ThermoFisher
SCIENTIFIC

Biopharma

Protein solutions eBook

thermo scientific

Protein therapeutics: At a glance

Protein therapeutics are a type of biotherapeutics derived from genetically engineered human proteins. They offer several advantages over small-molecule drugs, allowing for more precise targeting of complex functions.

Compared to chemical drug production, the production process for protein-based therapeutics is intricate and variable, with a higher risk of product degradation and contamination. The final product is fragile and can be affected by factors like temperature, storage duration, denaturants, solvents, oxygen, and pH changes.

As a result, protein biologics require strict controls in manufacturing, transport and storage. An array of analytical approaches and workflows facilitate comprehensive characterization, allowing for full product understanding which can save significant time and development costs.

Throughout process development and manufacturing, these analytical tools are required for reliable monitoring of product microheterogeneity ensuring drug safety and efficacy.

This eBook will provide a comprehensive explanation of the different workflows specifically designed for protein analysis, highlighting the benefits that each workflow offers.

Enabling every step in your protein therapeutics journey

Early discovery and research

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

Requires: High-throughput capabilities and versatile, advanced tools.

Process and analytical development

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

Requires: Robust and reproducible analyses to monitor molecular attribute changes with speed and confidence.

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.

Quality control and manufacturing

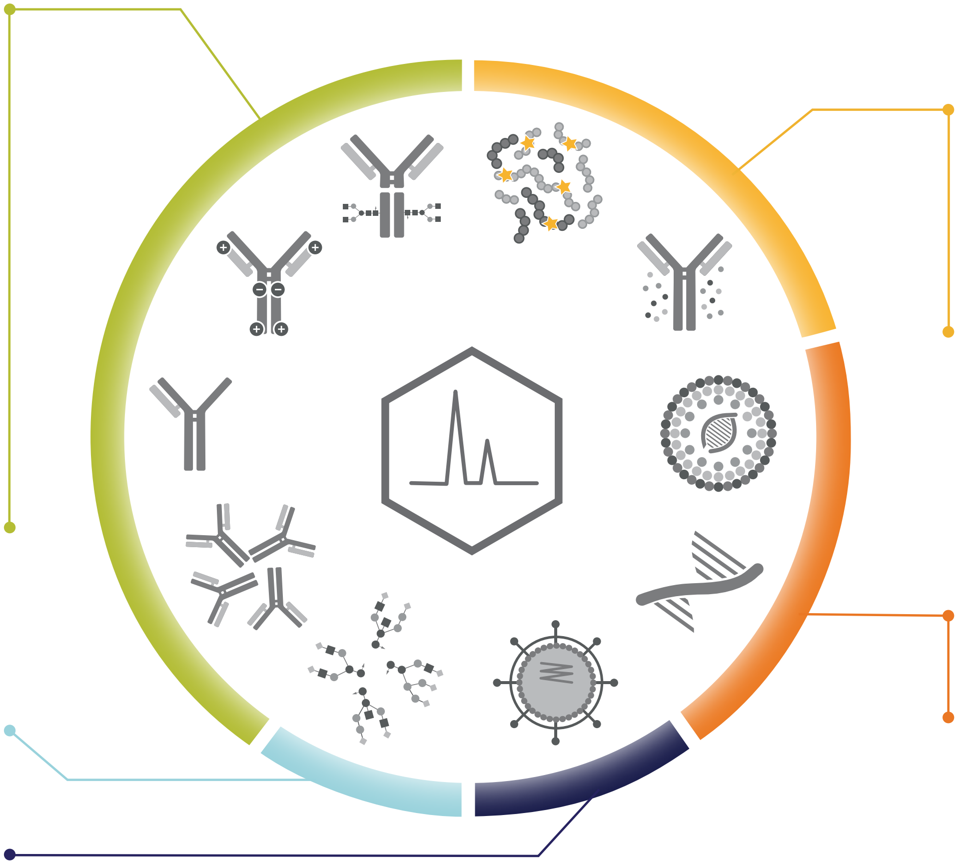
Small changes in manufacturing conditions can have a significant impact on product quality. To ensure the release of safe and effective products, advanced analytical methods are implemented to closely monitor CQAs for each batch.

Requires: Reproducible, robust, GMP compliance-ready methods.

From discovery to commercialization, Thermo Fisher Scientific provides expertise and a superior array of scalable tools, services, and support, designed to deliver high-quality results and accelerate your productivity and innovation.

Protein therapeutics workflows

Use the wheel to navigate to your area of interest.



Intact protein analysis
Native intact analysis
Aggregate analysis
Subunit analysis

Peptide mapping
Multi-attribute method
Host cell protein analysis

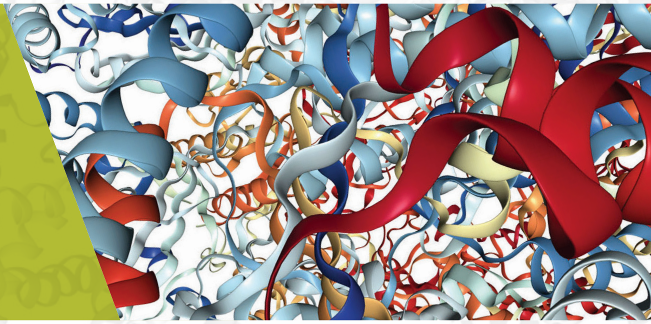
Charge variant analysis

Glycan analysis

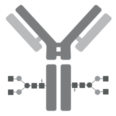
Higher order structure analysis

Intact protein analysis

Efficient intact protein analysis for rapid assessment enabling confident identification

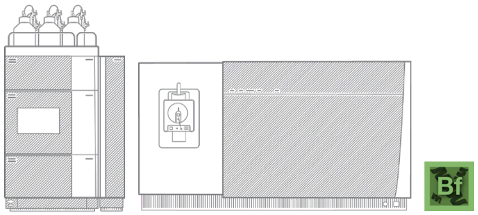


There is a need for a more efficient protein analysis approach that saves time on sample preparation and offers simple data analysis. The ideal solution provides high throughput, information-rich data, and fast assessment of post-translational modification levels.



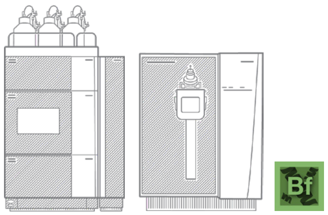
Biotherapeutic protein

Early discovery and research



Thermo Scientific™ Orbitrap Ascend™ Tribrid MS

CID and HCD, optionally with UVPD, ETD, PCTR, can be used in any MS stage, followed by mass analysis in either the ion trap or ultra-high resolution Orbitrap mass analyze

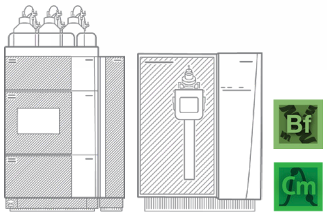


Thermo Scientific™ Orbitrap Exploris™ 480 MS

Intuitive instrument control for easy execution of analysis

Easy-to-use software for data processing

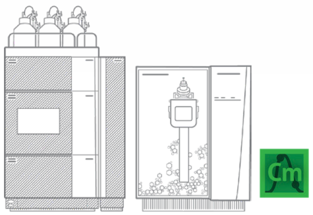
Development



Thermo Scientific™ Orbitrap Exploris™ 240 MS

Excellent sensitivity, allows the use of much less sample for high quality intact MS analysis

