Thermo Scientific
Vanquish Flex UHPLC System

Built for Biopharma

Characterization of biotherapeutics including:
Proteins
Monoclonal antibodies
Antibody drug conjugates

Thermo SCIENTIFIC

Engineered to build your

DRUG PIPELINE

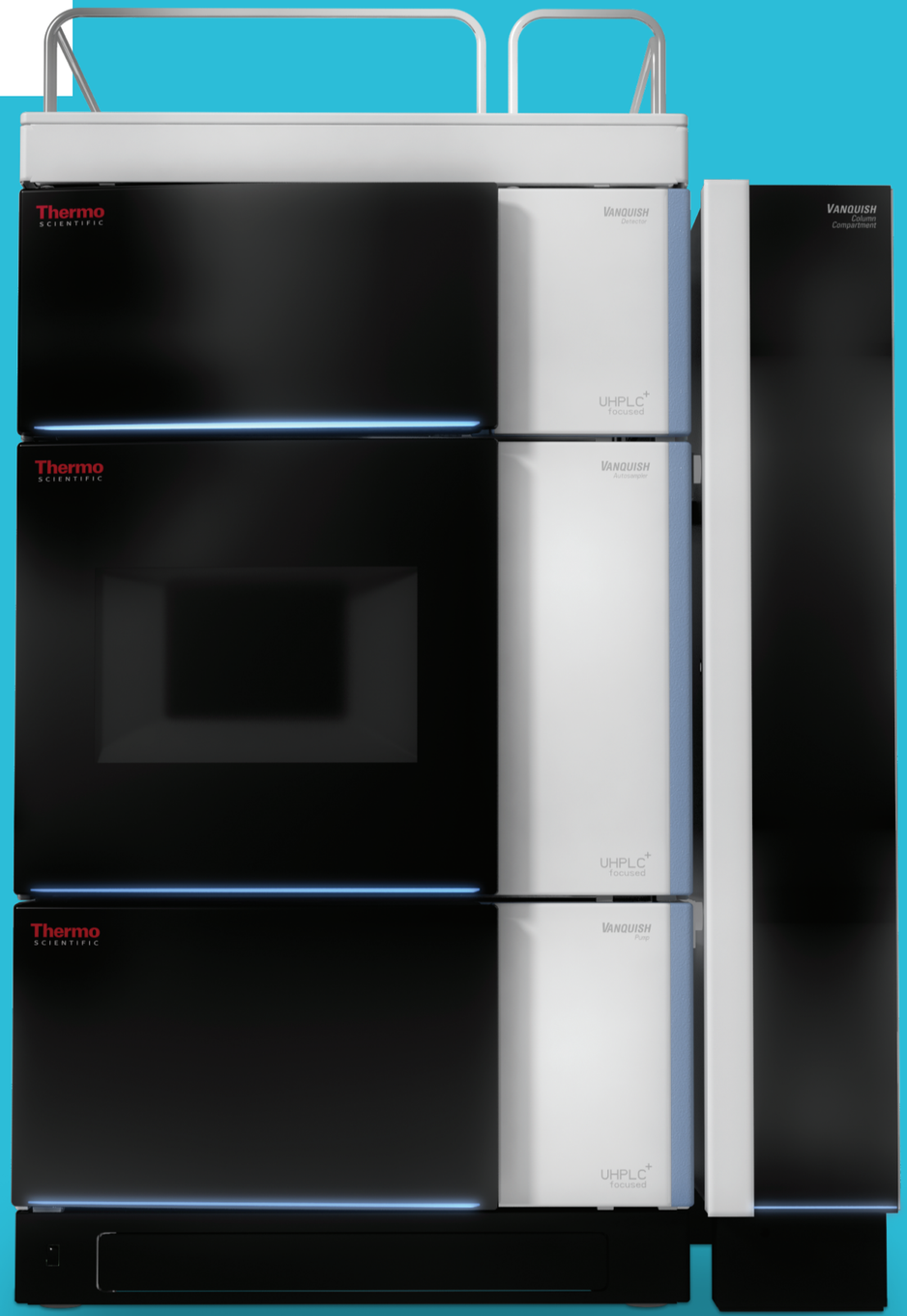
The world of biotherapeutic characterization is undergoing a revolution. Development of evermore complex protein-based therapeutics places rigorous demands on analytical technologies.

