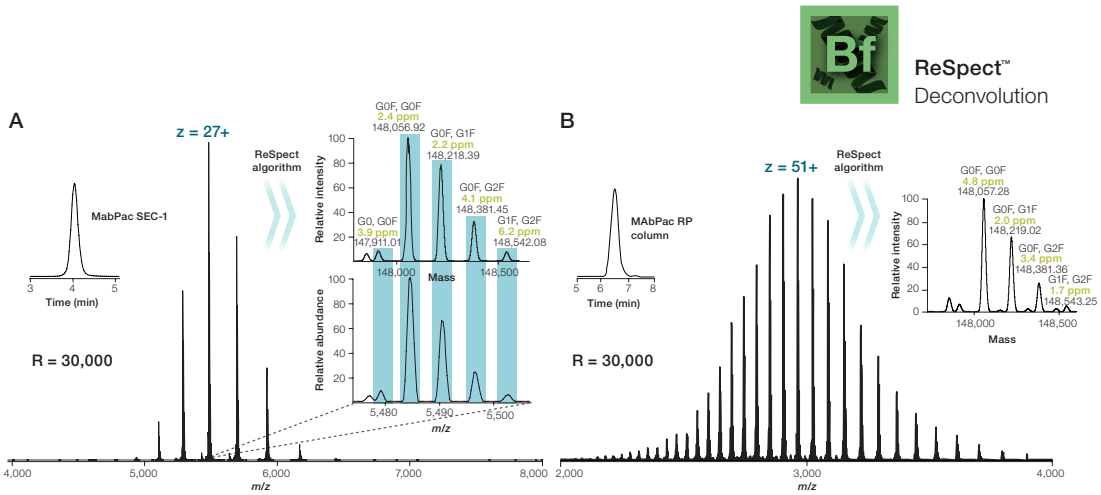
### Use less sample for reliable, high-sensitivity intact mass analysis acquired under native conditions




Size exclusion chromatography (SEC)-MS analysis of trastuzumab from 50 to 0.2 µg on-column using the BioPharma option to access the extended mass range to *m/z* 8,000 (left). Compared to denaturing conditions, intact native analysis, reduces the complexity of mass spectra (center). Consistent glycoform distributions are obtained (right), even at low sample loadings.

### Intact mass analysis under native and denaturing conditions

Native MS conditions allow molecules to retain their conformation in the gas phase resulting in ions in lower and fewer charge states. The detected mass signals appear at higher *m/z* and at higher intensity reducing the complexity of the overall protein spectra.




Denaturing solvent conditions result in protein unfolding and increased protonation. The use of salt-free, high organic solvents results in the detection of higher charge state peaks at lower *m/z* values.

Complete characterization of monoclonal antibodies under native and denaturing conditions

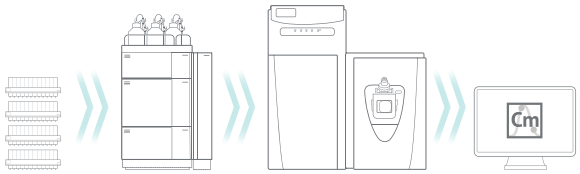
Intact Protein Analysis Workflows

# Intact mass analysis

## High throughput lead selection

In early discovery phases analytical methods need to support lead selection studies which require fast high confidence identification of the potential lead molecules. Combining information on aggregates and molecular mass with glycoform identifications in a single, rapid analysis provides several benefits:

- High quality data with rich information content
- Combination of 2 key analytical methods: Size Exclusion Chromatography (SEC) and MS
- No sample preparation required
- Very fast, robust and reproducible.



4 x 96-well plates

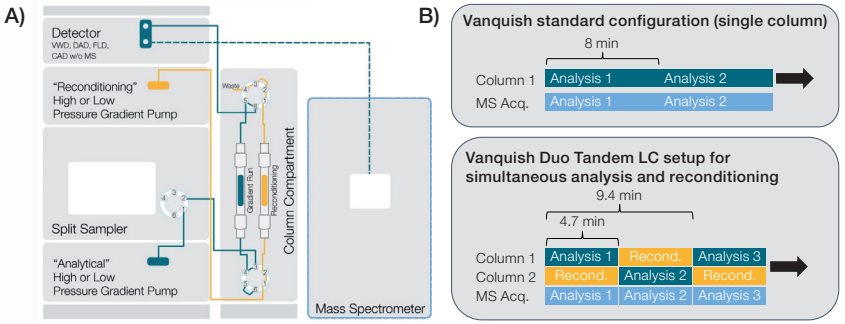
Vanquish Duo UHPLC System for Tandem LC or LC-MS

Orbitrap mass spectrometer with BioPharma Option

Instrument control and data processing in Chromeleon CDS

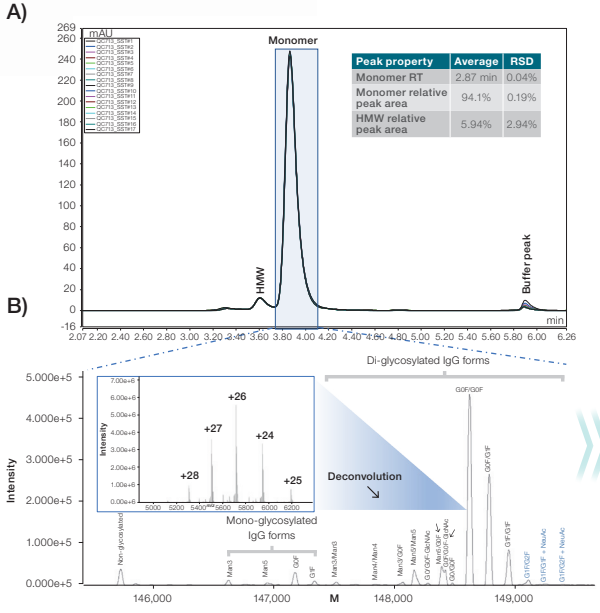
AN Increasing throughput of native SEC-MS in biopharmaceutical development using a tandem UHPLC setup

### Vanquish Duo LC analysis time

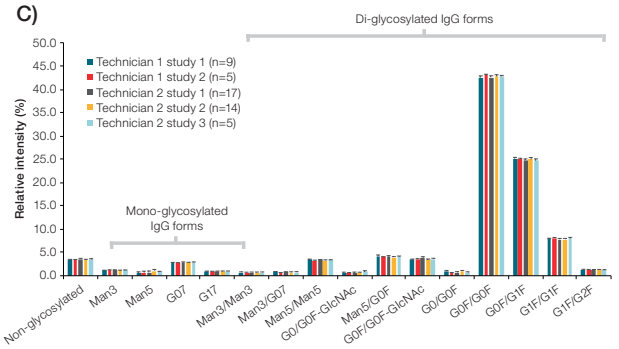



**A) In the tandem LC-MS configuration two columns are operated at the same time, with alternating column reconditioning.**

**B) In a standard single column configuration analysis time is 8 min per sample. In the tandem LC configuration analysis time is 4.7 min per sample.**




With the use of innovative UHPLC systems like the Vanquish Duo for tandem LC workflows, significant productivity can be gained, allowing for most efficient utilization of the high resolution accurate mass (HRAM) MS system.



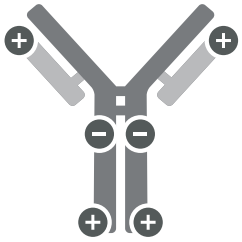

**Excellent system reproducibility and confident high resolution mass data**

**A) Overlay of several UV traces of Symphogen reference antibody shows excellent reproducibility.**
**B) Deconvolution of the reference antibody allows for confident assignment of glycoforms.**
**C) Average relative glycoform intensities of reference mAb from five independent studies showcase exceptional SEC-MS method reproducibility to allow reliable relative quantitation.**

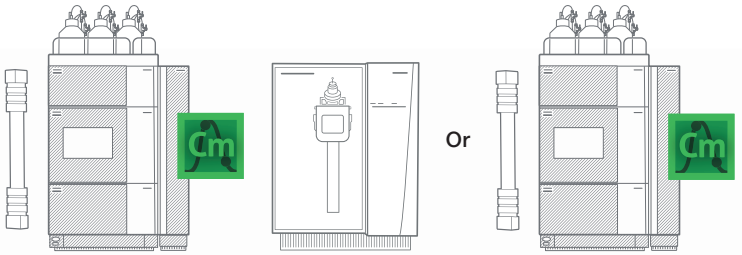
# Charge variant analysis

## Charge variant analysis (CVA)

Heterogeneity of monoclonal antibodies must be monitored and revealed by charge sensitive techniques. Separation by charge followed by mass spectrometry (CVA-MS) allows for characterization of PTMs at the intact protein level.



Protein variants




UHPLC-Orbitrap MS UHPLC

## CVA-MS for characterization of charge heterogeneity

Analyze multiple protein forms simultaneously, without the need for sample preparation, saving resource and time. Utilizing advanced software tools such as the sliding window algorithm available with BioPharma Finder software, one can improve the detection of low abundance species, providing more accurate qualitative and quantitative information and ultimately ensuring confidence in drug product quality.

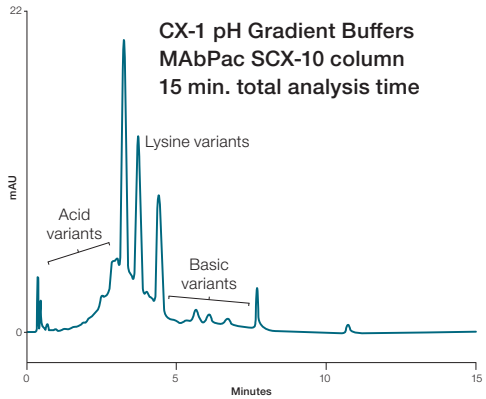
## Purpose-built tools for seamless charge variant monitoring

pH gradient ion-exchange chromatography provides rapid, highly robust and reproducible charge variant monitoring. Save time in method development, facilitate method transfer to QA/QC through a generic HPLC-based approach, and avoid the need for time-consuming mobile phase adjustments.

# Meeting your needs for charge variant profiling

## Charge variant analysis

Charge variants are monitored during drug development through to manufacturing because these variants can influence stability and biological activity of the product. Salt gradient cation-exchange chromatography can be used to monitor mAb charge variants. However, significant effort is often required to tailor the salt gradient method for each individual mAb. In the fast-paced drug development environment, a quick and robust platform method is desirable and can be achieved by using a pH gradient buffer.