QC



Thermo Scientific™ Orbitrap Exploris™ MX MS

Built for usage in the routine environment

Complemented by compliance-ready software Thermo Scientific™ Chromeleon™

Value-added workflow benefits

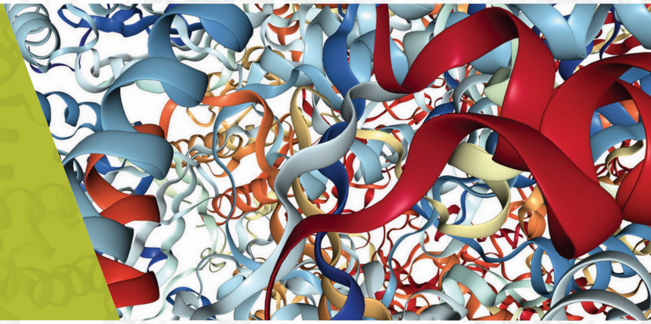
- Direct infusion techniques or separation allow intact protein analysis
- Screen, identify, and characterize intact proteins with higher productivity and confidence using the Intact Protein workflow in Thermo Scientific™ BioPharma Finder™ software
- BioPharma mass extension available for Orbitrap Exploris 240 and 480
- Quickly confirm intact protein molecular weight and determine glycoform heterogeneity of biopharmaceuticals

Learn more about intact protein analysis

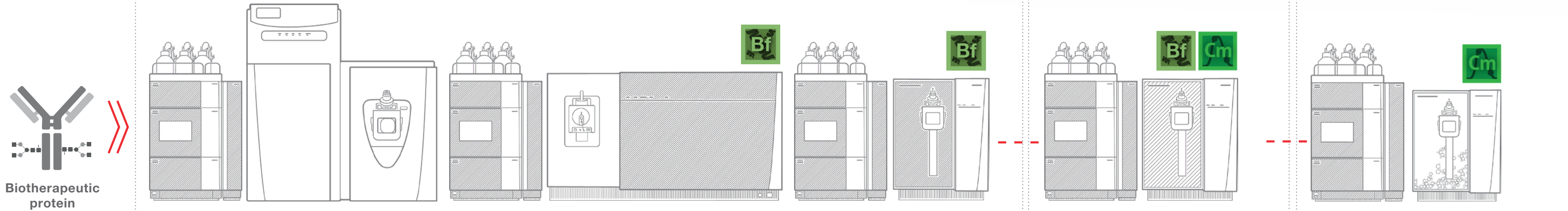
Webinar - Workflows for robust and sensitive separation of mAbs, intact proteins at subunit and peptide level using capillary chromatography by LC-MS

Intact protein analysis under near-native conditions

Analysis of intact proteins and protein-complexes, in their true biological states



Analyzing complex therapeutic proteins like antibody-drug conjugates (ADCs) is a significant challenge. The Thermo Scientific Orbitrap Exploris mass spectrometers, coupled with size exclusion or ion exchange chromatography and compatible buffers, enable routine intact protein analysis under native conditions.



Early discovery and research

Thermo Scientific™ Q Exactive™ UHMR MS
The first UHMR MS to combine substantially increased sensitivity and mass resolution at high m/z, MS2, and psuedo-MS3 capabilities in a single platform

Thermo Scientific™ Orbitrap Ascend™ Tribrid MS
Advanced MS capabilities and optional mass range of up to 16’000 m/z allow scientists to take advantage of versatile fragmentation techniques and advanced ion reaction techniques like proton transfer charge reduction

Thermo Scientific™ Orbitrap Exploris™ 480 MS
Intuitive instrument control for easy execution of analysis

Easy-to-use software for data processing

Development

Thermo Scientific™ Orbitrap Exploris™ 240 MS
Excellent sensitivity, allowing the use of much less sample for high quality native MS analysis

QC

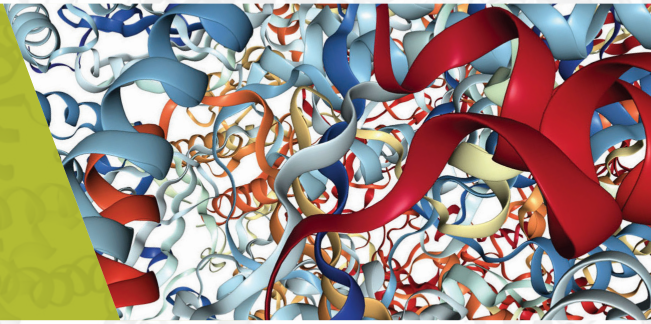
Thermo Scientific™ Orbitrap Exploris™ MX MS
Built for usage in the routine environment. Complemented by compliance-ready software Thermo Scientific™ Chromeleon™

Value-added workflow benefits

- Use Thermo Scientific MAbPac SEC-1 Size Exclusion Columns for high-resolution separation of monoclonal antibody (mAb) analysis, including monomers and aggregates
- BioPharma option to extend mass range available for the Orbitrap Exploris 240 and 480 MS systems
- Excellent mass spectrometric data, intuitive instrument control and easy-to-use software for data processing
- Q Exactive™ UHMR Hybrid Quadrupole Orbitrap™ Mass Spectrometer offers precise mass determination, peak interference mitigation, and the ability to study heterogeneous protein assemblies

Learn more about intact protein analysis

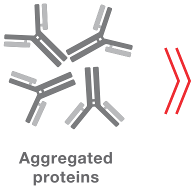
Webinar - NIBRT: Ion exchange chromatography hyphenated to mass spectrometry for fast and robust characterization of mAb variants under native conditions



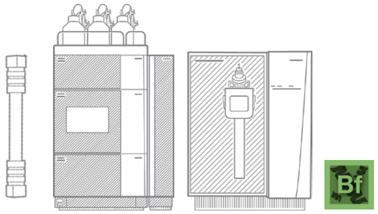
Aggregate analysis

High resolution separation of monomers and aggregate peaks

The consistent quality of biologic products must be ensured by monitoring protein aggregates throughout the production process, as aggregates can have serious negative effects. Failure to monitor protein aggregates can result in incorrect drug dosage, decreased solubility, and activity loss.



