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BioLC columns

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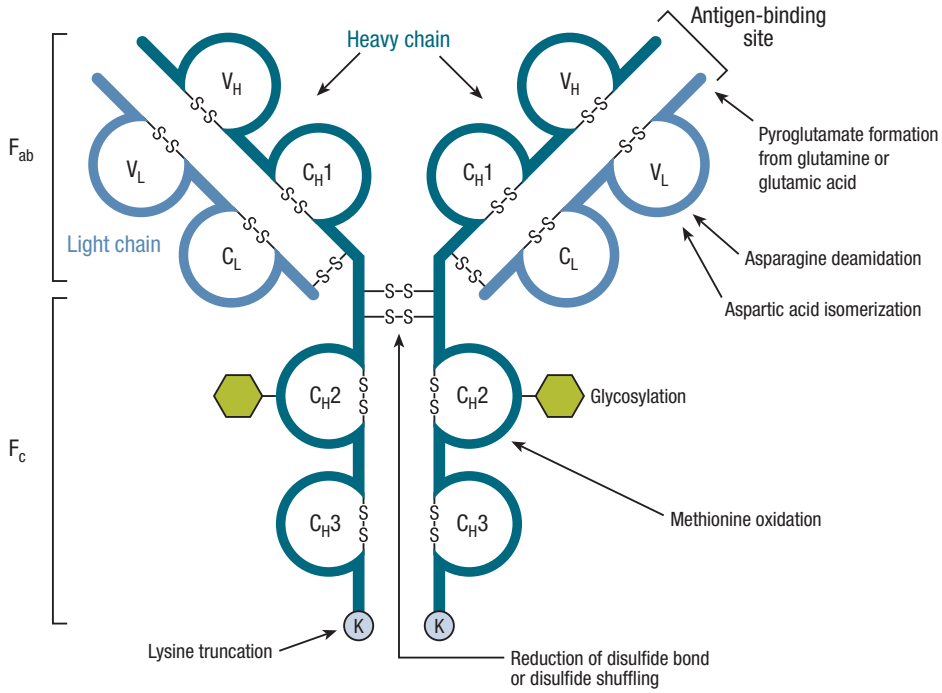
Contents

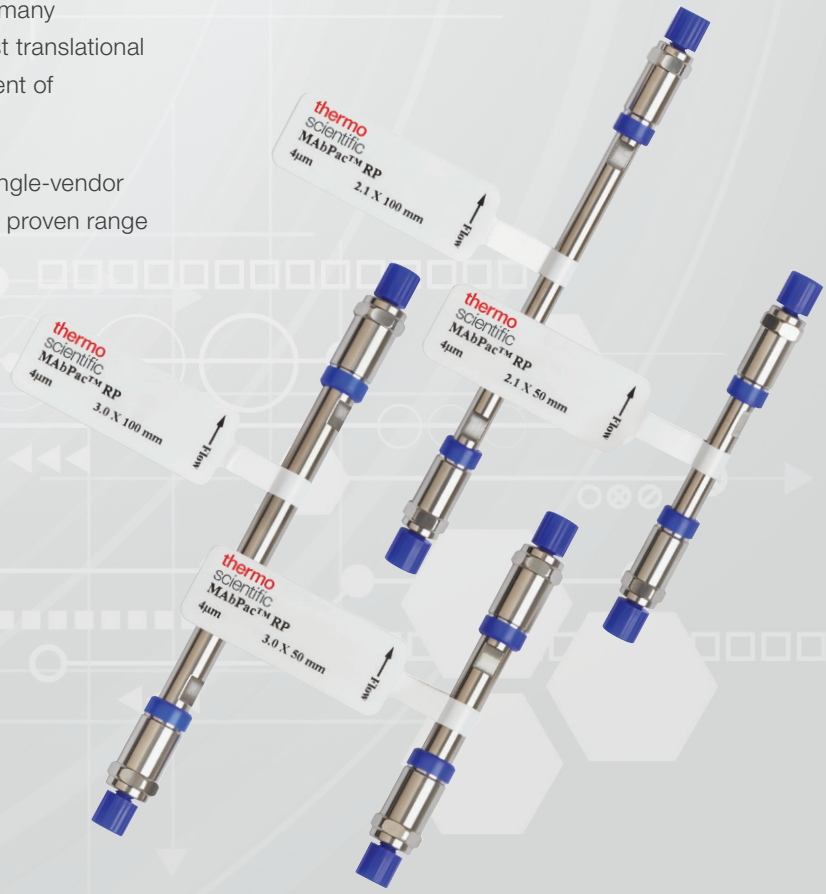
Monoclonal antibodies



Monoclonal antibodies have very complex structures, with many possible site-specific variations. With such potential for post translational modifications (PTMs), quality control and stability assessment of monoclonal antibodies are very challenging tasks.

Biopharmaceutical scientists can now take advantage of single-vendor analytical workflows using advanced instrumentation and a proven range of chemistries for comprehensive characterization.

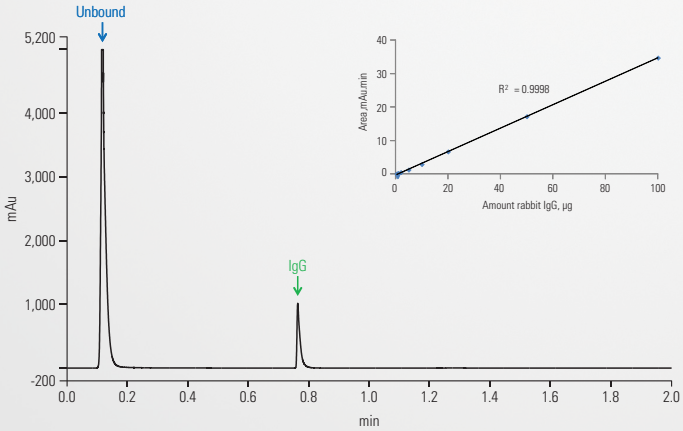
The Thermo Scientific portfolio offers a range of innovative chemistries, from our long lifetime polymer reversed phase column for intact and subunit separations to our first in class ion exchange Thermo Scientific™ ProPac™ and Thermo Scientific™ MAbPac™ lines for high resolution charge variant analysis.



Affinity titer determination

Early in the development of recombinant mAbs, a large number of harvest cell culture (HCC) samples must be screened for IgG titer. The high degree of specificity offered by affinity chromatography provides a powerful selective platform analysis due to its strong affinity to bind a wide range of antibodies.

Protein A ligand is often used due to its strong affinity to bind a wide range of antibodies.

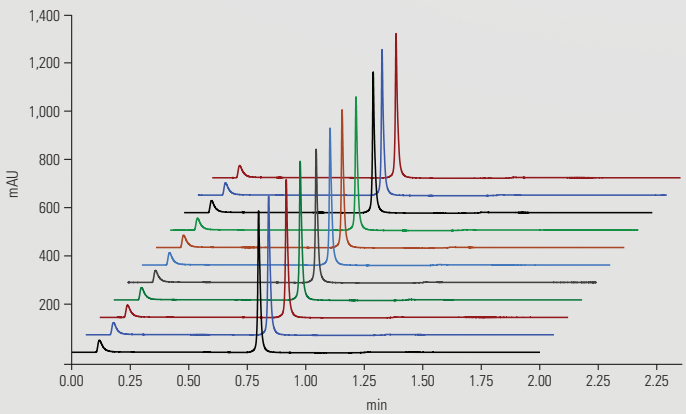


Column:	**MAbPac Protein A**
Format:	4.0 × 35 mm
Eluent A:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 7.5
Eluent B:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 2.5
Gradient:	0% B for 0.2 min, 100% B for 0.60 min 0% B for 1.20 min
Flow rate:	2 mL/min
Inj. volume:	10 µL
Temp.:	25 °C
Detection:	280 nm
Sample:	Harvest cell culture (HCC)

The challenge faced by analytical laboratories in the biopharmaceutical and pharmaceutical industries is the development of a high-throughput and robust titer assay. The MAbPac Protein A column has been engineered specifically for this application from a novel polymeric resin with a hydrophilic surface, covered with a covalently bound recombinant Protein A. The hydrophilic nature of the backbone minimizes nonspecific binding, enabling accurate titer determination. The MAbPac Protein A column format allows rapid automation of loading, binding, elution and collection using the biocompatible HPLC system, such as the Thermo Scientific™ Vanquish™ HPLC system.

MAbPac Protein A columns feature excellent binding efficiency, high loading capacity, and superior ruggedness. Lower backpressure enables fast analysis.

A simple step gradient from near-neutral to acidic pH is all that is required to elute the bound material.



Column:	**MAbPac Protein A**
Format:	4.0 × 35 mm
Eluent A:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 7.5
Eluent B:	50 mM sodium phosphate, 150 mM sodium chloride, 5% acetonitrile, pH 2.5
Gradient:	0% B for 0.2 min, 100% B for 0.60 min, 0% B for 1.20 min
Flow rate:	2 mL/min
Inj. volume:	20 µL
Temp.:	25 °C
Detection:	280 nm
Sample:	Rabbit IgG, 1 mg/mL

	t_R (min)	Area (mAu*min)	PWHH (min)
Average	0.80	7.76	0.01
% RSD	1.02	2.20	0.00

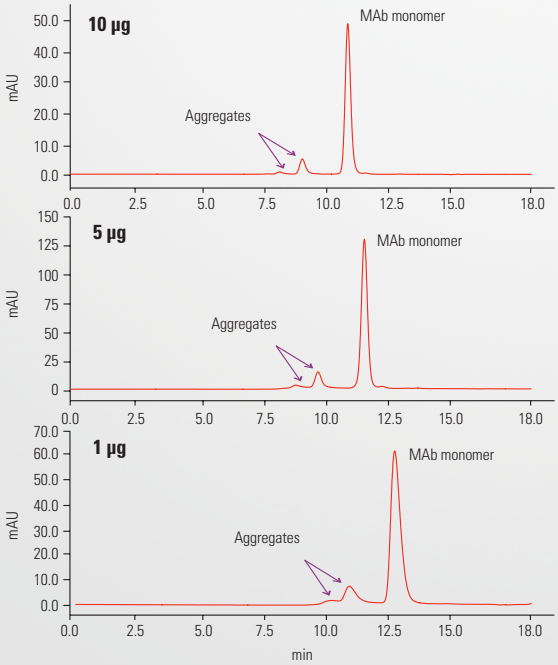
Aggregate separation

Monoclonal antibodies produced from mammalian cell culture may contain significant amounts of dimers and higher-order aggregates. Studies show that high levels of aggregation in drug products can cause severe immunogenic reactions. Thus, biopharmaceutical manufacturers are required to develop analytical methods to monitor size heterogeneity.

a

b

c



Column:	**MAbPac SEC-1, 5 µm,**
Format:	a. 7.8 × 300 mm
	b. 4.0 × 300 mm
	a. 2.1 × 300 mm
Mobile phase:	50 mM sodium phosphate pH 6.8, in 300 mM sodium chloride
Flow rate:	a. 760 µL/min
	b. 200 µL/min
	c. 50 µL/min
Inj. volume:	a. 10 µL
	b. 5 µL
	a. 1 µL
Temp.:	30 °C
Detection:	280 nm
Sample:	MAb (1 mg/mL)

Size-exclusion chromatography (SEC) is an ideal separation choice for aggregates. For SEC, the separation is based solely on the size of the molecule which does not interact with the stationary phase. Molecules of different sizes penetrate the pores to varying extents. This results in analytes of different sizes travelling through the media at different speeds. Larger molecules do not diffuse deep in the pores and elute first, whilst smaller entities diffuse deeper in the pores and elute later.

