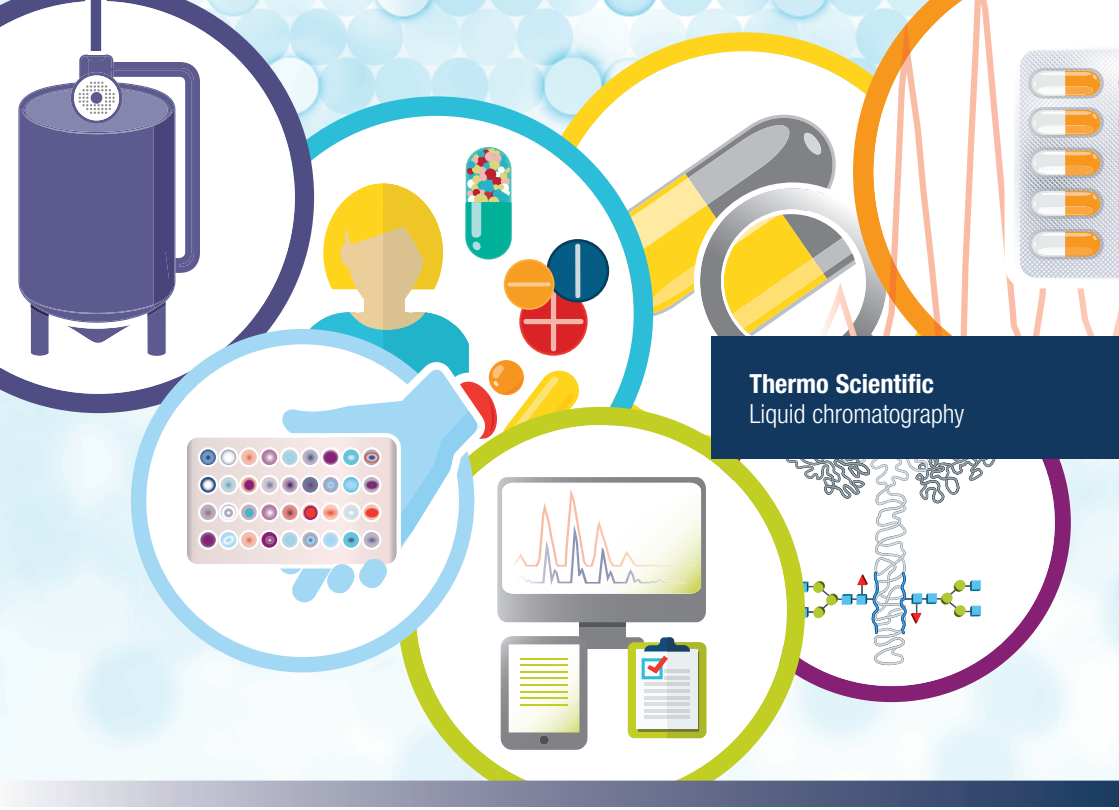
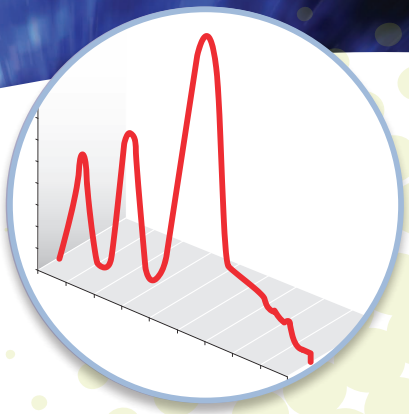


Liquid chromatography columns for every

biopharmaceutical analytical workflow

Thermo SCIENTIFIC



Aggregation

Antibodies are becoming increasingly important in the development and manufacture of drugs globally. During the manufacturing process, antibodies and other proteins can form aggregates – these occur when several structures join together to form larger, higher order structures. This can impact the efficacy of a drug, which ultimately can lead to severe side-effects for the consumer. Biopharmaceutical companies therefore have a requirement to accurately measure this aggregation to ensure that levels are minimized.