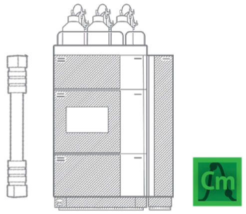
Characterization



Thermo Scientific™ Vanquish™ UHPLC System & Thermo Scientific™ Orbitrap Exploris 240 MS

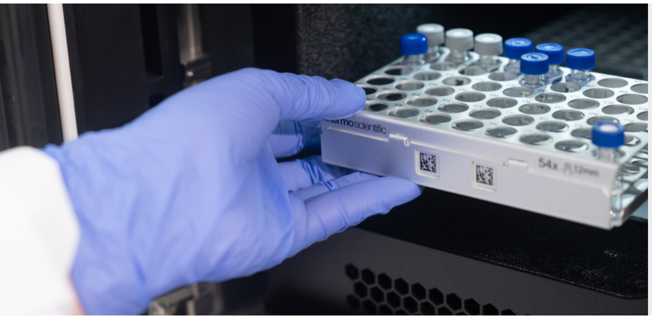
Aggregate analysis by HRAM MS facilitates the analysis of the proteins in their native form, without the need for sample preparation, while providing information on aggregation and fragmentation

Routine monitoring



Thermo Scientific™ Vanquish™ Duo UHPLC System

The Vanquish Duo UHPLC system provides two independent flow paths connected to two individual detectors. This unique system lets you run two analyses in parallel, either identical or not, on a single system to double throughput, accelerate method development, and improve sample characterization



Value-added workflow benefits

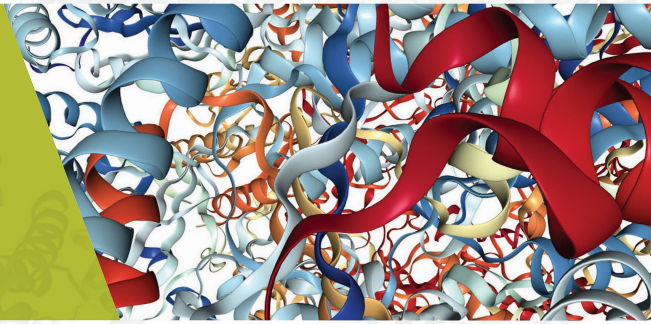
- High resolution separation of fragments, monomers and aggregates
- BioPharma option to extend mass range available for the Orbitrap Exploris 240 MS
- Thermo Scientific™ Vanquish™ Duo UHPLC system for Dual LC provides simple and rapid high-throughput analysis of aggregates
- Compliance-ready analytical setup using Thermo Scientific™ Chromeleon™ software for the monitoring stage

Learn more about aggregate analysis

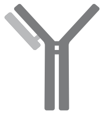
Video - Application Note Video - A universal Chromatography Method for Aggregate Analysis of Monoclonal Antibodies

Subunit analysis

Easy deconvolution by harnessing the power of Thermo Scientific BioPharma Finder Software

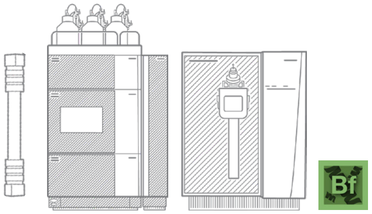


Monoclonal antibody (mAb) subunit analysis involves the antibody fragmentation through digestion and reduction of IgG antibody molecules. The cysteine protease produced by S.pyogenes, known as IdeS, is highly specific. IdeS digestion followed by reduction generates three subunits that are readily separated by reversed-phase (RP) UHPLC using Thermo Scientific MAbPac RP columns. Deconvolution of subunit mass spectrometry (MS) spectra is easy with powerful Thermo Scientific BioPharma Finder software.

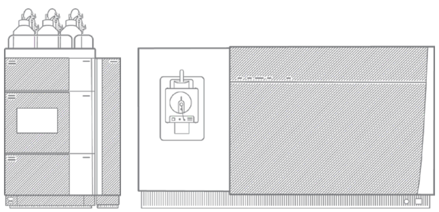


Subunits

Characterization

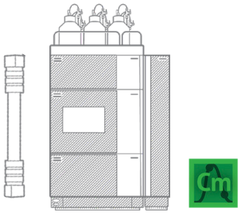


Vanquish UHPLC System & Orbitrap Exploris 240 MS
The extreme resolving power of the Thermo Scientific Orbitrap mass analyzer ensures isotopic resolution of monoclonal antibody subunits and facilitates middle-down sequencing.



Thermo Scientific™ Orbitrap Ascend™ Tribrid MS
Quantify more samples at lower concentrations using faster acquisition, while achieving greater coverage using a revolutionary new hardware design featuring dual ion routing multipoles

Routine monitoring



Thermo Scientific™ Vanquish UHPLC System
Take advantage of MAbPac SEC-1 columns which offer superior, reproducible separation of monomers, aggregates, and fragments resulting from proteolysis

Value-added workflow benefits

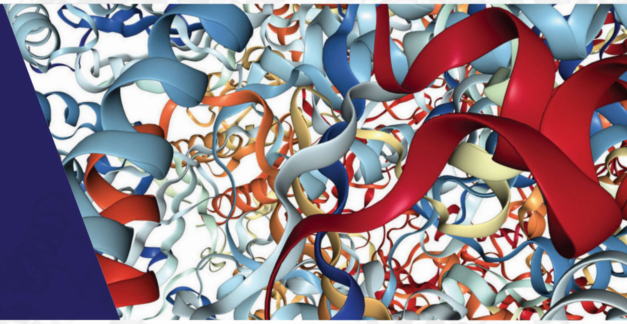
- Subunits are readily separated by reversed-phase (RP) UHPLC using Thermo Scientific MAbPac RP columns
- Deconvolution of subunit mass spectrometry (MS) spectra is easy with powerful Thermo Scientific BioPharma Finder software
- Thermo Scientific™ Pierce Fab Preparation Kit uses immobilized pepsin protease to digest human or mouse IgG antibodies to make separate Fab and Fc antibody fragments

Learn more about subunit analysis

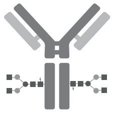
Webinar - Workflows for robust and sensitive separation of mAbs, intact proteins at subunit and peptide level using capillary chromatography by LC-MS

Higher order structure analysis

A robust method for the analysis of protein conformation, conformation dynamics, and protein-protein interactions

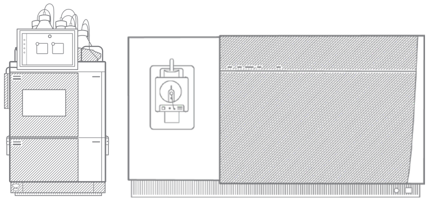


Full structural characterization is critical where local conformational changes can impact safety and efficacy. Hydrogen deuterium exchange (HDX) mass spectrometry (MS) is a powerful analytical approach for studying the dynamics of higher order structure of protein-based therapeutics. We offer a unique total solution for your HDX workflow.



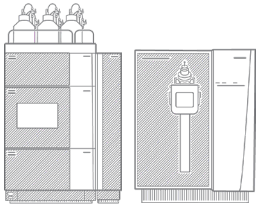
Biotherapeutic protein

Early discovery and research



Thermo Scientific™ Neo™ Nano LC & Orbitrap Ascend Tribrid MS

Delivering the ultimate flexibility to expand experimental scope, and with built-in intelligence, Orbitrap Ascend Tribrid Mass Spectrometer ensures the highest data quality for HDX-MS experiments

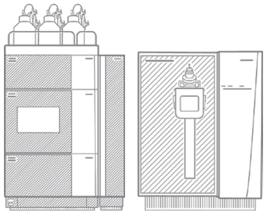


Orbitrap Exploris 480 MS

Intuitive instrument control for easy execution of analysis

Easy-to-use software for data processing

Development



Orbitrap Exploris 240 MS

Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer fast tracks your path to high-confidence data and identification

