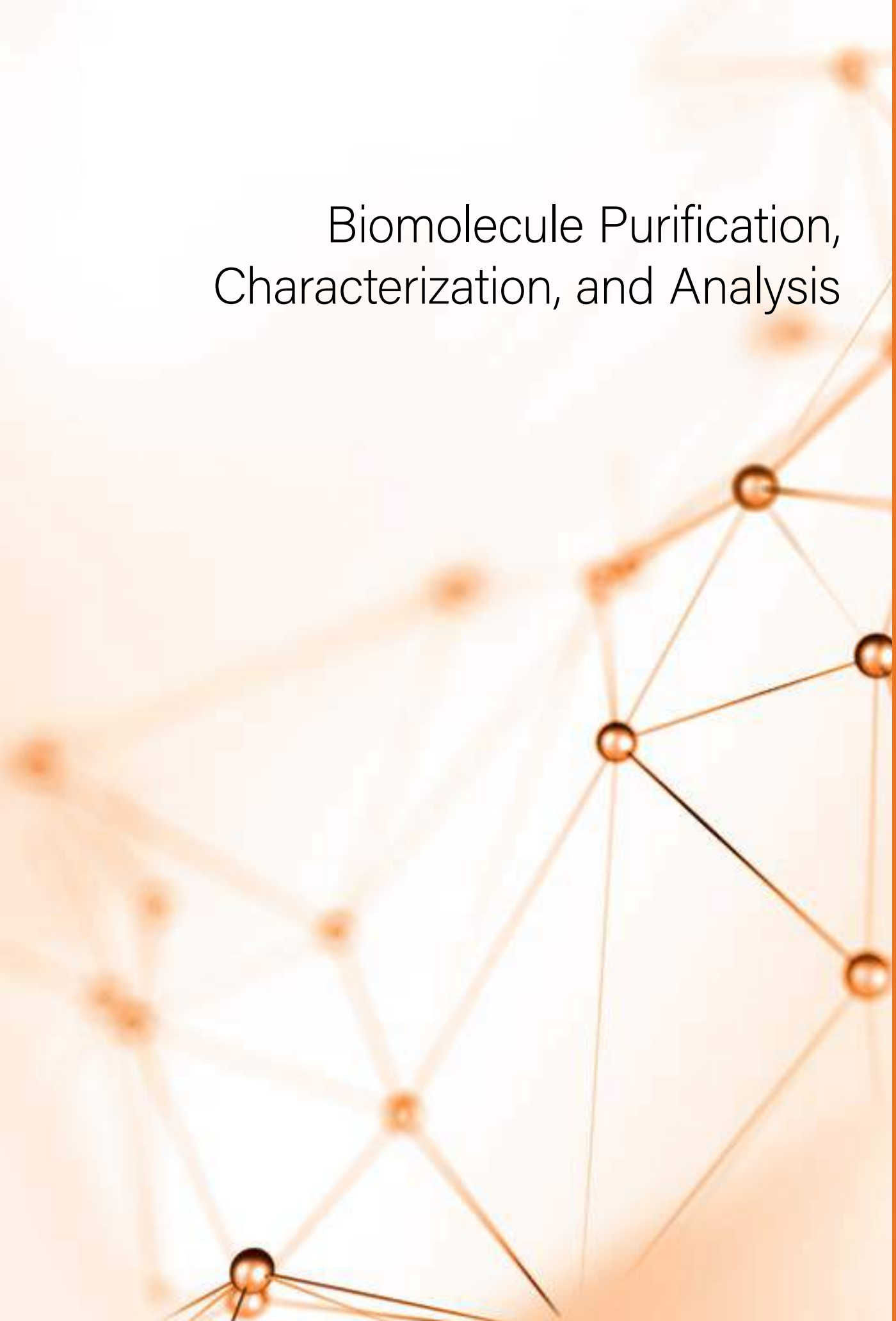
Biomolecule Purification, Characterization, and Analysis



Biomolecule Purification, Characterization, and Analysis

Contents

Biomolecule Purification, Characterization, and Analysis

Innovative Technologies in Separation Science and Analytical Biochemistry

Advances in the areas of genomics, proteomics, metabolomics, and molecular and system biology continue to revolutionize the diagnosis and treatment of diseases and increase our fundamental understanding of biological processes.

Beginning with a keen understanding of today's biomolecule-related challenges, Waters scientists and engineers continuously seek purposeful innovations that help deliver impactful solutions in applications ranging from proteomics and biomarker discovery through the commercialization of advanced biopharmaceuticals.

Waters comprehensive chemistry consumables family includes:

- BioResolve™ line of application specific columns that help streamline analytical challenges associated with mAb characterizations and associated biotherapeutic drug separations
- Scalable peptide columns for nano, capillary, analytical, and preparative applications
- Protein size-exclusion, ion-exchange, hydrophobic-interaction, hydrophilic-interaction, and reversed-phase columns for analytical HPLC, UHPLC, UPLC, and lab-scale purification applications
- AccQ•Tag™ Ultra Chemistry specific for Waters UPLC Amino Acid Analysis Solution, as well as Pico•Tag™ and AccQ•Tag for HPLC-based amino acid analyses
- Oligonucleotide columns for synthetic oligonucleotide and DNA/RNA fragment isolations and analyses
- GlycoWorks™ *Rapi*Fluor-MS™ sample preparation kits, columns and standards, and HILIC and Mixed-Mode Glycan Columns for the analysis of released glycans
- MaxPeak™ Premier High Performance Surface Technology available with many of the biomolecule chemistries reducing the need for long conditioning and significantly reducing non-specific binding and analyte loss
- IonHance™ LC-MS grade mobile phases and additives help deliver high-quality data
- Automation for many of the complex sample preparation workflows with the Biopharma Andrew+™ Pipetting Robot and scripts for Hamilton and Tecan.

In addition, several of our biomolecule separation offerings (e.g. ACQUITY™ Premier Protein SEC 250 Å, ACQUITY UPLC Glycoprotein BEH Amide, 300 Å offering, and Waters UPLC Amino Acid Analysis Solution) were developed for use on ACQUITY UPLC-based Systems to help obtain accurate, precise, and highly resolving quantitative analysis of various therapeutics.

Designed and QC tested with relevant biomolecules to help ensure reliable, high performing column-to-column consistency.

Bioseparations Columns

waters.com/biosep

Bioseparations Analytical Standards and Reagents

waters.com/biostds

BioAdvisor

Discover the best chemistry solutions for your application with this tool.

BioAdvisor enables you to select an appropriate UPLC/UHPLC or HPLC column and/or chemistry consumable for a desired application, all organized by molecule type.

For more information, visit **waters.com/BioAdvisor**

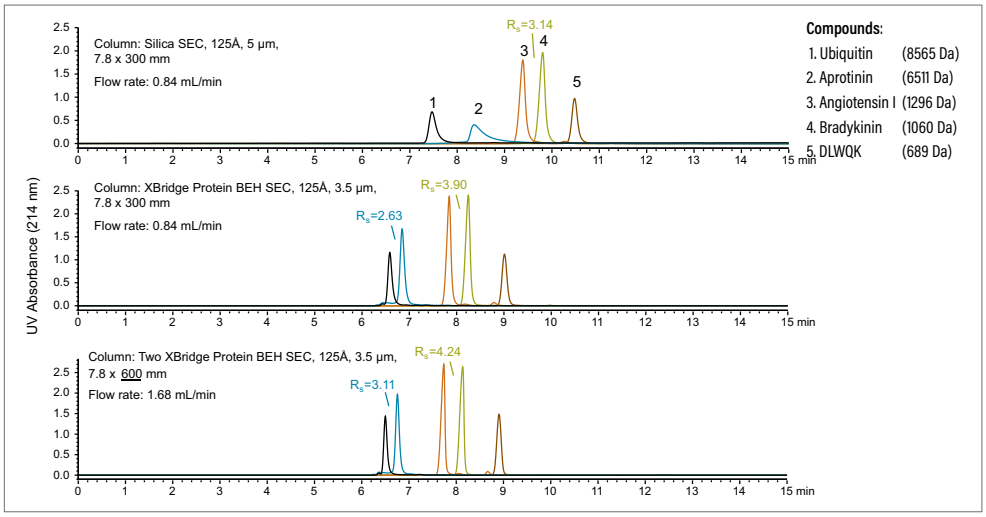
Factors to Consider when Investing and Using HPLC, UHPLC, or UPLC Columns for Bioseparations

Many factors can affect the quality of data obtained from LC-based separations of peptides, proteins, and other biomolecules. The following pages list just a few of the important factors to consider when selecting an appropriate HPLC, UHPLC, or UPLC column for analytical or lab-scale applications. Once an appropriate column is selected, time must be invested in developing a satisfactory separation, so we have also included a few useful method development "tips and tricks". We hope that these few examples will help chromatographers select a column and develop a method that matches their specific instrumentation and application needs.

PART 1: COLUMN SELECTION AND INSTALLATION

Effect of Particle Composition on SEC Peptide Separations

- Particle composition (e.g., silica, polymer, hybrid) influences desired LC separations
- These non-desired secondary interactions (i.e., ionic or hydrophobic) can be beneficial or detrimental
- Particle composition can also influence column life (e.g., silica-based at pH >7)

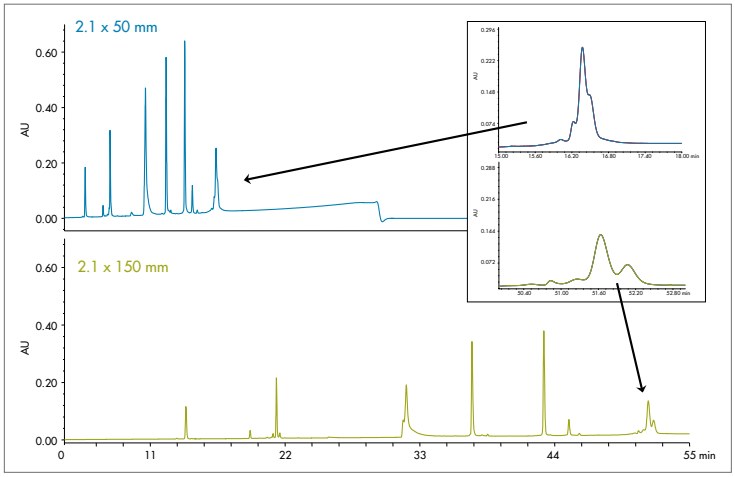


In the size-exclusion chromatographic (SEC) separation shown, a series of synthetic peptides are separated on a column containing 100% silica-based, diol-coated particles (top) vs. Waters diol-coated, bridged-ethylene hybrid (BEH Technology™) particles that have less-undesired-free silanols. Consequently, and as shown in this example, use of SEC columns that contain BEH particles results in comparatively less undesired secondary ionic interactions between the ubiquitin and aprotinin peaks and less peak tailing making quantitation of these peptides more reliable.

For more information, reference application note 720005369EN.

Effect of Column Length on Reversed-Phase Protein Separations

- Use of longer LC columns can translate into improved component resolution
- Analysis time increases as column length increases
- Separated peak volume increases as column length increases

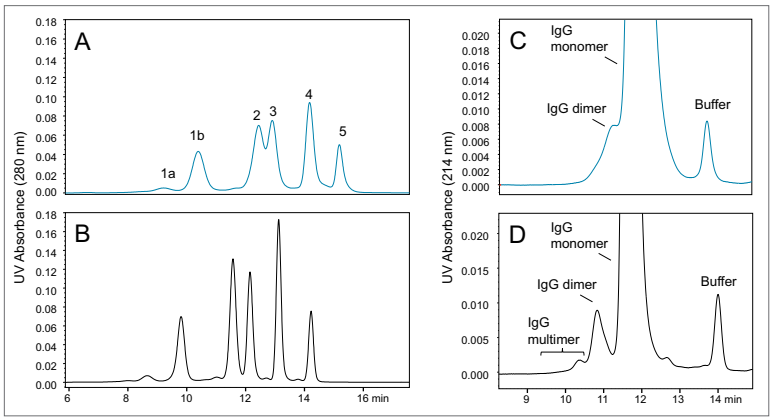


Increasing the length of the column will increase the resolving power for a separation. This is shown with the separation of a protein mixture. The additional small peaks surrounding the Phosphorylase b can be seen more readily on the longer column, as seen in the inset, but it comes at the cost of a 3-fold increase in run time and ~40% loss of sensitivity. Depending on the application objective, this may be a useful parameter to improve resolution.

For more information, reference application note 720003875EN.

Effect of Particle Size on SEC Protein Separations

- Well-packed columns containing small particles can improve a separation
- System back pressure will increase as particle size decreases
- Consequently, LC instrumentation can limit potential column use



A comparison of separations of Waters BEH450 SEC Protein Standard Mix (p/n: 186006842) and Intact mAb Mass Check Standard (p/n: 186006552, diluted to 1 mg/mL) on 450 Å, silica-based 8 µm (Frames A and C) and 450 Å, BEH 3.5 µm (Frames B and D) SEC columns. Both columns were the same dimensions (7.8 × 300 mm) and separations were performed with the same flow rate (0.84 mL/min) and with the same sample loads. Peak identities for chromatograms A and B are: 1a) thyroglobulin dimer (1.3 MDa), 1b) thyroglobulin (669 KDa), 2) IgG (150 KDa), 3) BSA (67 KDa), 4) myoglobin (14 KDa), and uracil (112 Da). For the chromatograms in frames C and D the molecular weights of the IgG monomer, dimer, and multimer are approximately 150 KDa 300 KDa, and ≥450 KDa, respectively.

For more information, reference application note 720005202EN.

Choosing an Analytical Column that Best Matches LC-based Instrumentation

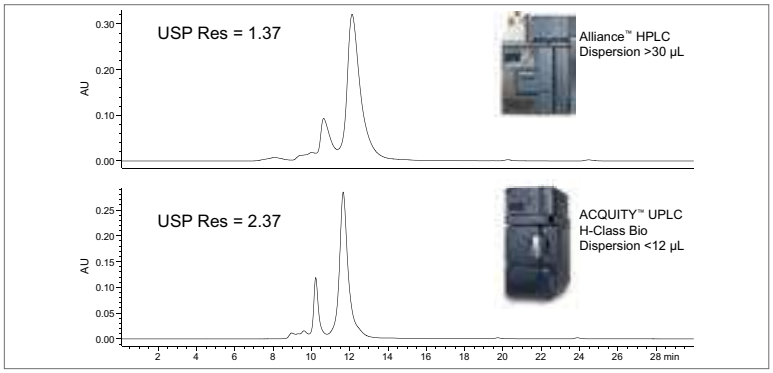
- Standard methods can be performed to measure system dispersion (band broadening)
- Different LC systems have different dispersion values (e.g., band broadening)
- Optimal chromatographic separations are obtained when the appropriate column (e.g., column I.D., particle size) and separation conditions (e.g., flow rate, temperature, gradient) are selected based on LC system design

HPLC	UHPLC	UPLC
Dispersion >30 µL	Dispersion 12–30 µL	Dispersion <12 µL
Columns: 3.0–4.6 mm I.D.; 3–10 µm particles	Columns: 2.1–4.6 mm I.D.; 1.7–5 µm particles	Columns: 1.0–4.6 mm I.D.; 1.6–5 µm particles
Recommended column: 4.6 mm I.D., 5 µm particles	Recommended column: 3.0 mm I.D., 2.5 µm particles	Recommended column: 2.1 mm I.D., 1.7 µm particles
Typical operating pressure: <6000 PSI	Typical operating pressure: <10,000 PSI	Typical operating pressure: <15,000 PSI

Dispersion – n. Broadening of an analyte band due to both on-column effects (diffusion and mass transfer kinetics which are both dependent on particle size and linear velocity) and system effects (tubing internal diameter [I.D.] and length, connections, detector flow cell volumes, etc.) True separation performance is governed by the system dispersion paired with a flow rate range that yields the highest possible efficiency for a given analytical column. Due to these dispersion levels, we can appropriately match the right type of column size (volume) with the system dispersion. UPLC, having a very low dispersion volume, provides the greatest flexibility in terms of the columns that can be run on the system.

