

Built for Biopharma

Thermo Scientific Vanquish Flex UHPLC Systems

Characterization of biotherapeutics including:

- Proteins
- Monoclonal antibodies
- Antibody drug conjugates

Engineered to build your

DRUG PIPELINE

The world of biotherapeutic characterization is undergoing a revolution. Development of evermore complex protein-based therapeutics places rigorous demands on analytical technologies.

Scientists require powerful and flexible solutions to fully characterize biotherapeutic proteins.

The innovative architecture of the new Thermo Scientific™ Vanquish™ Flex UHPLC systems is tailored to the specific and demanding analytical requirements that these essential drugs impose.

Vanquish Flex UHPLC systems have been designed and manufactured to exacting engineering standards to be truly

“Built for Biopharma.”





• • The ability to transfer the existing method to Vanquish easily in the beta test contributed in the purchase decision for multiple Vanquish systems. • •

Principle investigator, top biopharma producer

Vanquish Flex UHPLC delivers **CONFIDENCE**, with a fully biocompatible flow path and proven compliance. Achieve unprecedented **PERFORMANCE** in retention time stability, sensitivity and separation efficiency with the widest range of column chemistries for biotherapeutic proteins. Demand **VERSATILITY** through seamless integration with market-leading mass spectrometry, ultraviolet, fluorescence and charged aerosol detection. Gain operational **SIMPLICITY** with easy, freely available, one-click workflows via AppsLab and tool-free Viper fittings.



Innovative

ARCHITECTURE

BETTER RETENTION TIME STABILITY:

Vanquish Flex UHPLC offers SmartInject to give you better retention time stability, sharper peaks, and maximise column lifetime.

LESS MAINTENANCE AND LONGER COLUMN LIFE:

With a novel modular design and all-new ceramic valves, Vanquish Flex UHPLC is designed for ease-of-use and longevity.

1 Complete biological characterization:

Differing molecular characteristics of complex biomolecules necessitates multiple detection technologies. The system has been built to accommodate a range of analytical detectors, including mass spectrometry, diode array detection (DAD), charged aerosol detection (CAD), variable wavelength detection (VWD) and fluorescence detection (FLD).

2 Easier sample handling:

Automation of workflows via barcode reading.

3 Higher sample throughput:

High plate capacity as standard (4 plates vs 2 plates on some competitor systems). Increase capacity further with the addition of the Thermo Scientific™ Vanquish™ Charger Module

4 Accurate flow for more data confidence:

Improved data precision with up to 10 x better flow accuracy than some UHPLC systems (0.1% vs 1%), plus 10 x lower and 4 x higher flow rate capability than some systems.

5 Empowering workflow design:

The power and flexibility to deliver excellent binary, ternary and quaternary gradients, ideal for method scouting and buffer blending.



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TPFTSRLSIN
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TAALGCLVKD
LYSLSSVTV
VERSATILITY
VSHEDPEVKF
LTVLHQDWLN
DELTKNQVSL
PVLDSGDSFF
YTQKSLSLSP
TNIHWYQQRT
FTLSINSVES
PEPTIDE MAP
ESVTEQDSKD
RGA
NYGVHWVRQS
KDNSKSQVFF
STKGPSVFPL
PERFORMANCE
ICNVNHKPSN
GGPSVFLFPP
NAKTKPREEQ
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VARIANTS
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TFPAVLQSSG
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AGGREGATES
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YFPEPVTWSW
PSSSLGTQTY
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TKVDRVEPK
KPKDTLMISR
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ISKAKGQPRE
DIAVEWESNG
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ASESISGIPS
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LVQPSQSLSI
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QPENNYKTTP
SVMHEALHNN
FSCRASQSIG
RFSGSGSGTD
AAPSVFIFPP
DNALQSGNSQ
LSSPVTKSFN
TCTVSGFSLT
INTACT
QGTLTVSAA
YFPEPVTWSW
PSSSLGTQTY
CPPCPAPELL
NWWYDGVVH
GKEYKCKVSN
WORKFLOWS
FLEX UHPLC



EASIER LABORATORY INTEGRATION:

Compact footprint; shorter and narrower than competitor products.

AT-A-GLANCE STATUS:

Always know what your UHPLC is doing: Intuitive status indicator with clear, external color coded lighting.

6 Versatile column management:

Greater column capacity (2 x 30 cm columns or a greater number of shorter columns), plus class-leading temperature range (5 - 120 °C) and temperature stability (+/- 0.05 °C).

7 Versatile applications:

Analyse monoclonal antibodies (mAbs), antibody drug conjugates (ADCs), fusion proteins, and more with ease. Compatible with all Thermo Scientific™ Bio LC columns, including Thermo Scientific™ MAbPac™, Thermo Scientific™ GlycanPac™, Thermo Scientific™ ProPac™ and Thermo Scientific™ Accucore™ Vanquish™ columns.

8 Easier operation:

Vanquish systems use patented finger - tight Thermo Scientific™ Viper™ fittings throughout, offering near-zero dead volume and ergonomic design.

9 Precise sample handling:

SmartInject technology facilitates longer column lifetime, and 10 x lower injection volume (0.01 µL) than some competitor systems, with the highest precision (<0.25%) at low injection volumes (< 1 µL) and high reproducibility.