Value-added workflow benefits

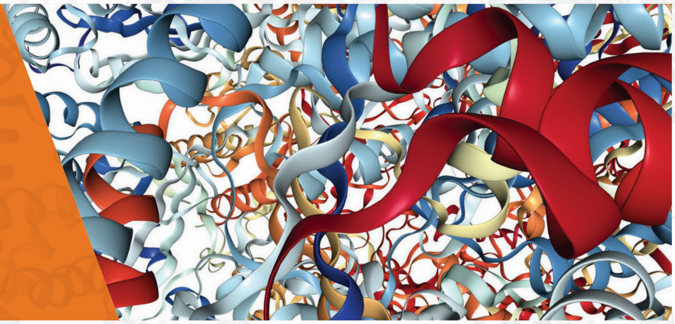
- Sample preparation and labelling is made easy by our partners, Trajan Scientific and Medical. Trajan's LEAP HDX sampler system enables automated labelling and digestion
- The Vanquish Neo UHPLC system combines an unrivaled degree of innovation to deliver 24/7 reproducible separations of complex mixtures at maximum performance for a variety of high-sensitivity LC-MS workflows
- Thermo Scientific BioPharma Finder software supports all HDX-MS data analysis, including peptide identification, PTM analysis and HDX-unique protection factor plots at the single residue level

Learn more about higher order structure analysis

Webinar - Dr Patrick Griffin - Hijacking Molecular Plasticity to Fine Tune Nuclear Receptor Signaling: Chemical Biology and Precision Therapeutics

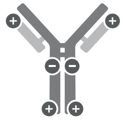
Charge variant analysis

Full solutions from mobile phase preparation to purpose-built software



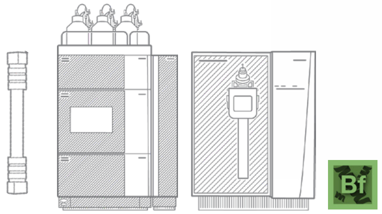
Heterogeneity of monoclonal antibodies must be monitored and revealed by charge sensitive techniques. Two workflows specific to your needs:

1. Separation by charge followed by mass spectrometry (CVA-MS) allows for characterization of post-translational modifications (PTMs) at the intact protein level.
2. Salt gradient cation-exchange chromatography can be used to monitor mAb charge variants.



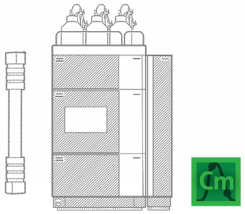
Biotherapeutic proteins

Characterization



Thermo Scientific™ Vanquish™ UHPLC System & Orbitrap Exploris 240 MS
Speed and accuracy are key criteria for laboratories performing charge variant profiling with an out of the box sample capacity of 216 samples. The Vanquish UHPLC system and the Orbitrap Exploris 240 MS offers the speed and accuracy required for charge variant profiling

Routine monitoring



Vanquish™ UHPLC System
Obtain new benchmarks in accuracy, precision and sensitivity in charge variant analysis with the Thermo Scientific™ Vanquish™ UHPLC systems. Providing biocompatibility with a state-of-the-art quaternary or binary high-pressure solvent blending, these ultra-high performance liquid chromatography systems share all Vanquish values, such as a design focused on uptime, robustness and reliability

Value-added workflow benefits

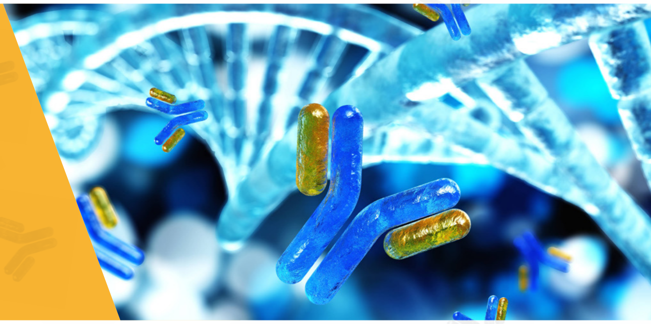
- Simplified and fast ion exchange analysis (IEX) of charge variants using a pH gradient
- Obtain fast and highly robust, reproducible HPLC gradients using Thermo Scientific™ CX-1 pH gradient buffer kits. The CX-1 buffers save time in method development, facilitate method transfer to QA/QC
- The Orbitrap Exploris 240 MS offers high sensitivity, allowing for the detection and quantification of low abundance charge variants
- Vanquish UHPLC systems offer dependable, flexible, and productive separations

Learn more about charge variant analysis

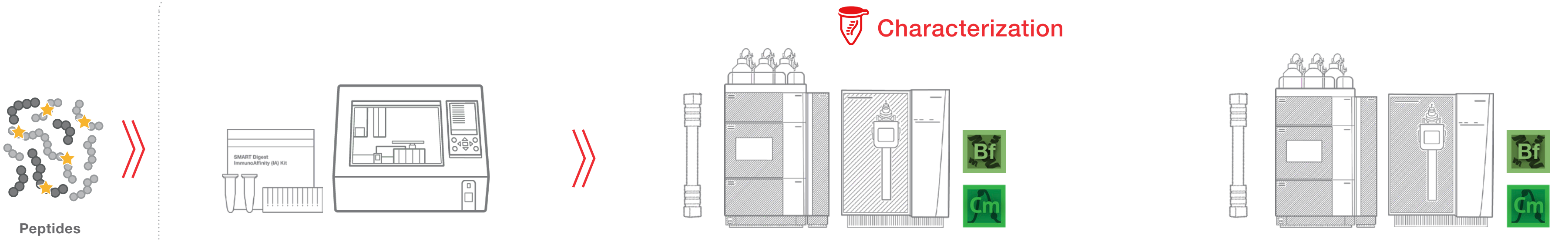
Webinar - Taking Charged Variant Analysis to the next level

Peptide mapping

High throughput peptide mapping with Vanquish Duo UHPLC system



Accurate peptide mapping is crucial for determining sequence coverage, identifying and quantitating post-translational modifications, determining disulphide bonds, identifying sequence variants, and performing de novo sequencing of proteins. Failure to accurately perform peptide mapping can result in incomplete or incorrect protein characterization, compromising the safety and efficacy of biologic products.



Thermo Scientific™ KingFisher™ Duo Prime Purification System
Thermo Scientific™ SMART Digest™ Kit provides fast and simple protein digestion with high reproducibility and sensitivity, in a format that's compatible with automation

Vanquish Duo UHPLC System & Orbitrap Exploris 240 MS
The Vanquish Duo allows for tandem LC-MS. With this approach, a greater number of sample injections can be performed in the same timeframe, enhancing throughput and significantly reducing MS idle time

Vanquish Duo UHPLC System & Orbitrap Exploris 480 MS
Accurate mass and resolving power provide the most effective way to accurately and confidently identify peptides.