Effect of LC System Dispersion on SEC Monoclonal Protein Separations

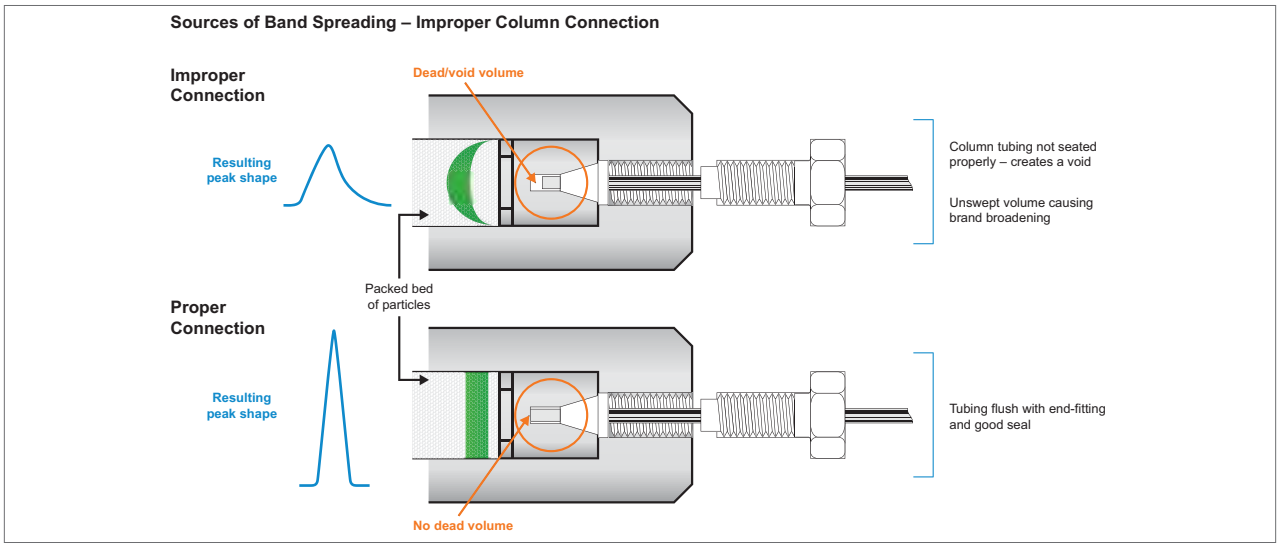
- LC Systems with lower system volumes better maintain column generated separations
- Isocratic-based SEC separations are most sensitive to deleterious band broadening effects



The extra system volume of the traditional HPLC System (top) caused the column separated peaks to "partially remix" resulting in a USP resolution factor of 1.37 vs. the superior 2.37 value obtained when the separation was performed on an ACQUITY UPLC System. This slide shows how the LC system's "band broadening" can adversely affect the quality of the mAb separation generated with the same XBridge™ Protein BEH SEC, 200 Å, 2.5 µm Column, SEC eluent, and sample.

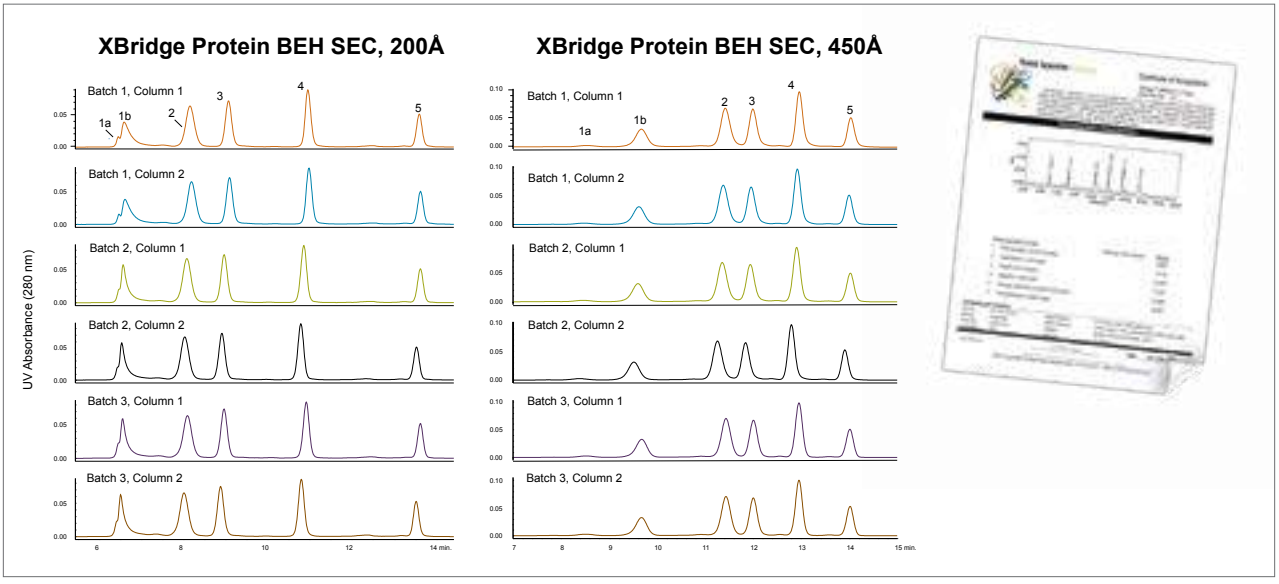
Importance of Making Good Connections from Column to LC System

- Poor column-to-instrument connections can degrade a chromatographic separation
- Perceived column leaking can also be caused by a poor connection



Importance of Batch-to-Batch and Column-to-Column Reproducibility

- Column reproducibility is a key attribute when selecting a column
- QC testing with relevant biological standards can help ensure consistency

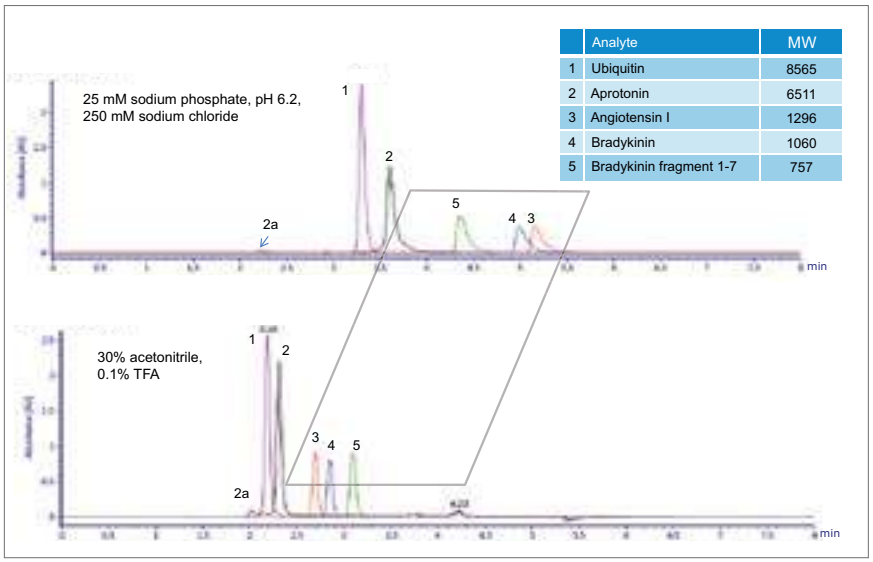


Shown are overlays of the separations of Waters BEH200 SEC Molecular Weight Standard (p/n: 186006518) on 200 Å and 450 Å BEH 3.5 µm SEC columns. Two columns (300 mm length × 7.8 mm I.D.) were packed from three individual manufactured batches of particles to evaluate both batch-to-batch and column-to-column reproducibility. Peak identities are: 1a) thyroglobulin dimer (1.34 MDa), 1b) thyroglobulin (669 KDa), 2) IgG (150 KDa), 3) BSA (67 KDa), 4) myoglobin (14 KDa), and 5) uracil (112 Da). Separations were performed on an ACQUITY UPLC H-Class Bio System.

PART 2: BIOSEPARATION METHOD DEVELOPMENT

Eluent Effect on SEC Peptide Separations

- Non-desired, secondary interactions can compromise LC separations
- Use of an appropriate LC eluent can minimize secondary interactions



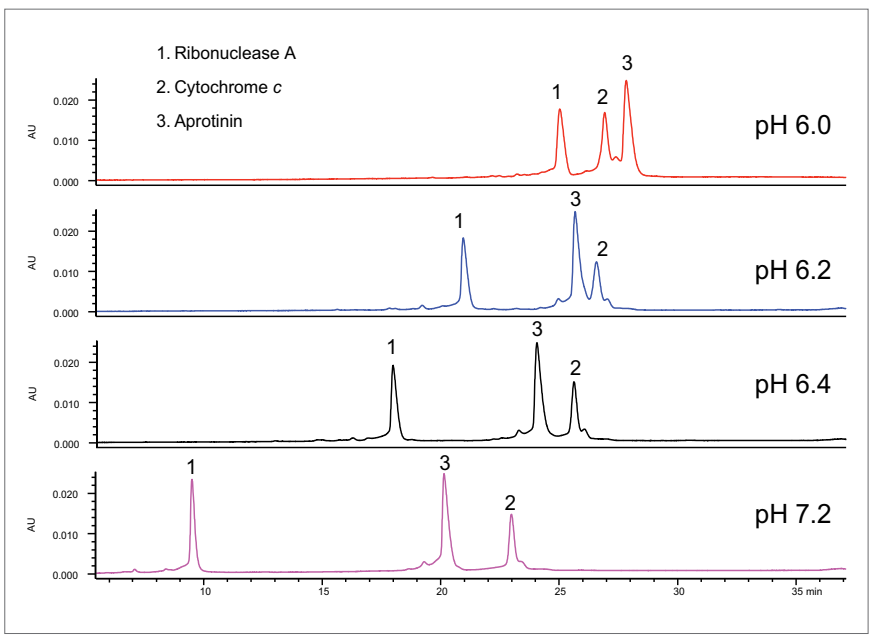
	Analyte	MW
1	Ubiquitin	8565
2	Aprotonin	6511
3	Angiotensin I	1296
4	Bradykinin	1060
5	Bradykinin fragment 1-7	757

Method development experiments evaluated the effect of mobile-phase pH and salt concentration. The results showed minimal effect of salt concentrations (150–350 mM) and mobile-phase pH (6.2–7.4) on retention time (data not shown). All of the aqueous mobile phases resulted in later than expected elution for most small peptides and proteins (<17,000 Da) as well as elution order that did not correspond to published molecular weight values. For example, bradykinin fragment 1–7 (MW 757) eluted before greater molecular weight peptides such as angiotensin I (MW 1296) and bradykinin (MW 1060). These results also suggest the non-ideal interactions of the tested peptides with the media is not solely due to an "ion-exchange" mechanism since increasing salt concentration had no significant impact on retention time.

For more information, reference application note 720004412EN.

Effect of pH on Ion-Exchange Protein Separations

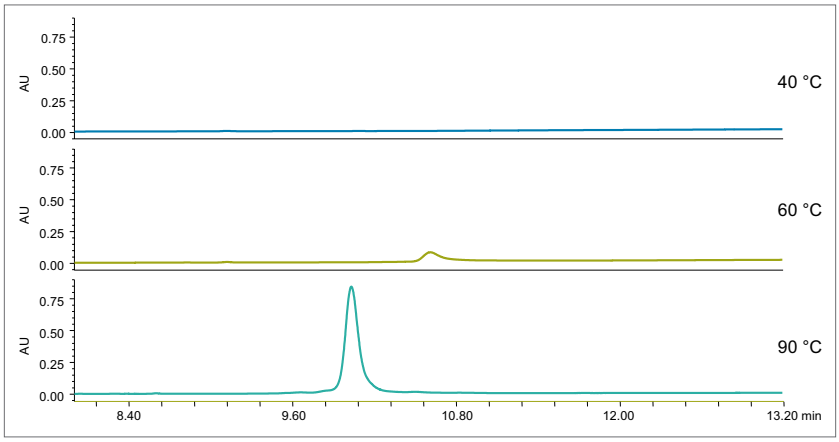
- pH has a significant influence on IEX separations
- Optimal pH for a separation is sample dependent



To illustrate the effect of buffer pH, a mixture of proteins was separated using weak cation-exchange chromatography at various pH values. At a pH of 6, different selectivity was observed for the most basic proteins vs. the separation at pH 6.2 and greater. At pH 6, ribonuclease A elutes before cytochrome c; this elution order is reversed when the separation was performed at pH 6.2 or greater, as shown in the figure. Sample: bovine, α-chymotryspinogen, bovine ribonucelase A, equine cyctochrome c. Column: Protein-Pak Hi Res CM 7 µm, 4.6 × 100 mm. Conditions: 20 mM buffer (MES or sodium phosphate) pH 6 to 7.2, 1 mL/min, 0 to 0.2 M NaCl in 34 minutes at 30 °C.

Temperature Effect on Reversed-Phase Protein Separations

- Use of “room temperature” is NOT always the ideal separation temperature
- Use of a column heater is strongly recommended for reproducible analyses

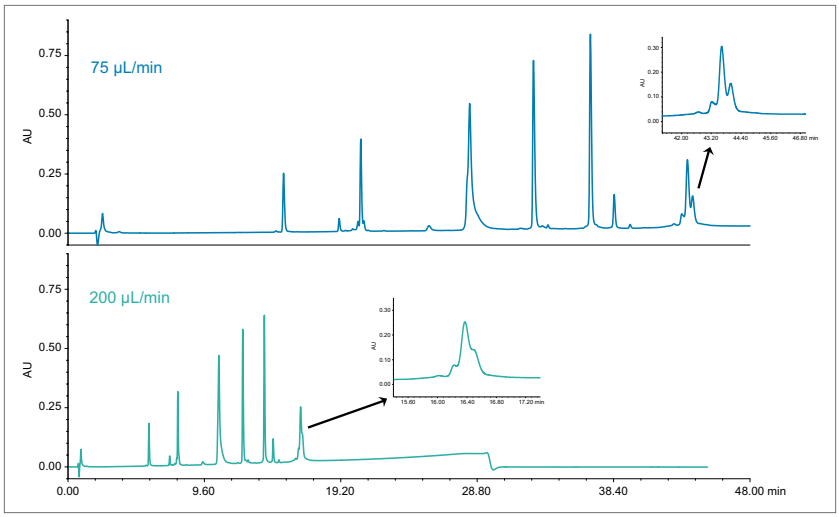


The intact IgG sample gave no observable peak at 40 °C, but recovery for the IgG sample improves with increasing temperature. There is not a measurable increase in recovery or improvement in peak shape above 80 °C. Column temperature has a large effect on reversed-phase separation of molecules. Changes in recovery and selectivity are not uncommon with small molecule separations. While increasing the temperature for proteins can significantly improve recovery, particularly for intact monoclonal antibodies, it doesn't generally affect the selectivity of the separation. However, not all proteins require higher temperatures for improved recovery. In fact, some protein separations have more desirable results with lower separation temperatures. Therefore, it is recommended that an evaluation of temperature be included in any method development strategy for new samples.