The MAbPac SEC-1 column is specially designed for the separation and characterization of monoclonal antibodies, their aggregates, and analysis of Fab and Fc fragments. Based on high-purity, spherical, porous (300 Å), 5 µm silica particles that are covalently modified with a hydrophilic layer, this column offers separations with very low levels of secondary interaction. The stationary phase is designed to handle different eluent conditions containing both high and low ionic strength mobile phases, as well as mass spectrometry friendly volatile eluents.

The three different column formats meet different analytical needs: the 7.8 mm ID column provides the highest resolution separation and accurate quantitation of mAbs and their aggregates; the 4.0 mm ID column enables baseline separation of mAb monomer and dimer, requiring only ¼ of sample compared to the 7.8 mm ID column; the 2.1 mm ID column uses low flow rate and low sample loading, making it the perfect format for MS detection.

Charge variant analysis

Ion Exchange Chromatography (IEC) is based on a stationary phase with a charged ligand on the surface interacting with analytes of the opposite charge. The technique is divided between anion exchange and cation exchange chromatography. In anion exchange, positively charged surface ligands interacts with negatively charged analytes, whilst in cation exchange negatively charged surface ligands interact with positively charged analytes.

Unsure if you need an anion or cation exchange column?

Separations are based on the accessible surface charge of the protein or glycoprotein with the column's functional group. When you are uncertain if you need an anion or cation exchange column, the protein's pI can help to determine which column to use.

If your protein pI is < 6, start with anion exchange columns. You may need to try both SAX and WAX options to determine which offers the best resolution. When uncertain, it is recommended to start with SAX for your separation.

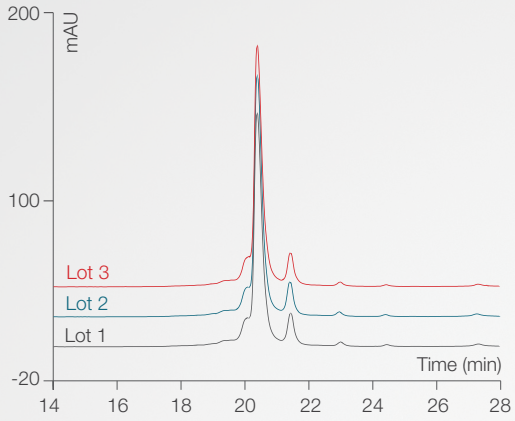
Thermo Scientific™ ProPac™ 3R column is our newest innovation providing robust separations at ultra-high resolution. The novel 3 µm monodisperse particle platform combined with state-of-the-art manufacturing processes delivers unrivalled efficiency and reproducibility. ProPac 3R is available both as SCX and SAX. The MAbPac SCX-10 family of columns offers a wide selection of column dimensions. ProPac Elite WCX columns provide complementary selectivity to the strong cation exchange columns. Separation profiles may be different between the weak and strong cation exchange columns, it is recommended to evaluate both to determine which offers the highest resolution for your charge variants. All our ion exchange columns offer the possibility to run salt or pH gradients.

Salt gradient method

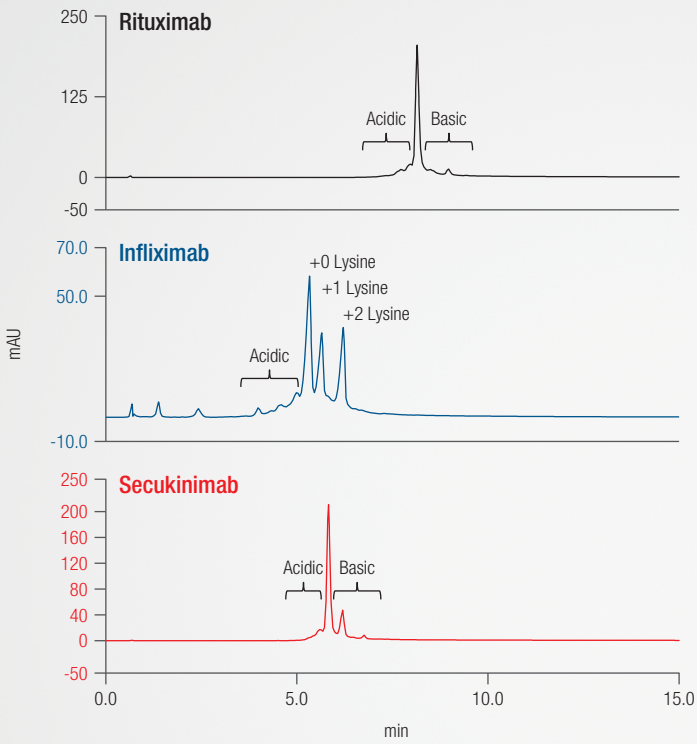
In salt gradient-based IEC, the pH of the buffer is fixed. In addition to choosing the appropriate pH of the starting buffer, the initial ionic strength is kept low since the affinity of proteins for IEC resins decreases as ionic strength increases.

The proteins are eluted by increasing the ionic strength (salt concentration) of the buffer to increase the competition between the buffer ions and proteins for charged groups on the IEC resin. As a result, the interaction between the IEC resin and proteins is reduced, causing the proteins to elute.

The pI of the majority of antibodies tends to be in the pH 6–10 region, which is the reason why cation exchange is the commonly chosen separation method for mAb charge variants.



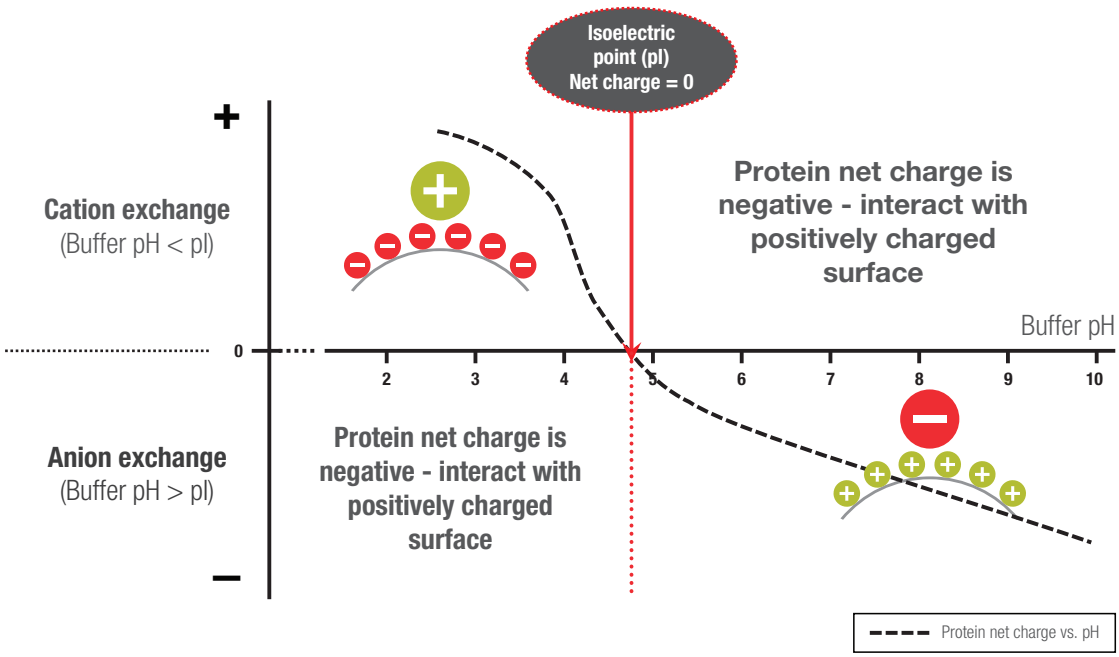
Column:	**ProPac 3R SCX column, 3 µm**		
Format:	4 × 100 mm		
Mobile phase A:	20 mM MES, pH 6.5		
Mobile phase B:	20 mM MES, pH 6.5 + 0.5 M NaCl		
Flow rate:	0.3 mL/min		
Inj. volume:	2 µL		
Temp.:	30 °C		
Detection:	UV, 280 nm		
Sample:	NISTmAb – 10 mg/mL)		
Gradient:	Time (min)	%A	%B
	0.0	90	10
	30.0	70	30
	30.1	20	80
	33.0	20	80
	33.1	90	10
	40.0	90	10



Column:	**ProPac Elite WCX**		
Format:	4 × 150 mm		
Eluents:	A: 20 mM MES pH 6.5 B: 20 mM MES pH 6.5 +0.5 M NaCl		
Gradient:	Time (min)	%A	%B
	0.0	95	5
	15.0	65	35
	15.1	60	50
	16.0	60	5
	16.1	95	5
	25.0	95	5
Flow rate:	1.0 mL/min		
Inj. volume:	3 µL		
Temperature:	30 °C		
Detection:	UV, 280 nm		
Sample:	Top: Rituximab, 5 mg/mL Middle: Infliximab, 5 mg/mL Bottom: Secukinimab, 5 mg/mL		

pH gradient method

In pH-gradient-based IEC, the starting buffer is held at a constant pH to ensure the proteins have a charge opposite to the stationary phase, thus binding to the column. As the gradient runs, the pH of the buffer is changed so the proteins transition to a net zero charge (the pI), and ultimately the same charge as the resin, thus the protein is released and eluted from the column.

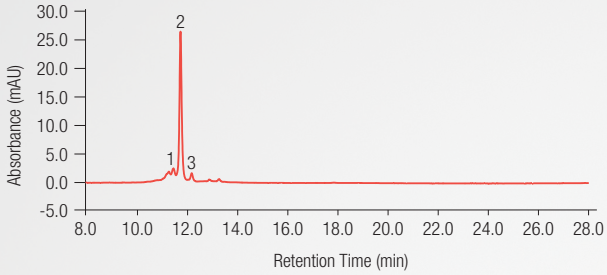


In the fast-paced drug development environment, a platform method to accommodate the majority of mAb analyses is desired. Considerable method development is required to optimize the salt gradient for charge variant separation of each mAb. Ion exchange separations by pH gradient provide the advantage of a generic platform approach, thus saving time on method development. As well, heavy salts are removed from LC systems, resulting in less cleaning and downtime.

One of the challenges in pH-gradient separations is choosing a buffer system able to cover a wide pH range whilst delivering a linear pH gradient. We offer a novel pH gradient method for cation-exchange chromatography. This method features a multi-component zwitterionic buffer system in which the linear gradient can be run from 100% eluent A (low pH buffer) to 100% eluent B (high pH buffer). The linearity of the pH gradient can be verified using a Thermo Scientific™ Dionex™ UltiMate™ PCM-3000 pH and conductivity online monitor, which is easily incorporated with Thermo Scientific liquid chromatography (LC) instrumentation.