10 Simple servability:

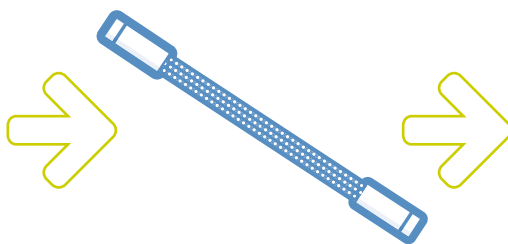
Modules can be serviced without de-stacking, each unit has handles for ease of manoeuvre, plus indicator lights in the pump module and tool - free user maintenance.

AGGREGATES

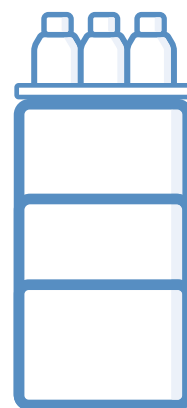
mAbs produced from mammalian cell culture may contain significant amounts of dimers and higher-order aggregates. Size exclusion chromatography (SEC) is a well-accepted technique for the detection and accurate quantification of protein aggregates in biological drug products. It is also routinely used for the characterization and quality control of mAb products. Regulatory bodies typically impose a limit on the acceptable degree of aggregation that a biotherapeutic may exhibit, therefore the confirmation of aggregation is vital throughout the drug development and production process. Our solutions for aggregation screening are rapid and reliable, giving you confidence in your ability to identify and separate higher order structures.



Dimers, trimers and further higher order structures affect clinical efficacy and must be determined and quantified, typically by size exclusion chromatography (SEC).

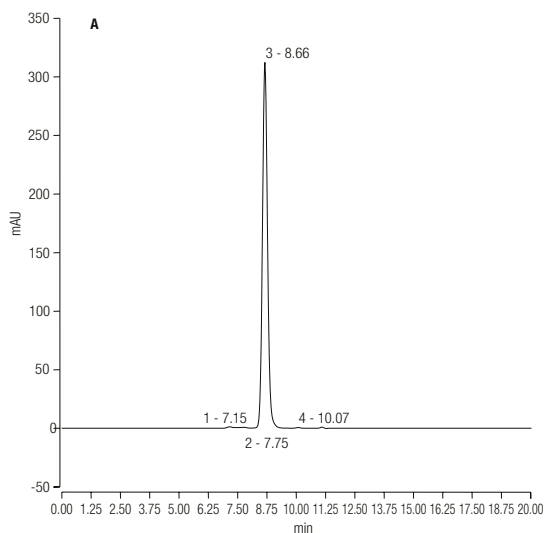


Our recommended SEC column, the MAbPac SEC-1, uses spherical, fully porous ultrapure silica, typically with a 5 μm particle size and 50-300mm column length.

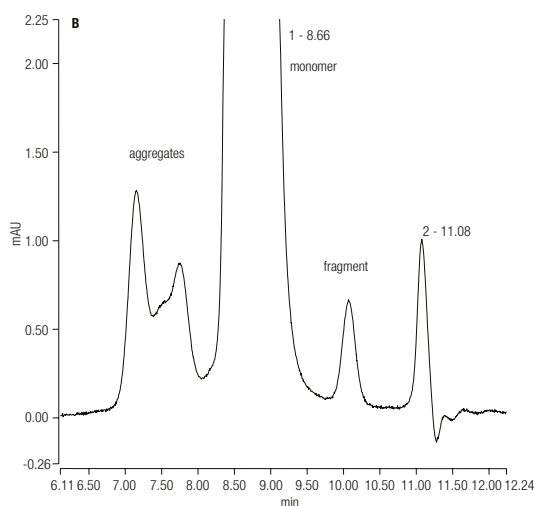


Vanquish Flex UHPLC provides high sensitivity, high resolution SEC in high- and low-salt mobile phases and volatile eluents.

Aggregation screening requires a powerful and flexible UHPLC. But column selection also plays a critical part in this workflow. MAbPac SEC-1 has a proprietary hydrophilic bonded layer that results in minimal non-desired interactions between the stationary phase and the biomolecules. Its nonmetallic and biocompatible PEEK housing eliminates metal contamination from the column.



Rituximab aggregate analysis on the MAbPac™ SEC-1, 5 μ m, 7.8 \times 300 mm SEC column. (A) Full range chromatogram (B) Expanded view.

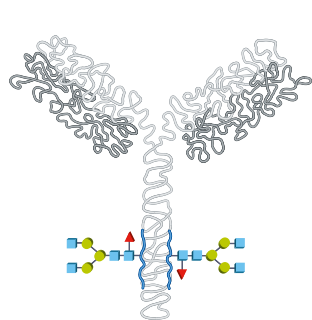


Compliant, simple, data management and reporting is performed using Thermo Scientific™ Chromeleon™ Chromatography Data Systems (CDS) software.

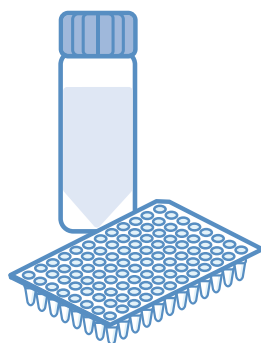


GLYCANS

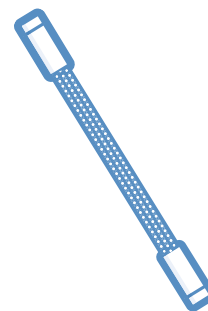
Even small changes in the type, composition or linkage of attached glycans can alter biotherapeutic efficacy, meaning that correct description is vital. We have a range of powerful and innovative techniques from sample labelling through ion chromatography and liquid chromatography to mass spectrometry.



Multiple glycosylation characterization techniques are possible; released glycans, glycopeptides, or intact protein analysis.



Released glycans are typically labelled for fluorescence detection (FLD).