For more information, reference application note 720003875EN.

Effect of Flow Rate on Reversed-Phase Protein Separations

- Use of lower flows can translate into improved component resolution
- Analysis time will increase as flow rate increases
- Sample complexity can influence selected separation flow rate

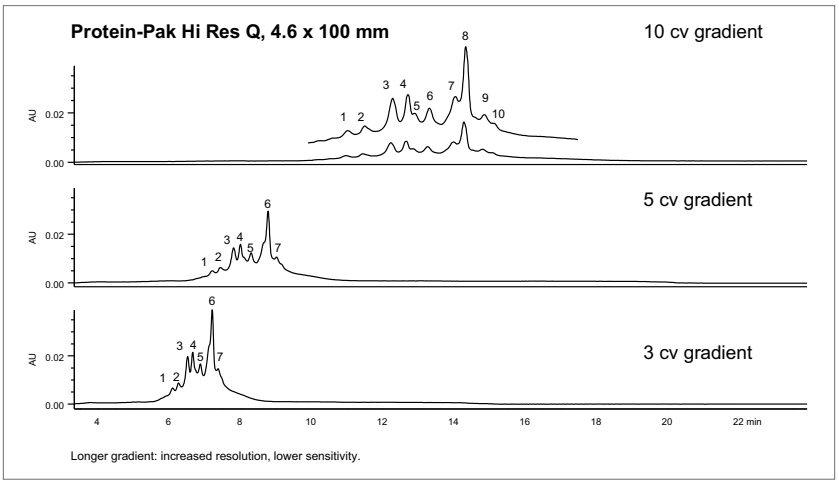


Decreasing the flow rate provides increased resolution without a compromise in the sensitivity, as seen in this separation of the protein mixture at 40 °C. The improved separation of the phosphorylase b sub-units can be seen (inset) at the lower flow rate. The run time of the analysis is increased proportionally to preserve the same gradient slope in both separations. Flow rate is seldom treated as an important parameter in method development except as an indirect modification of gradient slope. The impact of this variable is, however, more significant for larger molecules.

For more information, reference application note 720003875EN.

Effect of Gradient Duration on an IEX Protein Separation

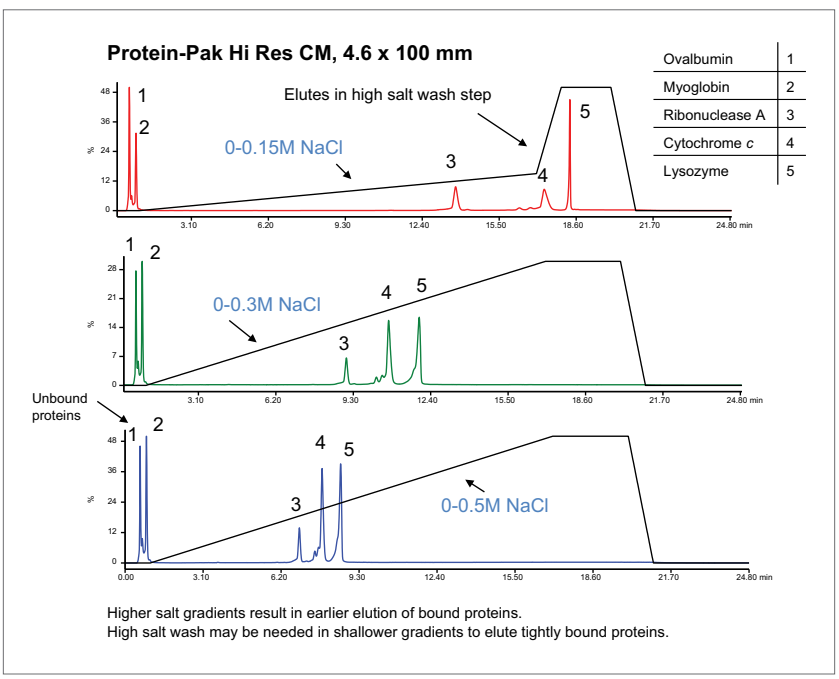
- Use of longer gradient time can translate into improved component resolution
- Analysis time will increase using longer gradients as will peak volume
- Sample complexity can influence selected gradient duration



The use of salt gradients can also be used to analyze variants of a single protein. In this example, chicken albumin was analyzed by anion-exchange chromatography. Three different gradient slopes were employed to analyze the variants of albumin formed by post transitional modifications, such as methylation, phosphorylation, and glycolysation. As can be observed in the chromatograms, the gradient slope can affect the number of variants detected, with shallower gradients reducing sensitivity but allowing for resolution of additional variants. Note: All gradients were performed at same flow rate from 0 to 0.5 M NaCl at same buffer pH.

Effect of Gradient Slope on an IEX Protein Separation

- Gradients of differing salt concentration affect in IEX protein separations
- Selected start and final salt concentration based on sample composition
- Analysis time and component resolution increases using increasingly shallow gradients

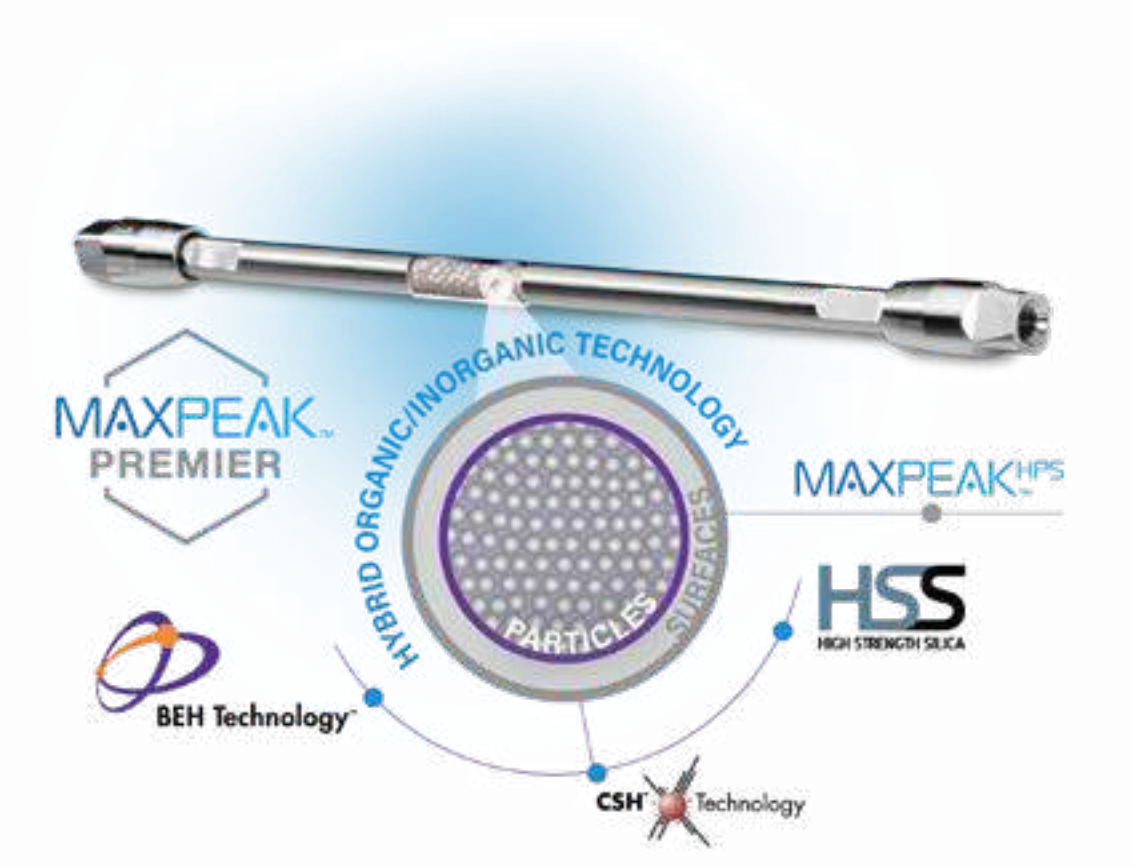


Gradients of differing salt concentration are frequently used in IEX protein separations to optimize retention time, component resolution, and overall analysis time. In this example, a protein mix was analyzed using three different salt gradients but keeping the run time identical. The higher salt gradient shown on the bottom of the chromatogram increases the salt concentration from 0–0.5 M NaCl, while the top chromatogram has a gradient which ends at 0.15 M NaCl, with the middle chromatogram ending at 0.3 M NaCl — note effect gradient has on separation. Flow rates same for all separations.

MaxPeak™ Premier Columns

Good Chromatography is as much about preventing the detrimental interactions you don't want, as it is creating the ones you do.

Waters™ MaxPeak Premier Columns enable scientists to have more control over their chromatographic separations by mitigating the loss of metal sensitive analytes, such as lipids, organic acids, acidic peptides, oligonucleotides, or other compounds containing phosphate or carboxylate functionalities. All MaxPeak Premier columns utilize MaxPeak High Performance Surfaces (HPS), new and innovative technologies designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses. MaxPeak HPS technology can also be found with Waters QuanRecovery™ plates and vial; for more information, please go to the QuanRecovery product information referenced on page 58.



MaxPeak Premier Columns provide:

- Reduced column conditioning and passivation times
- Improved sensitivity and peak shapes
- Simpler mobile phases, without complex additives
- Time savings in method development
- Reduced risk and greater confidence in data and decision making

Available with particle technologies and quality manufacturing you can trust for small molecule, protein, peptide, oligonucleotide, and glycan separations in both reversed-phase and HILIC separation modes.

Application-Specific Column Selections

PEPTIDE ANALYSIS

ACQUITY™ Premier BEH and XBridge™ Premier Particle Technology

- Outstanding peak capacity and superior peak shape in TFA, DFA, and FA
- Two pore sizes (130 Å and 300 Å) to provide different separation selectivities

ACQUITY Premier CSH C_{18}+ and XSelect™ CSH C_{18}+ Premier Particle Technology

- Accepts greater peptide mass loads for improved low-level detection of impurities
- Excellent performance with TFA for optical applications, FA for MS, and DFA for dual detection

ACQUITY Premier HSS T3 and XSelect HSS T3 Premier Particle Technology

- Ideal choice for the separation of small, polar peptides with greater retentivity than hybrid (BEH, CSH) particle technology columns

PROTEIN AGGREGATE, MONOMER, AND FRAGMENT ANALYSIS ANALYSIS

ACQUITY Premier Protein SEC and XBridge Premier Protein SEC 250 Å, 1.7 µm and 2.5 µm Particle Technology

- Efficiently separate protein size variants from simple to complex biotherapeutics (e.g., mAb, ADCs, bi-specifics, fusion proteins) that range from approximately 10,000 to 650,000 Daltons in a single SEC analysis for reliable component quantitation
- Mimize method development by using a single SEC buffer formulation without the need for co-solvents/additives for a variety of samples without sacrificing resolution
- Reduce the cost per analysis using MaxPeak Premier SEC 250 Å Guards that will not degrade the quality of challenging apllications

OLIGONUCLEOTIDE ANALYSIS

ACQUITY Premier BEH C_{18} and XBridge Premier BEH C_{18} Particle Technology

- Outstanding peak capacity and superior peak shape and lifetime in HFIP, HAA, and TEA
- Two pore sizes (130 Å and 300 Å) to provide different separation selectivities

GLYCAN ANALYSIS

ACQUITY Premier BEH Amide and XBridge Premier BEH Amide Particle Technology

- Best suited for the analysis of released, N-labeled glycans using pre-column labeling with 2-AB, 2-AA, or Waters innovative and enabling *Rapi*Fluor-MS™ reagent
- Two pore sizes (130 Å and 300 Å) to provide different selectivities from released glycans to large glycans, glycopeptides, and glycoproteins

ACQUITY Premier BEH C_{18} AX and XBridge Premier BEH C_{18} AX Particle Technology

- Charge-based separation of neutral-to-highly acidic released N-glycans
- Improved resolution and recovery for sialylated and phosphorylated glycans

INTACT AND SUBUNIT PROTEIN ANALYSIS

ACQUITY Premier Protein BEH C_4 and XBridge Premier Protein BEH C_4, 300 Å, 1.7 µm and 2.5 µm Particle Technology

- Separates proteins af various sizes, hydrophobicities, and isoelectric points
- tolerates extreme pH and temperature, and provides minimal secondary interactions
- Improves sensitivity for phosphorylated proteins and low-level intact and subunit mAb analysis

Ordering Information

ACQUITY Premier Columns

BEH C_{18}, 130 Å	Particle Size: 1.7 µm	
	Dimension	P/N
	2.1 × 50 mm	186009452
	2.1 × 100 mm	186009453
	2.1 × 150 mm	186009454

BEH C_{18}, 130 Å, VanGuard FIT	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009497
	2.1 × 100 mm	186009457
	2.1 × 150 mm	186009458

BEH Shield RP18, 130 Å	Particle Size: 1.8 µm	
	2.1 × 50 mm	186009490
	2.1 × 100 mm	186009498
	2.1 × 150 mm	186009499

BEH Shield RP18, 130 Å, VanGuard FIT	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009500
	2.1 × 100 mm	186009501
	2.1 × 150 mm	186009502

BEH Amide, 130 Å	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009504
	2.1 × 100 mm	186009505
	2.1 × 150 mm	186009506

BEH Amide, 130 Å, VanGuard FIT	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009507
	2.1 × 100 mm	186009508
	2.1 × 150 mm	186009509

CSH C_{18}, 130 Å	Particle Size: 1.7 µm	
	Dimension	P/N
	2.1 × 50 mm	186009460
	2.1 × 100 mm	186009461
	2.1 × 150 mm	186009462

CSH C_{18}, 130 Å, VanGuard FIT	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009463
	2.1 × 100 mm	186009464
	2.1 × 150 mm	186009465

CSH Phenyl Hexyl, 130 Å	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009474
	2.1 × 100 mm	186009475
	2.1 × 150 mm	186009476

CSH Phenyl Hexyl, 130 Å, VanGuard FIT	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009477
	2.1 × 100 mm	186009478
	2.1 × 150 mm	186009479

HSS T3, 100 Å	Particle Size: 1.8 µm	
	2.1 × 50 mm	186009467
	2.1 × 100 mm	186009468
	2.1 × 150 mm	186009469

HSS T3, 100 Å, VanGuard FIT	Particle Size: 1.8 µm	
	2.1 × 50 mm	186009470
	2.1 × 100 mm	186009471
	2.1 × 150 mm	186009472