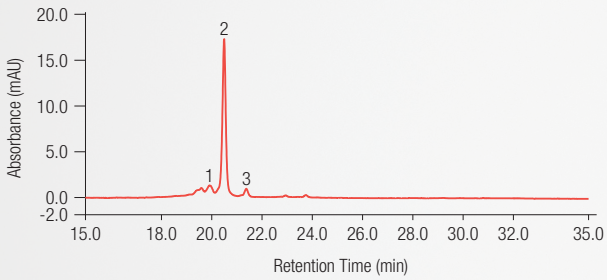
Once the approximate pH elution range of the target mAb has been established in the initial run, optimization of the separation is achieved by running a shallower pH gradient over a narrower pH range. Separation of bevacizumab variants is optimized using a pH gradient. When a broad pH gradient is applied from 0% to 100% B (pH 5.6 to 10.2), all variants are eluted in the pH range 7.0 to 8.0. Adjusting the gradient to 0–50% B (5.6–7.9 pH range, middle), resolution is further improved with minimal optimization steps. For comparison, bevacizumab variants separation by the salt gradient methods with a steeper (a) and a shallower gradient slope (b).

a pH Gradient 0–100% B



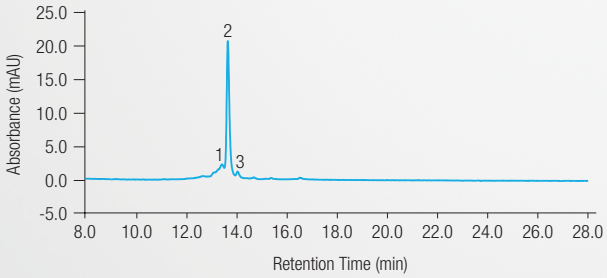
Column:	**MAbPac SCX-10, 10 µm**
Format:	4.0 × 250 mm
Mobile phase A:	1X CX-1 pH gradient buffer A, pH 5.6
Mobile phase B:	1X CX-1 pH gradient buffer B, pH 10.2
pH gradient:	0% B to 100% B from 1 to 31 min
Flow rate:	1.0 mL/min
Inj. volume:	5 µL
Temp.:	30 °C
Detection:	UV, 280 nm
Sample:	Bevacizumab (1 mg/mL)

b pH Gradient 0–50% B



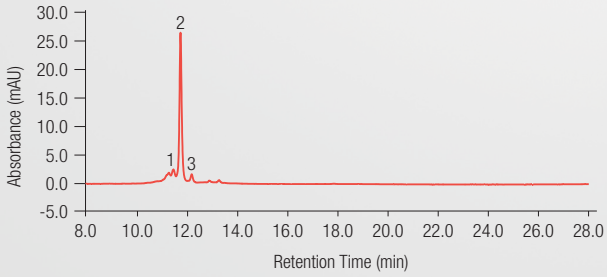
Column:	**MAbPac SCX-10, 10 µm**
Format:	4.0 × 250 mm
Mobile phase A:	1X CX-1 pH gradient buffer A, pH 5.6
Mobile phase B:	1X CX-1 pH gradient buffer B, pH 10.2
pH gradient:	0% B to 50% B from 1 to 31 min
Flow rate:	1.0 mL/min
Inj. volume:	5 µL
Temp.:	30 °C
Detection:	UV, 280 nm
Sample:	Bevacizumab (1 mg/mL)

c Salt Gradient 0–100% B



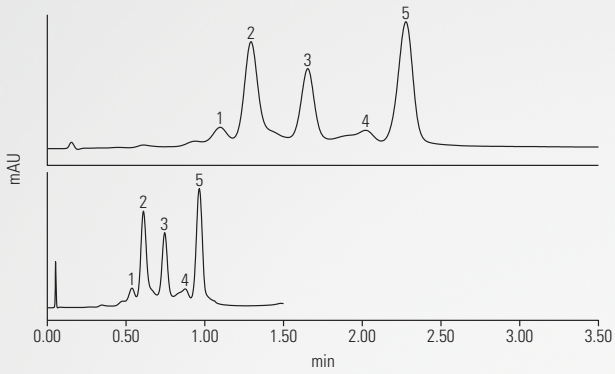
Column:	**MAbPac SCX-10, 10 µm**
Format:	4.0 × 250 mm
Mobile phase A:	20 mM MES (pH 5.6) + 60 mM sodium chloride
Mobile phase B:	20 mM MES (pH 5.6) + 300 mM sodium chloride
Salt gradient:	0% B to 100% B from 1 to 31 min
Flow rate:	1.0 mL/min
Inj. volume:	5 µL
Temp.:	30 °C
Detection:	UV, 280 nm
Sample:	Bevacizumab (1 mg/mL)

d Salt Gradient 0–50% B



Column:	**MAbPac SCX-10, 10 µm**
Format:	4.0 × 250 mm
Mobile phase A:	20 mM MES (pH 5.6) + 60 mM sodium chloride
Mobile phase B:	20 mM MES (pH 5.6) + 300 mM sodium chloride
Salt gradient:	0% B to 0% B from 1 to 31 min
Flow rate:	1.0 mL/min
Inj. volume:	5 µL
Temp.:	30 °C
Detection:	UV, 280 nm
Sample:	Bevacizumab (1 mg/mL)

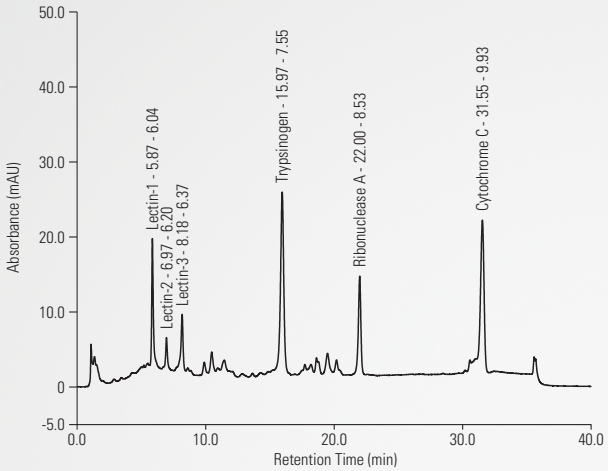
For those who desire high throughput methods, charge variant separations under 5 minutes can be achieved by running a pH gradient separation on a MAbPac SCX-10 RS (5 µm, 2.1 x 50 mm) column coupled to a Thermo Scientific™ Vanquish™ UHPLC system. The Vanquish UHPLC system is fully biocompatible, suitable for the analysis of intact proteins. The combination of low gradient delay volume and high precision gradient formation makes it the ideal system for high throughput analysis with gradient elution. An example shown here is an ultra fast separation of infliximab variants.



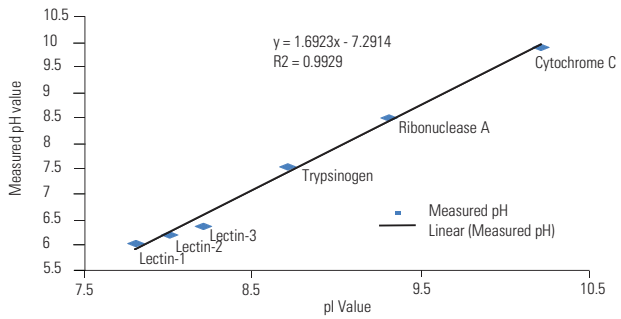
Column:	**MAbPac SCX-10 RS, 5 µm**
Format:	2.1 × 50 mm
Mobile phase A:	1X CX-1 pH gradient buffer A, pH 5.6
Mobile phase B:	1X CX-1 pH gradient buffer B, pH 10.2
pH gradient:	a. 20% B to 40% B in 5 min
	b. 18% B to 27% B in 0.8 min
Flow rate:	a. 0.5 mL/min
	b. 1.2 mL/min
Inj. volume:	4 µL
Temp.:	30 °C
Detection:	UV, 280 nm
Sample:	Infliximab (10 mg/mL)

The linearity of the pH gradient can be verified using the UltiMate 3000 pH and conductivity online monitor, which can be added to UltiMate 3000 BioRS system to serve as a platform for pH gradient ion exchange chromatography.

The UltiMate 3000 pH and conductivity monitor is a valuable tool for HPLC method development, particularly for protein and nucleic acid separations. It enables linking the mobile phase pH and/or conductivity with the elution time of the components. The pH and conductivity monitor allows for the monitoring of gradient formation, column equilibration, and understanding column buffering effects in pH gradient ion-exchange chromatography.



Column:	**MAbPac SCX-10, 10 µm**		
Format:	4.0 × 250 mm		
Mobile phase A:	1X CX-1 pH gradient buffer A, pH 5.6		
Mobile phase B:	1X CX-1 pH gradient buffer B, pH 10.2		
pH gradient:	Time (min)	%A	%B
	0.0	100	0
	1.0	100	0
	31.0	0	100
	34.0	0	100
	34.1	100	0
	40.0	100	0
Flow rate:	1.0 mL/min		
Inj. volume:	5 µL		
Temp.:	30 °C		
Detection:	UV, 280 nm		
Sample:	Protein standard		



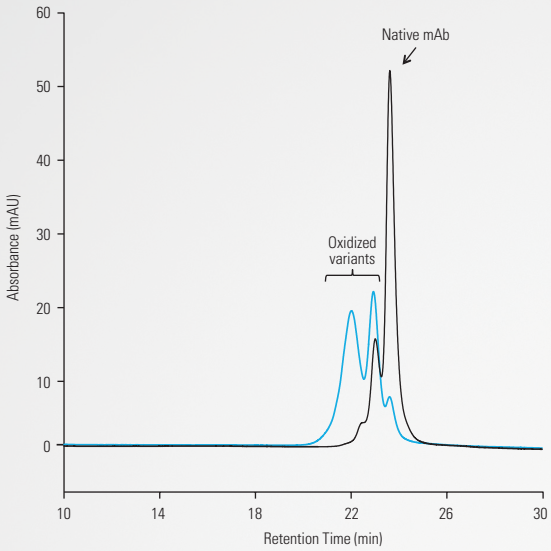
Monitoring the eluent pH during a pH gradient makes charge variant characterization more simple and predictable because proteins will only elute once the eluent pH is above the biomolecules pI. The measured pH values for six protein peaks (lentil lectin-1, lectin-2, lectin-3, trypsinogen, ribonuclease A, and cytochrome C), exhibit a strong linear correlation to the literature based pI values. This shows that the pI of a protein component can be estimated based on the peak retention time and measured pH.

Oxidation variant analysis

Using hydrophobic interaction chromatography (HIC), intact biomolecules can be separated by their degree of hydrophobicity. HIC is an important tool for protein chemists separating proteins under gentle, non-denaturing conditions. Biomolecules are separated by their degree of hydrophobicity. The mobile phase consists of a salting-out agent, which at high concentration retains the protein by increasing the hydrophobic interaction between proteins and the stationary phase. The bound proteins are eluted by decreasing the salt concentration.