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

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

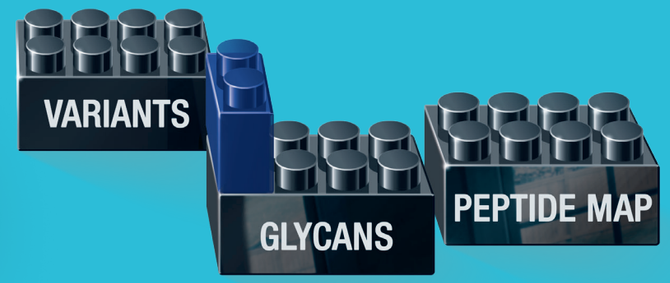
Vanquish Flex UHPLC has been designed and manufactured to exacting engineering standards to be truly

"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, 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 sample pre-compression to give you better retention time stability, sharper peaks, and minimizes column replacements.

LESS MAINTENANCE AND LONGER COLUMN LIFE:
With novel modular design and all-new ceramic valves, Vanquish Flex UHPLC is designed for longevity. It's also fully biocompatible and utilizes sample pre-compression to extend column lifetime.

1 Complete biological characterization:
Differing molecular characteristics of complex biomolecules necessitates multiple detector methodologies. The system has been built to utilize a wide 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 still via the optional charger.

4 Accurate flow for more data confidence:
Improved data precision with up to ten times 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.

QVQLKQSGPG
PGKGLEWLGV
KMNSLQSNDT
SIMPLICITY
NSGALTSGVH
TKVDKRVEPK
KPKDTLMISR
YNSTYRVVSV
ISKAKGQPRE
DIAVEWESNG
GLYCANS
LSVSPGERVS
ASESISGIPS
GTKLELKRTV
PREAKVQWKV
VYACEVTHQG
LVQPSQSLSI
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TAALGCLVKD
LYSLSSVVTV
VERSATILITY

CONFIDENCE
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TPEVTCVVVD
AGGREGATES
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SVMHEALHNH
FSCRASQSIG
RFSGSGSGTD
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LSSPVTKSFN
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INTACT
QGTLVTVSAA
YFPEPVTVSW
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CPPCPAPELL
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GKEYKCKVSN
WORKFLOWS
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GK
NGSPRLLIKY
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PERFORMANCE
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WORKFLOWS
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GLYCANS
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VERSATILITY
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DELTKNQVSL
VANQUISH

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PERFORMANCE
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DNALQSGNSQ
LSSPVTKSFN
TCTVSGFSLT
INTACT
QGTLVTVSAA
YFPEPVTVSW
PSSSLGTQTY
CPPCPAPELL
NWYVDGVEVH
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 (two 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:
Excels with mAbs, ADCs and fusion proteins. Compatible with all Thermo Scientific™ Bio LC columns, including Thermo Scientific™ MabPac™, Thermo Scientific™ GlycanPac™, Thermo Scientific™ ProPac™ and Thermo Scientific™ Accucore™ Vanquish.

8 Easier operation:
Uses patented finger tight Thermo Scientific™ Viper™ fittings with near-zero dead volume operation and ergonomic design.

9 Precise sample handling:
Has sample pre-compression for longer column lifetime, and ten times lower injection volume (0.01 µL) than some competitor systems and the highest precision (< 0.25%) at low injection volumes < 1 µL with high reproducibility.

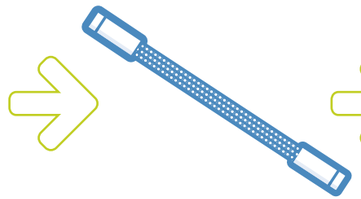
10 Simple servicability:
Modules can be serviced without de-stacking, each unit has handles for ease of manoeuvre, plus indicator lights in the pump module and tool free user maintenance.

AGGREGATES

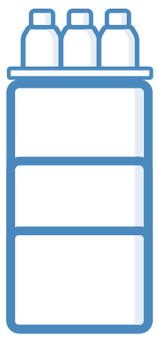
Regulatory bodies typically impose a limit on the acceptable degree of aggregation that a biotherapeutic may exhibit, and therefore the confirmation of aggregation is vital throughout the drug development and production process. Our solutions for aggregation screening are rapid and reliable, giving you confidence in your ability to identify and separate higher order structures.