Recommended Column

Thermo Scientific™ MAbPac™ SEC-1 5 µm, 7.8 x 300 mm column
Product code: 088460

- Features a proprietary hydrophilic bonded layer, designed to minimize the interactions between the biomolecules of interest and the stationary phase
- Provide accurate quantification of aggregation level within the drug
- Confirmation of drug safety within regulatory requirements

Sample Preparation

Thermo Scientific™ SOLAµ™
SPE Plates for increased sensitivity

Sample Handling

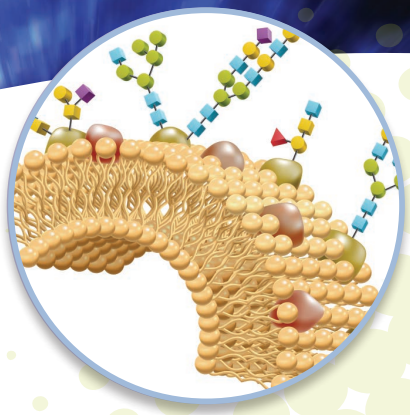
Thermo Scientific™ Virtuuso™ Vials
The highest level of sample integrity and sample information possible

Column

MAbPac SEC-1 BioLC Columns

UHPLC

Thermo Scientific™ Vanquish™
UHPLC Systems
Built for every biopharma workflow



Glycosylation

The majority of biotherapeutic proteins contain glycans. Glycans are sugars which are attached to the biotherapeutic compound i.e. they are glycosylated. Glycans are crucial for the function of the proteins and any changes or alterations, however small, can impact the performance and efficacy of the biotherapeutic. Regulatory bodies therefore mandate that full glycosylation characterization is carried out to determine the full pattern and confirm that the protein works as expected. As glycans can exist in both a released form and attached to the protein, multiple, complex measurements can be required.

Recommended Column

Thermo Scientific™ GlycanPac™ AXH-1
1.9 µm, 2.1 × 150 mm column
Product code: 082472

- Full glycosylation pattern as mandated by regulatory bodies
- Comprehensive Glycan profiling to confirm effectiveness of the biotherapeutic
- Complete characterization of both attached and released glycans by fluorescence and MS detection

Sample Preparation

SOLAµ SPE Plates for increased sensitivity

Sample Handling

Virtuoso Vials
The highest level of sample integrity and sample information possible

Column

GlycanPac AXH-1 Columns

UHPLC

Vanquish UHPLC Systems
Built for every biopharma workflow

Mass Spectrometry

Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometers
Quantify, confirm and characterize compounds in a single analysis

Charge Variants

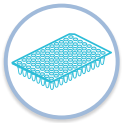
Biotherapeutic proteins consist of amino acids that can be either weakly acid or weakly basic. Proteins of this size and complexity, such as monoclonal antibodies, are far more heterogeneous than small-molecule drugs. The presence of the charged state can significantly impact the structure, stability, binding affinity and efficacy of the biotherapeutic drug. It is therefore necessary to understand the profile of the drug so that charge variants are identified and removed if necessary. Using this method, a wide range of antibodies can be targeted quickly and easily for determination of charge states.

Recommended Column

Thermo Scientific™ MAbPac™ SCX-10
10 µm, 4.0 × 250 mm column
Product code: 074625

- Characterization of charge variants, which can impact structure, stability, binding affinity and efficacy of biotherapeutics
- Comprehensive determination and quantification of compounds using sub-5 minute analysis times
- Used in conjunction with Thermo Scientific™ pH Gradient Buffers

Sample Preparation



SOLAµ SPE Plates for increased sensitivity

Sample Handling

Virtuoso Vials
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Column

MAbPac SCX-10 Columns

UHPLC

Vanquish UHPLC Systems
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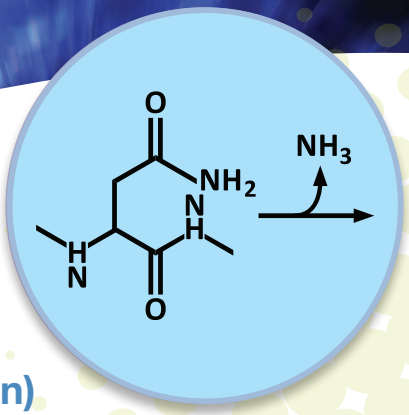
Mass Spectrometry

Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometers
Quantify, confirm and characterize compounds in a single analysis



Chemical Modification (deamidation and oxidation)

During the biotherapeutic protein production process, a large number of chemical and/or structural changes can occur. Two of the most common modifications are:

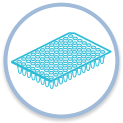
- Deamidation – damage of amide-containing side chains of amino acids such as asparagine and glutamine
- Methionine oxidation – which deactivates some protein activities