### The advantages of pH-based gradients

- Global applicability to monoclonal antibodies (mAb)
- Greatly simplified method development
- Significantly shorter analysis times: pH gradients (15 min.) vs. conventional ionic strength salt gradients (90 min.)

MAbPac SCX-10 LC Columns

### High-resolution, high efficiency mAb analysis

- Resolves isoforms that differ by a single charged residue
- High efficiency separations through rapid mass transfer
- Designed to eliminate hydrophobic interactions, resulting in very efficient separation.

MAbPac SCX-10 LC Columns

Charge variant analysis information

### CX-1 pH gradient buffer

- Robust, reproducible gradients compared to traditional salt-based gradients
- Ready to use – No need to formulate mobile phases
- Saving time in method development and easily transferable to QA/QC

ProPac Elite WCX LC Column

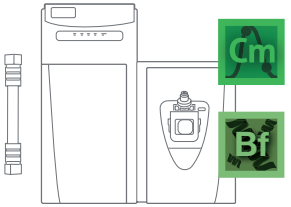
### Next generation cation exchange columns for method robustness and column lifetime

- Enhanced resolution separations for mAb variants
- Small particle sizes for faster separation and sharp peaks
- Ideal for quality control and assessment of mAbs

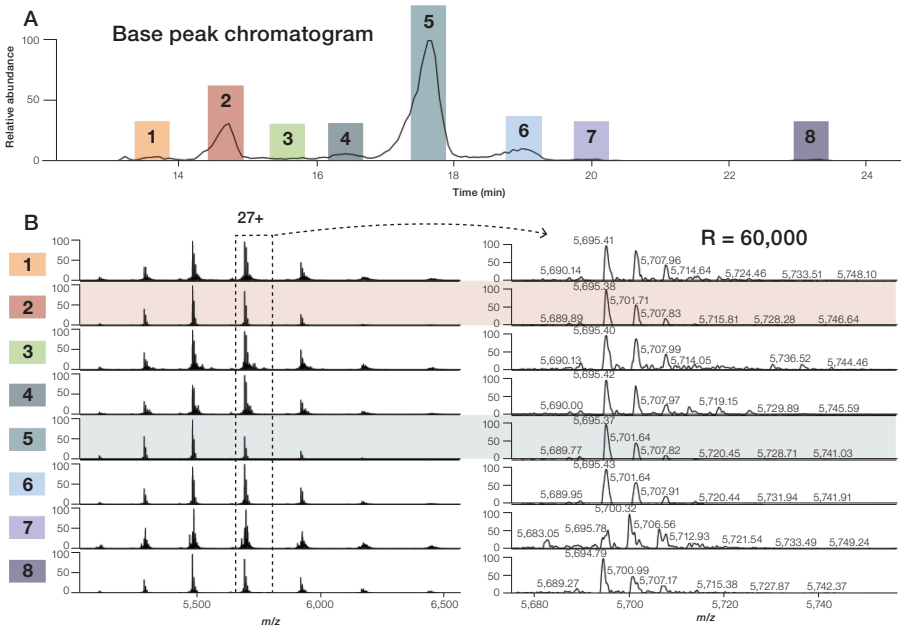
# Intact mass analysis under native conditions

## Charge variant analysis coupled to MS further expands intact protein characterization

For product characterization, understanding intact mAb heterogeneity is imperative. Combining the separation benefits of charge variant analysis (CVA) using ion-exchange chromatography (IEX) while obtaining mass spectral information is a powerful tool for analysis of complex mAb mixtures and for deeper insights when characterizing single mAb samples.




### Native MS using CVA-MS

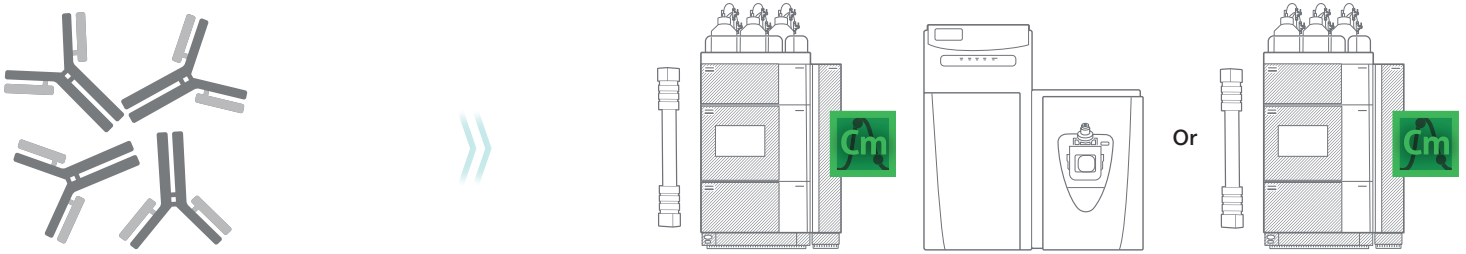



AN A global pH-gradient based charge variant analysis directly coupled to HRAM-MS (CVA-MS) for mAb analysis

**A) Base peak chromatogram from MS analysis of trastuzumab. 8 chromatographic peaks are separated that correspond to unique charge variants.**
**B) Averaged spectra corresponding to unique charge variants of trastuzumab. Spectra are highlighted by red and blue color shading which correspond to the two most abundant chromatographic peaks, peak 2 and peak 5.**
**C) Sliding Window ReSpect deconvolution in BioPharma Finder software displaying abundance traces of deconvolved components in peak 5, with top 3 glycoforms in both deamidated and unmodified forms.**

# Aggregate analysis

**Protein aggregation** gives rise to higher order structures which can significantly affect the efficacy of biologic drugs and cause harmful side effects and therefore needs to be monitored and controlled. Protein aggregates are commonly measured using liquid chromatography with either UV detection (HPLC-UV) or fluorescence detection (FLD) or followed by intact MS characterization under native conditions.




Proteins

UHPLC-Orbitrap MS

UHPLC

## SEC-MS for aggregate and intact protein analysis

Aggregate separation followed by HRAM MS facilitates the analysis of the proteins in their native form, without the need for sample preparation, while providing information on aggregation and fragmentation.

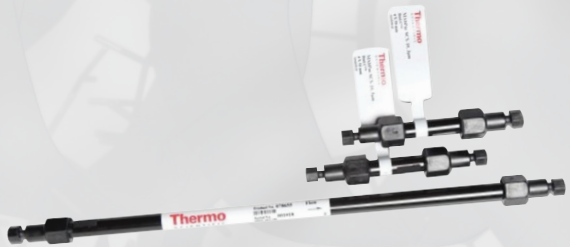
## Robust reproducible tools for aggregate monitoring

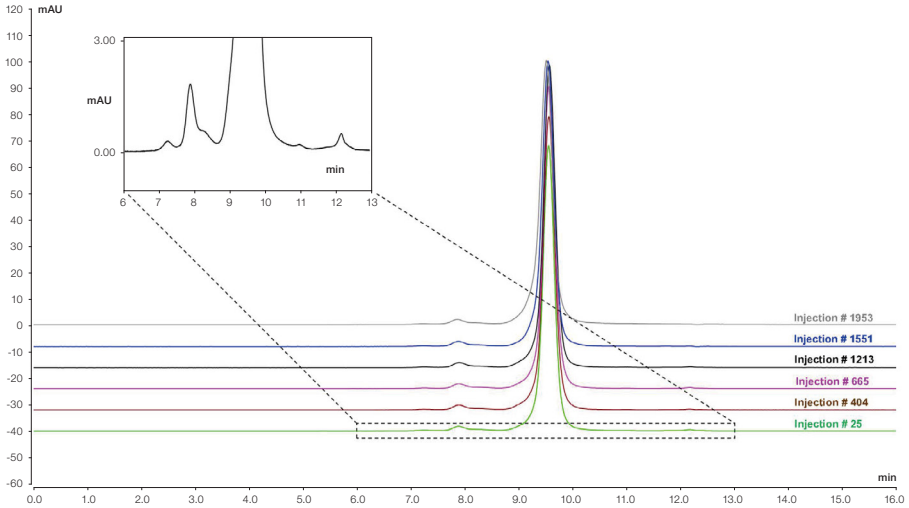
Ensure confident characterization of sub-visible protein aggregates in biotherapeutic products. Take advantage of MAbPac SEC-1 columns which offer superior, reproducible separation of monomers, aggregates, and fragments resulting from proteolysis. As a secondary confirmation, MAbPac HIC column family separates based on hydrophobicity in the native state, allowing for the detection of changes in protein structure and aggregates. Couple this to the biocompatible Vanquish Flex or Vanquish Horizon LC systems for the robust system to handle the high salts of HIC methods.

# Confident aggregate control

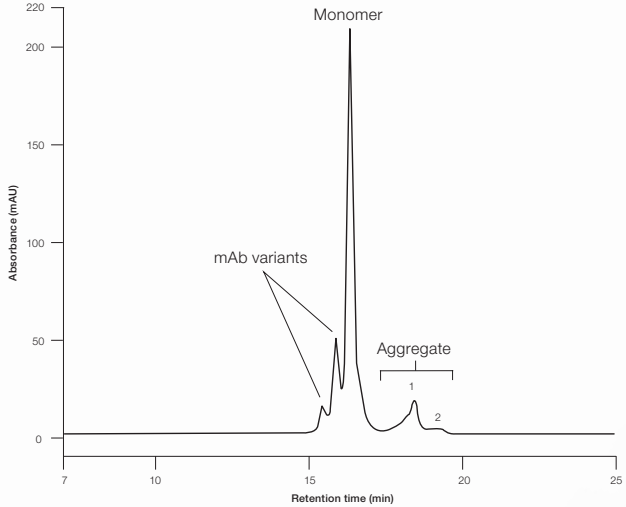
## Monitor aggregates confidently

Investigators routinely use **size-exclusion chromatography (SEC) and hydrophobic interaction chromatography (HIC),** because they are well-suited for separation of fragments and monomer peaks from aggregates. Robust, reproducible methods can be expected using the Vanquish UHPLC system coupled to the MAbPac SEC-1 column or MAbPac HIC-10 column.




Selection of representative UV-chromatograms showcasing excellent resolution and reproducibility of aggregates using the MAbPac SEC-1 column over 1,953 injections.




As an alternative to SEC, for separation of mAb aggregates and hydrophilic variants use the MAbPac HIC-10 column.