Oxidation of exposed amino acid residues such as methionine (Met) and tryptophan (Trp) is a major concern in therapeutic mAb stability studies. Oxidation of amino acid residues on a mAb can alter the hydrophobic nature of the protein by either an increase in polarity of the oxidized form or a conformational change. This can also have an effect on the activity of the therapeutic. Therefore hydrophobicity-based HPLC methods such as hydrophobic interaction chromatography are used to monitor oxidized products.

The MAbPac HIC-20 and MAbPac HIC-10 columns are high-resolution silica-based columns designed for the separation of mAbs and mAb variants. Each offers unique column chemistry and provides high resolution, rugged stability and desired selectivity for mAb and mAb variants.



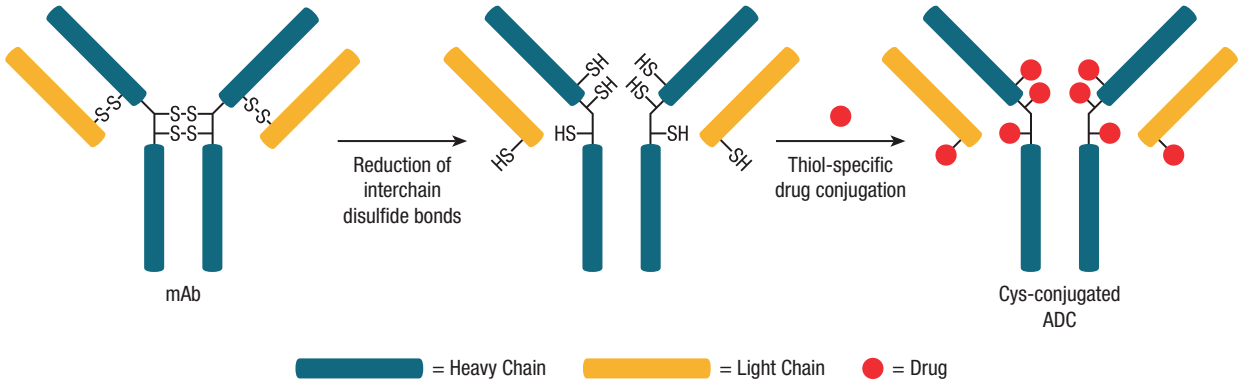
Column:	**MAbPac HIC-20, 5 µm**		
Format:	4.6 × 250 mm		
Mobile phase A:	2 M ammonium sulfate, 100 mM sodium phosphate, pH 7.0		
Mobile phase B:	100 mM sodium phosphate, pH 7.0		
Gradient:	Time (min)	%A	%B
	-6.0	50	50
	0.0	50	50
	2.0	50	50
	30.0	0	100
	35.0	0	100
Flow rate:	0.5 mL/min		
Temp.:	30 °C		
Detection:	UV, 280 nm		
Sample:	Untreated mAb (1.25 mg/mL) H_2O_2 oxidized mAb (1.25 mg/mL)		

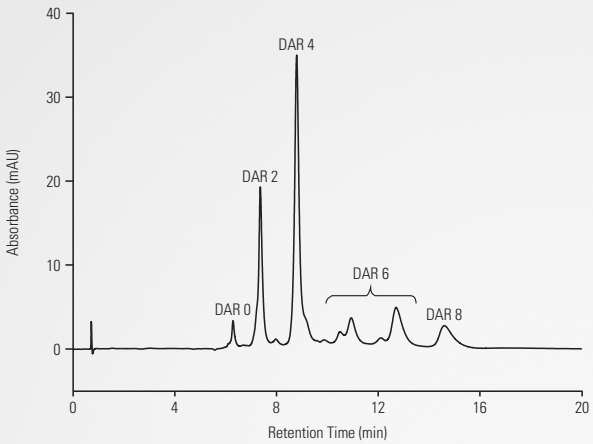
Antibody drug conjugate (ADCs) analysis

ADCs have gained tremendous interest among pharmaceutical companies due to their significantly improved clinical efficacy over native monoclonal antibodies. The conjugation of drugs often results in an ADC molecule that is heterogeneous with respect to both the distribution and loading of cytotoxic drugs on the mAb. The number of drugs attached to the mAb has been shown to directly affect the safety and the efficacy of the drug. Therefore, it is critical to fully characterize and monitor the heterogeneity of ADCs during development and production.

Hydrophobic interaction chromatography (HIC) and reversed-phase chromatography (RPC) are suitable for the separation of ADCs since attachment of toxic drugs alters the hydrophobicity of the antibody. Typically the least hydrophobic unconjugated antibody elutes first and as the number of drugs attached increases the elution time increases.

ADC analysis by hydrophobic interaction chromatography

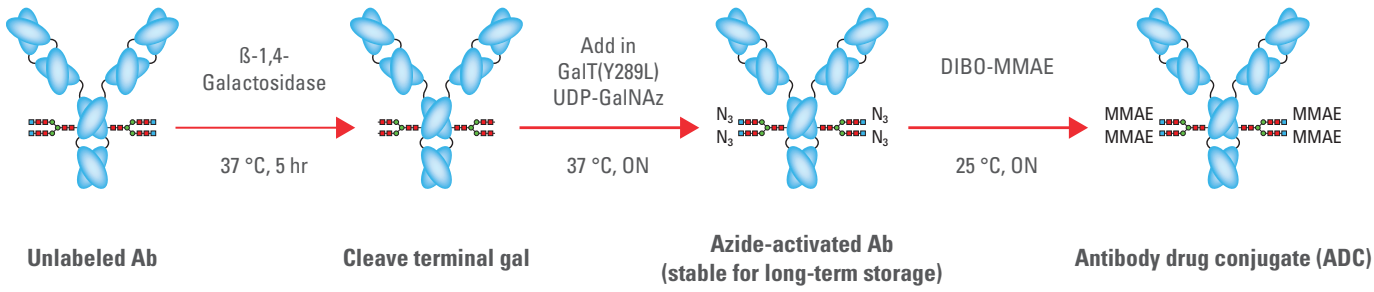




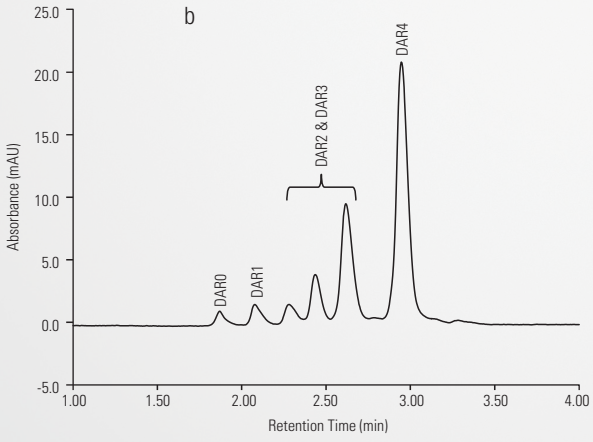
Column:	**MAbPac HIC-Butyl, 5 µm**		
Format:	4.6 × 100 mm		
Mobile phase A:	1.5 M ammonium sulfate, 50 mM sodium phosphate, pH 7.0/isopropanol (95:5 v/v)		
Mobile phase B:	50 mM sodium phosphate, pH 7.0/isopropanol (80:20 v/v)		
Gradient:	Time (min)	%A	%B
	-5.0	100	0
	0.0	100	0
	1.0	100	0
	15.0	0	100
	20.0	0	100
Flow rate:	1.0 mL/min		
Inj. volume:	5 µL (5 mg/mL)		
Temp.:	25 °C		
Detection:	UV, 280 nm		
Sample:	Cys-conjugated ADC mimic		

ADCs analysis by reversed-phase chromatography

The MAbPac RP column is based on supermacroporous 4 µm polymer particles. The hydrophobic phenyl based resin with its large pore size enables efficient separation of large molecules including mAbs and their conjugates. Shown in the chromatogram below is an ADC created using site-specific antibody labeling technology and analyzed using a MAbPac RP column. ADCs were prepared by enzymatically activating mAb Fc domain glycans with azides using the mutant beta-galactosyltransferase enzyme. The azide-activated antibodies were then conjugated with dibenzocyclooctyne (DIBO) -activated Val-Cit-PAB-Monomethyl Auristatin E (MMAE) toxin resulting in a mixture of drug-loaded antibody species with 0 to 4 MMAE molecules. The unmodified mAb and ADCs with DAR values ranging from 0 to 4 are well resolved by the MAbPac RP column.



Unlabeled Ab | **Cleave terminal gal** | **Azide-activated Ab (stable for long-term storage)** | **Antibody drug conjugate (ADC)**



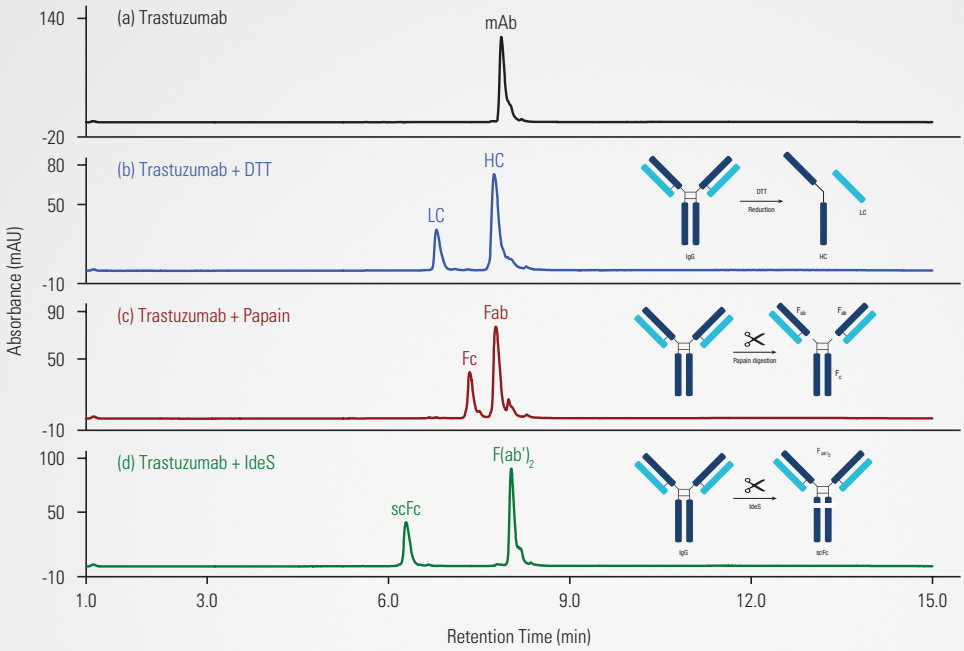
Column:	**MAbPac RP, 4 µm**		
Format:	2.1 × 50 mm		
Mobile phase A:	Water/trifluoroacetic acid (99.9 : 0.1 v/v)		
Mobile phase B:	Acetonitrile/water/trifluoroacetic acid (90: 9.9 :0.1 v/v/v)		
Gradient:	Time (min)	%A	%B
	0.0	65	35
	0.5	65	35
	4.5	45	55
	5.0	45	55
	5.5	65	35
	6.0	65	35
Flow rate:	0.6 mL/min		
Inj. volume:	2 µL		
Temp.:	80 °C		
Detection:	UV, 280 nm		
Sample:	Trastuzumab-MMAE		

Intact mAb and mAb fragments analysis

Reversed-phase chromatography has been used for many years as an excellent interface with mass spectrometry detection. The low concentrations of volatile buffers component combined with the high organic make-up of the mobile phase result in conditions ideal for stable and effective spray. Furthermore, the separation principle offers an alternative selectivity based on hydrophobicity, which provides orthogonal high-resolution separations to ion-exchange methods. The MAbPac RP uses supermacroporous resin which has large pore size and high loading capacity.