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.

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

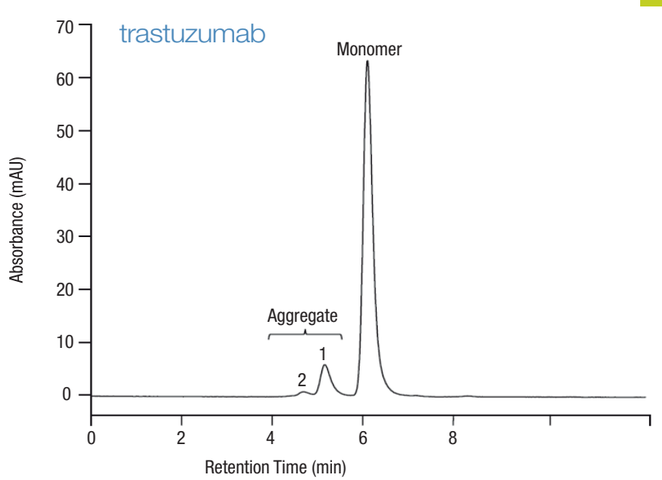


Our recommended SEC column, MAbPac SEC-1, uses spherical, fully porous ultrapure silica, typically with a 5 µm particle size and multiple column lengths.



Vanquish Flex UHPLC provides high sensitivity, high resolution SEC even under non-denaturing conditions, and in high- and low-salt mobile phases and volatile eluents.

Compliant, simple, data management and reporting is performed using Thermo Scientific™ Dionex™ Chromeleon™ CDS.



Baseline separation of trastuzumab monomer and aggregate with simultaneous detection of main compound and aggregates using MAbPac SEC-1.

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 characterization techniques are possible; as free gycans, as glycopeptides, or as intact proteins.

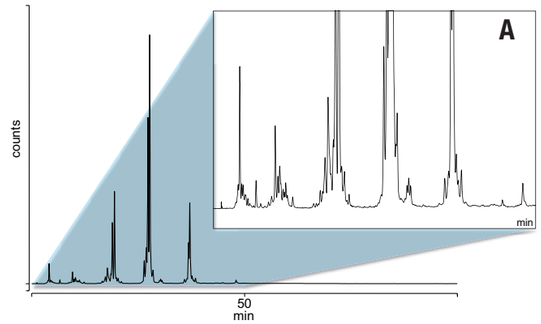
Glycans can be labelled for easier detection, a typical example being fluorescence labelling for detection using a fluorescence detector (FLD).

GlycanPac columns have innovative mixed-mode surface chemistry, combining WAX and reversed phase or HILIC functionality for separation according to charge, size, and in the case of GlycanPac AXR-1, isomerism.

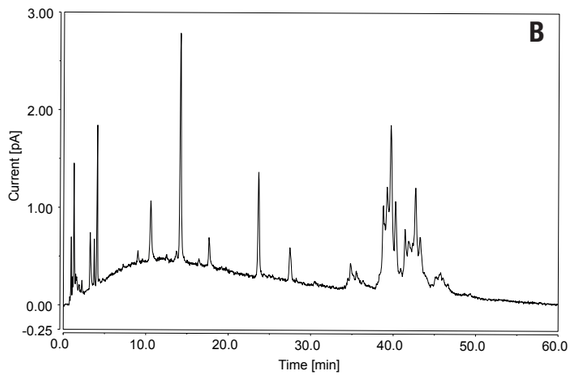
Vanquish Flex system is fitted with a fluorescence detector, which is perfect for labelled glycans at the lowest concentrations. It also has a CAD detector, ideal for unlabelled glycans.

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). However, 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).



Six replicates of separation of labelled N-glycans from fetuin using GlycanPac AXR-1 column.

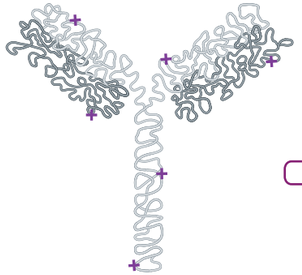


Fetuin O-linked native glycans analyzed by UHPLC-CAD using GlycanPac AXH-1.