AN LC Protein Aggregate Analysis

# Use ultra-high mass range to see your biotherapeutic aggregates for the first time

Native MS is a powerful tool for understanding the structure of large protein complexes. Technology limitations have prevented native MS for achieving its full potential until now.

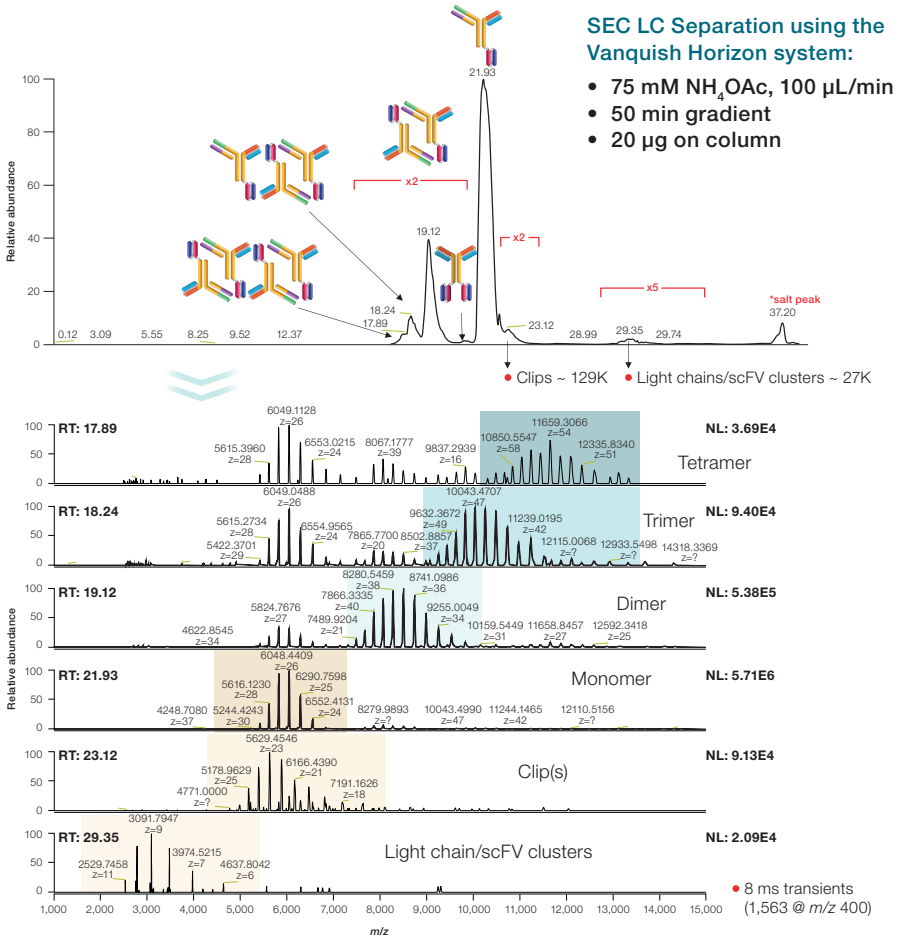
## The Q Exactive UHMR Hybrid Quadrupole Orbitrap MS

- Designed for scientists needing higher resolution and sensitivity in the ultra-high mass range and ability to perform highest quality native MS and native top-down experiments
- Unique combination of high resolution, high sensitivity and $MS^2$/pseudo-$MS^3$ capabilities makes the Q Exactive UHMR mass spectrometer ideal to further analyze complex macromolecules and aggregate forms of your biotherapeutic

### Instrument features

- HCD fragmentation
- In-source trapping
- Pseudo $MS^3$
- High mass range (*m/z* 80,000)
- Resolution 200,000 at *m/z* 200

> Due to increased complexity, there is a critical need for tools capable of resolving large protein complexes and accurately determine MW of aggregates of multispecific antibodies. The first instrument I have seen to make this possible is the Q Exactive UHMR. With its ability to efficiently transmit and detect ions of high *m/z* (>8,000), it provides a unique toolbox to tackle these complex problems.
>
> **Dr. Andrew Mahan, Principal Scientist,**
> **The Janssen Pharmaceutical Companies of Johnson & Johnson**




**Q Exactive UHMR MS allows for high quality native MS measurement and identification of different size protein fragments as small as ~27 kDa and aggregates up to ~630 kDa in size with remarkable resolution and clarity in a single analysis.**

# Oligonucleotide mapping

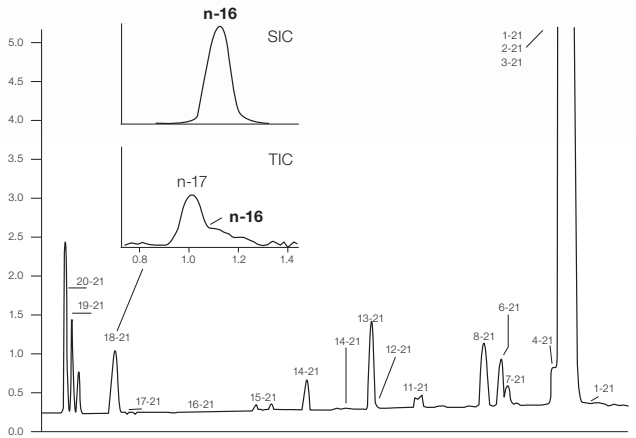
## Confident identification, mapping, and relative quantitation of oligonucleotides

Oligonucleotides are being developed as therapeutic agents and vaccines against a wide range of disease conditions. Advanced tools are indispensable for the characterization of oligonucleotides and their impurities.

We solve the common oligonucleotide challenges by offering:

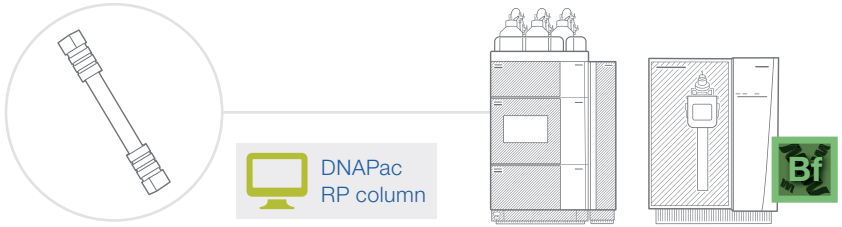
- High quality MS data for confident sequence confirmation
- Automated mass confirmation at both MS and MS/MS levels using BioPharma Finder software
- Robust and reproduction LC separation of oligonucleotides with DNAPac RP column and Vanquish UHPLC systems






**Excellent separation of 21 mer oligonucleotide and its impurities using the DNAPac RP column with the Vanquish Horizon UHPLC system coupled to the Orbitrap Exploris 240 MS.**

## Oligonucleotide analysis



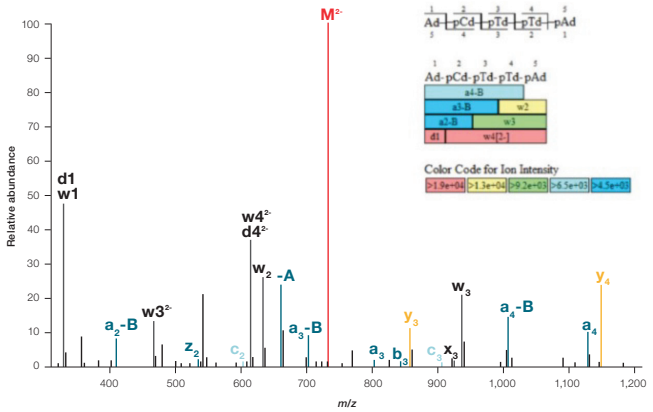

**Compatible with MS and separation of large and small oligonucleotides, our DNAPac RP column has high pH and temperature stability, excellent resolution, and exceptional column lifetime.**

**The biocompatibility of the Vanquish Flex UHPLC system allows for the use of ion-pairing modifying buffer conditions. HRAM based data-dependent tandem MS (ddMS2) method allows for confident identification and mapping of oligonucleotides and their impurities.**

## Oligonucleotide analysis workflow in BioPharma Finder software provides:

- A streamlined process from sequence creation and oligonucleotide mapping to relative quantitation of impurities and result reviewing.
- Fast processing and annotation of ddMS2 data.
- Comparative analysis tools allow easy optimization of ddMS2 and comparisons of data from different studies.

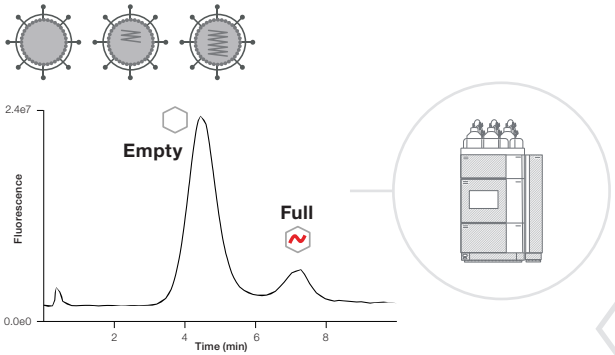
AN Identification and quantitation of oligonucleotides, impurities, and degradation products




**Total ion chromatogram of n-16 present, but not discernible, at a very low level. Using BioPharma Finder software, the impurity could be confidently identified by its MS² spectrum and a complete fragment coverage map.**

# Emerging gene therapies: fast, robust, and confident analysis from characterization through control

For gene therapy products, viruses such as Adeno-associated virus (AAV) are common vectors for gene delivery. AAV attributes need to be monitored, including purity, identity, and empty: full capsid ratio. With the right analytical tools scientists can expedite development of safe, high-quality gene therapy products while reducing time-to-market and reducing costs of manufacturing.




## Separation and ratio determination of empty and full AAV capsids

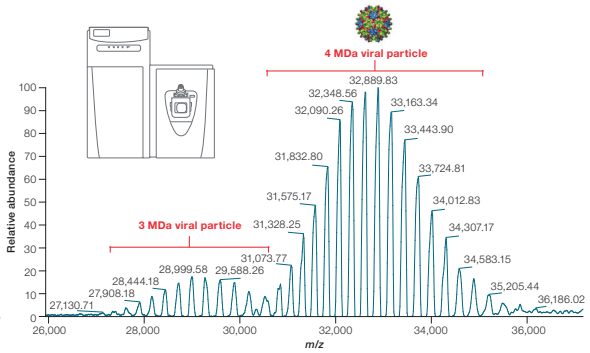
Empty or partial capsids are by-products of the AAV production and must be monitored because they can impact product efficacy. Fast and robust empty: full capsid separation can be accomplished using the Vanquish UHPLC system with ProPac SAX-10 column. The biocompatibility of the Vanquish Flex UHPLC system allows for the use of ion exchange and ion-pairing modifying buffer.