The polymeric nature of the column provides long column lifetime and stability, even at the elevated temperatures required for mAb separation. The wide pore size has an ideal benefit in antibody analysis, minimizing the chance of carryover and tailing, that could from proteins being stuck in smaller pores.

The excellent performance of the MAbPac RP HPLC columns for fragment analysis is shown here for the analysis of trastuzumab fragments: LC and HC, Fc and Fab, scFc and F(ab')2 are baseline separated using a 10-min gradient.



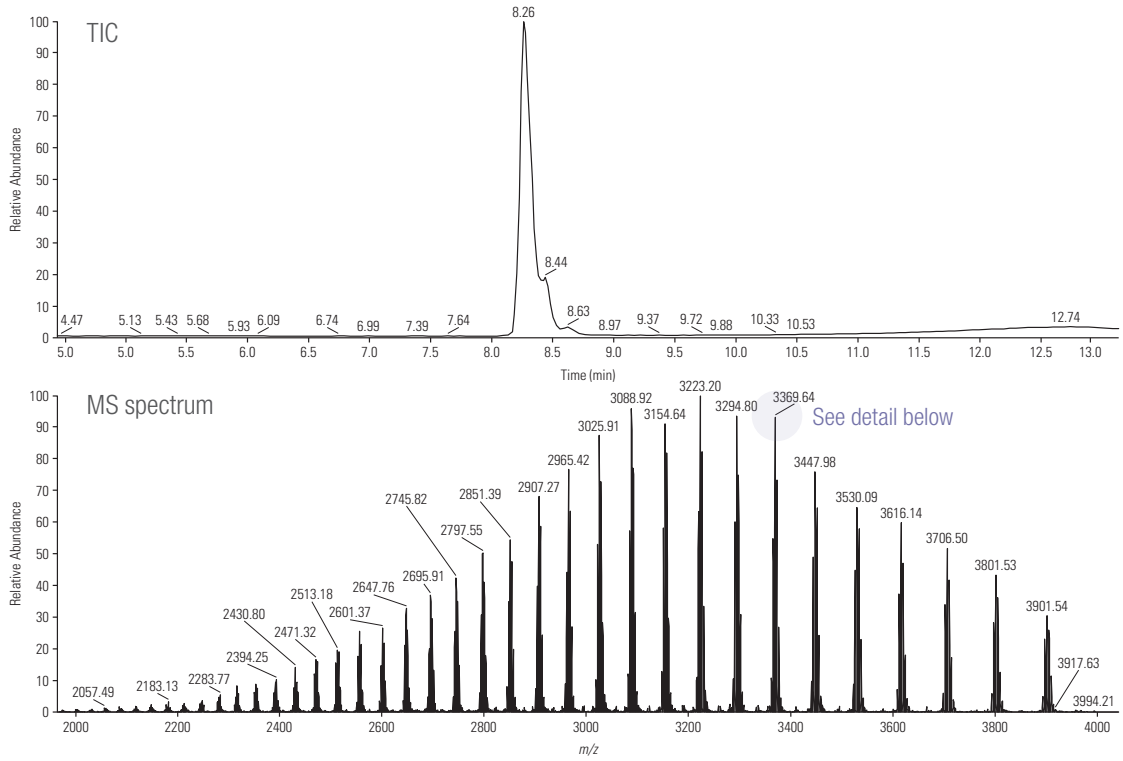
Column:	**MAbPac RP, 4 µm**
Format:	3.0 × 50 mm
Mobile phase A:	Water/formic acid/trifluoroacetic acid (99.88:0.1:0.02 v/v/v)
Mobile phase B:	Acetonitrile/water/formic acid/trifluoroacetic acid (90: 9.88:0.1:0.02v/v/v/v)

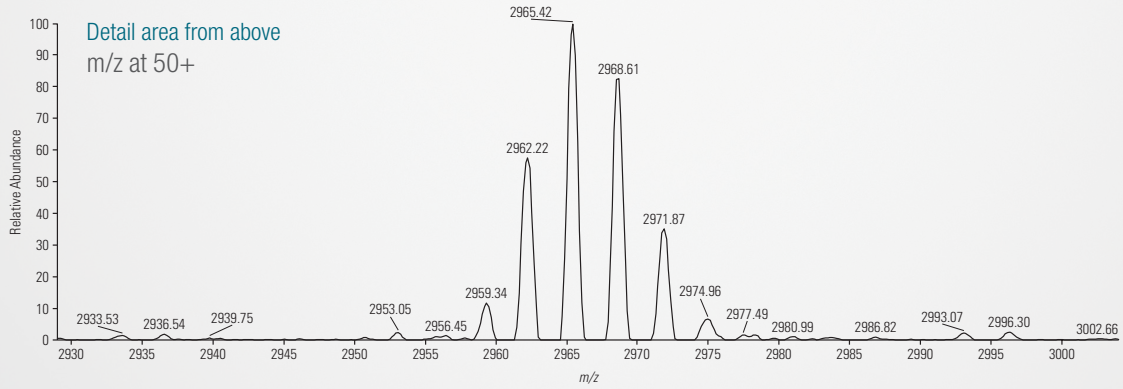
Gradient:

Time (min)	%A	%B
0.0	80	20
1.0	80	20
11.0	55	45
12.0	55	45
14.0	80	20
15.0	80	20

Flow rate:	0.5 mL/min
Inj. volume:	5 µL
Temp.:	80 °C
Detection:	UV, 280 nm
Sample:	(a) Trastuzumab (5 mg/mL) (b) Trastuzumab + DTT (4 mg/mL) (c) Trastuzumab + Papain (2 mg/mL) (d) Trastuzumab + IdeS (2 mg/mL)

The reversed-phase chromatographic analysis can be coupled to high resolution mass spectrometry systems to confirm the accurate molecular weight of the target product and identify variants. Here is an example of LC-MS analysis of trastuzumab using a Thermo Scientific™ Q Exactive™ Plus hybrid quadrupole-Orbitrap mass spectrometer. The top trace shows the total ion chromatogram and the bottom trace shows the mass spectrum.

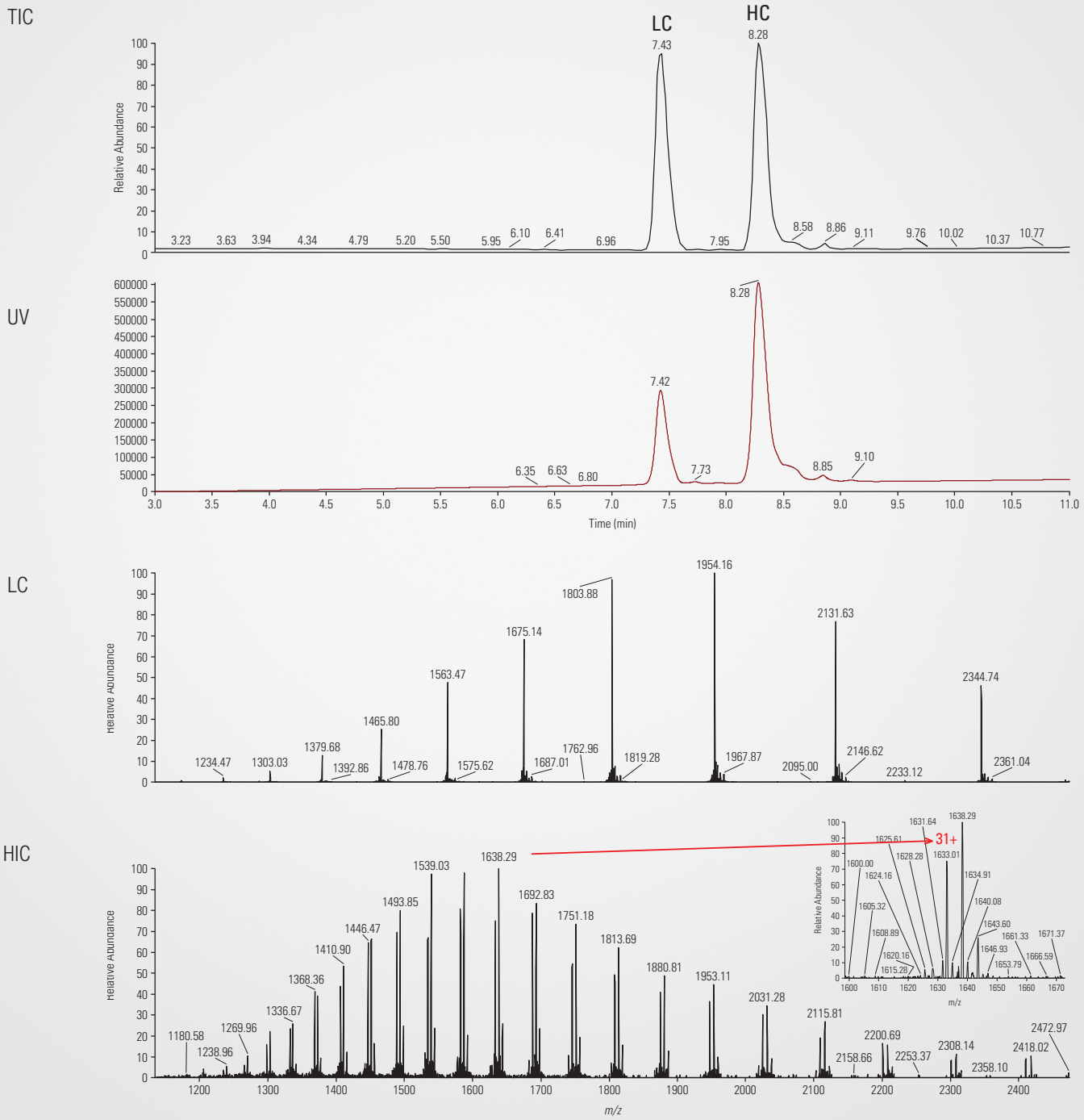




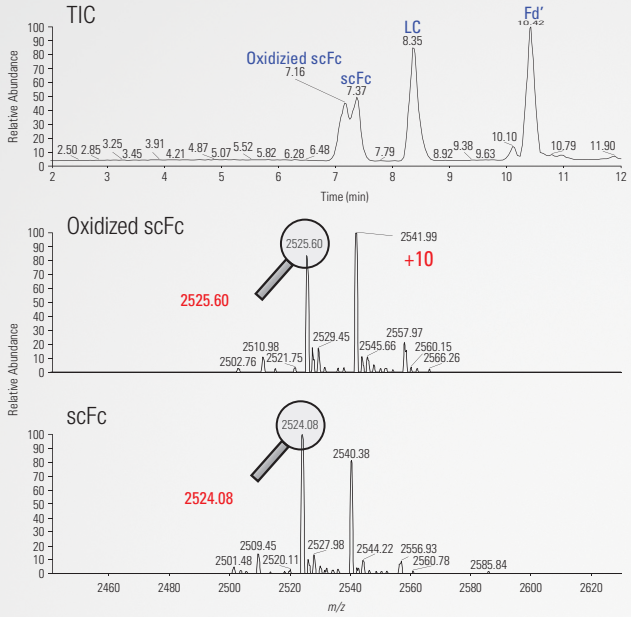
Column:	**MAbPac RP, 4 µm**		
Format:	3.0 × 50 mm		
Mobile phase A:	Water/formic acid/trifluoroacetic acid (99.88:0.1:0.02 v/v/v)		
Mobile phase B:	Acetonitrile/water/formic acid/trifluoroacetic acid (90:9.88:0.1:0.02 v/v/v/v)		
Gradient:	Time (min)	%A	%B
	0.0	80	20
	1.0	80	20
	11.0	55	45
	12.0	55	45
	14.0	80	20
	15.0	80	20