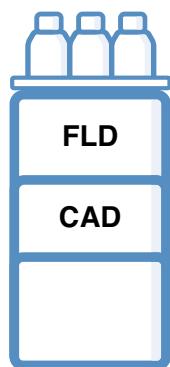
GlycanPac columns have innovative mixed-mode surface chemistry, combining weak anion exchange (WAX) and reversed phase (AXR) or HILIC (AXH) functionalities for separations according to charge, size, and in the case of GlycanPac AXR-1, isomerism.



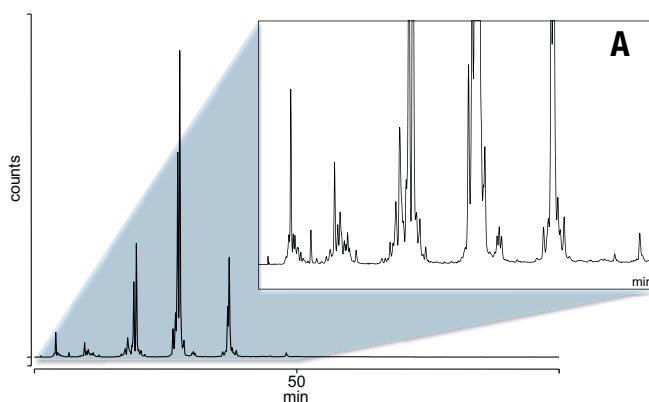


The Vanquish Flex CAD detector

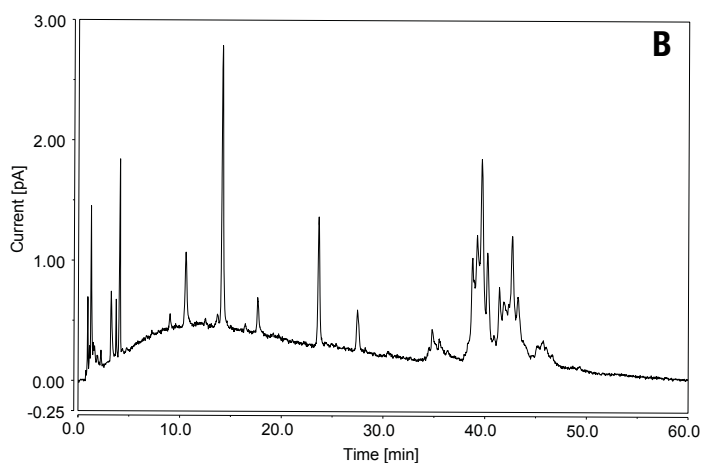
Detection of labelled glycans using FLD is well established, an example of this is shown for *N*-linked glycans in the upper of the two chromatograms below (A). It can also be an advantage to detect unlabelled glycans. The Vanquish Flex Charged Aerosol Detector (CAD), depicted above, offers label-free detection. It has consistent analytical response independent of chemical structure and a dynamic range of four orders of magnitude. CAD data of unlabelled *O*-linked glycans is shown in the lower of the two chromatograms below (B).



FLD is perfect for labelled glycans at the lowest concentrations. Charged aerosol detection (CAD) is ideal for unlabelled glycans.



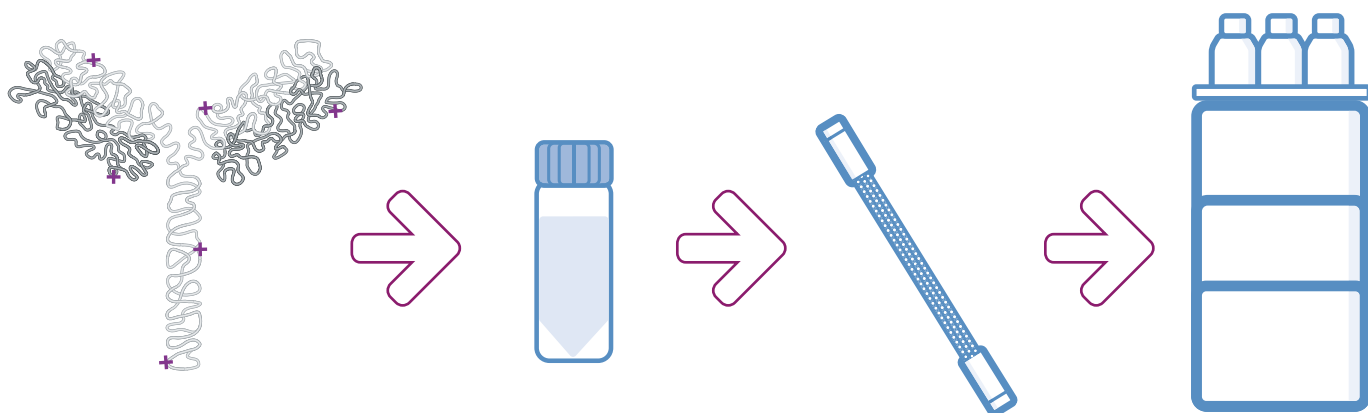
Six replicate injections of labelled *N*-glycans from fetuin using GlycanPac AXR-1 column.



Fetuin *O*-linked native glycans analyzed by UHPLC-CAD using GlycanPac AXH-1.

CHARGE VARIANTS

Protein charge homogeneity can have a significant effect on the structure, stability, binding affinity, and efficacy of a biotherapeutic drug. Ion exchange chromatography (IEX) is often used to profile charge variants, with either salt or pH gradient.