Empty/full capsid determination

Intact viral capsid characterization

Viral vectors analysis tools

Capsid protein characterization




## Intact capsid mass analysis

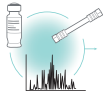
Mass confirmation of empty capsids can be performed with high confidence using the Q Exactive UHMR Hybrid Quadrupole-Orbitrap mass spectrometer. See how a mixture of 3 MDa and 4 MDa virus capsids analyzed under charge reducing conditions span a wide mass range between *m/z* 27,000 and 36,000.

## Capsid protein characterization

The correct AAV serotype is selected, then identity and purity are monitored; either on the intact protein level or by peptide mapping. Peptide mapping can be performed using pepsin SMART digest kit, with state-of-the-art consumables and instrumentation, to increase viral protein 1 (VP1) coverage for confidence in your characterization and monitoring of viral capsids.

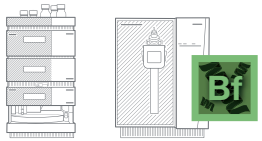
## Protein digestion

Thermo Scientific™ SMART Digest™ Pepsin Kit



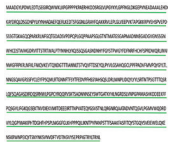
## Peptide separation

Thermo Scientific™ EASY-Spray column.




## Resolution and confidence

Thermo Scientific™ U3000 RSLC nano system and Orbitrap-based mass spectrometers.



This workflow solution offers a fast and reproducible solution to full capsid protein coverage.

# Higher order structure analysis with an all-in-one HDX solution

Full structural characterization is critical where local conformational changes can impact safety and efficacy. Hydrogen deuterium exchange (HDX) mass spectrometry (MS) is a powerful analytical approach for studying the dynamics of higher order structure of protein-based therapeutics. The rate of hydrogen-to-deuterium exchange within the amide hydrogen on the backbone of biotherapeutics provides solvent accessibility information and thus protein structure and conformation can be inferred. We offer a unique total solution for your HDX workflow.

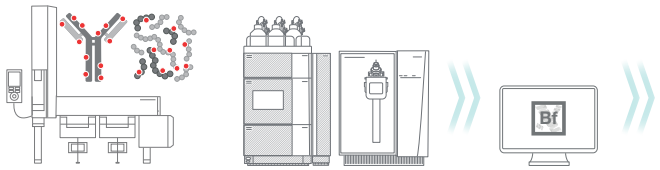
Hydrogen Deuterium Exchange (HDX)

## The Thermo Scientific HDX-MS workflow solutions

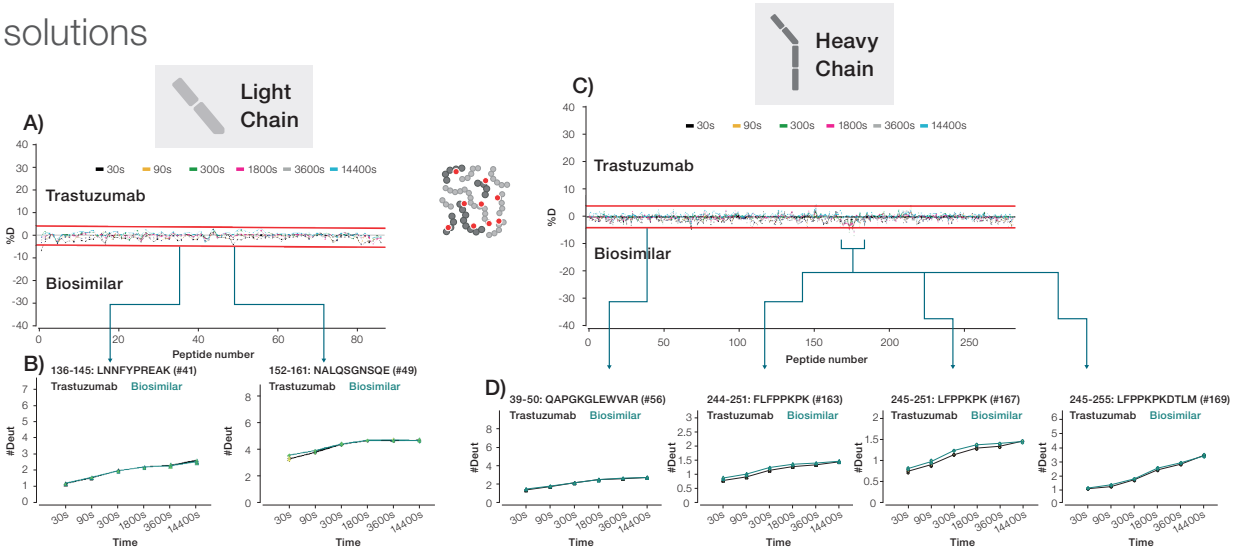
### Bottom-up HDX

Labelling and in-line digestion

Analysis by LC-MS



H/D-X PAL Hydrogen Deuterium Exchange autosampler coupled to MS

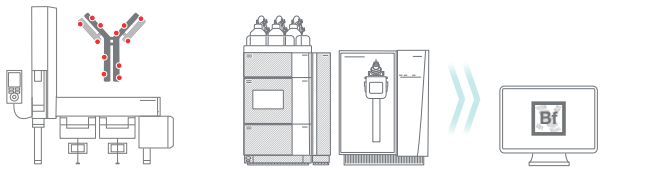



**Bottom-up HDX: Trastuzumab and its biosimilar deuterium uptake residual plots. A) Deuterium uptake comparison for peptides of the light chain. X-axis: number of peptides, Y-axis: deuterium uptake percent difference per peptide. B) specific peptides which have shown only around 4% differences in deuterium uptake between the originator and the biosimilar. C) Deuterium uptake comparison for peptides of the heavy chain. D) first panel: example of 100% match of peptide's deuterium uptake plots. D) last three panels: multiple peptides showed slightly different deuterium uptake curves.**

### Intact/top-down HDX

Labelling

Analysis by LC-MS



H/D-X PAL Hydrogen Deuterium Exchange autosampler coupled to MS

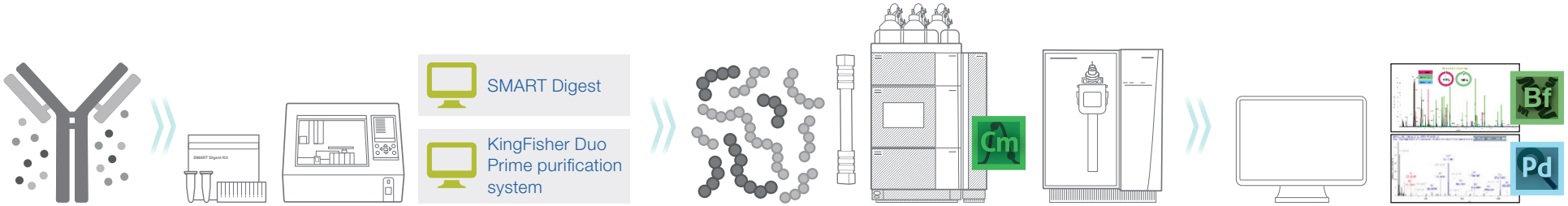
> HDX-MS can characterize a protein a lot better than other biophysical characterization methods. I believe in the future of HDX-MS as a must-have in the protein characterization toolkit.
>
> **Yoshitomo Hamuro, HDX-MS Expert, SGS**

AN Structural characterization of the therapeutic antibody and biosimilar product with hydrogen deuterium exchange mass spectrometry

# Simple, fast and sensitive host cell protein analysis rivals traditional techniques

HCPs are low-level, process-related protein impurities in drug products derived from the host organism during biotherapeutic manufacturing. Their levels need to be controlled and monitored.

The analytical solution for HCP monitoring must be extremely robust and relatively fast. As the need for increased process understanding grows, scientists are turning to an HCP analysis that utilizes robust and reliable UHPLC system coupled to an HRAM mass spectrometer to identify and quantify multiple HCPs in a single chromatographic separation, allowing for reliable HCP monitoring throughout the purification process.




**HCPs**

**Non-denaturing protein digestion with SMART Digest kit reduces intrasample dynamic range by removing the highly abundant expressed protein thereby increasing host cell protein (HCP) identifications. Automate the digestion process with the KingFisher Duo Prime purification system.**

**Efficient and reproducible chromatographic separation with Acclaim VANQUISH C18 column. High sensitivity detection over a large dynamic range, down to sub-1 ppm, using an Orbitrap-based mass spectrometer.**

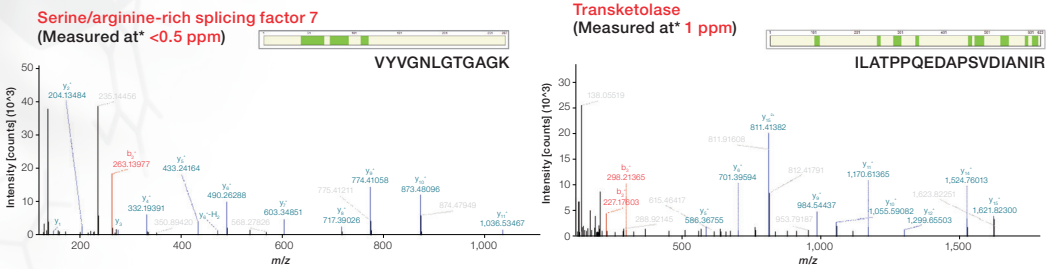
**HRAM MS data combined with Thermo Scientific™ Proteome Discoverer™ software provides confident HCP identifications.**

## LC-MS detection of low abundant HCPs using non-denaturing protein digestion

Detection of low abundant HCPs is facilitated by non-denaturing protein digestion. Under these conditions the dominating drug product is digested only to a small extent allowing for removal of remaining intact protein upon heat denaturation post digestion. This reduces the dynamic range of the sample making the low abundant HCPs more amenable for detection.