Flow rate:	0.5 mL/min
Inj. volume:	1 µL
Temp.:	80 °C
MS detection:	positive-ion mode
HPLC/mass spec:	Vanquish UHPLC system/Q Exactive Plus hybrid quadrupole-Orbitrap MS system
Sample:	Trastuzumab (5 mg/mL)

Further structural elucidation is obtained by reducing the trastuzumab into light chain and heavy chain fragments. Interface with Q Exactive Plus mass spectrometer allows the identification of different glycoforms in the heavy chain as shown below.

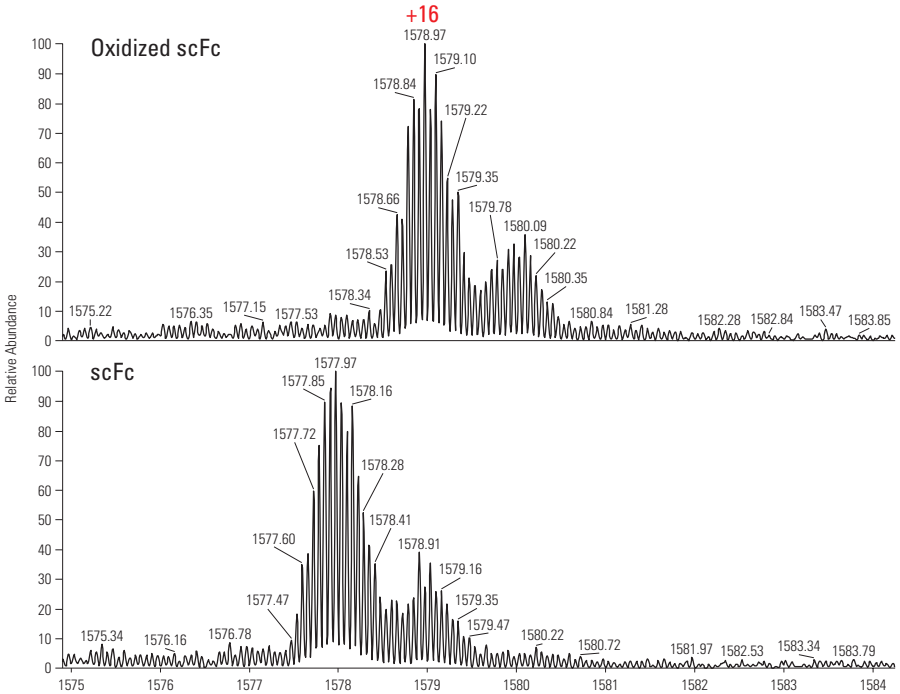


Methionine (Met) oxidation is one of the critical quality attributes required to be closely monitored. The two Met residues in the CH2-CH3 domain interface of recombinant humanized and fully human IgG1 antibodies were found susceptible to oxidation. It is desirable to monitor the progress of the Met oxidation without complete digestion of the mAb. This allows you to maintain site specific information to where the oxidation is occurring on the protein. A workflow was designed to first reduce mAb and then further digest it with IdeS resulting in smaller (25 kDa) fragments. Further digestion of the HC by IdeS resulted in two smaller fragments: scFc and Fd'. The MAbPac RP baseline separates scFc, LC, and Fd' fragments as shown below. The +10 charge state of the oxidized scFc and non-oxidized scFc are at *m/z* 2525.60 and at *m/z* 2524.08.



Column:	MAbPac RP, 4 µm		
Format:	3.0 × 50 mm		
Mobile phase A:	Water/formic acid/trifluoroacetic acid (99.88 : 0.1 : 0.02 v/v/v)		
Mobile phase B:	Acetonitrile/water/formic acid/trifluoroacetic acid (90 : 9.88 : 0.1 : 0.02 v/v/v/v)		
Gradient:	Time (min)	%A	%B
	0.0	75	25
	1.0	75	25
	11.0	63	37
	12.0	63	37
	14.0	75	25
	15.0	75	25
Flow rate:	0.5 mL/min		
Inj. volume:	2 µL		
Temp.:	80 °C		
MS detection:	positive-ion mode		
Mass spec:	Q Exactive Plus hybrid quadrupole-Orbitrap MS system		
Sample:	Oxidized trastuzumab, reduced by DTT and digested by IdeS (1 mg/mL)		

Data collected using 280K resolution shows isotopically resolved oxidized scFc and non-oxidized scFc at +16 charge state, see below.

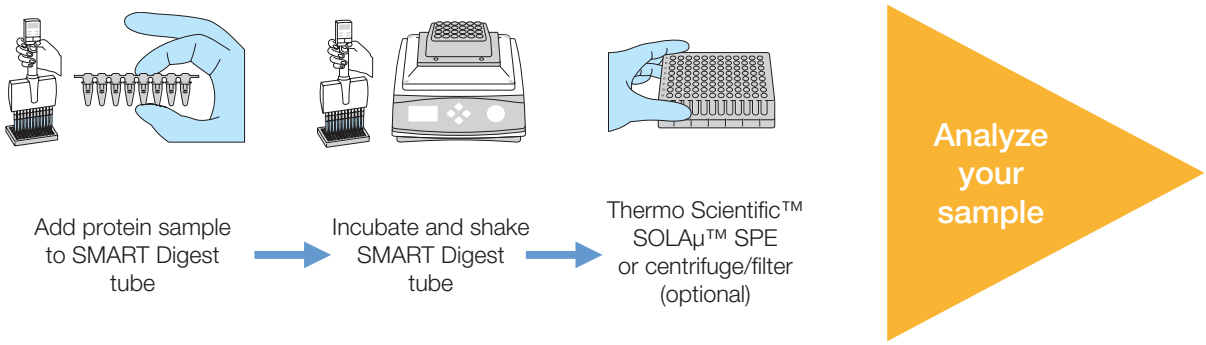


Peptide mapping

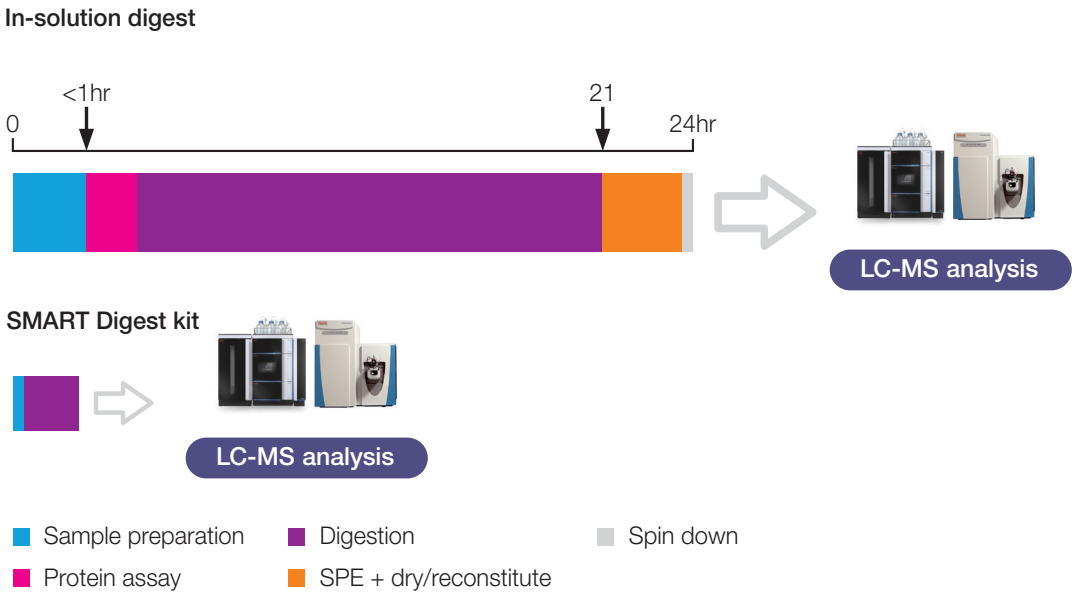
Reversed-phase liquid chromatography of peptides is used in the biopharmaceutical industry to provide information on the nature and quality of protein therapeutics. In the case of reverse phase chromatography with UV detection, the assignment of a peptide to a peak is based on retention time comparison between the investigated sample and a known reference sample. Since peak assignment is solely based on retention time, the highest run-to-run retention time precision is required in order to avoid incorrect peptide identification. This is complicated by the employment of in-solution digestion protocols which are time consuming (often taking in excess of 24 hours to complete) and highly irreproducible.

The Thermo Scientific™ SMART™ Digest Kits, which are based on an immobilized trypsin design, deliver highly reproducible digestions quickly and with increased sensitivity compared to in-solution digests.