ACQUITY Premier Van Guard FIT Cartridges

BEH C_{18}, 130 Å	Particle Size: 1.7 µm	
	Dimension	P/N
	2.1 × 5 mm	186009459

BEH Shield RP18, 130 Å	Particle Size: 1.7 µm	
	2.1 × 5 mm	186009503

BEH Amide, 130 Å	Particle Size: 1.8 µm	
	2.1 × 5 mm	186009510

CSH C_{18}, 130 Å	Particle Size: 1.7 µm	
	Dimension	P/N
	2.1 × 5 mm	186009466

CSH Phenyl Hexyl, 130 Å	Particle Size: 1.7 µm	
	2.1 × 5 mm	186009480

HSS T3, 100 Å	Particle Size: 1.8 µm	
	2.1 × 5 mm	186009473

ACQUITY Premier 1.7 µm Columns for Bioseparations

Glycan BEH C_{18} AX, 95 Å	Particle Size: 1.7 µm	
	Dimension	P/N
	2.1 × 50 mm	186009758
	2.1 × 100 mm	186009759
	2.1 × 150 mm	186009760

Glycan BEH C_{18} AX, 95 Å, VanGuard FIT	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009970
	2.1 × 100 mm	186009971
	2.1 × 150 mm	186009972

Glycan BEH Amide, 130 Å	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009522
	2.1 × 100 mm	186009523
	2.1 × 150 mm	186009524

Glycan BEH Amide, 130 Å, VanGuard FIT	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009974
	2.1 × 100 mm	186009975
	2.1 × 150 mm	186009976

Glycoprotein BEH Amide, 300 Å	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009547
	2.1 × 100 mm	186009548
	2.1 × 150 mm	186009549

Oligonucleotide BEH C_{18}, 130 Å	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009484
	2.1 × 100 mm	186009485
	2.1 × 150 mm	186009486

Peptide BEH C_{18}, 130 Å	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009481
	2.1 × 100 mm	186009482
	2.1 × 150 mm	186009483

Peptide BEH C_{18}, 130 Å	Particle Size: 1.7 µm	
	Dimension	P/N
	2.1 × 50 mm	186009493*
	2.1 × 100 mm	186009494*
	2.1 × 150 mm	186009495*

Peptide CSH C_{18}, 130 Å	Particle Size: 1.7 µm	
	2.1 × 50 mm	186009487
	2.1 × 100 mm	186009488
	2.1 × 150 mm	186009489

Peptide HSS T3, 100 Å	Particle Size: 1.8 µm	
	2.1 × 50 mm	186009490
	2.1 × 100 mm	186009491
	2.1 × 150 mm	186009492

Protein BEH C_4, 300 Å Column and Standard	Particle Size: 1.7 µm	
	2.1 × 50 mm	176005107**
	2.1 × 100 mm	176005108**
	2.1 × 150 mm	176005109**

Protein SEC, 250 Å Column and Standard	Particle Size: 1.7 µm	
	4.6 × 150 mm	176005071***
	4.6 × 300 mm	176005072***

Protein SEC, 250 Å Column, Standard, and Guard	Particle Size: 1.7 µm	
	4.6 × 150 mm	176004794***
	4.6 × 300 mm	176004795***

*Peptide BEH 300 Å columns may also be used for oligonucleotide analyses requiring wider pore sizes.

**MassPREP Protein Mix Standard p/n: 186004900

***mAb Size Variant Standard p/n: 186009429; MaxPeak Premier Protein SEC 250 Å, 2.5 µm, 4.6 × 30 mm Guard p/n: 186009969

ACQUITY Premier 1.7 µm Van Guard FIT Cartidges

Glycan BEH C_{18} AX, 95 Å	Particle Size: 1.7 µm	
	Dimension	P/N
	2.1 × 5 mm	186009973

Glycan BEH Amide, 130 Å	Particle Size: 1.7 µm	
	2.1 × 5 mm	186009977

XBridge Premier BEH C_{18}, 130 Å	Particle Size: 2.5 µm	
	Dimension	P/N
	2.1 × 50 mm	186009827
	2.1 × 100 mm	186009828
	2.1 × 150 mm	186009829
	4.6 × 50 mm	186009847
	4.6 × 100 mm	186009848
	4.6 × 150 mm	186009849

XBridge Premier BEH C_{18}, 130 Å, VanGuard FIT	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009843
	2.1 × 100 mm	186009844
	2.1 × 150 mm	186009845
	4.6 × 50 mm	186009850
	4.6 × 100 mm	186009851
	4.6 × 150 mm	186009852

XBridge Premier BEH Amide, 130 Å	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009928
	2.1 × 100 mm	186009929
	2.1 × 150 mm	186009930
	4.6 × 50 mm	186009935
	4.6 × 100 mm	186009936
	4.6 × 150 mm	186009937

XBridge Premier BEH Amide, 130 Å, VanGuard FIT	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009931
	2.1 × 100 mm	186009932
	2.1 × 150 mm	186009933
	4.6 × 50 mm	186009938
	4.6 × 100 mm	186009939
	4.6 × 150 mm	186009940

XBridge Premier BEH Shield RP18, 130 Å	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009914
	2.1 × 100 mm	186009915
	2.1 × 150 mm	186009916
	4.6 × 50 mm	186009921
	4.6 × 100 mm	186009922
	4.6 × 150 mm	186009923

XBridge Premier BEH Shield RP18, 130 Å, VanGuard FIT	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009917
	2.1 × 100 mm	186009918
	2.1 × 150 mm	186009919
	4.6 × 50 mm	186009924
	4.6 × 100 mm	186009925
	4.6 × 150 mm	186009926

XSelect Premier CSH C_{18}, 130 Å	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009865
	2.1 × 100 mm	186009866
	2.1 × 150 mm	186009867
	4.6 × 50 mm	186009872
	4.6 × 100 mm	186009873
	4.6 × 150 mm	186009874

XSelect Premier CSH C_{18}, 130 Å, VanGuard FIT	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009868
	2.1 × 100 mm	186009869
	2.1 × 150 mm	186009870
	4.6 × 50 mm	186009875
	4.6 × 100 mm	186009876
	4.6 × 150 mm	186009877

XSelect Premier CSH Phenyl Hexyl, 130 Å	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009879
	2.1 × 100 mm	186009880
	2.1 × 150 mm	186009881
	4.6 × 50 mm	186009886
	4.6 × 100 mm	186009887
	4.6 × 150 mm	186009888

XSelect Premier CSH Phenyl Hexyl, 130 Å, VanGuard FIT	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009882
	2.1 × 100 mm	186009883
	2.1 × 150 mm	186009884
	4.6 × 50 mm	186009889
	4.6 × 100 mm	186009890
	4.6 × 150 mm	186009891

XSelect Premier HSS T3, 100 Å	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009830
	2.1 × 100 mm	186009831
	2.1 × 150 mm	186009832
	4.6 × 50 mm	186009858
	4.6 × 100 mm	186009859
	4.6 × 150 mm	186009860

XSelect Premier HSS T3, 100 Å, VanGuard FIT	Particle Size: 2.5 µm	
	2.1 × 50 mm	186009854
	2.1 × 100 mm	186009855
	2.1 × 150 mm	186009856
	4.6 × 50 mm	186009861
	4.6 × 100 mm	186009862
	4.6 × 150 mm	186009863

MaxPeak Premier 2.5 µm Van Guard FIT Cartidges

XBridge BEH C_{18}, 130 Å	Particle Size: 2.5 µm
Dimension	P/N
2.1 × 5 mm	186009842
3.9 × 5 mm	186009846

XBridge BEH Amide, 130 Å	Particle Size: 2.5 µm
2.1 × 5 mm	186009927
3.9 × 5 mm	186009934

XBridge BEH Shield RP18, 130 Å	Particle Size: 2.5 µm
2.1 × 5 mm	186009913
3.9 × 5 mm	186009920

XSelect CSH C_{18}, 130 Å	Particle Size: 2.5 µm
Dimension	P/N
2.1 × 5 mm	186009864
3.9 × 5 mm	186009871

XSelect CSH Phenyl Hexyl, 130 Å	Particle Size: 2.5 µm
2.1 × 5 mm	186009878
3.9 × 5 mm	186009885

XSelect HSS T3, 100 Å	Particle Size: 2.5 µm
2.1 × 5 mm	186009853
3.9 × 5 mm	186009857

MaxPeak Premier 2.5 µm Columns for Bioseparations

XBridge Premier Glycan BEH C_{18} AX, 95 Å	Particle Size: 2.5 µm
Dimension	P/N
2.1 × 50 mm	186009947
2.1 × 100 mm	186009948
2.1 × 150 mm	186009949
4.6 × 50 mm	186009950
4.6 × 100 mm	186009951
4.6 × 150 mm	186009952

XBridge Premier Glycan BEH Amide, 130 Å	Particle Size: 2.5 µm
2.1 × 50 mm	186009941
2.1 × 100 mm	186009942
2.1 × 150 mm	186009943
4.6 × 50 mm	186009944
4.6 × 100 mm	186009945
4.6 × 150 mm	186009946

XBridge Premier Oligonucleotide BEH C_{18}, 130 Å	Particle Size: 2.5 µm
2.1 × 50 mm	186009836
2.1 × 100 mm	186009837
2.1 × 150 mm	186009838
4.6 × 50 mm	186009901
4.6 × 100 mm	186009902
4.6 × 150 mm	186009903

XBridge Premier Peptide BEH C_{18}, 130 Å	Particle Size: 2.5 µm
2.1 × 50 mm	186009733
2.1 × 100 mm	186009734
2.1 × 150 mm	186009835
4.6 × 50 mm	186009898
4.6 × 100 mm	186009899
4.6 × 150 mm	186009900

XBridge Premier Peptide BEH C_{18}, 300 Å	Particle Size: 2.5 µm
2.1 × 50 mm	186009892*
2.1 × 100 mm	186009893*
2.1 × 150 mm	186009894*
4.6 × 50 mm	186009895*
4.6 × 100 mm	186009896*
4.6 × 150 mm	186009897*

XBridge Premier Protein BEH C_4, 300 Å Column and Standard	Particle Size: 2.5 µm
2.1 × 50 mm	176005110**
2.1 × 100 mm	176005111**
2.1 × 150 mm	176005112**
4.6 × 50 mm	176005113**
4.6 × 100 mm	176005114**
4.6 × 150 mm	176005115**

XSelect Premier Peptide HSS T3, 100 Å	Particle Size: 2.5 µm
2.1 × 50 mm	186009839
2.1 × 100 mm	186009840
2.1 × 150 mm	186009841
4.6 × 50 mm	186009910
4.6 × 100 mm	186009911
4.6 × 150 mm	186009912

XBridge Premier Protein SEC 250 Å, Column and Standard	Particle Size: 2.5 µm
4.6 × 150 mm	176005067***
4.6 × 300 mm	176005068***
7.8 × 150 mm	176005069***
7.8 × 150 mm	176005070***

XBridge Premier Protein SEC 250 Å, Column, Standard, and Guard	Particle Size: 2.5 µm
4.6 × 150 mm	176004790***
4.6 × 300 mm	176004791***
7.8 × 150 mm	176004792***
7.8 × 150 mm	176004793***

*XBridge Premier Peptide BEH 300 Å Columns may also be used for oligonucleotide analyses requiring wider pore sizes.
**MassPREP Protein Mix Standard p/n: 186004900
***mAb Size Variant Standard p/n: 186009429; MaxPeak Premier Protein SEC 250 Å, 2.5 µm, 4.6 × 30 mm Guard p/n: 186009969

Atlantis Premier Columns

	Particle Size: 1.7 µm		Particle Size: 2.5 µm		Particle Size: 5 µm	
	Dimension	P/N	Dimension	P/N	Dimension	P/N
BEH C_{18} AX, 95 Å	2.1 × 30 mm	186009365	2.1 × 30 mm	186009389	2.1 × 50 mm	186009407
	2.1 × 50 mm	186009366	2.1 × 50 mm	186009390	2.1 × 100 mm	186009408
	2.1 × 75 mm	186009367	2.1 × 75 mm	186009391	2.1 × 150 mm	186009409
	2.1 × 100 mm	186009368	2.1 × 100 mm	186009392	4.6 × 50 mm	186009427
	2.1 × 150 mm	186009369	2.1 × 150 mm	186009393	4.6 × 100 mm	186009416
			4.6 × 50 mm	186009426	4.6 × 150 mm	186009417
			4.6 × 100 mm	186009397	4.6 × 250 mm	186009418
			4.6 × 150 mm	186009398		
BEH C_{18} AX, 95 Å, VanGuard FIT	2.1 × 30 mm	186009357	2.1 × 30 mm	186009374	2.1 × 50 mm	186009404
	2.1 × 50 mm	186009358	2.1 × 50 mm	186009375	2.1 × 100 mm	186009405
	2.1 × 75 mm	186009359	2.1 × 75 mm	186009376	2.1 × 150 mm	186009406
	2.1 × 100 mm	186009360	2.1 × 100 mm	186009378	4.6 × 50 mm	186009410
	2.1 × 150 mm	186009361	2.1 × 150 mm	186009379	4.6 × 100 mm	186009411
			4.6 × 50 mm	186009383	4.6 × 150 mm	186009412
			4.6 × 100 mm	186009384	4.6 × 250 mm	186009413
			4.6 × 150 mm	186009385		
BEH Z-HILIC, 95 Å	2.1 × 50 mm	186009978	2.1 × 50 mm	186009985	2.1 × 50 mm	186009999
	2.1 × 100 mm	186009979	2.1 × 100 mm	186009986	2.1 × 100 mm	186010000
	2.1 × 150 mm	186009980	2.1 × 150 mm	186009987	2.1 × 150 mm	186010001
			4.6 × 50 mm	186009992	4.6 × 50 mm	186010006
			4.6 × 100 mm	186009993	4.6 × 100 mm	186010007
			4.6 × 150 mm	186009994	4.6 × 150 mm	186010008
					4.6 × 250 mm	186010009
BEH Z-HILIC, 95 Å, VanGuard FIT	2.1 × 50 mm	186009981	2.1 × 50 mm	186009988	2.1 × 50 mm	186010002
	2.1 × 100 mm	186009982	2.1 × 100 mm	186009989	2.1 × 100 mm	186010003
	2.1 × 150 mm	186009983	2.1 × 150 mm	186009990	2.1 × 150 mm	186010004
			4.6 × 50 mm	186009995	4.6 × 50 mm	186010010
			4.6 × 100 mm	186009996	4.6 × 100 mm	186010011
			4.6 × 150 mm	186009997	4.6 × 150 mm	186010012
					4.6 × 250 mm	186010013

Atlantis Premier Van Guard FIT Cartidges

	Particle Size: 1.7 µm		Particle Size: 2.5 µm		Particle Size: 5 µm	
	Dimension	P/N	Dimension	P/N	Dimension	P/N
BEH C_{18} AX, 95 Å	2.1 × 5 mm	186009373	2.1 × 5 mm	186009402	2.1 × 5 mm	186009421
			3.9 × 5 mm	186009403	3.9 × 5 mm	186009422
BEH Z-HILIC, 95 Å	2.1 × 5 mm	186009984	2.1 × 5 mm	186009991	2.1 × 5 mm	186010005
			3.9 × 5 mm	186009998	3.9 × 5 mm	186010014

Amino Acid Analysis

AccQ•Tag™ Ultra
UPLC® Amino Acid Analysis

Amino acids are the constituents of proteins and are the intermediates in many metabolic pathways. Qualitative and quantitative Amino Acid Analysis (AAA) is used to determine the concentration of proteins, identify proteins, and detect structural variants. Amino acid composition is a critical component of the nutritional value of foods and feeds. The same analytical tools are used to monitor cell culture and fermentation processes. AAA is also used as a clinical diagnostic tool for assessing inborn errors of metabolism and nutritional status. For LC-MS based physiological amino acid analysis solution, please refer to Kairos in Application Specific Columns, Kits, and Spare Parts chapter.