AN Residual host cell protein analysis of NISTmAb: From simplified sample preparation to reliable results

Host cell protein analysis




**Detection of HCPs ranging in concentrations from <0.5 ppm to 200 ppm**

Thanks to the excellent sensitivity and dynamic range capabilities of Orbitrap MS systems, many HCPs can be identified with high confidence in samples with at least two peptides where these HCPs are present from ~200 ppm to <0.5 ppm.

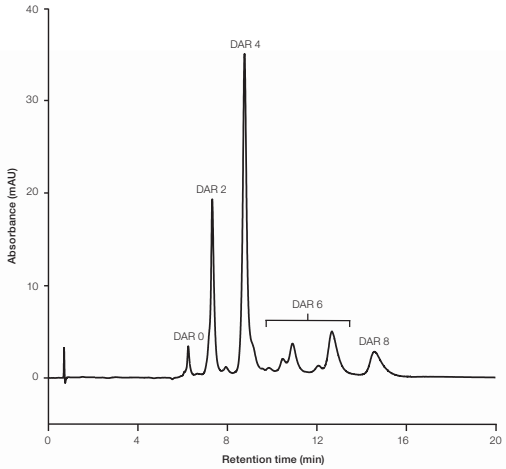
# Antibody-drug conjugates

## DAR confirmation and monitoring

### Leveraging hydrophobic interactions for separation

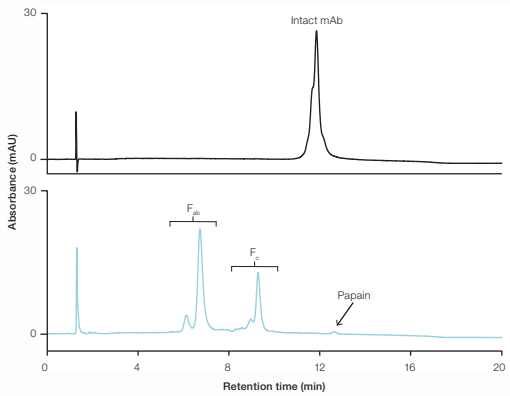
Hydrophobic interaction chromatography (HIC) takes advantage of the altered hydrophobicity of the antibody due to attachment of cytotoxic small molecules.

Attain high resolution separation of mAbs and ADCs using MAbPac HIC family of columns.




Chromatogram shows outstanding separation of a Cys-conjugated ADC mimic on a MAbPac HIC-Butyl column.

Separation of mAb fragments



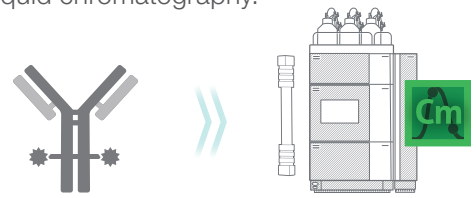

HIC can provide chromatographic resolution required for separation of Fab and Fc fragments and related variants during subunit analysis. The MAbPac HIC-20 column efficiently separates the fragments and other variants.

PN MAb separation with HIC

BR MAbPac HIC Columns

### ADC analysis

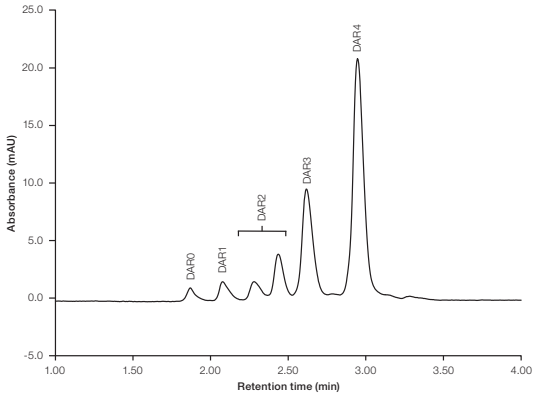
For antibody drug conjugates (ADC) the drug load must be monitored, as the distribution of drug can impact the therapeutic efficacy of an ADC. This complexity presents a great analytical challenge which requires high resolution liquid chromatography.



Antibody-Drug Conjugate (ADC)

### Leveraging reverse phase chromatography

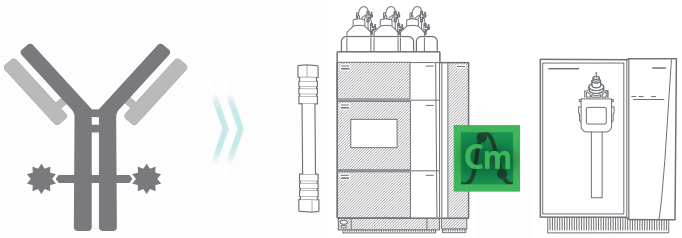
RP-LC is often the quickest monitoring assay of DARs for ADCs. The MAbPac RP column offers fast and high resolution separation.



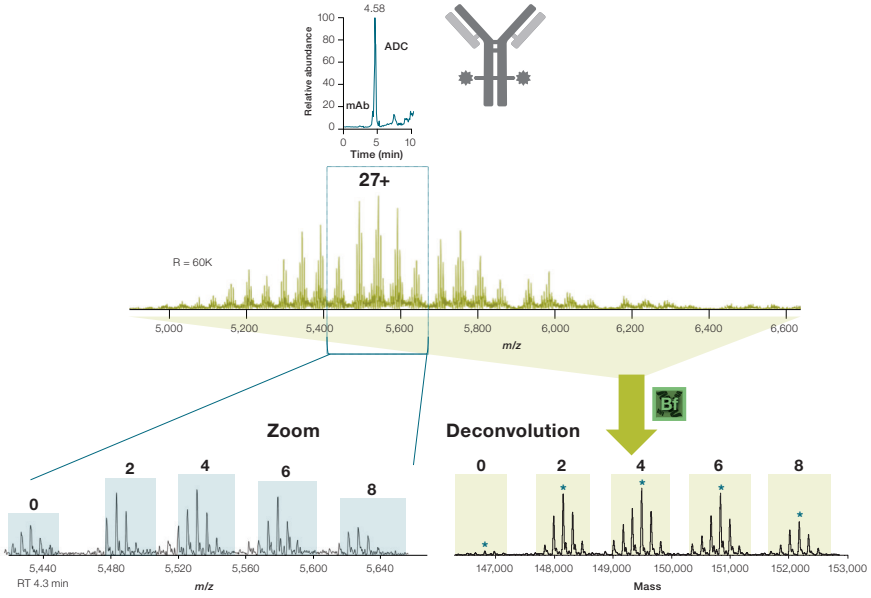

For the analysis of the MMAE Modified trastuzumab ADC - DAR values ranging from 0 to 4 are well resolved on the MAbPac RP column.

## Calculating Drug to Antibody Ratio (DAR) of Antibody-Drug Conjugates (ADCs) using High Resolution Native MS

High resolution accurate mass MS systems fit for unique ADC challenges – deeper structural insights, accurate determination drug-to-antibody ratios, and conjugation isoform and modification characterization.




Antibody-Drug Conjugate (ADC) UHPLC-Orbitrap MS




Analysis of ADC mimic under native conditions

Native intact MS allows for greater spatial separation between sequential charge states. For ADC samples, this allows low and high abundant DAR forms with varying degrees of glycosylation to be better separated spatially and detected more accurately.

| G0F/G1F DAR | Mass accuracy (ppm) | Relative abundance (%) |
|---|---|---|
| DAR0 | 6.5 | 9.19 |
| DAR2 | 4.4 | 85.74 |
| DAR4 | 2.1 | 100 |
| DAR6 | 7.8 | 88.66 |
| DAR8 | 11.4 | 49.25 |

DAR calculation of Sigma ADC based on most abundant glycoform (G0F/G1F).

Average Drug-to-Antibody Ratio (DAR)
**4.56**

Heterogeneity with varying degrees of glycosylation and drug to antibody ratios results in complex spectra. With the resolution power of the Orbitrap Exploris MS systems, DAR determination is straightforward in a very fast simple native MS analysis with no prior sample preparation. No need for deglycosylation to simplify spectra, as all glycoforms are resolved.

Antibody Drug Conjugates (ADCs)

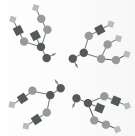
PO Comprehensive Characterization of Monoclonal Antibody and Antibody Drug Conjugate on a Hybrid Quadrupole

# Biopharmaceutical glycan analysis

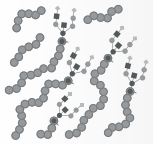
Biotherapeutics with complex glycosylation patterns have the potential to easily fall out of specification with changes in biomanufacturing processes. To meet regulatory demands, manufacturers must carefully characterize and monitor glycosylation of proteins throughout the development and manufacturing process. The complete analysis of a glycoprotein provides information on the primary structure of the oligosaccharides as well as their variation at individual glycosylation sites. Several complete analytical solutions for glycan profiling of biotherapeutic proteins may need to be applied.

Intact glycoform mass analysis

Released glycan analysis using fluorescence labels



Unlabeled released glycan analysis



Glycopeptide analysis

Monosaccharide and sialic acid analysis

# Glycan characterization and monitoring

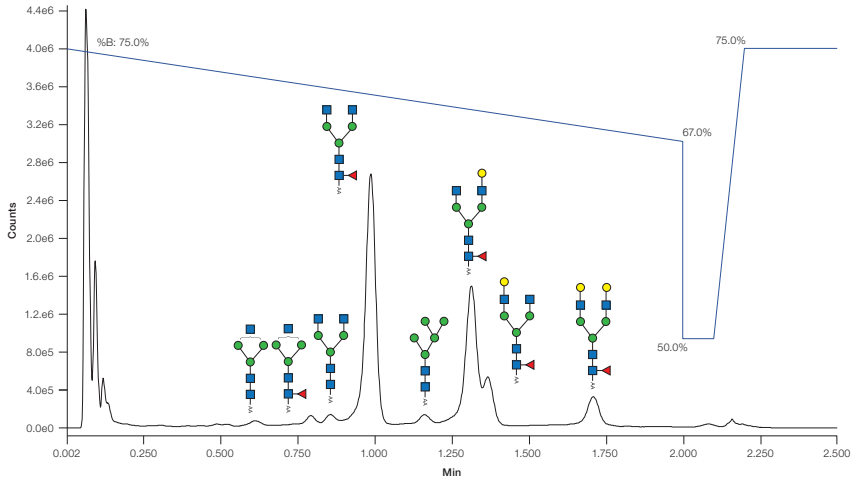
## Labelled N-glycan analysis

It is crucial when analyzing glycans to be able to efficiently separate all isomeric and branching variants present within the sample to achieve maximum structural elucidation. The separation must be rapid but with sufficient resolution to allow batch-to-batch differences in the glycan profile to be identified and subsequently characterized by high-resolution accurate-mass (HRAM) mass spectrometry.