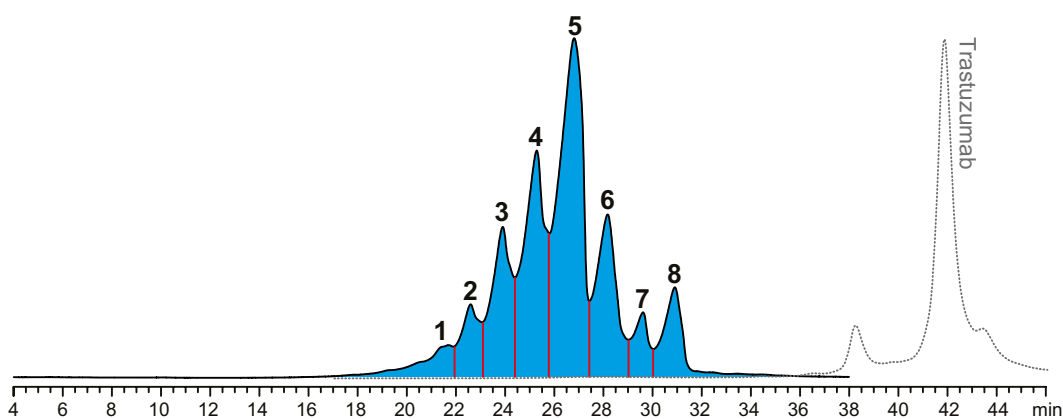
Antibody charge variants must be characterized. Separation according to charge is required, usually by IEX.

Our dedicated Thermo Scientific™ CX-1 pH buffer kits allow set up of reproducible and linear pH gradients with ease.

MAbPac SCX-10 is a strong cation exchange column designed specifically for high-resolution separations of antibodies and associated variants by IEX.

Vanquish Flex UHPLC is ideal for routine, high throughput ion exchange chromatography employing generic and/or steep gradients, being fast, robust and reliable and having twice the sample capacity of many UHPLC systems.

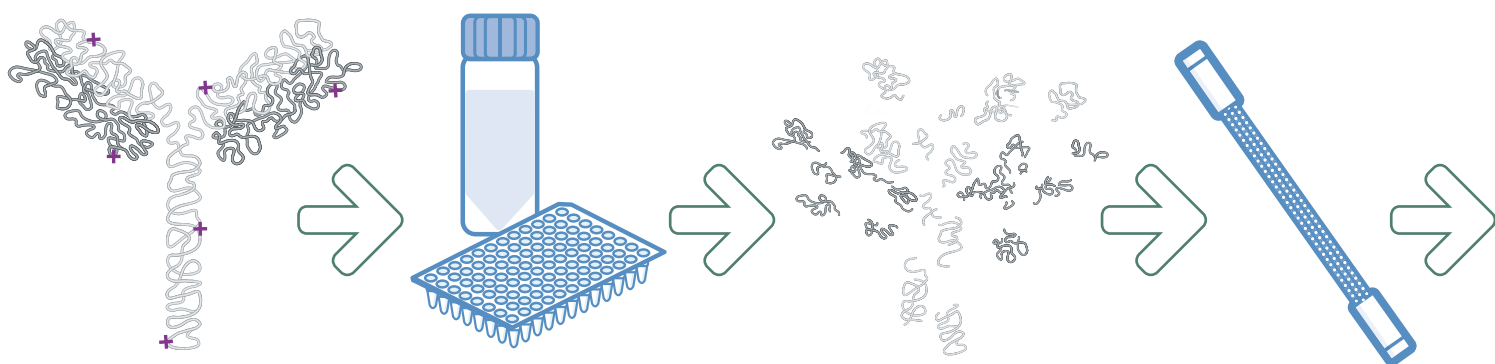
Identification and separation of charge variants is performed rapidly and reliably. The use of a pH gradient-based system can be more robust and less challenging than a salt-based system. The unique nonporous pellicular resin used in MAbPac SCX-10 provides high resolving power, permitting the separation of monoclonal antibody variants that differ by as little as one charge.



Charge variant profile of cetuximab as analysed on the MAbPac SCX-10 column. Cetuximab presents a very complex charge variant profile due to heterogeneity arising from N-glycosylation in both Fc and Fab regions bearing sialylation and additional modifications such as C-terminal lysines.

PEPTIDE MAPPING

Peptide maps that detail the entire protein (100% sequence coverage) are required to prove molecular structure as well as determine post-translational modifications (PTMs). Complex protein digests require high peak capacity and high-resolution separations. Our peptide mapping workflow solution includes a fast, reproducible digestion system and bespoke peptide identification software, with Vanquish Flex UHPLC at its heart.



Prior to peptide mapping, proteins must be digested into their constituent peptides, typically using an enzyme.

Thermo Scientific™ SMART Digest™ kits are designed for biopharmaceutical applications that require highly reproducible, sensitive and fast analyses, with digests taking <60 minutes, for high-throughput routine workflows.

UV and MS detection are both widely used in peptide mapping. UV is often used in routine environments.