Confident and complete sequence coverage with versatile Thermo Scientific™ BioPharma Finder™ software

Value-added workflow benefits

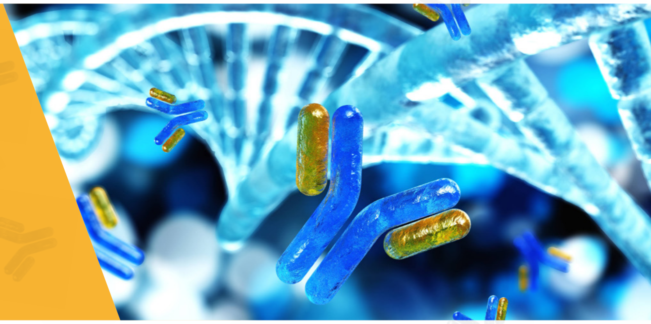
- Automation options and kit-based protein digestion for the reproducible results
- High throughput peptide mapping with Vanquish Duo UHPLC system
- Complete product characterization: Sequence coverage, PTMs, Disulfide bonds and Sequence variants

Learn more about peptide mapping

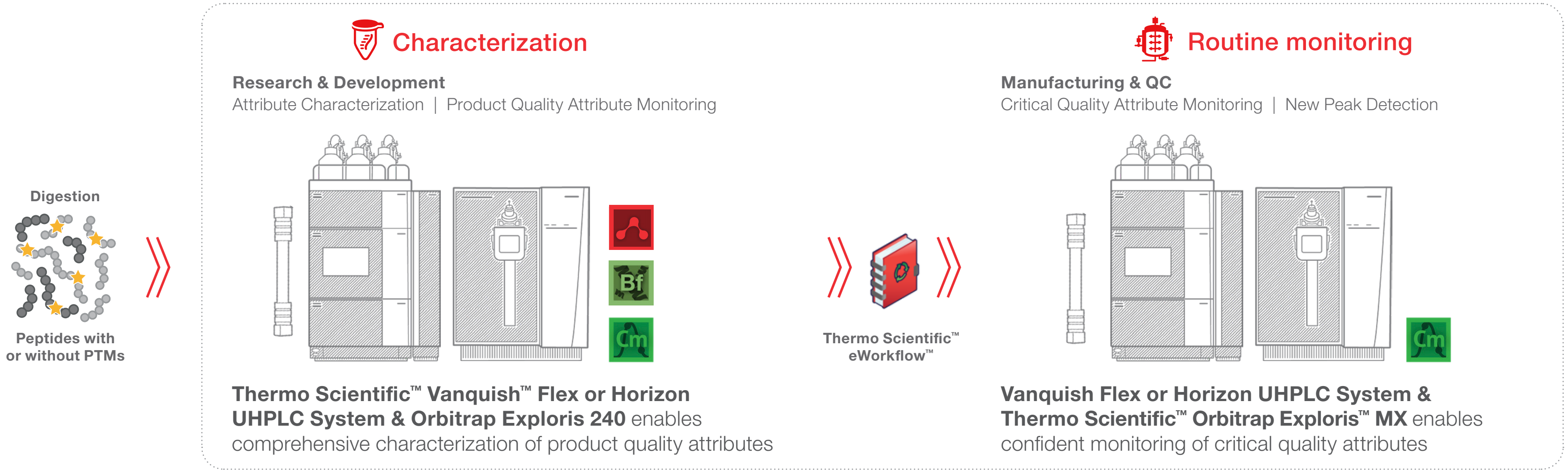
Webinar - Rapid Automated Peptide Mapping

Multi-Attribute Method

A platform from discovery and development to QC



The Multi-Attribute Method (MAM) is a peptide mapping-based method used to quantify multiple potential CQAs simultaneously. Thermo Scientific™ MAM 2.0 is a powerful high resolution accurate mass-based workflow that enables comprehensive characterization and monitoring of quality attributes from research to quality control (QC). MAM is in alignment with the QbD approach to the development of biopharmaceuticals, which is advocated by regulatory agencies and is being adopted by biopharmaceutical companies.



Characterization

Research & Development
Attribute Characterization | Product Quality Attribute Monitoring

Thermo Scientific™ Vanquish™ Flex or Horizon UHPLC System & Orbitrap Exploris 240 enables comprehensive characterization of product quality attributes

Routine monitoring

Manufacturing & QC
Critical Quality Attribute Monitoring | New Peak Detection

Vanquish Flex or Horizon UHPLC System & Thermo Scientific™ Orbitrap Exploris™ MX enables confident monitoring of critical quality attributes

Value-added workflow benefits

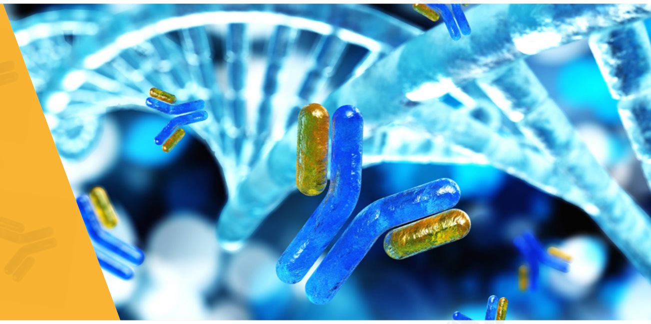
- Seamless and direct method transfer from lab-to-lab
- End-to-end, compliance-ready platform for deployment from development to QC
- Enterprise software platform for connectivity at global scale leveraging the power of enterprise Chromeleon and Ardia Platform software.
- Confident and consistent attribute identification and monitoring with high resolution and accurate mass data

Learn more about Multi-Attribute Method

Webinar - Pfizer: Evaluation of the Orbitrap Exploris MX Mass Detector

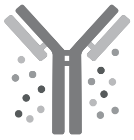
Host cell protein analysis

Fast analysis achieved by harnessing the power of Orbitrap technology

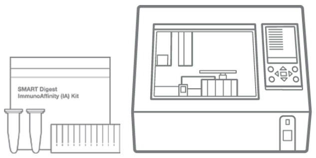


The analytical solution for Host Cell Protein (HCP) monitoring must be both robust and fast, and capable of identifying and quantifying multiple HCPs in a single chromatographic separation. To meet the growing demand for increased process understanding, scientists are turning to reliable UHPLC systems coupled with HRAM mass spectrometers for accurate and reliable HCP monitoring throughout the purification process.

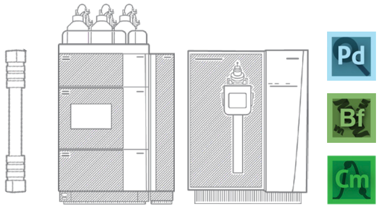
Characterization



HCPs

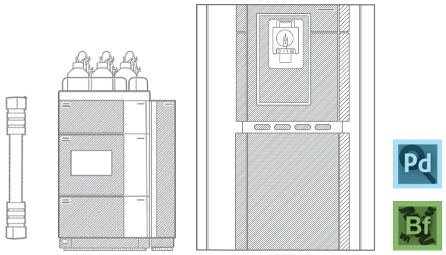


KingFisher Duo Prime Purification System
Non-denaturing protein digestion with SMART Digest kit reduces intra-sample dynamic range and increases host cell protein (HCP) identifications



Vanquish™ Duo UHPLC System & Orbitrap Exploris 480 MS
Detection of HCPs ranging in concentrations from <0.5 ppm to 200 ppm

Identify and quantify multiple HCPs in a single chromatographic separation, allowing for reliable HCP monitoring throughout the purification process



Vanquish Neo Nano LC & Thermo Scientific™ Orbitrap™ Astral™ MS
The Orbitrap Astral Mass Spectrometer helps overcome the challenges of insufficient throughput, low abundant HCP ID and provides sub ppm quantification