### The Accucore™ 150-Amide-HILIC HPLC phase is designed for the separation of hydrophilic biomolecules

- High-throughput screening method for unambiguous identification of glycoforms
- 20 times reduction in separation time compared to standard profiling methods
- Excellent batch-to-batch reproducibility for method development of novel mAbs and biosimilar products

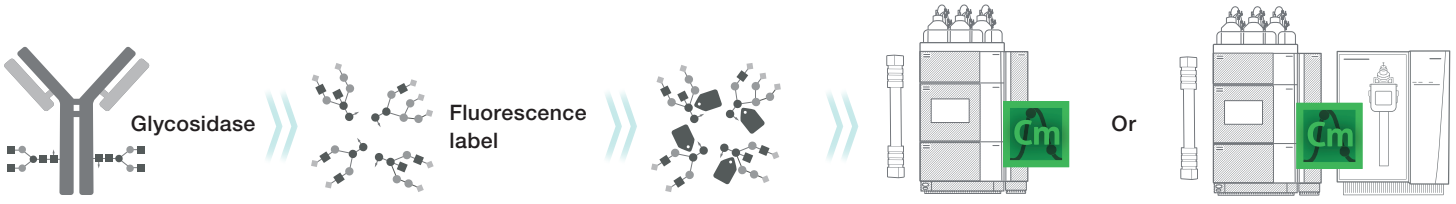
> Thermo Scientific Accucore 150-Amide-HILIC column provides excellent separation for n-glycoforms from biotherapeutics with excellent retention time precision. Coupled with the Vanquish Horizon UHPLC platform and the Vanquish FLD is a powerful tool for the analysis of 2AA-labelled glycans
> **Dr. Stefan Mittermayr, Biopharmaceutical Applications Scientist, NIBRT**




**Chromatographic separation of commercial chimeric IgG1 mAb (rituximab) 2AA-labelled N-glycans on an Accucore 150-Amide-HILIC column (2.1 × 50 mm, 2.6 µm).**

AN An ultrafast, batch-to-batch comparison of monoclonal antibody glycosylation

Labeled Glycan Workflow




The Applied Biosystems™ GlycanAssure™ HyPerformance APTS Kit is a magnetic bead–based sample preparation solution offering multiple fluorescent dyes for high-throughput analysis.

Accucore 150-Amide-HILIC column for robustness, reproducibility, accuracy, and precision needed for quantitation of released glycans.

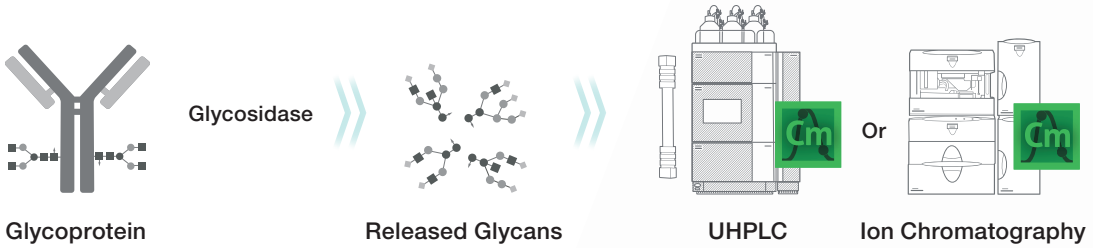
Accucore 150 Amide HILIC HPLC Columns

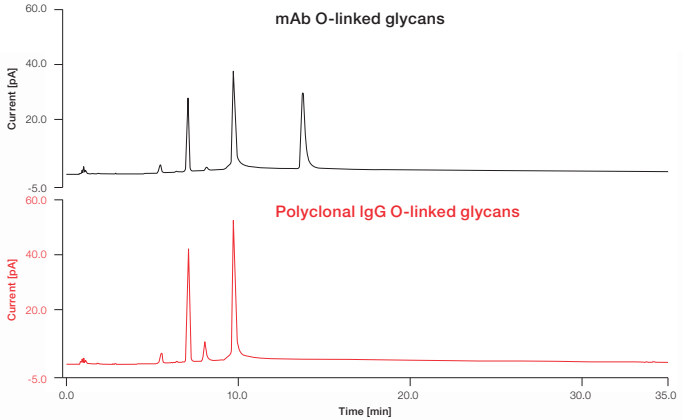
# Glycan characterization and monitoring

## Released N- & O-glycans without labeling

### Save time and money and avoid complex labeling routines

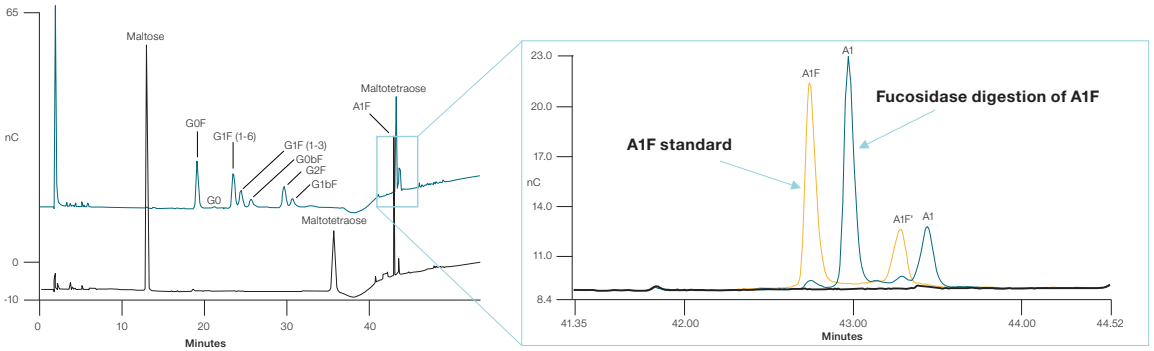
Analysis of unlabeled glycans not only eliminates an extra reaction step and cumbersome cleanup methods during labeling, but also retains the original glycan profiles without adding further ambiguity imposed by labeling reactions. This analysis can be performed using ion chromatography (IC) with high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) or liquid chromatography (LC) with charged aerosol detection (CAD).




Direct and accurate quantitation of O-linked glycans using LC-CAD

## See more. Label-free.




Label-free ion chromatography with HPAE-PAD detection

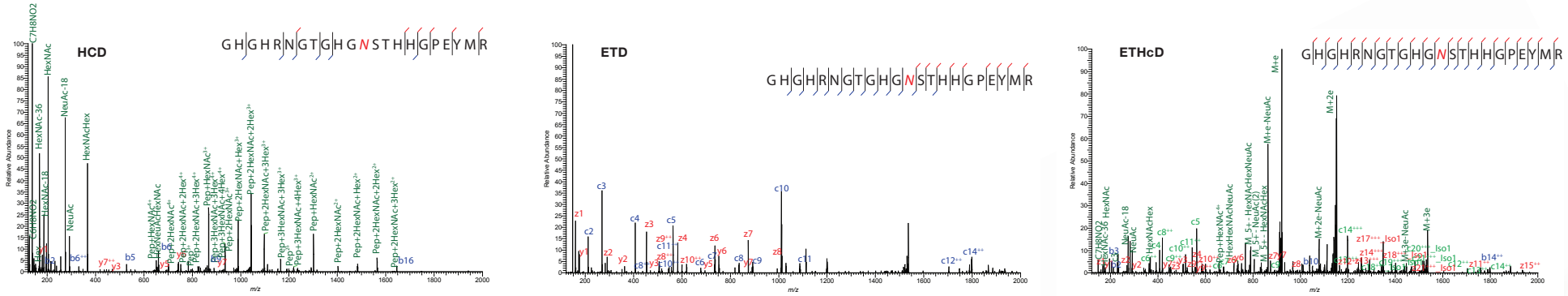
- GlycanPac AXH-1 HPLC Columns
- CarboPac PA200 IC Column
- CarboPac PA300 IC Column

# Glycan characterization and monitoring

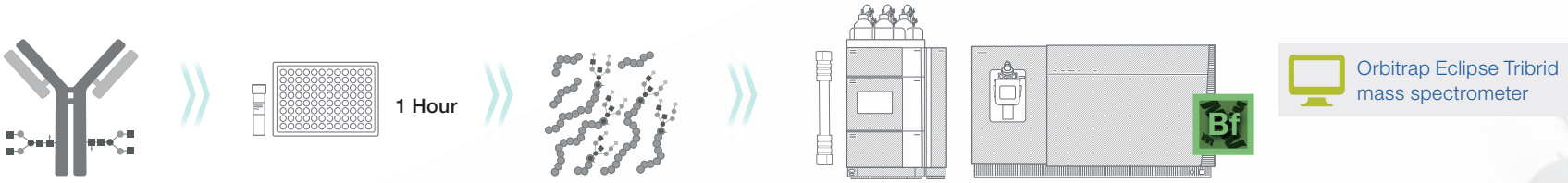
## Glycopeptide analysis and site location

The use of glycoengineering to produce therapeutic proteins with specific glycoforms may be required to achieve the desired therapeutic efficacy. Glycan site location is a critical characterization step. Thermo Scientific™ Tribrid™ MS systems can perform novel fragmentation such as electron transfer dissociation (ETD), and electron-transfer/higher-energy collisional dissociation (EThcD) which is especially advantageous for N-linked glycopeptides, enabling higher sequence coverage and glycosylation site localization.

The Orbitrap Eclipse Tribrid mass spectrometer offers unique versatility in fragmentation options and $MS^n$ capabilities that enable the user to go beyond of what is possible in glycan and glycopeptide analyses. Glycan analysis performed at the peptide level can provide both glycan composition and peptide sequence at the site of glycosylation.




**EThcD fragmentation technique enables unambiguous assignment of glycosylation site**




**Glycoprotein** | **SMART Digest Kits** | **Glycopeptides** | **UHPLC-Tribrid MS**

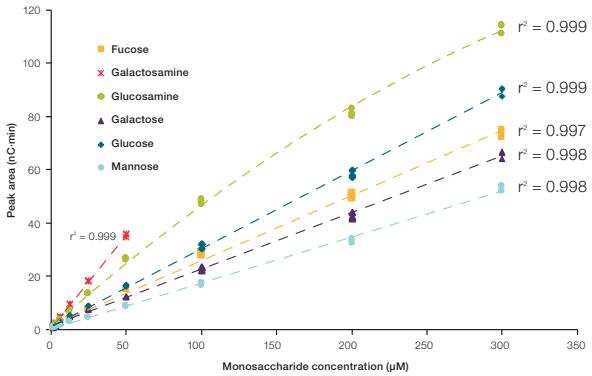
Acclaim 120 C18 columns are recommended for peptide mapping of biotherapeutic glycoproteins, offering excellent separation with superb peak shapes.