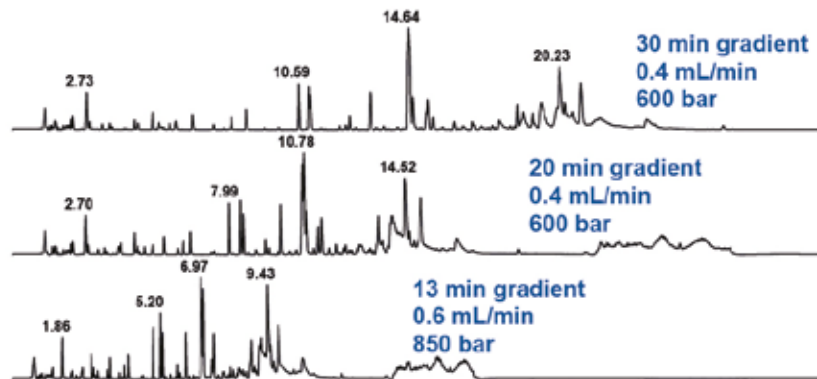
The Acclaim VANQUISH C18 column has an ultrapure silica substrate with extremely low metal content to minimize tailing effects and deliver the sharp, symmetrical peak shapes needed in a peptide map.



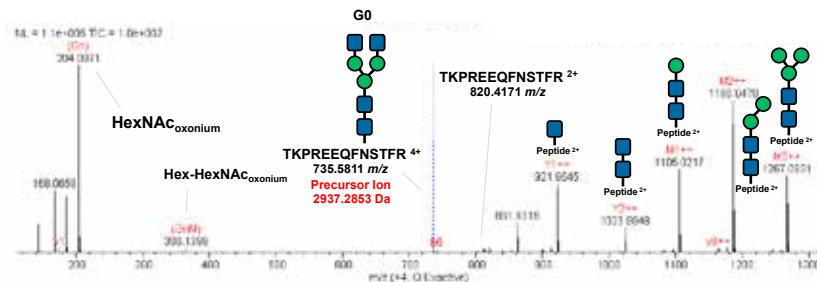
MASS SPECTROMETRY

Thermo Scientific Orbitrap™ mass spectrometers and Thermo Scientific™ BioPharma Finder™ Mass Informatics platform software provide accurate identification, in-depth characterization, and relative quantitation of therapeutic proteins.

For the ultimate in peptide mapping Orbitrap™ mass spectrometry is the gold standard.



Total ion chromatograms obtained from peptide mapping experiments of rituximab applying gradient lengths of 30, 20 and 13 minutes.

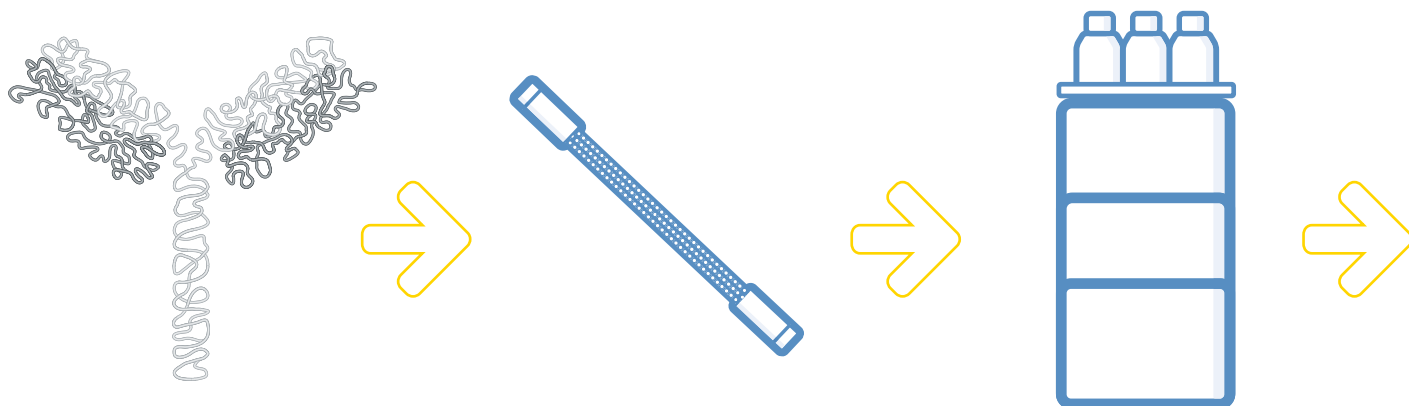


MS/MS spectrum of the rituximab glycopeptide aa 290-302 (TKPREEQFN*STFR, *=G0) with the typical fragmentation pattern: the two oxonium ions 204 (HexNAc), 366 (Hex-HexNAc), and the sequence ladder of the fragmented glycan attached to the intact peptide.

Vanquish Flex has a 1000 bar upper pressure limit and SmartInject technology to ensure high peak capacity, retention time stability and peak area precision, ideal for peptide mapping applications.

INTACT

There remains a need to characterize biotherapeutic proteins in the intact level, particularly where there is likelihood of structural information such as isomerism that may not be observed by other techniques. It is also important in some of the novel biotherapeutic classes such as ADCs. Our range of hydrophobic interaction chromatography (HIC) columns are particularly well suited to the characterization of ADCs, allowing drug-to-antibody ratio (DAR) determination in minutes.



mAbs and ADCs require thorough characterization, including glycoforms, higher order structures and PTMs.

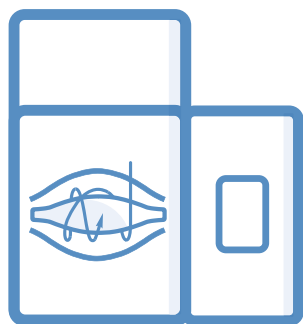
MAbPac RP is a new reversed phase column that uses a resin based on hydrophobic supermacroporous 4 μm polymer particles. It is ideal for the efficient separation of protein molecules with very low carry over.

UHPLC is the most effective technique to ensure separation of protein variants and unwanted interferents.