CHARGE VARIANTS

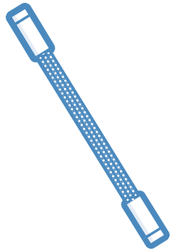
Protein charge homogeneity can have a significant effect on the structure, stability, binding affinity, and efficacy of a biotherapeutic drug. Ion exchange chromatography (IEX) is often used to profile charge variants, either by salt gradient or more recently by pH gradient.

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.

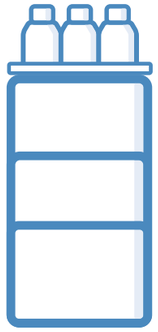


Antibody variants may have different charge states, meaning separation according to charge is required, usually by IEX.

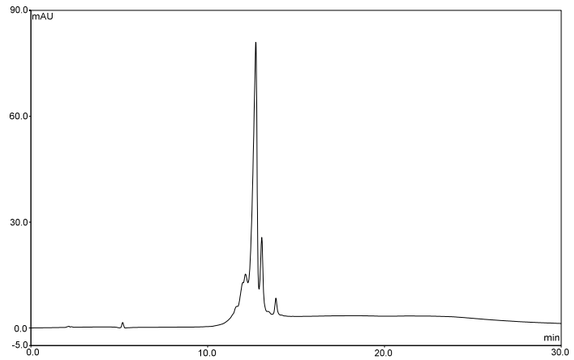
Our dedicated buffer kits allow set up of pH gradients with ease.



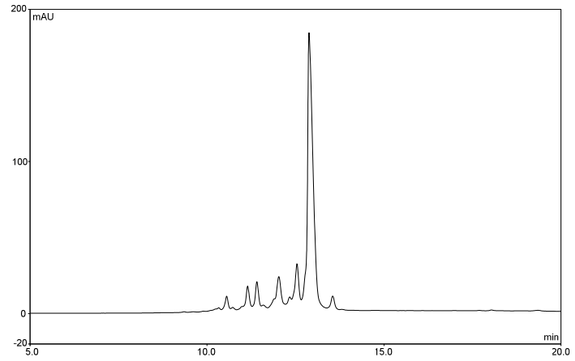
MAbPac SCX-10 is a strong cation exchange column designed specifically for high-resolution separations of antibodies and associated variants by IEX.



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



Generic linear pH gradient of a monoclonal antibody with MAbPac SCX-10.



Optimized salt gradient for monitoring deamidation of ribonuclease with ProPac WCX-10.

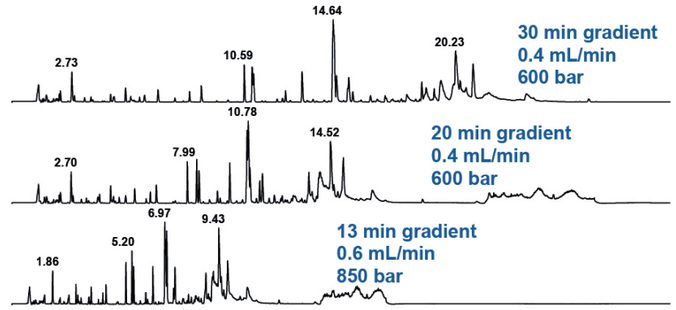
PEPTIDE MAPPING

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 peptide 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.

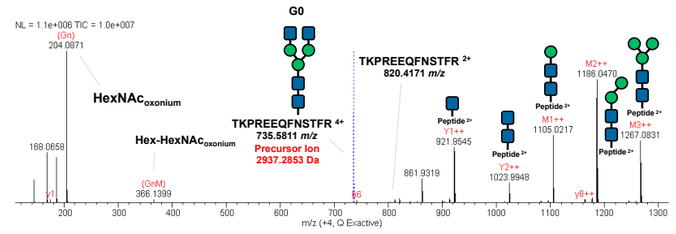
For the ultimate in peptide mapping fine structure, mass spectrometry is often utilized.