# Monosaccharides and sialic acids

Determination and monitoring of monosaccharide and sialic acid composition is a key during process optimization and quality control for glycoprotein-based therapeutics. Thermo Fisher Scientific provides sensitive workflows based on high performance anion exchange chromatography with pulsed amperometric detection (HPAE-PAD).

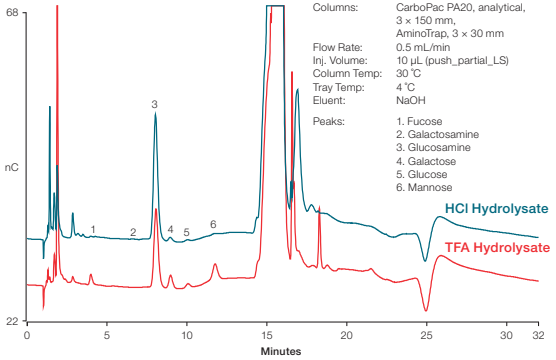
## Benefits of HPAE-PAD for determining monosaccharides, sialic acids and other carbohydrates

- Allows for direct detection without sample derivatization, reducing time and cost
- It is a selective technique with sensitive detection
- Fast separations without resolution loss using Thermo Scientific™ Dionex™ CarboPac™ PA20 columns



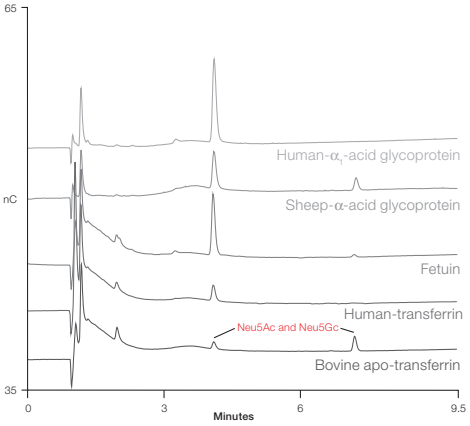

**HPAE-PAD method shows excellent linearity for each monosaccharide measured.**


## Monosaccharide analysis




**Human serum IgG trifluoroacetic acid (TFA) and hydrochloric acid (HCl) hydrolysates. Although human serum IgG has lower carbohydrate content compared to most mammalian glycoproteins, HPAE-PAD is sensitive enough to determine monosaccharides without derivatization.**

## Sialic acid analysis




**Direct and accurate quantitation of sialic acids in glycoprotein hydrolysates. Method shows good separation of the two most commonly analyzed forms of sialic acid.**

AN Improved Profiling of Sialylated N-Linked Glycans by Ion Chromatography-Orbitrap Mass Spectrometry

Analyzing carbohydrates by HPAE-PAD ion chromatography

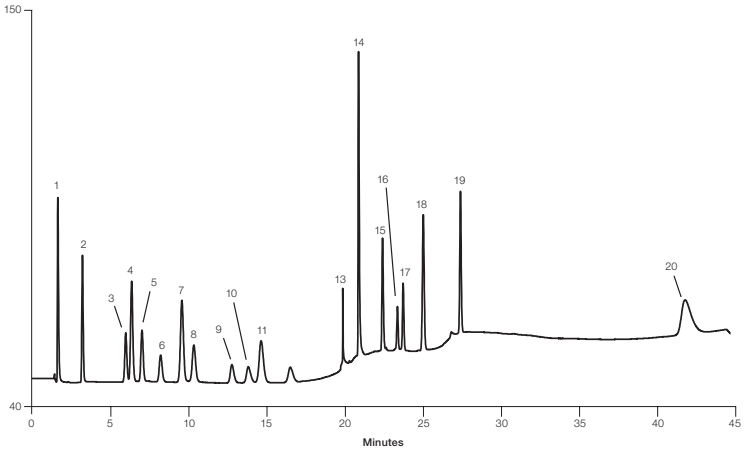
TN Glycoprotein monosaccharide analysis using HPAE-PAD with manually prepared eluent

# Amino acids

Amino acids typically require derivatization prior to analysis by UV-Vis detection due to weak chromophoric properties. This is typically a laborious and time-consuming step during sample preparation. Thermo Fisher Scientific has solutions to automate in-line or eradicate the step altogether reducing time and cost of analysis.

## Automate the post-column derivatization with robust IC separation

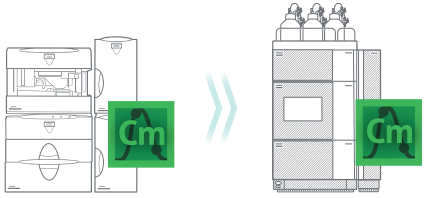
Thermo Fisher Scientific ion chromatography (IC) systems have a totally inert flow path that provides a robust and corrosion resistant platform for separations with high salt content, while allowing use of popular post-column derivatization agents, automated through Chromeleon CDS.




**Separation of amino acid standards (NISTmAb, supplemented with tryptophan) using HPAE-PAD for direct amino acid detection without derivatization.**

TN Determination of the Amino Acid Content of Peptides

Amino acid separation by ion chromatography

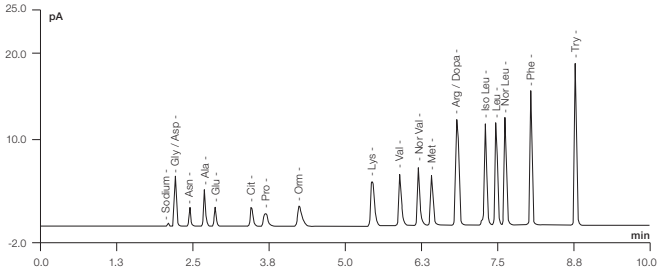


Ion Chromatography

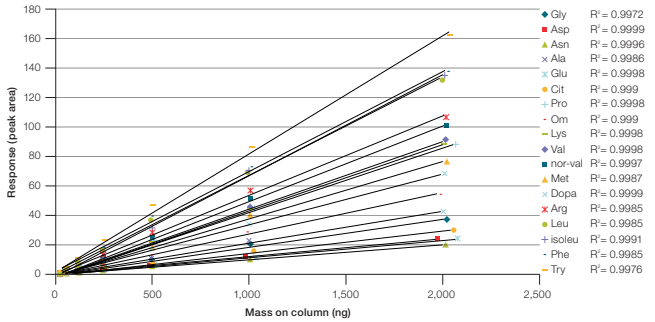
UV/Vis detection

## Eliminate the need for derivatization with rapid CAD detection

Combining rapid ion-paired UHPLC separation and near-universal detection capabilities of Thermo Fisher Scientific charged aerosol detection (CAD) speeds up amino acid analysis and removes the need to derivatize pre- or post-separation, removing manual sample preparation steps.



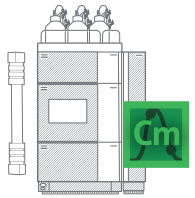

**Use of ion-paring agent can assist chromatographic retention on reverse phase columns whilst providing narrow peak widths, increasing sensitivity.**




**Linear calibration curves for all measured amino acids can be achieved with CAD detection by optimization of the user defined power function values (PFV).**

HPLC and UHPLC Charged Aerosol Detectors

AN Enhancement of linearity and response in Charged Aerosol Detection



UHPLC-CAD

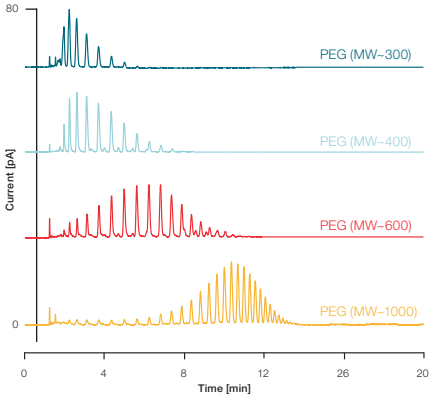
# Confident formulation analysis

There are many instances where the biotherapeutic agent is UV active, but UV-transparent excipients and critical non-chromophoric modifications must be detected and analyzed.

Charged aerosol detector (CAD) is a near universal detection technology that can be used to detect non-volatile and some semi-volatile compounds with or without a strong UV chromophore, no derivatization required.

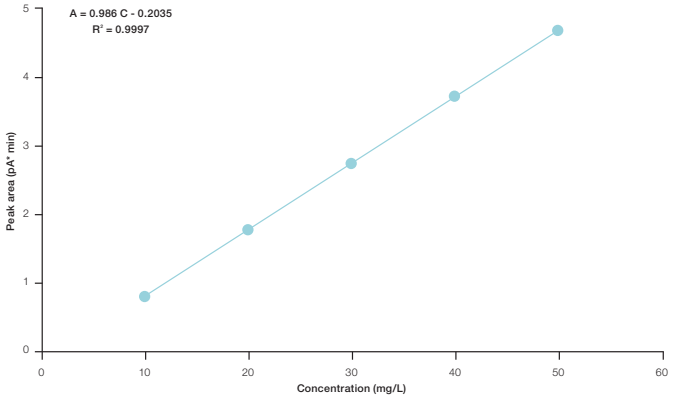
Thermo Scientific™ Vanquish™ Charged Aerosol Detector

## Profiling surfactants is made possible with CAD




**Example of surfactant analysis for PEGs shows the power of CAD. Quantitation methods are also available.**

## Great linearity achieved for Tween 80




**Calibration curve of Tween 80 (10–50 mg/L)**

Polysorbates are non-ionic surfactants commonly used in biotherapeutic formulations to prevent surface adsorption and stabilize proteins against aggregation induced by stress.

For quality control purposes, their concentration needs to be determined. The quantitative analysis of polysorbate can be challenging since polysorbate is a complex mixture of many different species, which lack natural UV chromophores. CAD can help you to get the results you need.