Value-added workflow benefits

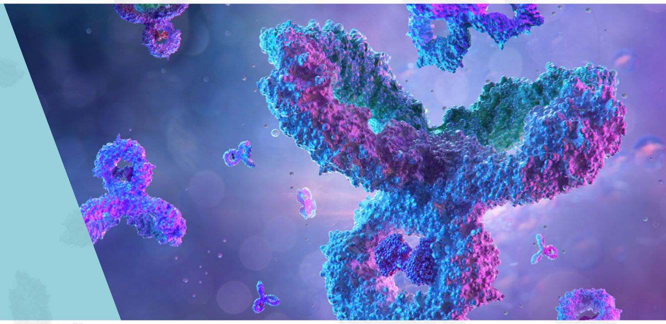
- Ready-to-use single informatics workflow for peptide mapping and HCP data processing and visualization
- HRAM MS data combined with Thermo Scientific™ Proteome Discoverer™ software provides confident HCP identifications
- Increased sensitivity enabled by micro-flow rate separation allows detection of low abundant HCPs

Learn more about host cell protein analysis

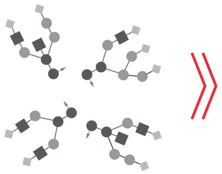
Video - Optimized sample preparation for low ppm detection of Host Cell Proteins (HCPs) in biotherapeutics with Orbitrap-based MS detection

Glycan analysis

Comprehensive toolbox for glycan analysis

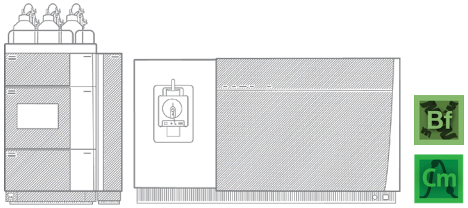


Analysis of glycans can be complex as no single method will provide all information, but there are many different approaches for glycan analysis. Thermo Fisher Scientific offer full solutions to analyze glycans utilizing UHPLC, UHPLC-MS and IC for analysis of glycoforms.



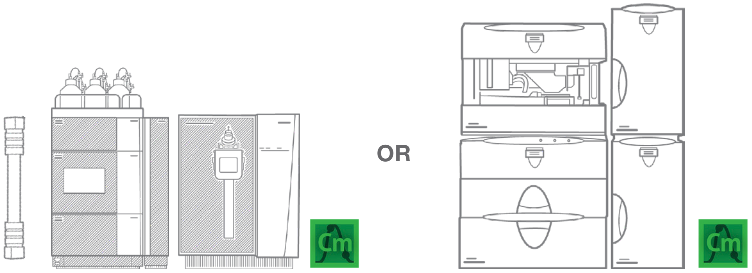
Glycans

Characterization



Thermo Scientific™ Orbitrap Ascend™ Tribrid MS
Quantify more samples at lower concentrations using faster acquisition, while achieving greater coverage using a revolutionary new hardware design featuring dual ion routing multipoles

Routine monitoring



Vanquish UHPLC System & Orbitrap Exploris Platform or Thermo Scientific™ ICS-6000 ™ Ion Chromatography system
Monitor released N- & O-glycan without labelling

Flexibility: Ion chromatography with pulsed amperometric detection (HPAE-PAD) OR LC with charged aerosol detection (CAD)

Value-added workflow benefits

- Flexible solutions for all analytical strategies
- Fit for purpose sample preparation options
- HRAM MS for outstanding intact protein or glycopeptide analysis
- Easy-to use fit for purpose software capabilities from discovery to control

Learn more about glycan analysis

Webinar - Advancing carbohydrate analysis - Glycans

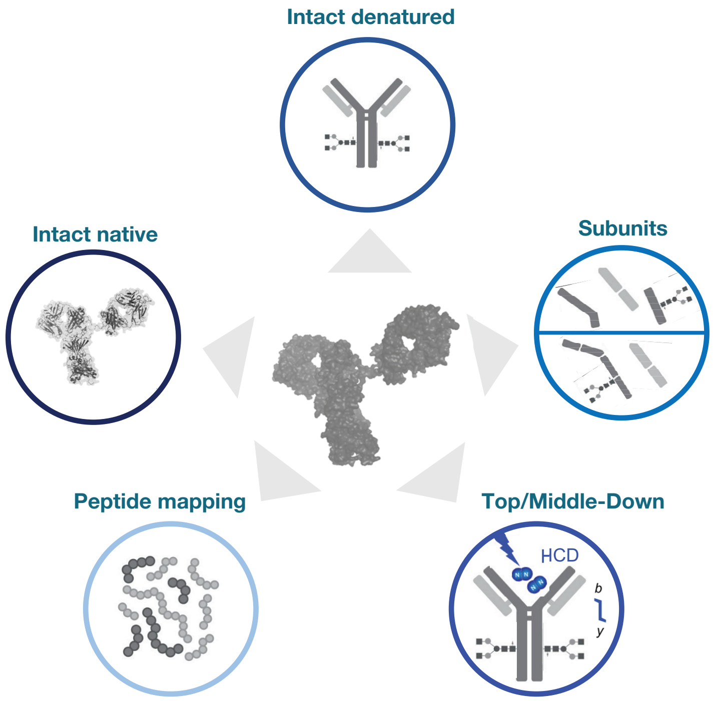
Application note spotlight

Complete characterization of monoclonal antibodies under native and denaturing conditions

Application Note - View the PDF

This document provides a comprehensive overview of the use of the Thermo Scientific Orbitrap Exploris 480 mass spectrometer equipped with BioPharma Option for the full characterization of antibody samples. The primary objective is to provide optimal LC-MS conditions to achieve conclusive information on the molecular weight and proteoform heterogeneity at the intact mAb, subunit, and peptide levels.

A key challenge in analyzing therapeutic monoclonal antibodies (mAbs) is the macro-heterogeneity deriving from various N-linked glycan species and structural heterogeneity deriving from various endogenous modifications. These modifications contribute to micro-heterogenous proteoform mixtures of covalently assembled molecules, which vary in size, charge, and hydrophobicity and could impact mAb chemical properties.The solution to this challenge is the application of different workflows for characterizing biopharmaceuticals on the new LC-MS platform, including intact mass analysis under native and denaturing conditions, subunit analysis complemented with Middle-Down analysis, and peptide mapping. The Orbitrap Exploris 480 mass spectrometer equipped with the BioPharma Option is used to achieve these analyses with high sensitivity, scan speed, and resolution to achieve results with the highest confidence.