MASS SPECTROMETRY

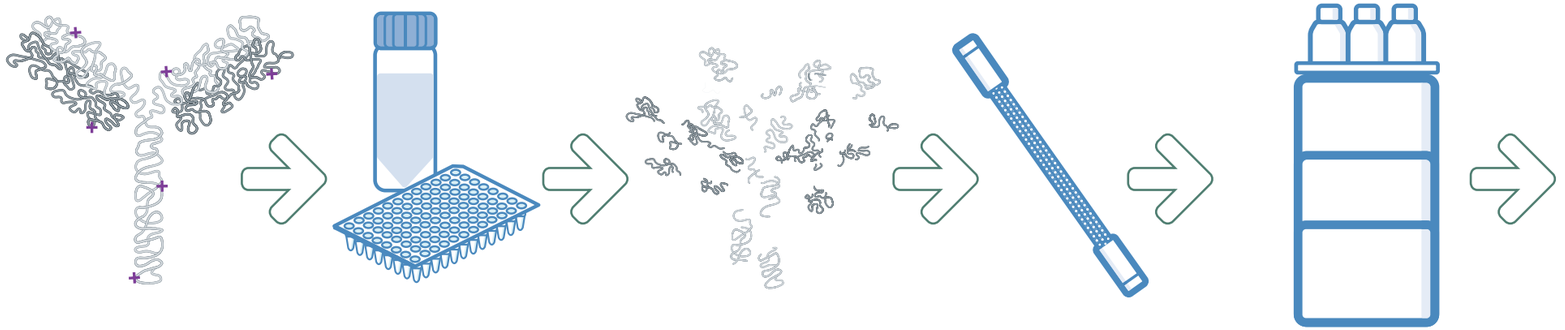
Thermo Scientific Orbitrap™ mass spectrometers utilize PepFinder™ software to provide accurate identification, in-depth characterization, and relative quantitation of biotherapeutic and other proteins from mass spectrometric data.



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-Hex-NAc), and the sequence ladder of the fragmented glycan attached to the intact peptide.



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 and is the workflow described here.

Acclaim RSLC 120 C18 is an excellent choice of column, it has an ultrapure silica substrate with extremely low metal content to minimize tailing effects and deliver the sharp, symmetrical peak shapes needed in this workflow.

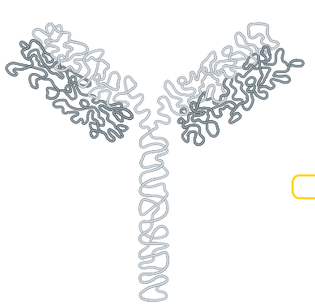
Vanquish Flex has a very high pressure flow path and sample pressurization prior to injection to ensure high peak capacity, retention time stability and peak area precision, ideal for peptide mapping applications.

INTACT

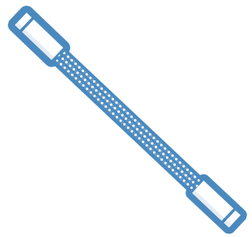
There remains a need to characterize biotherapeutic proteins in an intact form, particularly where there is likelihood of structural information such as isomerism that may not be observed by other techniques. It is also important in some of the novel biotherapeutic classes such as antibody drug conjugates.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

Vanquish Flex system readily hyphenates to the full range of Thermo Scientific mass spectrometers including gold standard Orbitrap mass analyzer instruments. In addition to the HRAM capabilities required for intact protein characterization, systems such as the Thermo Scientific Q Exactive™ range deliver accurate glycan profiling.

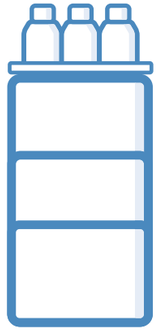
Reproducibility of protein separation is as important as resolution and sensitivity. Vanquish Flex UHPLC produces excellent separation reproducibility giving you the confidence you need.



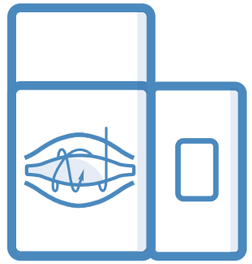
mAbs and ADCs require thorough characterization, including glycoforms, higher order structures and PTMs.



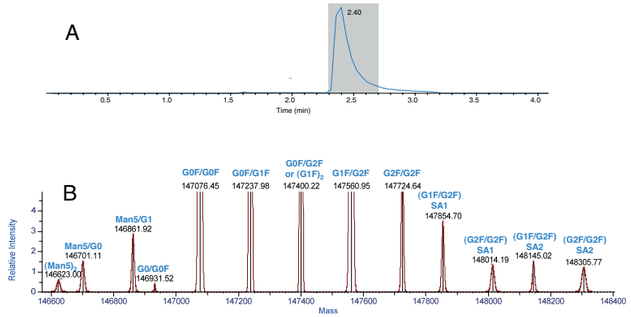
MAbPac RP is a new reversed phase column that uses a resin based on hydrophobic supermacroporous 4µm polymer particles. It is ideal for the efficient separation of protein molecules with very low carry over.