The accurate identification and quantification of amino acids in biological research and in the development and commercialization of food, beverage, and biotherapeutic products is challenging. This set of analytes covers a wide range of chemical properties (e.g., acidic, basic, neutral), yet resolution of individual pairs having only minor structural differences is required. Analysis is further complicated by the absence of common chromophores, necessitating use of a derivatization chemistry to enable analyte detection.

Reversed-phase chromatography provides good selectivity for separating amino acids. The most common approach to reversed-phase AAA includes pre-column derivatization. The derivatized amino acids retain better on the reversed-phase column and can be more easily separated. Most common derivatization reagents react with the amines. Some reagents react only with primary amines, but the most useful ones also react with secondary amines such that proline and hydroxyproline are also measured. In addition to improving chromatography, derivatization can make the amino acids readily detectable by UV absorbance or fluorescence.

For more than 50 years, Waters has provided reversed-phase chromatographic solutions that have successfully addressed a variety of organic compound analytical needs, including amino acid analysis. Hundreds of published papers have positively testified to the successful application of one of Waters pre-column amino acid derivatization chemistries that are used prior to the reversed-phase separation with on-line detection of resolved peaks using either UV absorbance or fluorescence. Waters offers three distinct methods that utilize pre-column derivatization and reversed-phase chromatography for accurate identification and quantitation of free or bound amino acids: Pico•Tag, AccQ•Tag, and AccQ•Tag Ultra C_{18}.

Pico•Tag Method	AccQ•Tag Method	AccQ•Tag Ultra C_{18} Chemistry Package
1980's	1990's	2006
▪ Designed for use with HPLC systems ▪ Applicable to any sample including protein hydrolysates, physiologic fluids, feeds, foods, and pharmaceutical preparations ▪ Based on the coupling reaction of the well known Edman Degradation, the reaction of phenylisothiocyanate (PITC) with both primary and secondary amino acids to form phenylthiocarbamyl (PTC) derivatives ▪ QC tested for use on HPLC with UV detection	▪ Designed for use with HPLC systems ▪ Suitable for protein and peptide identification and quantitation, monitoring cell culture media and nutritional content of food and feed ▪ Based on AccQ•Tag derivatization of primary and secondary amino acids in aqueous conditions ▪ QC tested for use on HPLC with fluorescence detection	▪ Designed specifically for use with the UPLC Amino Acid Analysis Solution ▪ AccQ•Tag Ultra C_{18} Chemistry Package is part of a complete solution that includes instrument, software, and support for amino acid analysis of protein hydrolysates, cell culture media, foods, and feeds ▪ Based on AccQ•Tag derivatization of primary and secondary amino acids in aqueous conditions ▪ Reagents, columns, and eluents QC tested with an amino acid separation

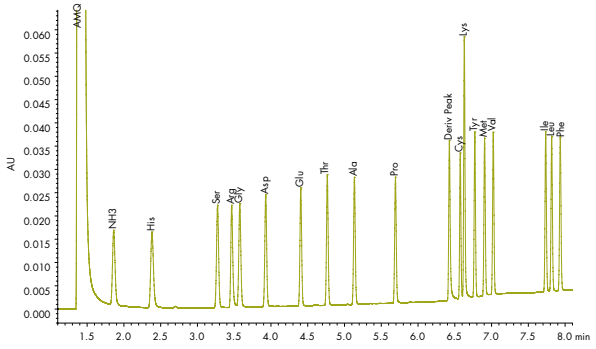
ACCURATE AMINO ACID ANALYSES FROM VARIED SAMPLE MATRICES

The UPLC Amino Acid Analysis Solution includes two complete methods using the same instrumentation and chemistries. The first is suitable for the amino acids derived from protein hydrolysates. The second is suitable for the larger number of free amino acids found in process samples such as cell culture or fermentation broths. The methods differ in the dilution of the AccQ•Tag Ultra Eluent A and the separation column temperature. There are no user adjustments of pH or modifications of composition for either Eluent A or Eluent B.

Hydrolysate Standard 10 pmol/µL

Conditions

Sample:	10 pmol Hydrolysate Standard
Column:	AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 100 mm (p/n: 186003837)
Temp.:	55 °C
Eluent A:	AccQ•Tag Ultra Eluent A – 1:20 Dilution
Eluent B:	AccQ•Tag Ultra Eluent B
Flow rate:	0.7 mL/min
Gradient:	UPLC Amino Acid Analysis Solution Hydrolysate Gradient (provided in the UPLC Amino Acid Analysis Solution System Guide)
UV detection:	260 nm

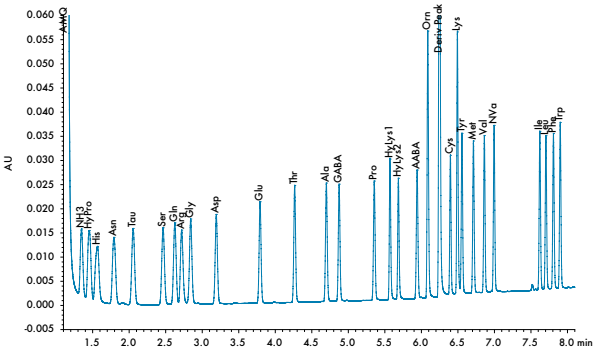


Separation of standard amino acids using the UPLC Amino Acid Analysis Solution Hydrolysate Method.

Cell Culture Standard 10 pmol/µL

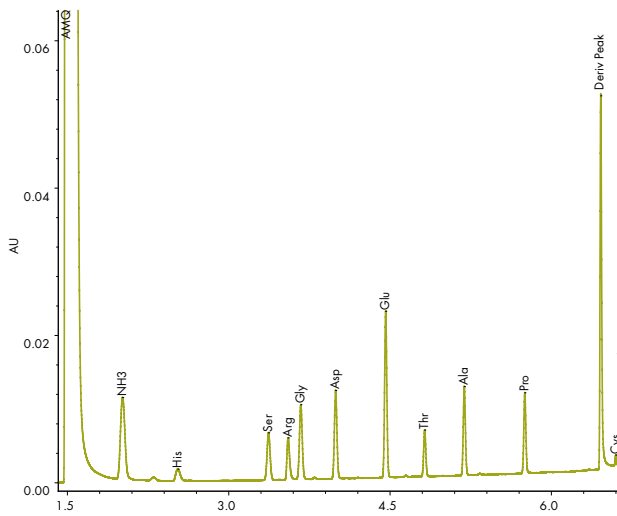
Conditions

Sample:	10 pmol Cell Culture Standard
Column:	AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 100 mm (p/n: 186003837)
Temp.:	60 °C
Eluent A:	AccQ•Tag Ultra Eluent A – 1:10 Dilution
Eluent B:	AccQ•Tag Ultra Eluent B
Flow rate:	0.7 mL/min
Gradient:	UPLC Amino Acid Analysis Solution Cell Culture Gradient (provided in the UPLC Amino Acid Analysis Solution System Guide)
UV detection:	260 nm



Separation of the larger set of standard amino acids using the UPLC Amino Acid Analysis Solution Cell Culture Method. No modification of the mobile phase pH or composition is required.

Amino Acid Analysis of Hydrolyzed Poultry Diet

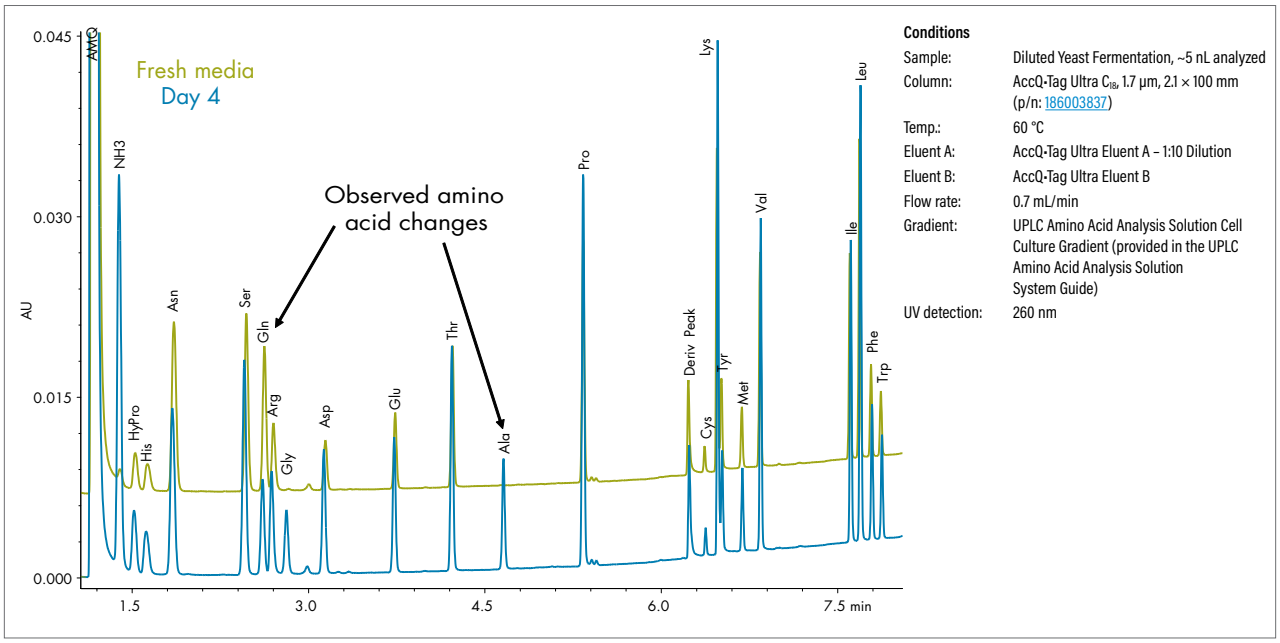


Conditions

Sample:	Acid Hydrolysate of Poultry Diet, ~6 ng
Column:	AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 100 mm (p/n: 186003837)
Temp.:	55 °C
Eluent A:	AccQ•Tag Ultra Eluent A – 1:20 Dilution
Eluent B:	AccQ•Tag Ultra Eluent B
Flow rate:	0.7 mL/min
Gradient:	UPLC Amino Acid Analysis Solution Hydrolysate Gradient (provided in the UPLC Amino Acid Analysis Solution System Guide)
UV detection:	260 nm

The 75 replicate analyses of a poultry diet mixed feed gives reproducible measurements of the weight percentage of the growth-limiting amino acids, typically 1% or better. The high sensitivity of the method ensures that only a very small aliquot of sample is required, thereby minimizing interferences.

Amino Acid Analysis of Cell Culture Media



Amino acid levels in a growing cell culture change over a relatively short period shown here as a decrease in glutamine accompanied by an increase in alanine. The supplied methods were used without modification and no sample prep beyond dilution was required.

UPLC: AccQ•Tag ULTRA C_{18} AMINO ACID ANALYSIS SOLUTION

Waters' UPLC Amino Acid Analysis Application Solution is the product of over 25 years of experience in amino acid analysis, highlighted by the development and industry-wide acceptance of the innovative and proven Pico•Tag and AccQ•Tag pre-column derivatization chemistries. The UPLC Amino Acid Analysis Solution is holistically designed to offer a total application solution that is optimized for accurate, reliable, and reproducible analysis of amino acids. The solution leverages Waters experience in separation science, derivatization chemistries, and information management to ensure accurate and precise qualitative and quantitative results. Our solution also provides performance-qualified methodologies that are designed to be rugged and reliable, assuring reproducible results day-to-day, instrument-to-instrument, lab-to-lab, around the world—with the expert support that scientists have come to expect from Waters. Users can feel confident with assured performance in the areas of protein characterization, cell culture monitoring, and nutritional analysis of foods and feeds.

The UPLC Amino Acid Analysis Solution consists of:

- ACQUITY UPLC H-Class (quaternary*) System with a tunable UV detector for enhanced chromatographic resolution and maximum-sensitivity detection
- AccQ•Tag Ultra C_{18} derivatization chemistries including quality-controlled 1.7 µm columns, reagents, and eluents
- Empower™ 3 pre-configured projects, methods, and report templates
- Installation and application training and support
- Connections INSIGHT™ ISDP instrument diagnostics to ensure continuous, consistent, and reliable operation
- Standards and kits to validate and troubleshoot

** Amino acid analysis can be performed on other systems such as ACQUITY UPLC H-Class PLUS Binary and ACQUITY Premier Binary systems. These are not considered as a total solution.*

UHPLC AND HPLC: AccQ•Tag ULTRA C_{18} AMINO ACID ANALYSIS

In 2022, Waters expanded its Amino Acid Analysis offerings with the introduction of the same BEH-based, C_{18} columns (as used in UPLC-based applications) but using 2.5 µm particles all amino analysis batch tested to work on UHPLC and HPLC systems with <70 µl dispersion with UV detection. These columns combined with the AccQ•Tag Ultra C_{18} pre-column derivatization kit for 250 analyses, completes this flexible portfolio to help scientists quickly and accurately obtain accurate quantitative data in half the time compared to use of legacy HPLC methods with FLR detection. Samples can now be successfully analyzed in under an hour.