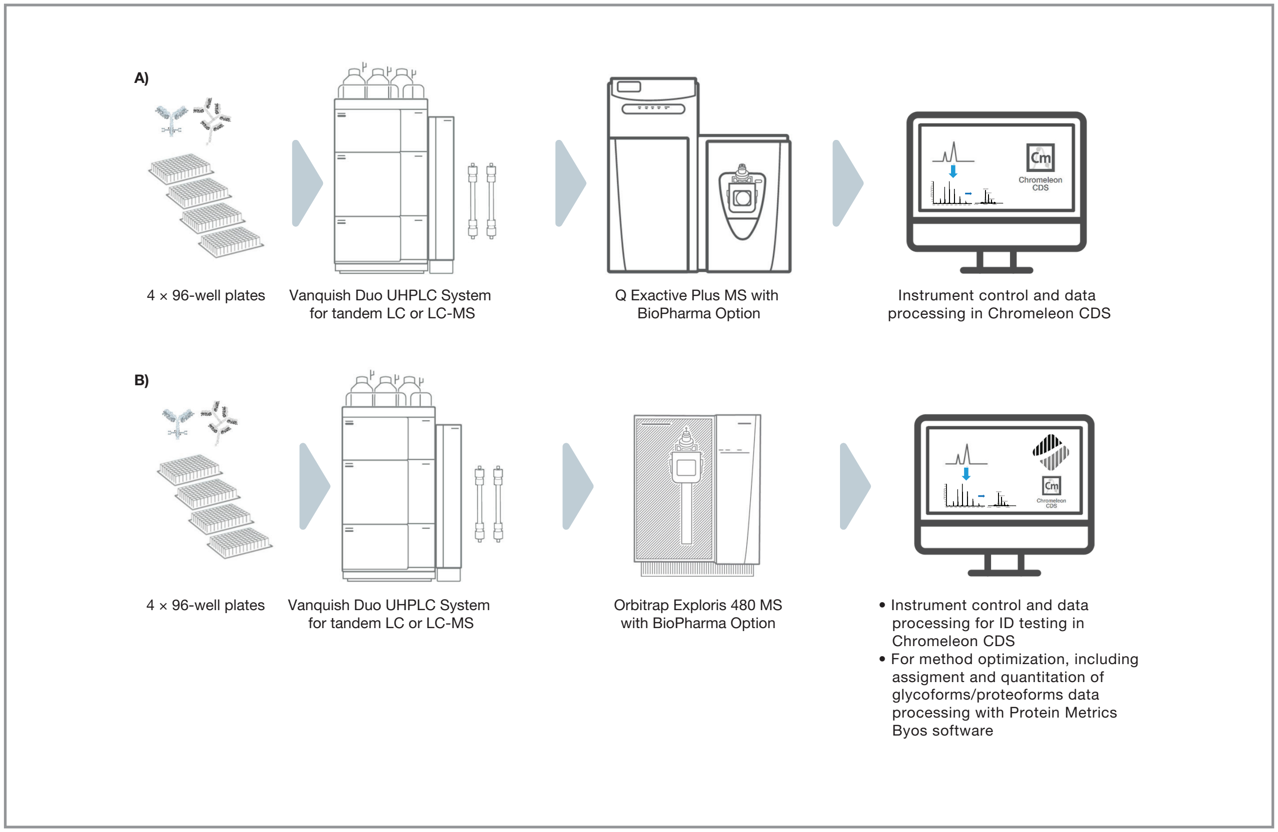
Application note spotlight

Seamless LC-MS method transfer in a biopharmaceutical development laboratory

Application Note - View the PDF

Symphogen, a Denmark-based biopharmaceutical company, employs an LC-MS system, made up of a Thermo Scientific Vanquish Horizon Duo UHPLC and Thermo Scientific Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer, for the development and lead selection studies of complex therapeutic proteins and protein mixtures. Recently, Symphogen aimed to augment their laboratory capabilities by introducing a new generation benchtop Orbitrap-based high-resolution accurate mass (HRAM) mass spectrometer, the Thermo Scientific Orbitrap Exploris 480 mass spectrometer.

- The primary challenge was to ensure the transfer of the execution of the native mass analysis measurements hyphenated to size exclusion chromatography (SEC-MS) from the Q Exactive Plus MS to the new Orbitrap Exploris 480 MS. The new MS platform had to deliver consistent high-quality data, comparable to the existing Q Exactive Plus MS system, across hundreds of samples in each lead selection study.
- The solution involved a systematic evaluation and optimization of method parameters on the Orbitrap Exploris 480 MS to generate high-quality data comparable to the Q Exactive Plus MS platform. Figure 6 shows the assessment of robustness through the investigation of glycoform level determination reproducibility. The results demonstrate the new platform's robustness, with remarkably low variability and %CV values, thus ensuring a seamless method transfer between the two platforms.



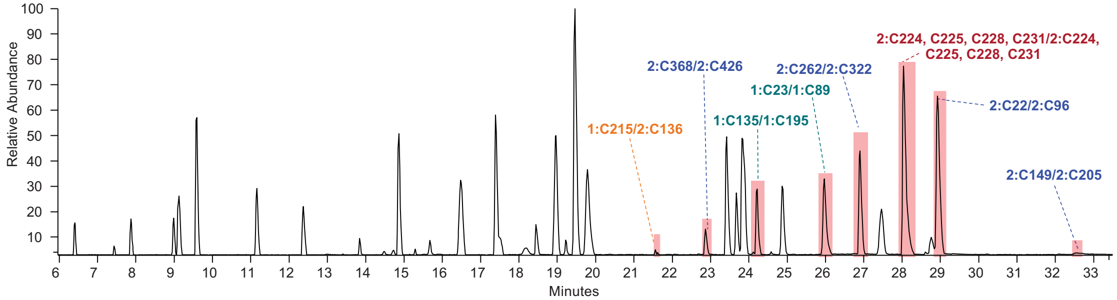
Application note spotlight

Confident peptide mapping and disulfide bond analysis of an IgG2 monoclonal antibody

Application Note - View the PDF

The document discusses an application brief related to peptide mapping and disulfide bond analysis of an IgG2 monoclonal antibody, denosumab, using the Thermo Scientific Orbitrap Exploris 240 mass spectrometer. The primary goal was to demonstrate the utility and capability of the mass spectrometer for the routine characterization of biotherapeutics through LC-MS peptide mapping. This procedure allows for the full sequence coverage, high confidence in low level post-translational modifications (PTMs) identification, and ease in interpreting complex data

- The major challenge in the biopharmaceutical industry is ensuring the quality, efficacy, and safety of biotherapeutic proteins, such as monoclonal antibodies, due to variations in production processes and the intrinsic complexity of these proteins. There are many critical quality attributes (CQAs) that must be monitored, including the detection of inter- and intrachain disulfide bonds and low-level post-translational modifications (PTMs). Additionally, the industry requires instruments and analytical methods that can be transferred and adopted routinely across organizations.
- The Orbitrap Exploris 240 mass spectrometer provides operational simplicity and flexibility, enabling a wide range of characterization assays to be performed with high confidence. It was used to perform peptide mapping analysis to confirm 100% sequence coverage of denosumab, enabling the detection of low-level PTMs and the location of disulfide bonds.