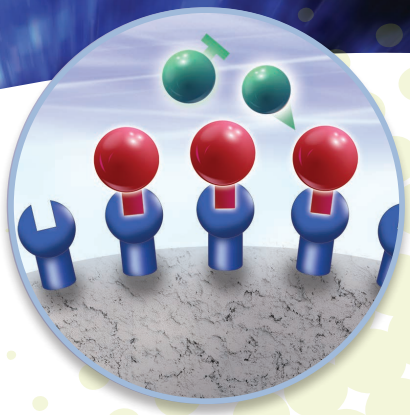
These chemical changes can reduce or even negate the effect of the protein. During protein characterization, confirmation of these modifications is required.

Recommended Column

Thermo Scientific™ MAbPac™ HIC-20 5 µm, 4.6 × 100 mm column
Product code: 088553

- Identification of unwanted chemical modifications, resultant of the protein production process; these modifications can affect the efficacy of the biotherapeutic protein
- Separation of mAb fragments from oxidized and damaged compounds

Sample Preparation	Sample Handling	Column	UHPLC	Mass Spectrometry
				
SOLAµ SPE Plates for increased sensitivity	Virtuoso Vials The highest level of sample integrity and sample information possible	MAbPac HIC-20 Columns	Vanquish UHPLC Systems Built for every biopharma workflow	Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometers Quantify, confirm and characterize compounds in a single analysis



Titer (Affinity)

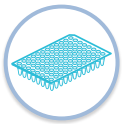
Affinity chromatography is based on the biospecific interaction between a biomolecule and its ligand. This interaction is reversible and therefore specific compounds can be isolated from complex matrices such as cell culture used for mAb production. By doing this, the concentration, or titer, of a biotherapeutic protein during the manufacturing process can be determined. Affinity titer is an extremely important technique in the biopharmaceutical industry as it is used during several stages of the biotechnological protein production process.

Recommended Column

Thermo Scientific™ MAbPac™ Protein A 12 µm, 4.0 × 35 mm column
Product code: 082539

- Full titer analysis in less than 2 minutes
- Linearity over a sample concentration range of 0.025 to 5mg/mL

Sample Preparation



SOLAµ SPE Plates for increased sensitivity

Sample Handling

Virtuoso Vials
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Column

MAbPac Protein A Columns

UHPLC

Vanquish UHPLC Systems
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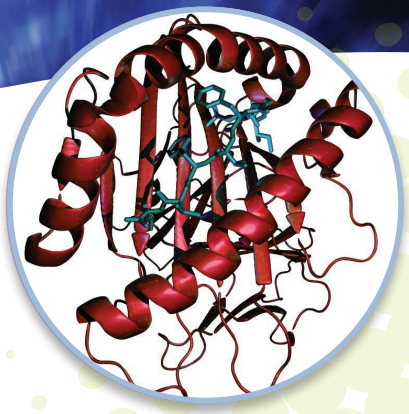


Peptide Mapping

Biotherapeutic proteins, like all proteins, can be digested into a number of smaller fragments called peptides. Mapping these peptides via peptide mapping is a commonly used identity test for proteins. Peptide mapping is used to verify the identity of the therapeutic protein and to monitor its structural integrity and or the presence of modifications. In conjunction with mass spectrometry, a full structural survey of the peptides and their modifications can be created which will reveal more about the protein than intact characterization. Peptide digestion can be made significantly easier using Thermo Scientific™ SMART Digest™ Kits.

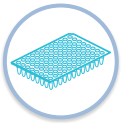
Recommended Column

Thermo Scientific™ Acclaim™ RSLC 120 C18
2.2 µm, 2.1 × 250 mm column
Product code: 074812

Acclaim PepMap RSLC C18
75 µm × 500 mm column
Product code: 164942

- Comprehensive separation and identification of peptide fragments
- Confirmation of the structural integrity of the therapeutic protein through identification of any modifications
- Reveals more information about the protein than intact characterization

Sample Preparation



SMART Digest Kits for rapid and reproducible digestion, SOLAµ SPE Plates for increased sensitivity

Sample Handling

Virtuoso Vials
The highest level of sample integrity and sample information possible