** This is not a full system solution but a detailed care and use manual is available to help successfully use this offering on appropriate LC Systems.*

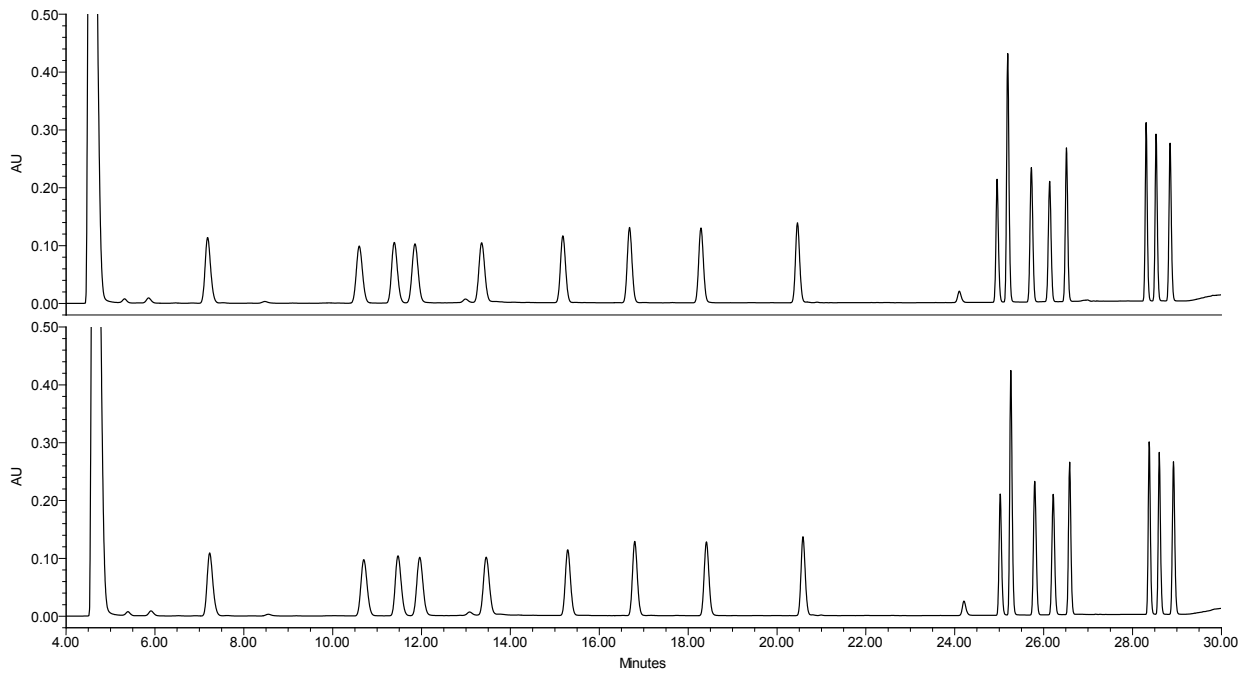
Hydrolysate Standard (500 µm) Chromatographic Comparison

Conditions

LC systems:	Waters Arc HPLC with 2998 Detector (10 mm HPLC Analytical Flow Cell) and 30 cm CHC with Passive Preheater. Note: Total LC System Dispersion without column or guard > 70 µL.
Detection:	UV @ 260 nm
Column:	AccQ•Tag Ultra C_{18}, 2.5 µm Column 4.6 × 150 mm (p/n: 186010407)
Guard:	AccQ•Tag Ultra C_{18}, 2.5 µm VanGuard Cartridge 3.9 × 5 mm (p/n: 186010407[e])
Column temp.:	43 °C
Sample temp.:	20 °C
Injection volume:	5.0 µL
Flow rate:	1.5 mL/min
Mobile phase A:	AccQ•Tag Eluent A (p/n: 186003838)
Mobile phase B:	90:10 (v/v) Water:AccQ•Tag Ultra Eluent B
Mobile phase C:	Milli-Q Water
Mobile phase D:	AccQ•Tag Eluent B (p/n: 186003839)

Gradient

	Time (min)	Flow (mL/min)	%A	%B	%C	%D	Curve
1	Initial	1.500	10.0	0.0	90.0	0.0	Initial
2	0.86	1.500	10.0	0.0	90.0	0.0	11
3	20.17	1.500	9.0	80.0	11.0	0.0	7
4	25.57	1.500	8.0	16.0	60.0	16.0	7
5	26.24	1.500	8.0	16.0	58.0	18.0	6
6	27.55	1.500	7.8	0.0	70.9	21.3	6
7	28.70	1.500	4.0	0.0	36.3	59.7	6
8	30.58	1.500	4.0	0.0	36.3	59.7	6
9	30.88	1.500	10.0	0.0	90.0	0.0	6
10	35.98	1.500	10.0	0.0	90.0	0.0	6



Analysis of Protein Hydrolysate standard on the AccQ•Tag Ultra C_{18}, 2.5 µm 4.6 × 150 mm Column with (top) and without AccQ•Tag Ultra C_{18}, 2.5 µm VanGuard Cartridge 3.9 × 5 mm (bottom) installed on a Waters Arc HPLC with 2898 Detector. 1) AMQ, 2) His, 3) Ser, 4) Arg, 5) Gly, 6) Asp, 7) Glu, 8) Thr, 9) Ala, 10) Pro, 11) Derivatization peak, 12) Cys, 13) Lys, 15) Tyr, 15) Met, 16) Val, 17) Ile, 18) Leu, 19) Phe.

ACCQ•TAG ULTRA C_{18} HPLC CHEMISTRY

The AccQ•Tag Ultra C_{18} Chemistry is an integral component of the Waters UPLC Amino Acid Analysis Application Solution. This application solution is an integrated combination of instrumentation, derivatization chemistry, separation column and eluents, methods and software. Analysts are assured of accurate and precise amino acid analyses with the complete application solution. The use of the AccQ•Tag Ultra C_{18} Chemistry without the rest of the application solution is not supported as an Amino Acid Analysis method.

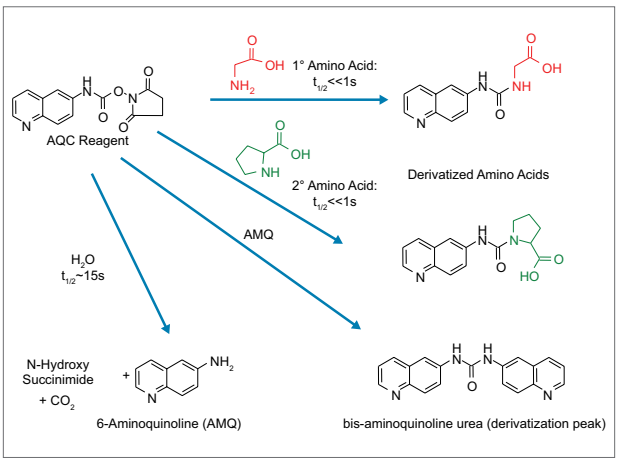
AccQ•Tag Ultra C_{18} Chemistry is different from the AccQ•Tag HPLC method, that uses an HPLC Column containing 100% Silica-based C_{18}, 4 µm particles, described later in this chapter. Although the components of the two derivatization kits are the same, the QC tests are based on the specific separation and detection protocols. Both methods begin with the same derivatization chemistry but differ in all the other details such that components cannot be interchanged. Most importantly, the AccQ•Tag Ultra C_{18} 1.7 µm and 2.5 µm Guard and Columns have a completely different chemistry from the AccQ•Tag HPLC Column. The AccQ•Tag Ultra C_{18} Columns leverage Waters 1.7 µm and 2.5 µm hybrid-silica BEH Technology particles that deliver excellent column efficiency and resolution. The AccQ•Tag Ultra C_{18} 1.7 µm Column is designed for use on Waters ACQUITY™ UPLC Systems and include use of Waters eCord™ Intelligent Chip Technology that is permanently attached to the column to easily track use history. The mobile phases used in the AccQ•Tag Ultra C_{18} method is different from that used for the AccQ•Tag HPLC method, each being optimized for the specific column and detection technique.

Compared to traditional HPLC methods, Waters UPLC Amino Acid Analysis Solution, that uses the AccQ•Tag Ultra C_{18}, 1.7 µm Column, results in peaks that are much sharper and better resolved. This improved resolution results in a rugged method where there is no ambiguity in peak identification and it simplifies quantitation. The better resolution provides a precise, reliable method. The dramatically higher throughput (3 to 5 times faster) with UPLC Technology enables users to make more informed decisions faster and to perform more analyses per day.

AccQ•Tag Derivatization Reaction

- Utilizes AccQ•Tag Ultra C_{18} Reagent Powder
  - 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC)
  - US Patent #5,296,599 and European Patent #EP 0 533 200 B1
- AQC reacts rapidly with both primary and secondary amines
- Excess reagent reacts more slowly with water to form 6-aminoquinoline (AMQ)
- AMQ reacts slowly with excess AQC reagent to form a bisurea
- Derivatized amino acids are separated chromatographically from the byproducts
- Requires no vacuum drying, sample prep, or extraction

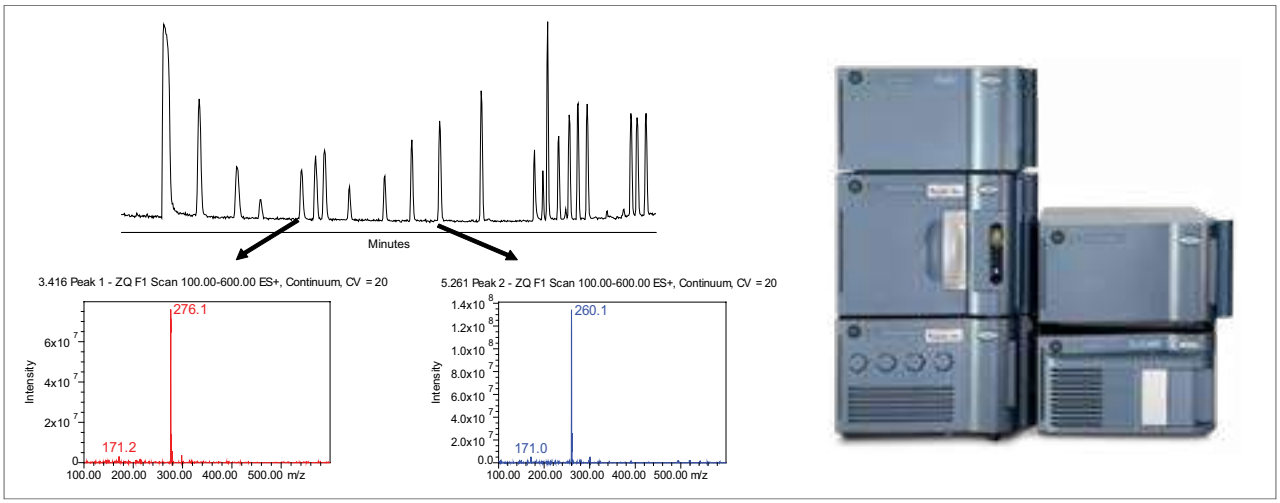
Chemistry of the AccQ•Tag Derivatization Reaction



MS Compatible

The UPLC Amino Acid Analysis Application Solution is directly compatible with electrospray mass spectrometry. No adjustment is required to have an MS TIC that exactly matches the UV trace. MS is extremely useful for any samples that may have an extra, unknown, or unexpected peak, since the identification of amino acids can be confirmed by their molecular weight. Although MS is not required for routine peak identification and does not provide additional useful sensitivity, the use of MS-compatible mobile phases makes using MS detection simple.

Direct Flow into Source at 700 µL/min



The UPLC Amino Acid Analysis Application Solution is directly compatible with electrospray mass spectrometry.

Amino Acid Analysis Standard

Amino acid analysis is required in many applications in pharmaceutical and food and feed industries. A variety of standards containing free amino acids are offered for qualitative and quantitative determination of amino acids, method development, and troubleshooting of the AccQ•Tag™ Ultra C_{18} or AccQ•Tag HPLC methods.

Ordering Information

Amino Acid Standard

Description	P/N
Amino Acid Standard 10 × 1 mL ampules of unlabeled amino acid standards	WAT088122
Amino Acid Cell Culture Standard Kit Kit contains: 2 vials contain 17 amino acids 8 vials contain 9 cell culture supplemental amino acids	186009300
Amino Acid Food and Feed Standard Kit Kit contains: 2 vials contain 17 amino acids 8 vials contain 4 food and feed supplemental amino acids	186009299
Amino Acid Internal Standard – Norvaline 1 vial	186009301

List of Amino Acids in Each Amino Acid Standard Amino Acid Standard

Amino Acid	Amino Acid Standard	Cell Culture Standard Kit	Food and Feed Standard Kit	Internal Standard
	p/n: WAT088122	p/n: 186009300	p/n: 186009299	p/n: 186009301
Alanine	■	■	■	—
Arginine	■	■	■	—
Aspartic acid	■	■	■	—
Cystine	■	■	■	—
Glutamic acid	■	■	■	—
Glycine	■	■	■	—
Histidine	■	■	■	—
Isoleucine	■	■	■	—
Leucine	■	■	■	—
Lysine	■	■	■	—
Methionine	■	■	■	—
Phenylalanine	■	■	■	—
Proline	■	■	■	—
Serine	■	■	■	—
Threonine	■	■	■	—
Tyrosine	■	■	■	—
Valine	■	■	■	—
Taurine	—	■	■	—
HydroxyProline	—	■	—	—
Asparagine	—	■	—	—
Glutamine	—	■	—	—
GABA (γ-Aminobutyric acid)	—	■	—	—
Tryptophan	—	■	—	—
Ornithine	—	■	—	—
AABA (α-Aminobutyric acid)	—	■	■	—
HydroxyLysine	—	■	—	—
Methionine Sulfone	—	—	■	—
Cysteic Acid	—	—	■	—
Norvaline	—	—	—	■

UPLC: AccQ•Tag Ultra C_{18} Amino Acid Analysis Kits and Accessories designed for use on a Waters, low dispersion, ACQUITY UPLCs

Description	Qty.	P/N
UPLC AAA H-Class Applications Kit		176002983
This kit is intended to enable existing ACQUITY UPLC H-Class Systems for AAA applications.		
Kit contains:		
AccQ•Tag Ultra Derivatization Kit, 250 analyses		
AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 100 mm Column		
AccQ•Tag Ultra Eluent A, concentrate	1 L	
AccQ•Tag Ultra Eluent B	1 L	
Amino acid standard, hydrolysate	10 × 1 mL	
Total recovery vials	3 × 100/pk	
Tube inlet 0.0025 I.D. PEEK nut PDA assembly		
Column In-line filter kit		
UPLC AAA H-Class solution information set		
AAA application and familiarization service		
AccQ•Tag Ultra Chemistry Kit		176001235
The refill kit is intended to recharge the AccQ•Tag Ultra chemistries that are part of the application kit. This kit should be purchased by those that have already purchased the AccQ•Tag Ultra Application Solution. This kit is applicable to both ACQUITY UPLC and ACQUITY UPLC H-Class AAA Application Solutions, and should not be purchased as part of an initial system.		
Kit contains:		
AccQ•Tag Ultra Derivatization Kit, 250 analyses		
AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 100 mm Column		
AccQ•Tag Ultra Eluent A, concentrate	1 L	
AccQ•Tag Ultra Eluent B	1 L	
Amino acid standard, hydrolysate	10 × 1 mL	
Sample tubes	4 × 72/pk	
Total recovery vials with caps	3 × 100/pk	
AccQ•Tag Ultra Derivatization Kit, 250 Analyses		186003836
AccQ•Tag Ultra Borate Buffer	5 × 6 mL	
AccQ•Tag Ultra Derivatization Reagent Powder	5 × 3 mg	
AccQ•Tag Ultra Reagent Diluent	5 × 4 mL	
AccQTag Ultra Borate Buffer – 10 mL		186009283
Amino Acid Standard, Hydrolysate (AccQ•Tag, Pico•Tag, AccQ•Tag Ultra)	10 × 1 mL	WAT088122
A standard mixture containing 18 amino acids (17 hydrolysate amino acids each at 2.5 mM and cystine at 1.25 mM)		
Sample Tubes	4 × 72/pk	WAT007571
Total Recovery Vials with Caps	3 × 100/pk	186000384C
AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 100 mm Column		186003837
AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 50 mm Column, 1/pk		186009953
AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 150 mm Column, 1/pk		186009954
AccQ•Tag Ultra C_{18}, 1.7 µm, VanGuard Pre-Column, 2.1 × 5 mm, 3/Pk		186009955
AccQ•Tag Ultra Eluent A, concentrate	1 L	186003838
AccQ•Tag Ultra Eluent B	1 L	186003839
Hydrolysis Primer, Amino Acid Analysis		715006455

UHPLC and HPLC: AccQ•Tag Ultra C_{18} Amino Acid Analysis Kit

Description	Qty.	P/N
UHPLC and HPLC: AccQ•Tag Ultra C_{18} Amino Acid Analysis Kit		176005152
The kit is intended to provide all the materials needed in order to get started running the AccQ•Tag Ultra chemistries on a UHPLC and HPLC system.		
Kit contains:		
AccQ•Tag Ultra Derivatization Kit, 250 analyses		
AccQ•Tag Ultra C_{18}, 2.5 µm, 4.6 × 150 mm Column		
AccQ•Tag Ultra Eluent A, concentrate	1 L	
AccQ•Tag Ultra Eluent B	1 L	
Amino acid standard, hydrolysate	10 × 1 mL	
Total recovery vials	3 × 100/pk	

UPLC-based Amino Acid Analysis

Description	P/N
AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 50 mm Column	186009953
AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 100 mm Column	186003837
AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 150 mm Column	186009954
AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 × 5 mm VanGuard Pre-Column	186009955

UHPLC and HPLC-based Amino Acid Analysis

Description	P/N
AccQ•Tag Ultra C_{18}, 2.5 µm, 4.6 × 50 mm Column	186010405
AccQ•Tag Ultra C_{18}, 2.5 µm, 4.6 × 100 mm Column	186010406
AccQ•Tag Ultra C_{18}, 2.5 µm, 4.6 × 150 mm Column	186010406
AccQ•Tag Ultra C_{18}, 2.5 µm, 4.6 × 5 mm VanGuard Cartridge*, 3/pk	186010408

* Requires use of VanGuard 3.9 mm ID Cartridge Holder: p/n 186007949

Amino Acid Primer

Description	P/N
Hydrolysis Primer, Amino Acid	715006455

Amino Acid Analysis Automation

Automation increases efficiency, repeatability and avoids contamination and human errors. Amino acid analysis automation is enabled through the automation derivatization kit and verified automation scripts on Andrew+, Tecan, or Hamilton automation platforms. The automation derivatization kit is system agnostic and designed in a 32 × 3 format for up to 96 sample preparation. It has a larger volume per sample than the manual derivatization kit to accommodate the residual volumes required by automation workflow. The script includes barcode scanning, linearity calibration, sample dilution, derivatization, heating, shaking functions, which allow analysts to walk away during sample preparations, and 96 samples are prepared in less than an hour.