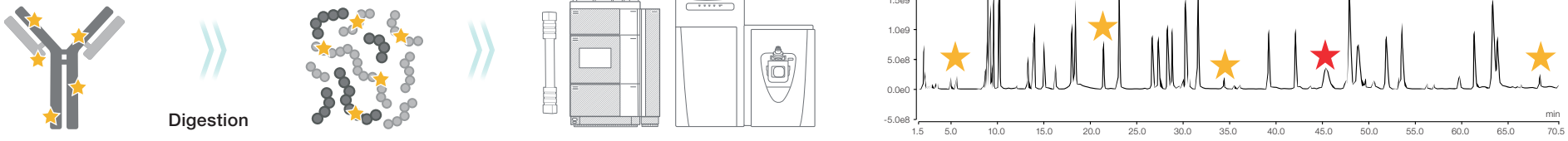
HPLC and UHPLC Charged Aerosol Detectors

Why Choose Charged Aerosol Detection for Your HPLC Analysis?

The Vanquish CAD system is essential in situations where more conventional detection techniques are not optimal. It adds an extra dimension to our detection capabilities.

# High Resolution Multi-Attribute Method:
# Resolution matters from research to routine

The Multi-Attribute Method (MAM) is a peptide mapping-based method used to monitor and quantify multiple potential CQAs simultaneously. HR Multi-Attribute Method (MAM) is a powerful high resolution accurate mass (HRAM)-based workflow that enables comprehensive characterization and monitoring of quality attributes from research to quality control (QC). MAM is in alignment with the Quality by Design (QbD) approach to the development of biopharmaceuticals, which is advocated by regulatory agencies and is being adopted by biopharmaceutical companies.

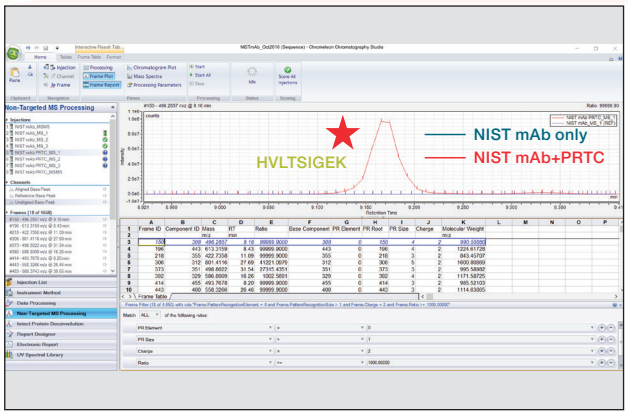



Biotherapeutic protein

Peptides with or without PTMs

LC-HRAM MS

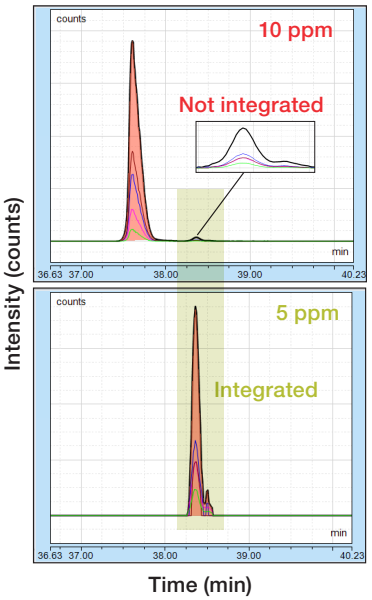
Peptides separated with LC and detected with MS


**HR MAM workflow enables characterization of biologics with ease and allows potential CQAs to be monitored throughout the drug development process while also providing purity testing with the feature of New Peak Detection (NPD). Data processing performed with Chromeleon CDS.**

**10 ppm mass tolerance**
**– low abundant deamidated peptide not integrated due to abundant wild type form (if peak integration based on intensity)**

**5 ppm mass tolerance**
**– deamidated peptide correctly integrated Illustrates the importance of high mass accuracy for peak integration and accurate quantitation**



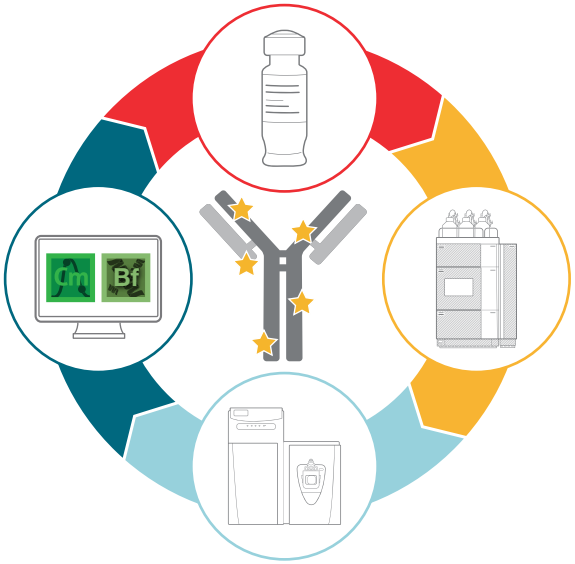
# An end-to-end analytical workflow verified and fully supported by the world leader in serving science

The HR MAM consists of industry-leading hardware and compliance-ready software as well as a system suitability test specifically developed for the workflow.

## HR MAM provides:

- High resolution accurate mass detection
- Robust instrument performance
- Reproducible and accurate CQA quantitation
- New Peak Detection for impurity analysis
- Advanced user friendly data analysis tools
- System suitability test
- Professional support from service and application teams

## Fully verified protocol




**System suitability test using the Pierce™ BSA Protein Digest standard.**

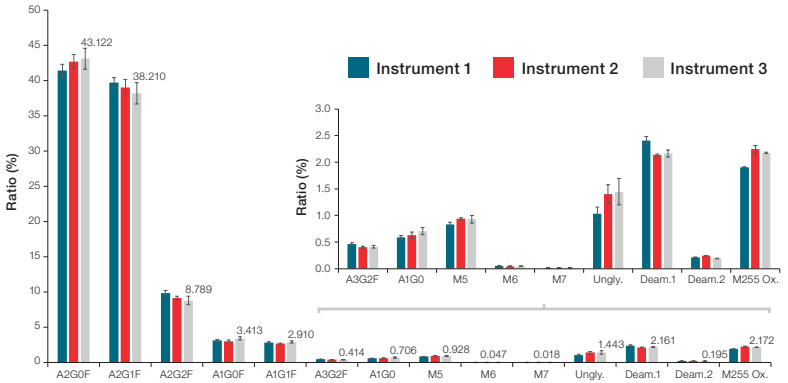
**Verify the suitability of the LC/MS system for the HR MAM with defined acceptance criteria in Chromeleon CDS.**

**Fisher Scientific LC-MS grade solvents are used for UHPLC separation**

Release your product with
**fewer tests** and **more confidence**

## Additional benefits of HR MAM:

- Potential reduction in cost and time, as well as fewer SOPs and instruments to maintain.
- A standardized workflow for your analytical processes on multiple instruments across different development sites, from research and development to manufacturing and QC.
- Global environment – workflow standardization offers peace of mind when preparing regulatory filings.
- Full workflow support, globally, from a dedicated team of Thermo Fisher Scientific MAM experts.



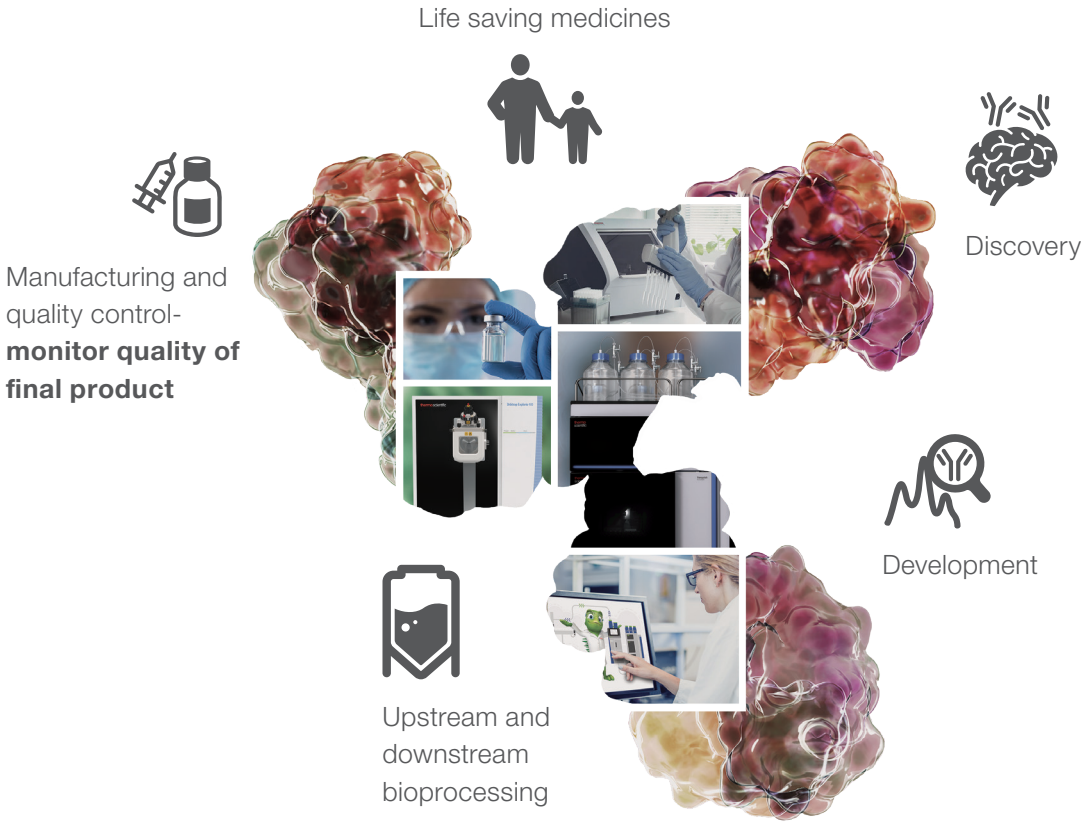

**Reproducibility demonstrated on different instruments**
Average ratios for the same NISTmAb digest analyzed on three different Q Exactive Plus mass spectrometers.

CS MAM Case Study with Rich Rogers – MAM Consortium

BR An end-to-end analytical solution, verified and supported by a single vendor

thermo scientific

**Be Confident...** as **your partner, Thermo Fisher Scientific** provides all the right products and solutions for your **characterization** and **control** challenges with exceptional separation, reproducibility, and high-quality data providing deeper insights in a compliance-ready data environment.

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