UHPLC is the most effective technique to ensure separation of higher order structures and unwanted interferents.



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



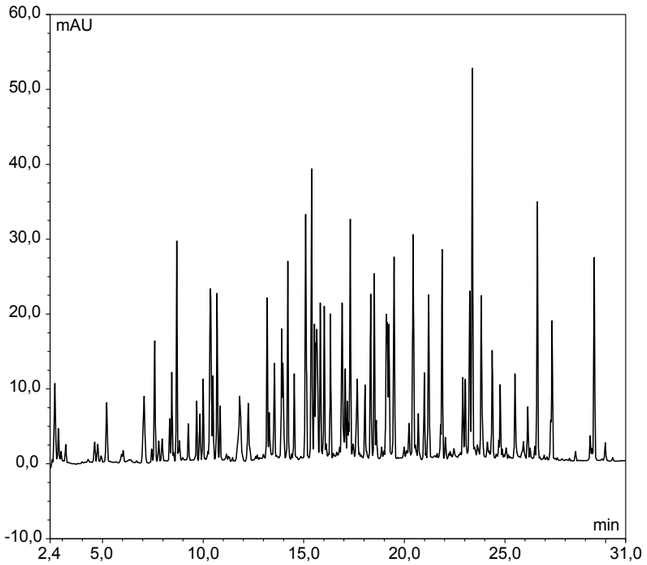
MS analysis of 100 ng of the mAb Rituximab, depicting (A) LC chromatogram using Thermo Scientific MSPac™ DS-10 Desalter Cartridge, and (B) the deconvoluted spectrum and annotated glycoforms.

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.

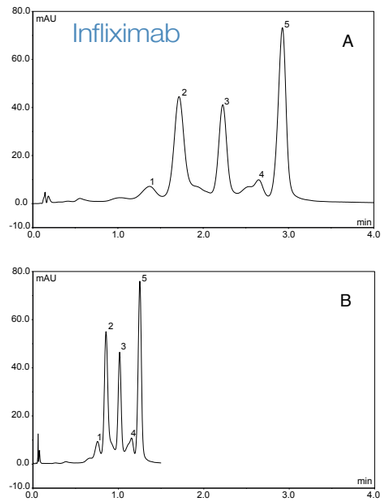
Retention Time (min)	Retention Time RSD (%)	RSD Area (%)
3.171	0.077	0.61
7.601	0.077	0.31
10.702	0.042	0.22
14.217	0.028	0.24
18.345	0.036	0.77
22.912	0.018	0.79
26.137	0.013	0.20
29.438	0.007	2.10

Rentention 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 sample pre-compression to give you better 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.

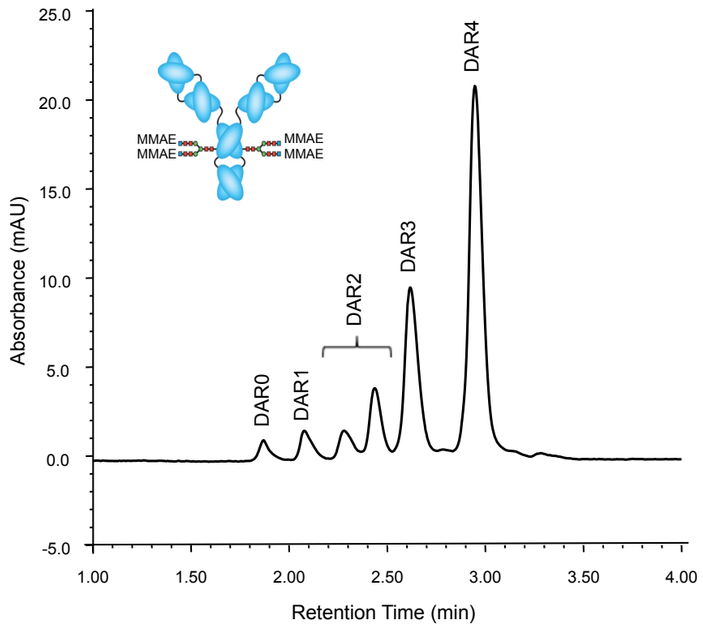
The ability to separate charge variants quickly and confidently is extremely useful. Here, pH range and gradient slope were modified to reduce gradient time to under two minutes.
Separation A contains a pH range of 6.5 to 7.4 and a gradient range of 20-40% B whereas separation B shows a pH range of 6.4 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 biotherapeutic characterization.