Ordering Information

Automation: AccQ•Tag Ultra C_{18} Amino Acid Analysis Kits and Accessories

Description	P/N
AccQ•Tag Ultra C_{18} Derivatization Kit – Automation, 96 analyses	186009232
AccQ•Tag Borate Buffer – 10 mL	186009283
96-Well Sample Collection Plate, 800 µL Round Well, 50/pk	186002481
Cap Mat, 5/pk	186006332
AccQ•Tag Ultra Cell Culture Chemistry Kit – Automation	176004534
AccQ•Tag Ultra Food and Feed Chemistry Kit – Automation	176004533
AccQ•Tag Ultra Hydrolysates Chemistry Kit – Automation	176004542
AccQ•Tag Ultra Cell Culture Tecan Script Starter Kit – CD	176004543
AccQ•Tag Ultra Cell Culture Tecan Script Starter Kit – USB	176004544
AccQ•Tag Ultra Cell Culture Hamilton Script Starter Kit – CD	176004545
AccQ•Tag Ultra Cell Culture Hamilton Script Starter Kit – USB	176004546

AccQ•Tag Ultra C_{18} Derivatization Automation Kit

AccQ•Tag Ultra Amino Acid Analysis Automation Kits for Andrew+

Description	P/N
Andrew+ Pipetting Robot Andrew+ Pipetting Robot, waste base, waste container, power supply, cables, and 1 × each single and multi-channel pipette adaptors	176004567
Andrew+ Startup Kit Intended for all new Andrew+ systems and includes Dominos, pipette adaptors, and lab kit with consumables for system installation	176004568
Pipette Kit for AccQ•Tag Includes 3× Andrew Alliance Pipettes	176004583
Domino Kit for AccQ•Tag Includes additional dominos and connected devices for Amino Acid OneLab protocol with Andrew+ automation	176004582
AccQ•Tag Ultra Derivatization Kit – Automation Provides simplified tools to enhance high throughput amino acid automation, enabling processing of up to 96 samples in 3 × 32 sample batches	186009232
Roller for Cap mats Helps to smooth out the cap mat before putting it on system for injection	186002633

AccQ•Tag Ultra Amino Acid Analysis Optional Accessories for Andrew+

Description	P/N
Amino Acid Cell Culture Standard Kit Contains 26 amino acids monitored in cell culture media or other matrices. The standard is designed for both ID and quantitative amino acid analysis	186009300
Amino Acid Food and Feed Standard Kit Contains 21 amino acids analyzed in food and feed matrix. The standard is designed for both ID and quantitative amino acid analysis	186009299
Amino Acid Internal Standard – Norvaline Compensates for the variability generated in sample hydrolysis and amino acid analysis	186009301
AccQ•Tag Ultra 1.7 µm, 2.1 × 100 mm Column Separates the amino acid derivatives produced in the reaction with Waters AccQ•Tag Ultra Derivatization Reagent	186003837
AccQ•Tag Ultra Eluent A Mobile phase eluents for reversed phase separation of amino acid derivatives	186003838
AccQ•Tag Ultra Eluent B Mobile-phase eluents for reversed phase separation of amino acid derivatives	186003839

HPLC: AccQ•Tag AMINO ACID ANALYSIS SOLUTION

The HPLC-based AccQ•Tag Method utilizes the same pre-column derivatization step as used for the AccQ•Tag Ultra C_{18} Method but uses a 100% silica-based, C_{18}, 4 µm column resulting in a 60 min analysis time using fluorescent detection. The AccQ•Fluor™ Reagent, 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC), derivatizes primary and secondary amines in a simple, single-step reaction to yield highly stable, fluorescent adducts. We offer the AccQ•Tag Method as a system package consisting of pre-packaged reagents and extensive documentation.

The AccQ•Tag chemistry package contains the items you need for up to 250 analyses of protein and peptide hydrolysate amino acids.

AccQ•Tag Derivatization Kit

The AccQ•Tag Derivatization Kit contains five sets of the derivatizing reagents. Each set of reagents includes one vial each of:

- AccQ•Fluor Borate Buffer – The buffer is added to the samples to ensure the optimum pH for derivatization.
- AccQ•Fluor Reagent Powder – The reagent powder is the 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) derivatizing reagent. It is shipped dry for maximum stability.
- AccQ•Fluor Reagent Diluent – This diluent, acetonitrile, is used reconstitute the reagent for derivatization.

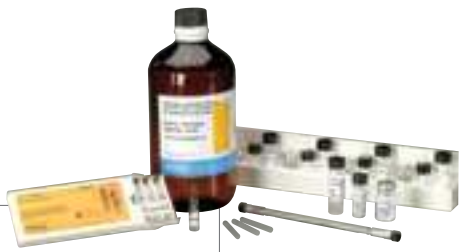
AccQ•Tag Amino Acid Analysis Column

The AccQ•Tag Column is a high-efficiency HPLC column specifically certified for use with the AccQ•Tag Method. This column separates the amino acid derivatives produced by the AccQ•Fluor derivatization reaction.

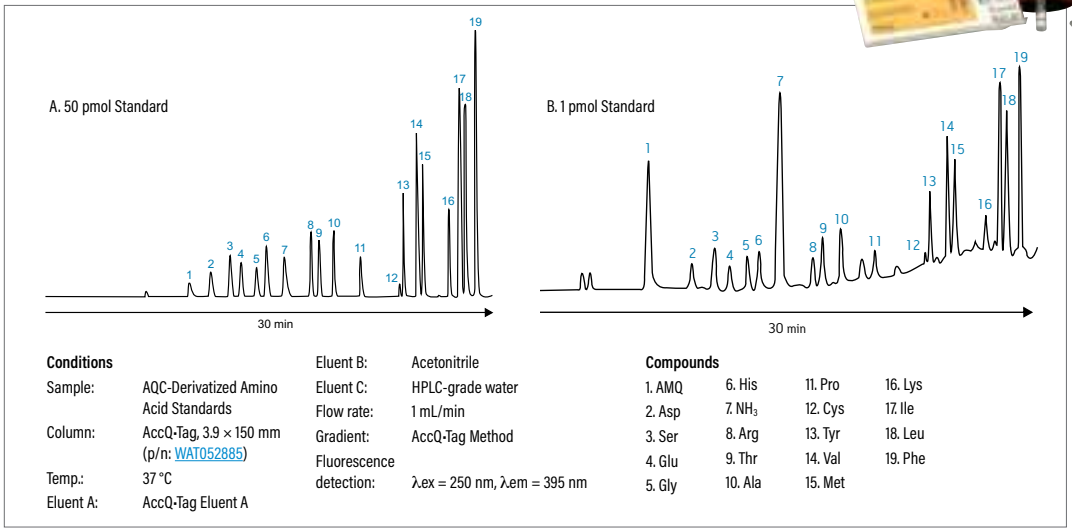
Ordering Information

AccQ•Tag Amino Acid Analysis Kits and Accessories for HPLC and UHPLC AAA Analysis

Description	Qty.	P/N
AccQ•Tag Chemistry Kit		WAT052875
Kit is for up to 250 analyses and contains:		
AccQ•Fluor Reagent 1	5 × 6 mL	
AccQ•Fluor Reagent 2A	5 × 3 mg	
AccQ•Fluor Reagent 2B	5 × 3 mL	
AccQ•Tag Column, 3.9 × 150 mm		
AccQ•Tag Eluent A, concentrate	2 × 1 L	
Sample tubes	4 × 72/pk	
Amino acid standard, hydrolysate	10 × 1 mL	
AccQ•Tag User Guide		
Amino Acid Standard, Hydrolysate	10 × 1 mL	WAT088122
A standard mixture containing 18 amino acids (17 hydrolysate amino acids each at 2.5 mM and cystine at 1.25 mM).		
AccQ•Tag Eluent A	1 L	WAT052890
Concentrate		
AccQ•Tag Eluent B	1 L	WAT052895
AccQ•Fluor Reagent Kit		WAT052880
Kit contains:		
AccQ•Fluor Reagent 1	5 × 6 mL	
AccQ•Fluor Reagent 2A	5 × 3 mg	
AccQ•Fluor Reagent 2B	5 × 4 mL	
The components of this kit are not available separately		
AccQ•Tag Column, 3.9 × 150 mm		WAT052885
AccQ•Tag User Guide		WAT052874



AccQ•Tag Analysis of Hydrolysate Amino Acids



Conditions

Sample:	AQC-Derivatized Amino Acid Standards
Column:	AccQ•Tag, 3.9 × 150 mm (p/n: WAT052885)
Temp.:	37 °C
Eluent A:	AccQ•Tag Eluent A
Eluent B:	Acetonitrile
Eluent C:	HPLC-grade water
Flow rate:	1 mL/min
Gradient:	AccQ•Tag Method
Fluorescence detection:	λex = 250 nm, λem = 395 nm

Compounds

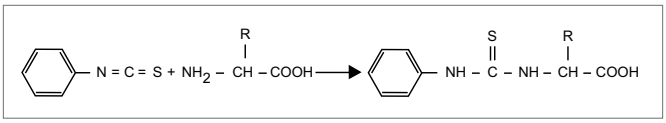
1. AMQ	6. His	11. Pro	16. Lys
2. Asp	7. NH_3	12. Cys	17. Ile
3. Ser	8. Arg	13. Tyr	18. Leu
4. Glu	9. Thr	14. Val	19. Phe
5. Gly	10. Ala	15. Met	

Application of the AccQ•Tag Method to the analysis of hydrolysate amino acids is illustrated. The high purity reagents provided in the AccQ•Tag chemistry package enable high sensitivity analysis by minimizing background amino acid content. AMQ (6-aminoquinoline).

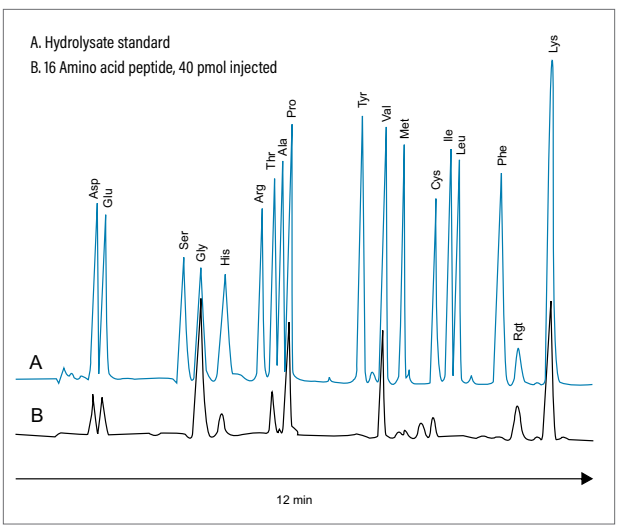
HPLC: Pico•Tag METHOD

Waters Pico•Tag Method is a widely-used technique for HPLC amino acid analysis. This method is applicable to any sample including protein hydrolysates, physiologic fluids, feeds, foods, and pharmaceutical preparations. Pre-column derivatization relies on the coupling reaction of the well-known Edman Degradation, the reaction of phenylisothiocyanate (PITC) with both primary and secondary amino acids to form phenylthiocarbamyl (PTC) derivatives. The PTC-amino acid adducts are stable and easily separated by reversed-phase HPLC. A single product is formed for each amino acid. Most reaction by-products and all derivatization reagents are volatile, so they may be removed from the sample by vacuum drying.

Pico•Tag Derivatization Reaction

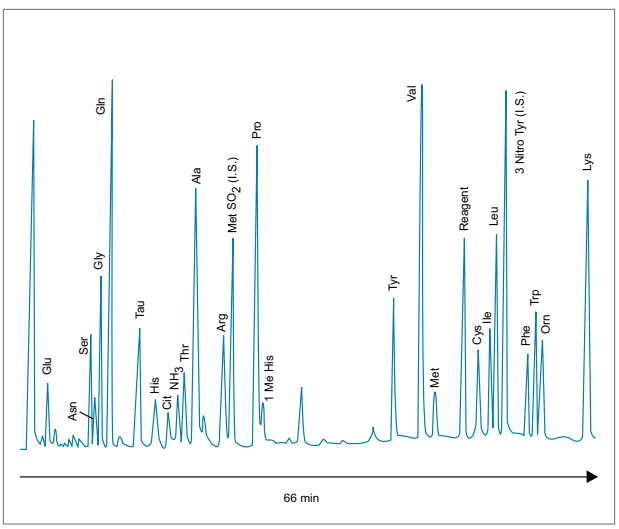


Peptide Hydrolysate Amino Acid Analysis Using the Pico•Tag Method



This 12-minute analysis using Waters Pico•Tag Amino Acid Analysis Method provides identification and accurate quantitation of the amino acid composition.

Plasma Amino Acid Profile Using the Pico•Tag Method



Reproducible and reliable plasma amino acid profiles are obtained in 66 minutes using Waters Pico•Tag Method. In this analysis, 100 µL plasma was diluted with an internal standard, deproteinized by centrifugal ultrafiltration, and derivatized. The methionine sulfone (internal standard) peak represents 25 picomoles. Courtesy of A.S. Feste, R.W. Drummond, and S.J. Dudrich, Nutritional Support Service, St. Luke Episcopal Hospital, Houston, Texas.