SMART Digest kit

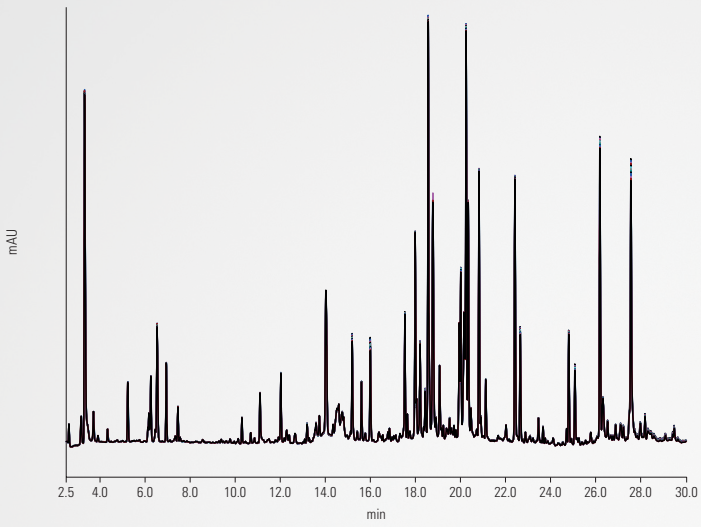


SMART Digest kit vs. a conventional in-solution digest protocol

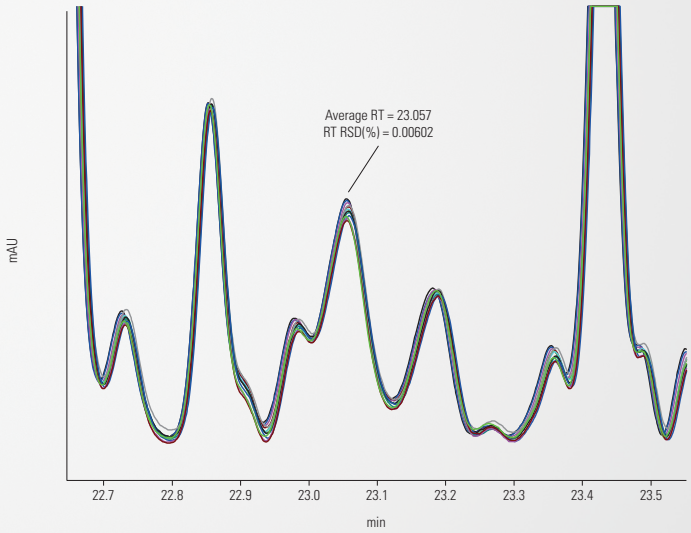


This is complemented by the Vanquish UHPLC system featuring a binary pump with extremely low pulsation ripple, which allows highly stable flow delivery and highly precise gradient formation. The Vanquish UHPLC system is capable of providing unmatched retention time precision for gradient separations.

The Thermo Scientific™ Acclaim™ Vanquish™ C18 analytical column (2.2 µm, 2.1 × 250 mm) provides the high resolution separation required by the method.



Column:	**Acclaim Vanquish 120 C18 analytical column (2.2 µm)**
Format:	2.1 × 250 mm
Mobile phase A:	0.05% trifluoroacetic acid in water
Mobile phase B:	0.04% trifluoroacetic acid in 8/2 acetonitrile/water (v/v)
Gradient:	4% B to 50% B in 30 minutes
Flow rate:	0.4 mL/min
Inj. volume:	5 µL
Temp.:	80 °C
Detection:	UV, 214 nm
Sample:	mAb tryptic digest (lower image, zoomed-in view: overlay of 10 injections)

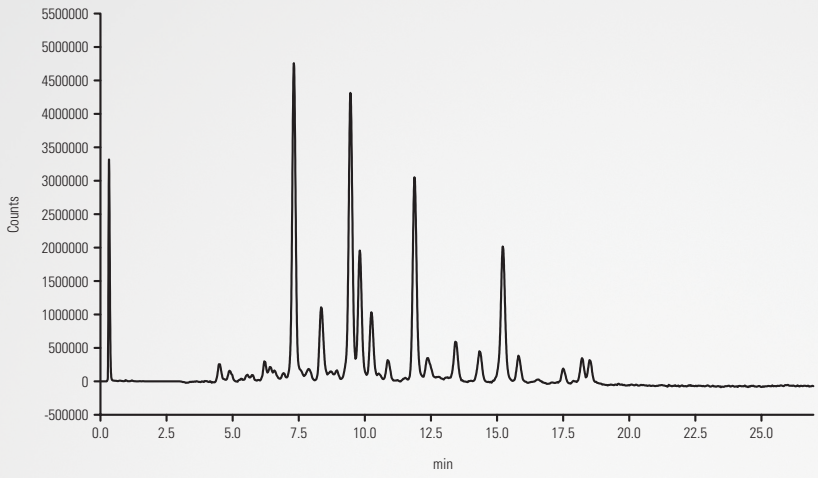


Glycan analysis

In order to meet the challenges of glycan analysis, we offer three different column chemistries with different selectivity.

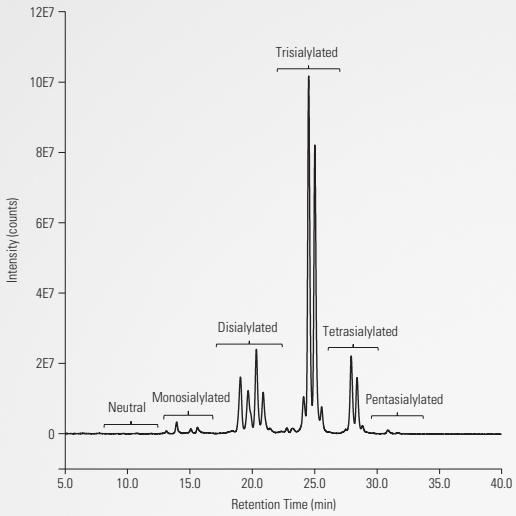
	Thermo Scientific™ GlycanPac™ AXR-1 HPLC columns	Thermo Scientific™ GlycanPac™ AXH-1 LC columns	Thermo Scientific™ Accucore™ 150 Amide HILIC LC columns
Column chemistry	Alkyl chain with tertiary amine	Polar groups with amine	Polyamide
Retention mechanism	WAX/RP mixed-mode	WAX/HILIC mixed-mode	HILIC
Silica substrate	High-purity totally porous spherical silica (1.9 and 3.0 µm)	High-purity totally porous spherical silica (1.9 and 3.0 µm)	High-purity superficially porous spherical silica (2.6 µm)
Feature	Ultra-high resolution (size, charge and isomerism based separation)	High-resolution with added structural information (size and charge based separation)	High-resolution (size-based separation)
Applications	Charged glycan species (native or labeled)	Neutral or charged glycans (native or labeled)	Neutral glycans with labeling

HILIC columns commonly used for glycan analysis are based on amide, amine, or zwitterionic packing materials. These columns separate glycans mainly by hydrogen bonding, resulting in separations based on size and composition. Thermo Scientific™ Accucore™ 150 Amide HILIC HPLC phase is designed for the separation of hydrophilic, neutral glycans. Here is an example of human IgG glycans analyzed on an Accucore 150 Amide HILIC HPLC columns.



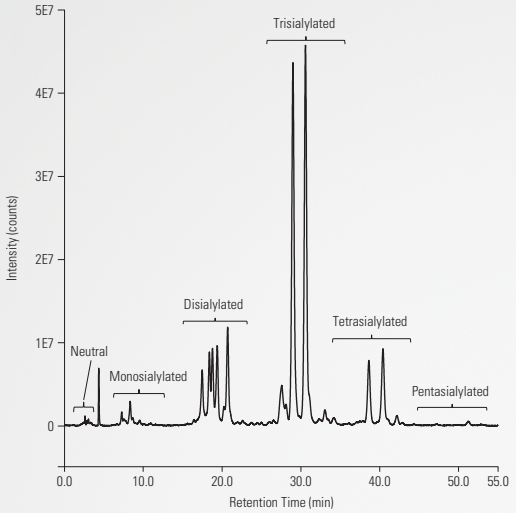
Column:	**Accucore 150 Amide HILIC (2.6 µm)**
Format:	100 × 2.1 mm
Mobile phase A:	Acetonitrile
Mobile phase B:	50 mM ammonium formate pH 4.4 (prepared from LS-N-BUFFX40, Ludger Ltd)
Gradient:	Time (min) %A
	0 20
	26 40
	27 50
Flow rate:	1 mL/min
Inj. volume:	5 µL in water, 50 µL loop
Temp.:	60 ºC
Backpressure:	300 bar
Injection wash solvent:	Acetonitrile/water (78:22 v/v)
Detection:	Fluorescence at 320/420 nm

GlycanPac AXH-1 column combines both weak anion exchange (WAX) and HILIC retention mechanisms for optimal selectivity and high resolving power. The WAX functionality provides retention and selectivity for negatively charged glycans, while the HILIC mode facilitates the separation of glycans of the same charge according to their polarity and size. GlycanPac AXH-1 is designed for neutral and charged glycans analysis, in their native or labeled form. Neutral and acidic 2AB labeled N-glycan from bovine fetuin are separated using a GlycanPac AXH-1 column (1.9 µm, 2.1 × 150 mm). In total, 35 glycan peaks are resolved and detected.



Column:	**GlycanPac AXH-1, 1.9 µm**				
Format:	2.1 × 150 mm				
Mobile phase A:	Acetonitrile				
Mobile phase B:	D.I. water				
Mobile phase C:	Ammonium formate (100 mM, pH4.4)				
Gradient:	Time (min)	A%	B%	C%	Curve
	-10	78	20	2	5
	0	78	20	2	5
	1	78	20	2	5
	35	60	20	20	5
	40	50	20	30	5
	45	50	20	30	5
Flow rate:	0.4 mL/min				
Inj. volume:	100 pmoles				
Temp.:	30 ºC				
Detection:	Fluorescence at 320/420 nm				
Sample:	2AB labeled *N*-glycan from bovine fetuin				

The GlycanPac AXR-1 column is based on novel mixed-mode column chemistry, combining the retention mechanisms of both WAX and reversed-phase (RP) properties for optimal selectivity and resolution. The WAX functionality provides retention and selectivity for negatively charged glycans, while the reversed-phase mode facilitates the separation of glycans of the same charge according to their isomeric structure, and size. As a result, the GlycanPac AXR-1 column provides the highest resolution of charged 2AB-labeled N-linked glycans. 2AB-labeled N-linked glycans from bovine fetuin are separated using a GlycanPac AXR-1 column (1.9 µm, 150 × 2.1 mm). In total, 59 glycan peaks are resolved and detected.



Column:	**GlycanPac AXR-1, 1.9 µm**				
Format:	2.1 × 150 mm				
Mobile phase A:	Acetonitrile				
Mobile phase B:	D.I. water				
Mobile phase C:	Ammonium formate (100 mM, pH4.4)				
Gradient:	Time (min)	A%	B%	C%	Curve
	-10	0	93	7	5
	0	0	93	7	5
	1	0	93	7	5
	55	0	30	70	5
	56	70	0	30	5
	70	70	0	30	5
Flow rate:	0.4 mL/min				
Inj. volume:	100 pmoles				
Temp.:	30 oC				
Detection:	Fluorescence at 320/420 nm				
Sample:	2AB labeled *N*-glycan from bovine fetuin				

Application notes

Visit the AppsLab Library of Analytical Applications at **thermofisher.com/appslab** for detailed method information, chromatograms and related compound information.
More application notes and resources are available on **thermofisher.com/NIBRT**.