S-S bond type		Peptide sequence	Position	Δ ppm	RT
Intrachain	**LC1**	ATLSCR / LEPEDFAVFYCQQYGSSPR	**1:C23/1:C89**	**-1.2**	25.95
	LC2	SGTASVVCLLNNFYPR / HKVYACEVTHQGLSSPVTK	**1:C135/1:C195**	**-2.1**	24.15
	HC1	LSCAASGFTFSSYAMSWVR / AEDTAVYYCAK	**2:C22/2:C96**	**-2.7**	28.95
	HC2	STSESTAALGCLVK / DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTK	**2:C149/2:C205**	**-0.9**	32.54
	HC3	TPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAK / CK	**2:C262/2:C322**	**-0.6**	26.76
	HC4	NQVSLTCLVK / WQQGNVFSCSVMHEALHNHYTQK	**2:C368/2:C426**	**0.4**	22.87
Interchain	**LC-HC**	GEC / GPSVFPLAPCSR	**1:C215/2:C136**	**-2.5**	21.86
	Hinge	KCCVECPPCPAPPVAGPSVFLFPPKPK / KCCVECPPCPAPPVAGPSVFLFPPKPK	**2:C224, C225, C228, C231/ 2:C224, C225, C228, C231**	**-0.02**	28.05

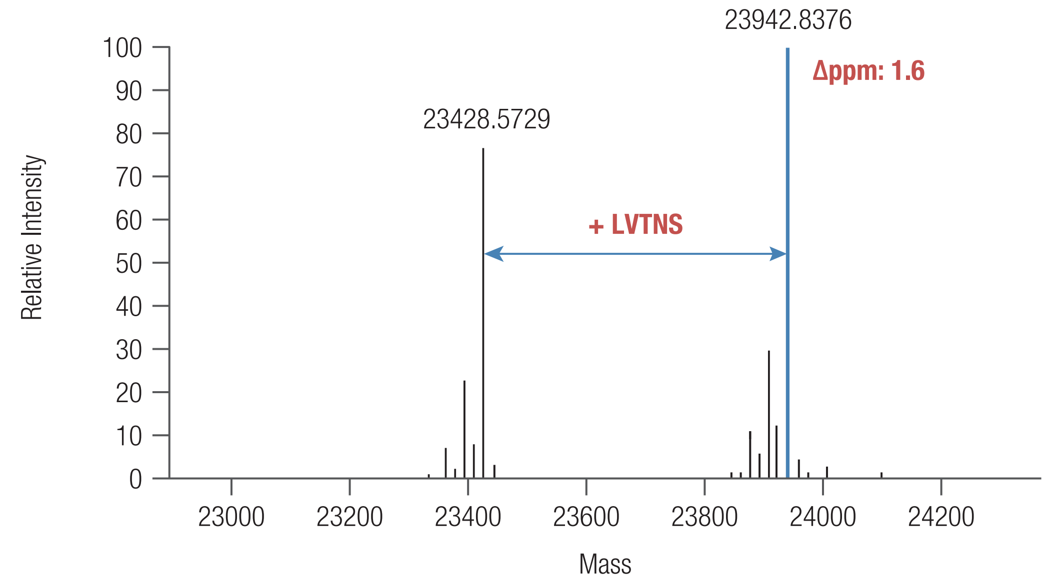
Application note spotlight

Application Note - View the PDF

IdeS-cleaved mAb subunit analysis with LC-HRAM-MS: a quick and accurate comparison of biosimilar and originator biotherapeutics

The document discusses the application of a middle-up approach for the characterization of biotherapeutics, using the Thermo Scientific Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer. This technique is crucial for the fast and reliable characterization of monoclonal antibody variants and modifications, which is of paramount importance in the biopharmaceutical industry.

- The key challenge in the biopharmaceutical industry is to establish the quality and safety of biosimilar therapeutics, which are drugs with minimal variations from their originator. These variations can occur due to multiple factors, including expression system, growing conditions, purification steps, or final formulations. Therefore, it is crucial to monitor and quantify these variations to correlate them to any potentially different in vivo activity, such as different clearance time or other interactions within the patient.
- The solution to this challenge is the use of a middle-up approach, which involves the use of liquid chromatography hyphenated with high-resolution, accurate-mass spectrometry (LC-HRAM-MS) for the comparison of monoclonal antibody drug substances and their respective biosimilars. This approach minimizes sample handling and artifacts and provides quicker or complementary information. As illustrated in Figure 2, the LC-MS analysis of IdeS digested bevacizumab biosimilar provided high quality data, which allowed for a confident identification and sequence verification of light chain and Fd region and a rapid analysis of Fc region variants, including glycoform and N-terminal lysine loss.



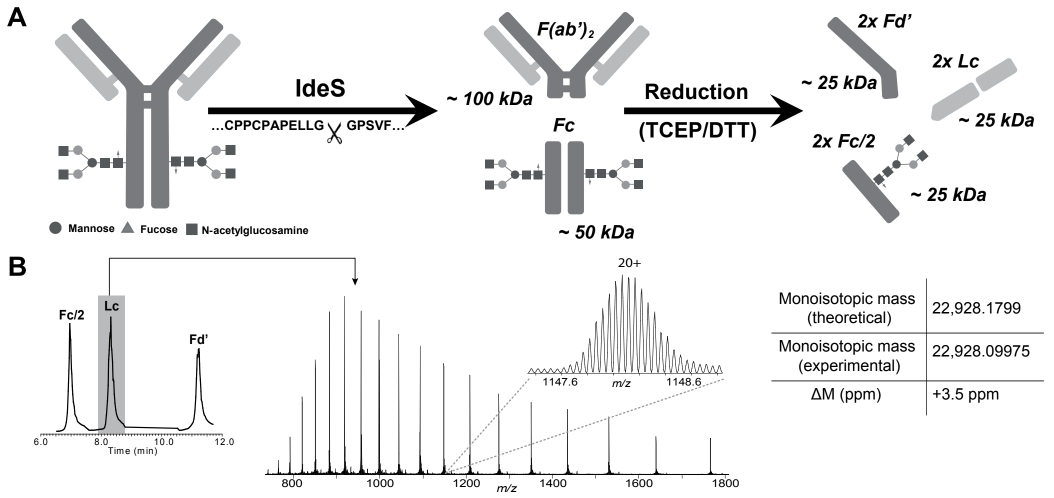
Application note spotlight

Application Note - View the PDF

Proton transfer charge reduction (PTCR)

This document provides a comprehensive overview of the experiments conducted for the analysis of monoclonal antibody (mAb) subunits under denaturing conditions. The experiments utilize Liquid Chromatography (LC) and Mass Spectrometry (MS) analysis with various reagents, columns, solvents, and flow rates. The document details the LC conditions, MS conditions, data analysis, results, and discussion.

- The primary challenge in this context is the characterization of the primary sequence of biotherapeutics, specifically monoclonal antibodies (mAbs). Traditional methods such as Bottom-Up Mass Spectrometry (BU MS) and Top-Down Mass Spectrometry (TD MS) have limitations in terms of sequence coverage and the complexity of MS2 spectra, especially for larger mAb subunits.
- The solution proposed in this document is the application of Proton Transfer Charge Reduction (PTCR) in conjunction with Electron Transfer Dissociation (ETD) for middle-down analysis of mAbs. This approach simplifies fragment ion spectra, leading to improved sequence coverage and increased confidence in product ion matching.



Monoisotopic mass (theoretical)	22,928.1799
Monoisotopic mass (experimental)	22,928.09975
ΔM (ppm)	+3.5 ppm

Resources

BioPharma learning center

Peptide mapping

Glycan analysis

Host cell protein analysis

Subunit analysis

Native intact mass analysis

Protein aggregate analysis

Charged variant analysis

Multi-attribute method

Higher order structure analysis

Protein homepage

Find the column for your application

Biopharma webinar portal, searchable by application

Learn more at **thermofisher.com/byyourside**

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