Column

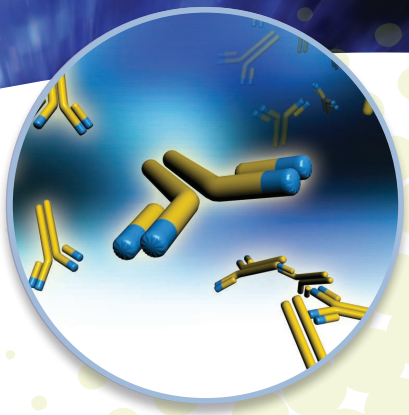
Acclaim RSLC 120 Columns and Acclaim PepMap RSLC C18 Columns

UHPLC

Vanquish UHPLC Systems
Built for every biopharma workflow

Mass Spectrometry

Q Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometers
Quantify, confirm and characterize compounds in a single analysis



Intact Mass

A significant amount of information about a protein can be determined using peptide mapping. However, additional information can also be found through characterization of the whole protein (or light and heavy chain fragments). Accurate mass and, to some degree, the heterogeneity of the protein can be determined. Intact mass is also an important step after manufacture, to ensure the stability of the biotherapeutic drug after storage. Intact mass characterization is sometimes referred to as “top down” for the proteins and is challenging compared to peptide mapping, due to the large mass and complex nature of the samples.

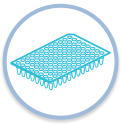
Recommended Column

Thermo Scientific™ MAbPac™ RP 4 µm, 2.1 × 50 mm column
Product code: 088648

Thermo Scientific™ ProSwift™ RP-5H 100 µm × 500 mm column
Product code: 164928

- Simplified process for the characterization of large and complex protein samples
- Reveals information not determined during peptide mapping, such as accurate mass and heterogeneity of protein
- Confirms stability of biotherapeutic drug after manufacturing

Sample Preparation



SOLAµ SPE Plates
for increased sensitivity

Sample Handling

Virtuoso Vials
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Column

MAbPac RP and
ProSwift RP-5H Columns

UHPLC

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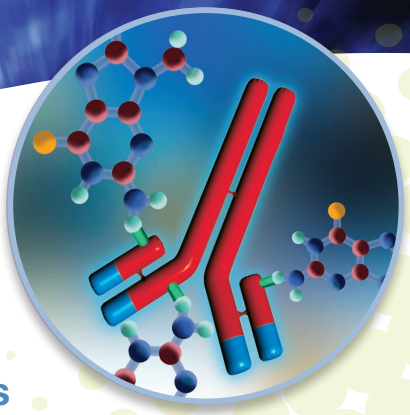
Mass Spectrometry

Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap Mass Spectrometers
Quantify, confirm and characterize compounds in a single analysis



Antibody Drug Conjugates

Antibody Drug Conjugates (ADCs) are a new and exciting class of drugs designed for the targeted treatment of certain cancers. They consist of a monoclonal antibody with a synthetic small molecule drug or drugs joined (conjugated) to them. Combining the capabilities of the monoclonal antibody with a cancer-killing drug allows sensitive discrimination between healthy and diseased cells. Development of these biotherapeutics is even harder than with monoclonal antibodies due to the potency of the attached drug, sometimes called the "payload" or "warhead". In addition to the analysis carried out on standard antibodies, further characterization is needed to determine how much of the drug is attached to the antibody. This is known as Drug to Antibody Ratio (DAR).

Recommended Column

Thermo Scientific™ MAbPac™ HIC-Butyl 5µm, 4.6 × 100 mm column
Product code: 088558

- Antibody drug conjugates (ADC) require extended characterization to determine the amount of drug attached to monoclonal antibody due to potency of attached drug
- Confirmation of Drug to Antibody Ratio (DAR) for ADC
- Stable at wide pH range 2-12

Sample Preparation

SOLAµ SPE Plates for increased sensitivity

Sample Handling

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Column

MAbPac HIC-Butyl Columns

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Mass Spectrometry

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Watson LIMS™ and Data Manager
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SOLAμ SPE Plates
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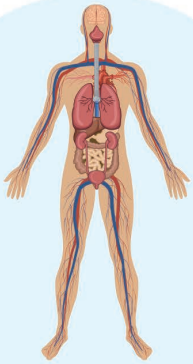
Dionex™ Chromeleon™
Chromatography Data System
Streamlining laboratory workflow



BioLC/UHPLC Columns

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Tribrid Mass Spectrometers
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