Column	Application note	Literature code
MAbPac Protein A	A novel affinity Protein A column for monoclonal antibody (mAb) titer analysis	**AN20813**
MAbPac SEC-1	Lifetime stability of size-exclusion chromatography columns for protein aggregate analysis	**AN72362**
	Analysis of monoclonal antibodies and their fragments by size-exclusion chromatography coupled with an Orbitrap mass spectrometer	**AN20940**
ProPac Elite WCX	Separation of IgG2 and IgG4 therapeutics using weak cation exchange chromatography	**AN21843**
	Salt gradient analysis of IgG1 monoclonal antibodies using a 5 µm WCX chromatography column	**AN21844**
MAbPac SCX-10	A global pH-gradient based charge variant analysis directly coupled to HRAM-MS (CVA-MS) for mAb analysis	**AN21917**
	High throughput, high resolution monoclonal antibody analysis with small particle size HPLC columns	**AN21008**
CX-1 pH gradient buffer	A novel pH gradient separation platform for monoclonal antibody (mAb) charge variant analysis	**AN20784**
	pH gradient analysis of IgG1 therapeutic monoclonal antibodies using a 5 µm WCX column	**AN21845**
	A fast and robust linear pH gradient separation platform for monoclonal antibody (mAb) charge variant analysis	**AN20946**
	Separation of intact monoclonal antibody sialylation isoforms by pH gradient ion-exchange chromatography	**AN71062**
	High resolution charge variant analysis for top-selling monoclonal antibody therapeutics using linear pH gradient separation platform	**AN21092**
MAbPac RP	Confident monoclonal antibody sequence verification by complementary LC-MS techniques	**AN21919**
	Fast analysis of therapeutic monoclonal antibody fragments using a supermacroporous, reversed-phase chromatography column	**AN21200**
MAbPac HIC-10	High resolution separation of a fusion protein on MAbPac HIC-10 column	**AN71205**
	HIC as a complementary, confirmatory tool to SEC for the analysis of mAb aggregates	**AN21206**
MAbPac HIC-20	High resolution separation of mAb fragments on MAbPac HIC-20 column	**AN21047**
	High resolution separation of monoclonal antibody (mAb) oxidation variants on the MAbPac HIC-20 column	**AN21069**
MAbPac HIC-butyl	High resolution separation of cysteine-conjugated antibody drug mimics using hydrophobic interaction chromatography	**AN21079**
GlycanPac AXH-1	Separation of 2AB labeled N-glycans from bovine fetuin on a novel mixed-mode stationary phase	**AN20754**
	Structural analysis of native N-glycans released from proteins using a novel mixed-mode column and a hybrid quadrupole-orbitrap mass spectrometer	**AN20827**
GlycanPac AXR-1	Separation of 2AB-labeled N-linked glycans from bovine fetuin on a novel ultra high resolution mixed-mode column	**AN20908**
	Separation of 2AA-labeled N-linked glycans from human IgG on a high resolution mixed-mode column	**AN20909**
	Separation of 2AA-labeled N-linked glycans from glycoproteins on a high resolution mixed-mode column	**AN20910**
Acclaim VANQUISH C18	Easy, fast and reproducible analysis of host cell protein (HCP) in monoclonal antibody preparations	**AN21918**
Accucore 150 Amide HILIC	Analysis of human IgG glycans on a solid core amide HILIC stationary phase	**AN20703**

Ordering information

Affinity columns

MAbPac Protein A

Particle size (µm)	Format	Length (mm)	4.0 mm ID
12	HPLC column	35	063655

Size-exclusion columns

MAbPac SEC-1

Particle size (µm)	Format	Length (mm)	2.1 mm ID	4.0 mm ID	7.8 mm ID
5	HPLC column	50	–	074697	–
		150	088790	075592	–
		250	088789	074696	88460

Ion exchange columns

ProPac 3R SCX

Particle size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID
3	HPLC column	50	43103-052068	43103-054068
		100	43103-102068	43103-104068

MAbPac SCX-10

Particle size (µm)	Format	Length (mm)	2.1 mm ID	4.0 mm ID	4.6 mm ID	9.0 mm ID
3	HPLC column	50	–	075603	–	–
5	HPLC column	50	082675	078656	082674	–
		150	088242	085198	085209	–
		250	082515	078655	082673	–
10	HPLC column	50	–	075603	–	–
		150	–	075602	–	–
		250	075604	074625	–	088784
	HPLC column Lot select 3 column	250 (1 lot of resin)	–	088782	–	–
	HPLC column	250 (3 lot of resin)	–	088783	–	–

ProPac Elite WCX

Particle size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID
5	HPLC column	50	303028	302973
		150	303027	302972
		250	303026	303025
	HPLC column Lot select 3 column	150 (1 lot of resin)	–	302976
	HPLC column Lot select 3 column	150 (3 lot of resin)	–	302977
	HPLC column Lot select 3 column	250 (1 lot of resin)	–	303061
	HPLC column Lot select 3 column	250m (3 lot of resin)	–	303062

Ordering information continued

Ion exchange columns (continued)

ProPac WCX-10

Particle size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22 mm ID
10	Guard column	50	063480	054994	–	–
	HPLC column	50	–	074600	–	–
		100	–	088778	–	–
		150	–	088779	–	–
		250	063472	054993	063474	088766

ProPac SCX-10

Particle size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22 mm ID
10	Guard column	50	063462	079930	–	–
	HPLC column	250	063456	054995	063700	088769

ProPac SCX-20

Particle size (µm)	Format	Length (mm)	4.0 mm ID
10	Guard column	50	074643
	HPLC column	250	074628

ProPac WAX-10

Particle size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22 mm ID
10	Guard column	50	063470	055150	–	–
	HPLC column	250	063464	054999	063707	088771

ProPac 3R SAX

Particle size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID
3	HPLC column	50	43203-052068	43203-054068
		100	43203-102068	43203-104068

ProPac SAX-10

Particle size (µm)	Format	Length (mm)	2.0 mm ID	4.0 mm ID	9.0 mm ID	22 mm ID
10	Guard column	50	063454	054998	–	–
	HPLC column	50	–	078990	–	–
		250	063448	054997	063703	088770

Gradient buffers

Description	pH	Quantity	Part no.
CX-1 pH gradient buffer A	5.6	125 mL	083273
CX-1 pH gradient buffer B	10.2	125 mL	083275
CX-1 pH pH gradient buffer A	5.6	250 mL	085346
CX-1 pH pH gradient buffer B	10.2	250 mL	085348
CX-1 pH pH gradient buffer A	5.6	500 mL	302779
CX-1 pH pH gradient bufferr B	10.2	500 mL	302780
CX-1 pH pH gradient buffer A	5.6	1 L	303274
CX-1 pH pH gradient buffer B	10.2	1 L	303275

Ordering information continued

Hydrophobic interaction columns

MAbPac HIC family

Description	Particle size (µm)	Format	Length (mm)	4.6 mm ID
MAbPac HIC-10	5	HPLC column	100	088480
			250	088481
		Guard cartridges (2/pk)*	10	088482
MAbPac HIC-20	5	HPLC column	100	088553
			250	088554
		Guard cartridges (2/pk)*	10	088555
MAbPac HIC-Butyl	5	HPLC column	100	088558
		Guard cartridges (2/pk)*	10	088559

*Standard guard cartridge holder required (P/N 069580)

ProPac HIC-10

Particle size (µm)	Format	Length (mm)	2.1 mm ID	4.6 mm ID	7.8 mm ID
5	HPLC column	75	–	–	063665
		150	063653	063655	–
		250	–	074197	–

Reversed-phase protein columns

MAbPac RP

Particle size (µm)	Format	Length (mm)	1.0 mm ID	2.1 mm ID	3.0 mm ID
4	HPLC column	50	303182	088648	088645
		100	303183	088647	088644
		150	303184	303270	303269
	Guard cartridges (2/pk)*	10	–	088649	088646

*Standard guard cartridge holder required (P/N 069580)

Thermo Scientific™ ProSwift™ Monolithic

Packing	Format	Functional group	Length (mm)	1.0 mm ID	4.6 mm ID
Monolithic	HPLC column	RP-1S	50	–	064297
		RP-2H	50	–	064296
		RP-3U	50	–	064298
		RP-4H	50	069477	–
			250	066640	–

Ordering information continued

Reversed-phase peptide columns

Acclaim RSLC 120 C18, Thermo Scientific™ Acclaim™ Polar Advantage II (PA2)

Particle size (µm)	Format	Length (mm)	ID (mm)	120 C18	Polar Advantage II
2.2	RSLC column	30	2.1	071400	071402
			3.0	–	–
		50	2.1	068981	068989
			3.0	071605	071608
		100	2.1	068982	068990
			3.0	071604	071607
		150	2.1	071399	071401
		250	2.1	074812	074814
	Vanquish	150	2.1	071399-V	–
		200	2.1	074812-V	–
	PA2	150	2.1	071401-V	–
		200	2.1	074814-V	–

Acclaim 300 C18

Particle size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
3	HPLC column	50	060263	–	060265
		150	060264	063684	060266
5	Guard cartridges (2/pk)*	10	069690	075721	069697

*Standard guard cartridge holder required (P/N 069580)

Accucore 150 C18

Particle size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID	0.075 mm ID
2.6	HPLC column	30	16126-032130	–	16126-034630	–
		50	16126-052130	–	–	–
		100	16126-102130	–	–	–
		150	16126-152130	17126-153030	16126-154630	
		500	–	–	–	164942
	Guard cartridges (2/pk)*	10	16126-012105	16126-013005	16126-014005	–

*Guard cartridge holder (P/N 852-00)

SMART Digest kits

Description	Cat. no.
SMART Digest Trypsin Kit, with Collection plate	60109-101
SMART Digest Trypsin Kit, Magnetic Bulk Resin option	60109-101-MB
SMART Digest Trypsin Kit, with SOLAµ/collection plate	60109-103
SMART Digest Trypsin Kit Magnetic Bulk Resin option with SOLAµ/collection plate	60109-103-MB
SMART Digest Trypsin Kit, with Filter/Collection plate	60109-102
SMART Digest Trypsin Kit, Magnetic Bulk Resin option with Filter/Collection plate	60109-102-MB
SMART Digest IA Kit, Streptavidin non-magnetic	60110-101
SMART Digest IA Kit, Streptavidin magnetic	60110-104
SMART Digest IA Kit, Protein A non-magnetic	60111-101
SMART Digest IA Kit, Protein A magnetic	60111-104
SMART Digest IA Kit, Protein G non-magnetic	60112-101
SMART Digest IA Kit, Protein G magnetic	60112-104

Ordering information continued

Glycan analysis columns

GlycanPac AXH-1

Particle size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
1.9	HPLC column	250	082521	–	–
		150	082472	–	–
		100	082473	–	–
3		150	082470	082469	082468
3	Guard cartridges (2/pk)*	10	082476	082475	082474

Standard guard cartridge holder required (P/N 069580)

GlycanPac AXR-1

Particle size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
1.9	HPLC column	150	088136	–	–
		250	088135	–	–
3		150	088136	088136	088136

*Standard guard cartridge holder required (P/N 069580)

Accucore VANQUISH C18+ UHPLC

Particle size (µm)		Length (mm)	2.1 mm ID
1.5		50	27101-052130
		150	27101-152130
		100	27101-102130

Accucore 150-Amide-HILIC

Particle size (µm)	Format	Length (mm)	2.1 mm ID	3.0 mm ID	4.6 mm ID
2.6	HPLC column	50	16726-052130	16726-053030	–
		100	16726-102130	16726-103030	16726-104630
		150	16726-152130	16726-153030	16726-154630
		250	16726-252130	–	–
	Guard cartridges (2/pk)*	10	16726-012105	–	–

*Guard cartridge holder (P/N 852-00)

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