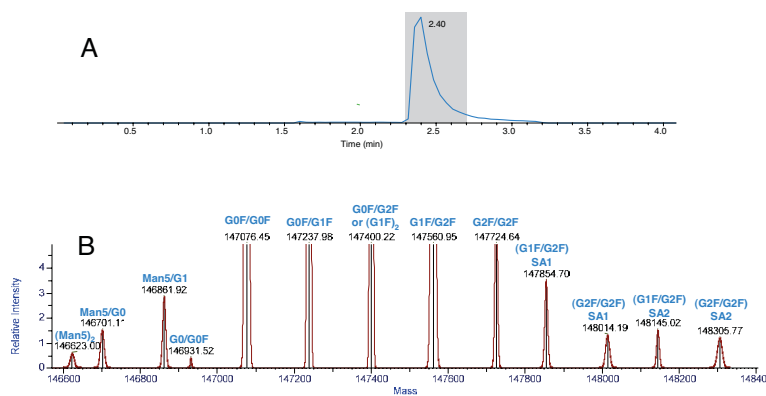


The Vanquish Flex UHPLC systems readily hyphenate to the full range of Thermo Scientific mass spectrometers, including the gold - standard in HRAM, the Orbitrap. In addition to the HRAM capabilities required for intact protein characterization, the Q Exactive BioPharma Platform offers an extended high mass range (HMR) mode up to 8000 Da, perfect for the analysis of native intact proteins. When combined with BioPharma Finder Mass Informatics platform, the Vanquish Flex UHPLC and Q Exactive BioPharma is a biopharmaceutical characterization workhorse.

Reproducibility of protein separation is as important as resolution and sensitivity. Vanquish Flex UHPLC produces excellent separation reproducibility giving you the confidence you need.



MS techniques for intact proteins should ideally be high resolution accurate mass (HRAM) for maximum quantification and structural elucidation possibilities.



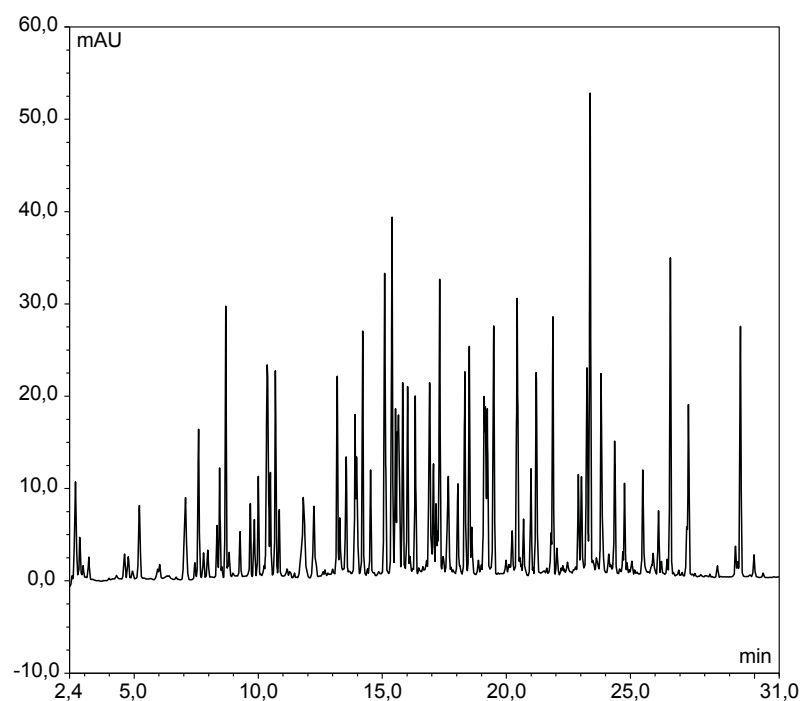
MS analysis of 100 ng of the mAb Rituximab, depicting (A) LC chromatogram using Thermo Scientific™ MSPac™ DS-10 Desalter Cartridge, and (B) the deconvoluted spectrum and annotated glycoforms for the lower intensity peaks.

CONFIDENCE

Development of Vanquish Flex UHPLC utilized a Quality by Design philosophy to maximise the key pharmaceutical attributes of robustness and reliability. Engineered with exceptional quality components and exacting manufacturing standards, to deliver the highest performance and retention time stability.

Our world-wide network of demonstration and application facilities offer highly qualified application scientists to ensure that you are confident to utilise Vanquish Flex UHPLC to its full potential.

Unity Lab Services is one of the largest scientific support networks in the world. It has the reach to ensure maximum up-time no matter where your research and manufacturing facilities are located.



Overlay of 13 subsequent injections of a trypsin digested mAb.

Retention Time (min)	Retention Time RSD (%)	RSD Area (%)
3.171	0.077	0.61
7.601	0.077	0.31
10.702	0.042	0.22
14.217	0.028	0.24
18.345	0.036	0.77
22.912	0.018	0.79
26.137	0.013	0.20
29.438	0.007	2.10

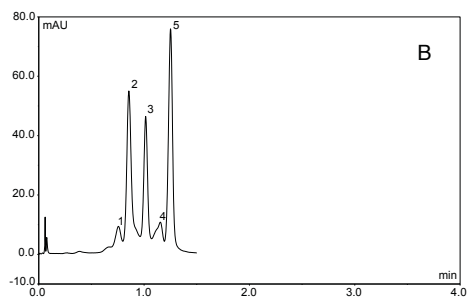
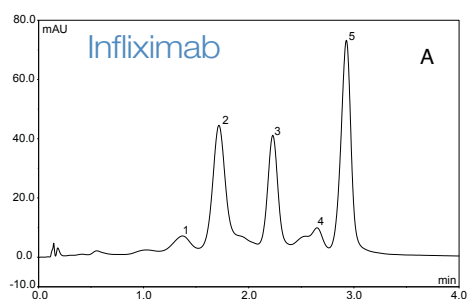
Retention time precision for the major peaks in the same mAb digest.