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

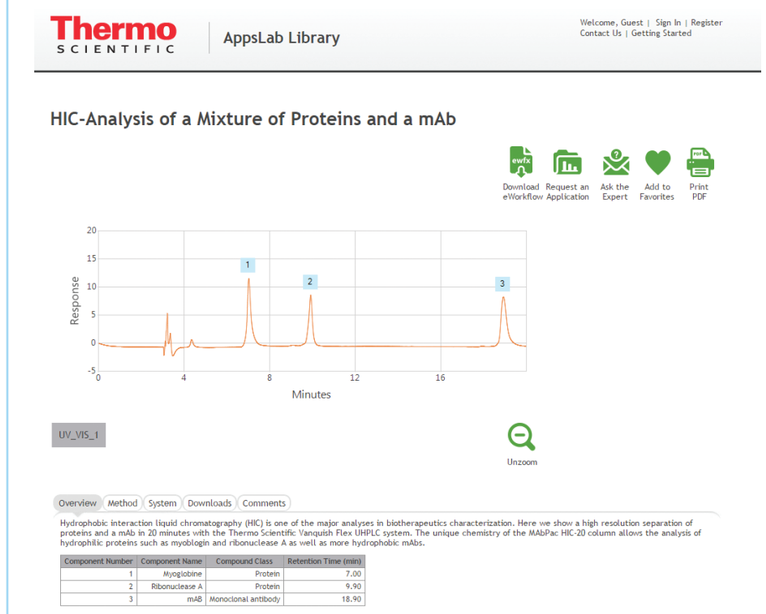
Vanquish Flex system can be interfaced to a wide range of detectors, from CAD to fluorescence detection to our high performance DAD LightPipe detector. It also has double the capacity of well plates of its competitors, and unrivalled temperature control for fully optimized characterization conditions.



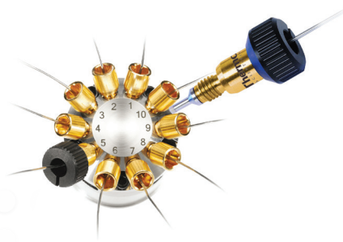
Showing the versatility of UHPLC, the new Thermo Scientific™ MAbPac™ RP column is used for Drug Antibody Ratio (DAR) measurements of an antibody drug conjugate in under four minutes.

SIMPLICITY

Thermo Scientific™ AppsLab Library™ one-click workflows for Chromeleon can significantly reduce method development time. The AppsLab library of analytical applications is a full 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.



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, ideal to minimize experimental transfer times.

COMPLIANCE

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

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

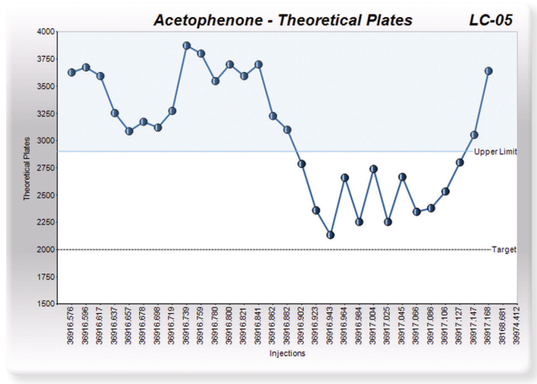
Chromeleon is also compliance ready; it supports **GxP** and **21 CFR Part 11.** It is robust, secure, and administrator-controlled. Furthermore, it allows for file storage that is fully secure.

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 mass spectrometry along with gas, liquid and ion chromatography.

NEW! APPSLAB APPLICATIONS RESOURCE LIBRARY:

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.
www.appslab.com



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 bio-inert systems that are built for biopharma

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