PERFORMANCE

Vanquish Flex has a fully biocompatible flow path, for the lowest possible carryover and SmartInject to provide the ultimate retention time stability and sharper peaks.



Significantly increase your throughput with the optional Vanquish Charger module. This robotic unit is fully integrated into Chromeleon for environmentally controlled sample management and automated sample loading into the Vanquish system.



Sub - two minute gradient of the monoclonal antibody infliximab using MAbPac SCX-10.

The ability to separate charge variants quickly and confidently is extremely useful. Here, pH range and gradient slope were modified to reduce gradient time to under two minutes.

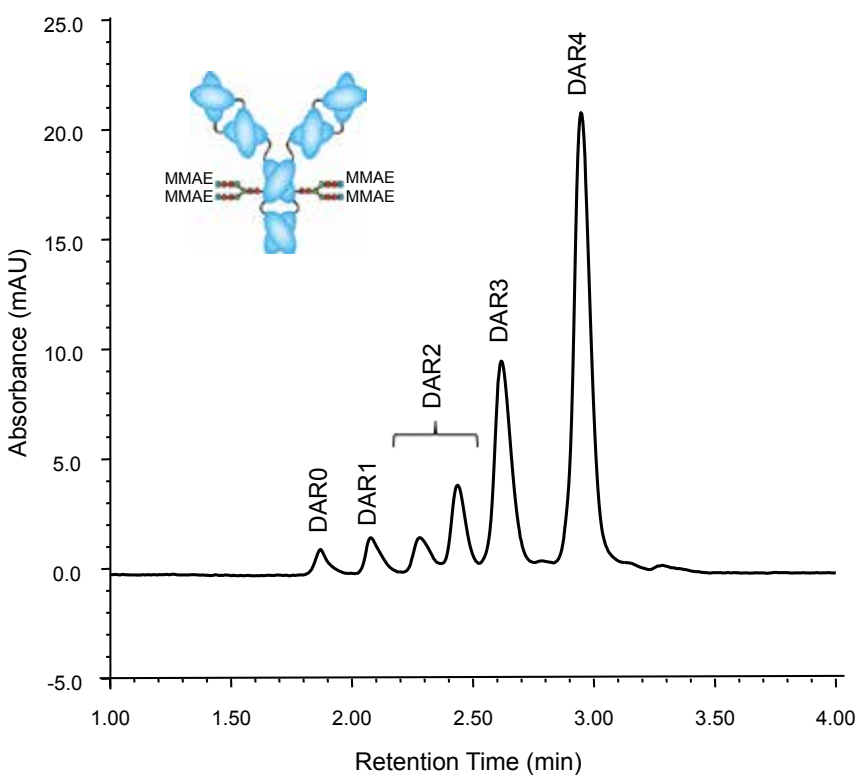
Separation A contains a pH range of 6.5 to 7.4 and a gradient range of 20-40% B whereas separation B shows a pH range of 6.4 to 6.8 and a gradient range of 18-27% B. The sample is infliximab, and the number of variants resolved remains the same.

VERSATILITY

From charge variance to aggregation, from glycosylation to intact proteins, Vanquish Flex UHPLC has the versatility to address the specific needs of biopharmaceutical characterization.

To create powerful biopharmaceutical workflows, Vanquish Flex leverages the entire Thermo Scientific range of column chemistries. ProPac, Acclaim and MAbPac are market-leading stationary phase technologies that can be directly applied to specific biomolecular entities.

Vanquish Flex systems can be interfaced to a wide range of detectors, from CAD and fluorescence detection to our high performance DAD with Lightpipe technology. It also has double the capacity of well plates of its competitors, and unrivalled temperature control for fully optimized characterization conditions.



Showing the versatility of UHPLC, the new Thermo Scientific MAbPac RP column is used for Drug Antibody Ratio (DAR) measurements of an antibody drug conjugate in under four minutes.

SIMPLICITY

Thermo Scientific™ AppsLab Library of Analytical Applications one-click workflows for Chromeleon can significantly reduce method development time. The Library of Analytical Applications is a fully searchable on-line method repository where you can find applications with detailed method information, chromatograms and related compound information.



AppsLab methods are easily searched on-line, with clearly identified expected peaks.



The Vanquish Flex system is tool-free, meaning rapid and painless day-to-day usage. It utilizes Viper near-zero dead volume fittings, for genuine plug-and-play operation.

The column compartment has a slim, vertical design. It's stackable for multiple columns, has multiple heating modes, and can take long column lengths, offering simple and fast method transfer.

COMPLIANCE

In the heavily regulated environment of a biopharmaceutical production facility, ensuring compliance with regulatory standards is vitally important.

Chromeleon CDS provides full integration of Vanquish Flex UHPLC with MS solutions, allowing you to quickly and easily process and report UV, FLD, CAD and MS data in one application. It streamlines your data processing and reporting and simplifies instrumental control, working under an enterprise-client environment.



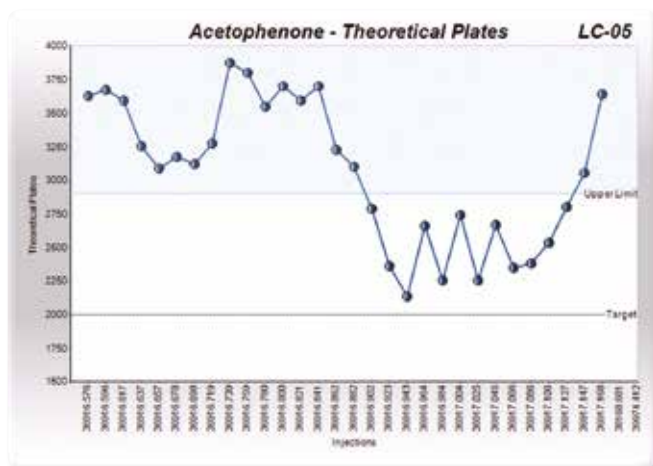
Chromeleon is compliance ready; it supports **GxP** and **21 CFR Part 11**. It is robust, secure, and administrator-controlled. Furthermore, it allows for file storage that is fully secure.

Chromeleon also minimises operational errors. **“Right first time”** analysis allows intelligent run control to get you from sample to result even faster. Import and export functions allows the seamless transfer of data between laboratory systems. It even allows you to trace all actions performed in the software, quickly and easily, using Chromeleon’s built in audit trails.

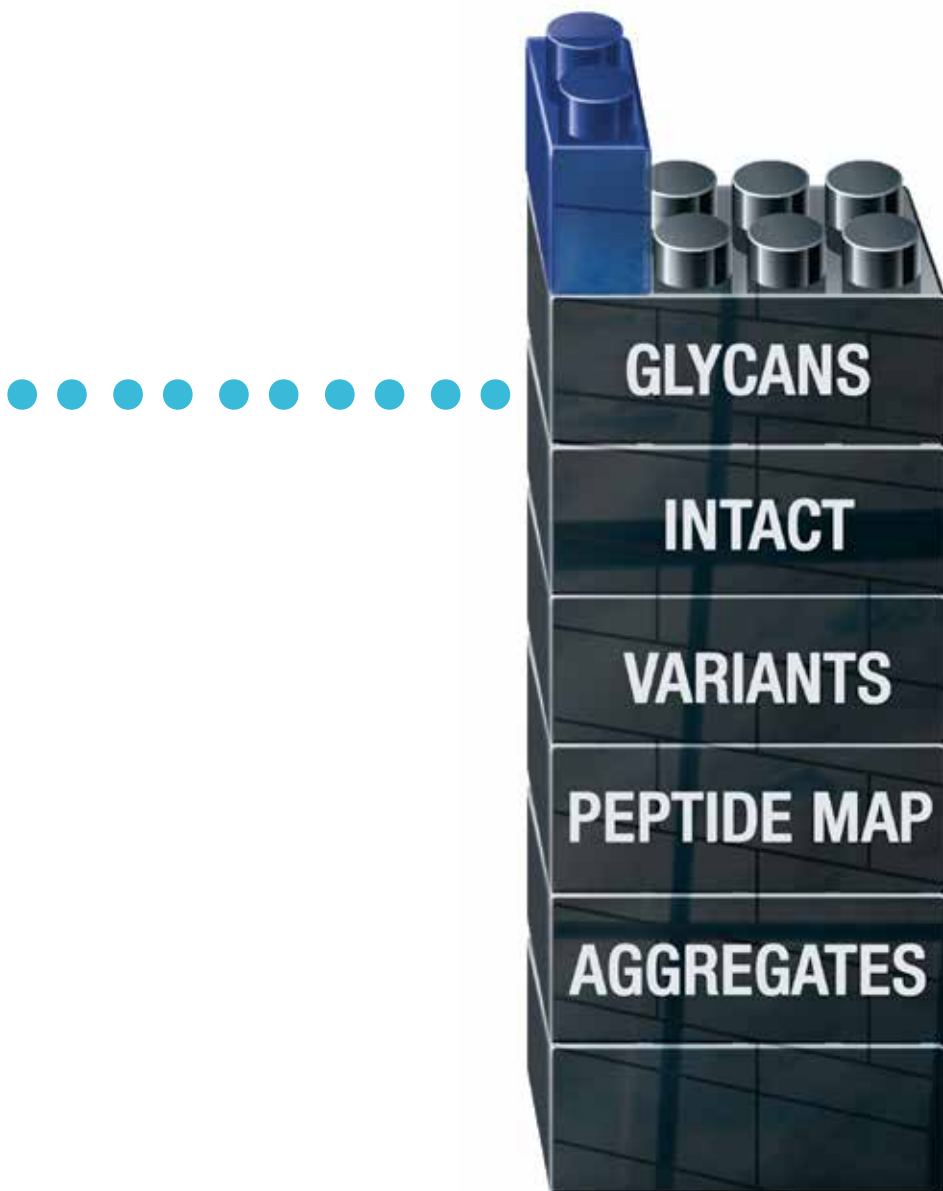
Chromeleon CDS supports MS along with gas, liquid and ion chromatography.

THERMO SCIENTIFIC APPSLAB LIBRARY OF ANALYTICAL APPLICATIONS

Find the best solution to your separation challenges; easily download **ONE-CLICK** workflows for use with Chromeleon CDS, working within the same fully-compliant regime as Chromeleon. It has an ever-expanding database of field-tested workflows that you can freely access.
thermofisher.com/Appslab



Chromeleon System Suitability Tests are used for trend monitoring. Chromeleon includes tools that facilitate generating control charts to monitor chromatographic results over time. These charts can be used to identify trends or anomalies in the performance of systems, methods or users.



Find out about our biocompatible systems that are built for biopharma
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