Ordering Information

Pico•Tag Amino Acid Analysis of Physiologic Amino Acids

Description	Qty.	P/N
Chemistry Package for Amino Acid Analysis of Physiologic Amino Acids		WAT091681
Kit contains: Free Amino Acid Analysis Column, 3.9 × 300 mm		
Pico•Tag Reagent Kit		
Pico•Tag Eluent 1	4 × 1 L	
Pico•Tag Eluent 2	4 × 1 L	
Pico•Tag Diluent	100 mL	
Manual, column heater inserts, and sample tubes		
Pico•Tag Reagent Kit (PITC, TEA, and standards A/N and B)		WAT010947
Amino Acid Analysis Column, 3.9 × 300 mm		WAT010950
Pico•Tag Eluent 1	4 × 1 L	WAT010960
Pico•Tag Eluent 2	4 × 1 L	WAT010965
Pico•Tag Diluent	100 mL	WAT088119
Pico•Tag Eluent 2	1 L	WAT010985

Pico•Tag Amino Acid Analysis for Protein Hydrolysates

Description	Qty.	P/N
Chemistry Package for Amino Acid Analysis of Protein Hydrolysates		WAT007360
Kit contains: Pico•Tag Column, 3.9 × 150 mm		
Pico•Tag Reagent Kit (includes PITC, TEA, and standards)		
Pico•Tag Eluent A	4 × 1 L	
Pico•Tag Eluent B	4 × 1 L	
Pico•Tag Diluent	100 mL	
Manual, column heater inserts, and sample tubes		
Pico•Tag Column, 3.9 × 150 mm		WAT088131
Pico•Tag Reagent Kit (PITC, TEA, and standards)		WAT088123
Pico•Tag Eluent A	4 × 1 L	WAT088108
Pico•Tag Eluent B	4 × 1 L	WAT088112
Pico•Tag Diluent	100 mL	WAT088119
Pico•Tag Eluent B	1 L	WAT010983

[AUTOMATION]

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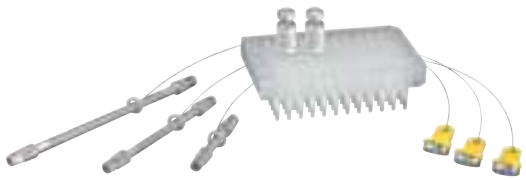
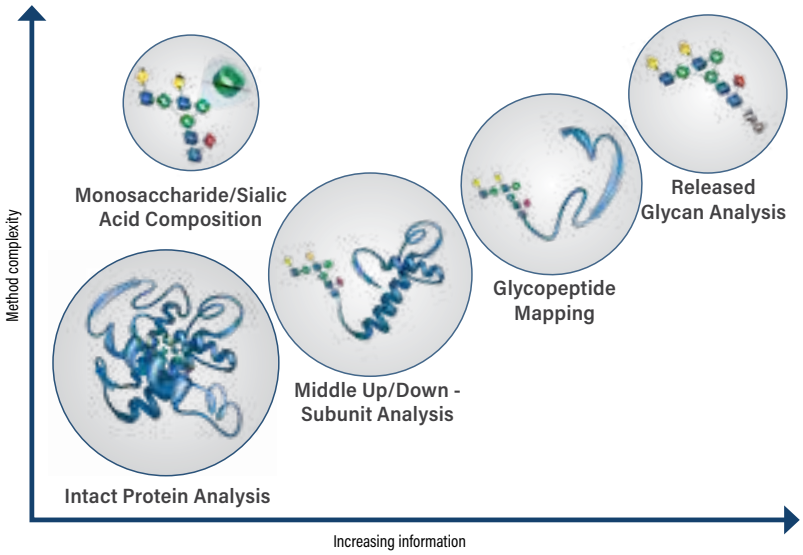
Glycan and Glycoprotein Analysis

More than two thirds of recombinant biopharmaceutical products on the market are glycoproteins, and nearly every stage of their manufacture is carefully monitored and regulated to ensure consistency in quality, safety, and effectiveness. Consequently, international regulatory agencies require use of state-of-the-art glycan analyses methods to help ensure the successful development and commercialization of effective and safe glycosylated biotherapeutics. To address this need, Waters offers a variety of robust, reproducible, complementary, information-rich analytical methods for this application.



CONSOLIDATING COMPLEMENTARY TECHNIQUES TO STREAMLINE GLYCAN ANALYSIS



For analyzing all structural levels of glycoproteins, we offer complete approaches according to workflow:

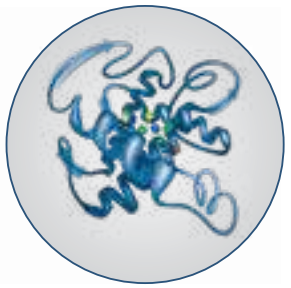
- Intact glycoprotein profiling (e.g., glycan occupancy determination)
- Middle up/down - subunit analysis
- Glycopeptide mapping
- Released and labeled glycan analysis
- Monosaccharide/sialic acid composition

Glycoprotein and Glycopeptide Analysis

Intact glycoprotein profiling, subunit analysis, and glycopeptide mapping are means of characterizing protein glycosylation and are valuable orthogonal methods that provide accurate mass confirmation, glycan identification, and elucidate sites of glycan occupancy. Waters ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm Column is a powerful, single column chemistry that can run multiple complimentary, glycoprotein analyses methods.

- Optimized, large-pore, HILIC stationary phase for resolving the glycoforms of intact and digested glycoproteins
- Unprecedented separation selectivity and orthogonality to reversed phase
- High resolution glycopeptide mapping without limitations due to peptide/glycan size or composition
- Improved resolution in separations of large, released N-glycans (EPO, Factor IX)
- MaxPeak Premier column format reduces sample adsorption onto metal surfaces and delivers the representative performance from the first injection.

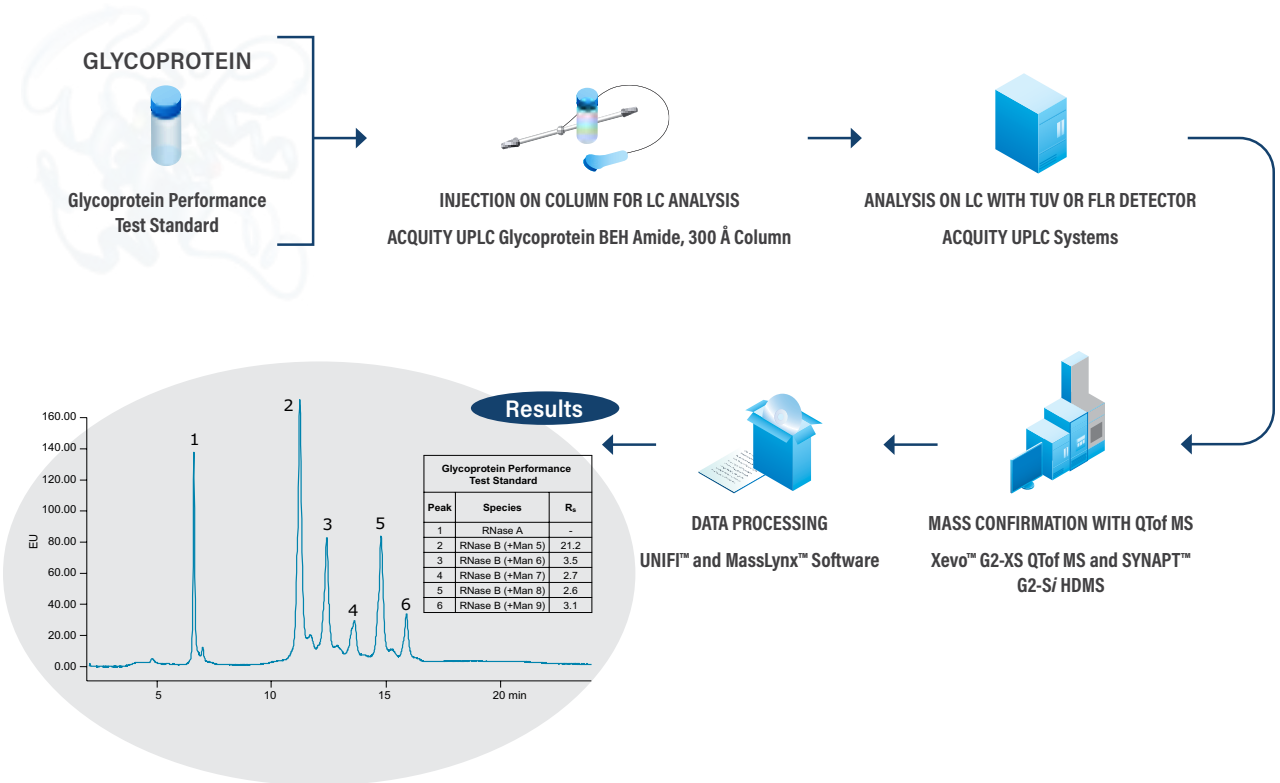
INTACT GLYCOPROTEIN ANALYSIS



Waters ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm Column separates individual intact protein glycoforms as well as delivers information about glycan occupancy. Using elevated 80 ˚C column temperature, TFA ion pairing, and an HFIP mobile-phase additive, one is able to successfully enhance the solubility of 150,000 Dalton, Intact IgGs for this HILIC-based separation that uses an initial high organic solvent concentration. The figure on the next page shows the HILIC fluorescence chromatograms resulting from a separation of a native Intact mAb Mass Check Standard (a murine IgG1 mAb) and its partially as well as completely deglycosylated isoforms.

- Measure glycan occupancy of an intact therapeutic mAb
- Relative abundance of aglycosylated forms (-2 and -1 N glycans moetites) can be monitored by fluorescence
- Wide-pore phase facilitates the development of previously unimagined separations that includes an orthogonal separation of mAb fragments compared to well-established, reversed-phase chromatography

Intact Protein Analysis Workflow



ACQUITY UPLC Glycan vs. Glycoprotein BEH Amide Analyses of Intact mAb vs. Partially- and Fully-Deglycosylated Species

LC Conditions

System: ACQUITY UPLC H-Class Bio

Columns: ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm, 2.1 × 100 mm (p/n: 176003701) that contains Glycoprotein Performance Test Standard (p/n: 186008010)
ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 150 mm (p/n: 186004742)

Mobile phase A: 0.1% TFA, 0.3% HFIP in water

Mobile phase B: 0.1% TFA, 0.3% HFIP in acetonitrile

Column temp.: 80 °C

Flow rate: 0.2 mL/min

Injection vol.: ≤1 µL (aqueous diluents)

Gradient:

Time	%A	%B	Curve
0.0	20	80	6
10.0	50	50	6
11.0	100	0	6
14.0	100	0	6
15.0	20	80	6
25.0	20	80	6

MS Conditions

System: Xevo G2 QTof

Ionization mode: ESI+

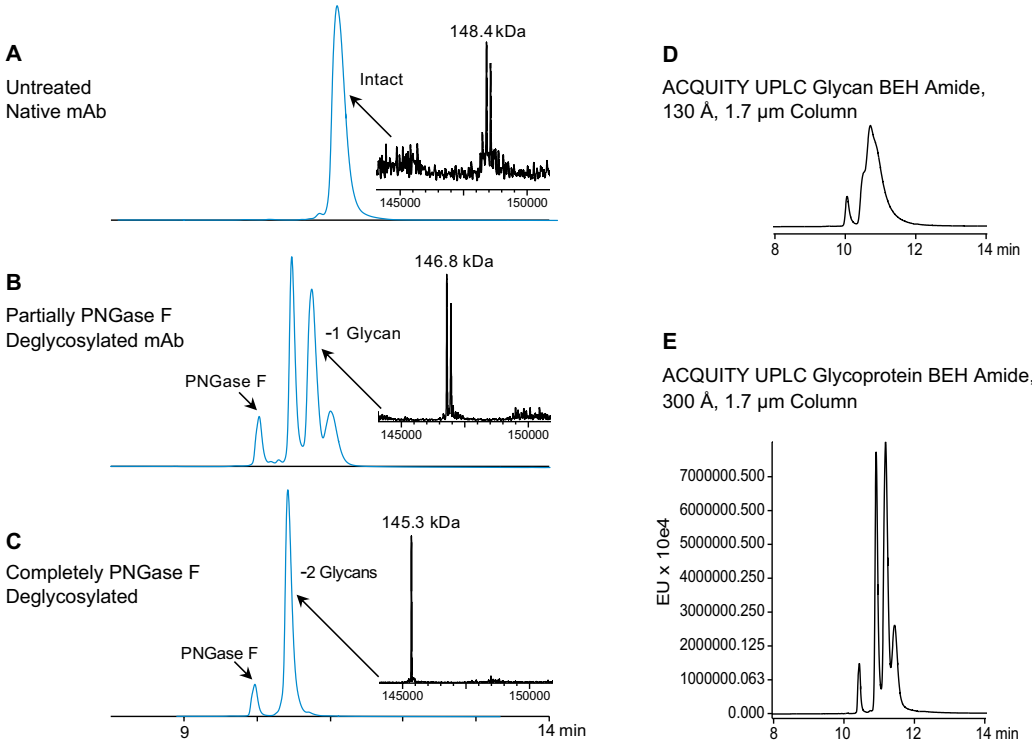
Analyzer mode: Resolution (~20 K)

Capillary voltage: 3.0 kV

Cone voltage: 45 V

Source temp.: 150 °C

Desolvation temp.: 350 °C



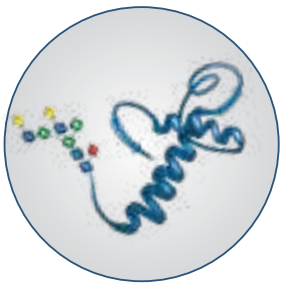
Glycoprotein BEH Amide, 300 Å, 1.7 µm Column analyses of Waters mAb Mass Check Standard showing native (A), partially deglycosylated (B), and completely deglycosylated (C) samples. Also showing HILIC fluorescence profiles of partially deglycosylated Intact mAb Mass Check Standard using two ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 150 mm Columns in series (D) versus two ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm, 2.1 × 150 mm Columns in series (E).

Ordering Information

ACQUITY UPLC Glycoprotein BEH Amide Columns, Kits, and Standards

Description	P/N
ACQUITY UPLC Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 50 mm, 1/pk with Standard	176003700
ACQUITY UPLC Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 100 mm, 1/pk with Standard	176003701
ACQUITY UPLC Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 150 mm, 1/pk with Standard	176003702
ACQUITY UPLC Glycoprotein BEH Amide VanGuard Pre-Column, 300 Å, 1.7 µm, 2.1 × 5 mm, 3/pk with Standard	176003699
ACQUITY UPLC Glycoprotein BEH Amide Method Validation Kit, 300 Å, 1.7 µm, 2.1 × 100 mm, 3/pk with Standard	176003703
Glycoprotein Performance Test Standard	186008010
Intact mAb Mass Check Standard	186006552
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 50 mm, 1/pk	186009547
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 100 mm, 1/pk	186009548
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 150 mm, 1/pk	186009549
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 50 mm, 1/pk with Glycoprotein Performance Test Standard	176004866
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 100 mm, 1/pk with Glycoprotein Performance Test Standard	176004867
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 150 mm, 1/pk with Glycoprotein Performance Test Standard	176004868

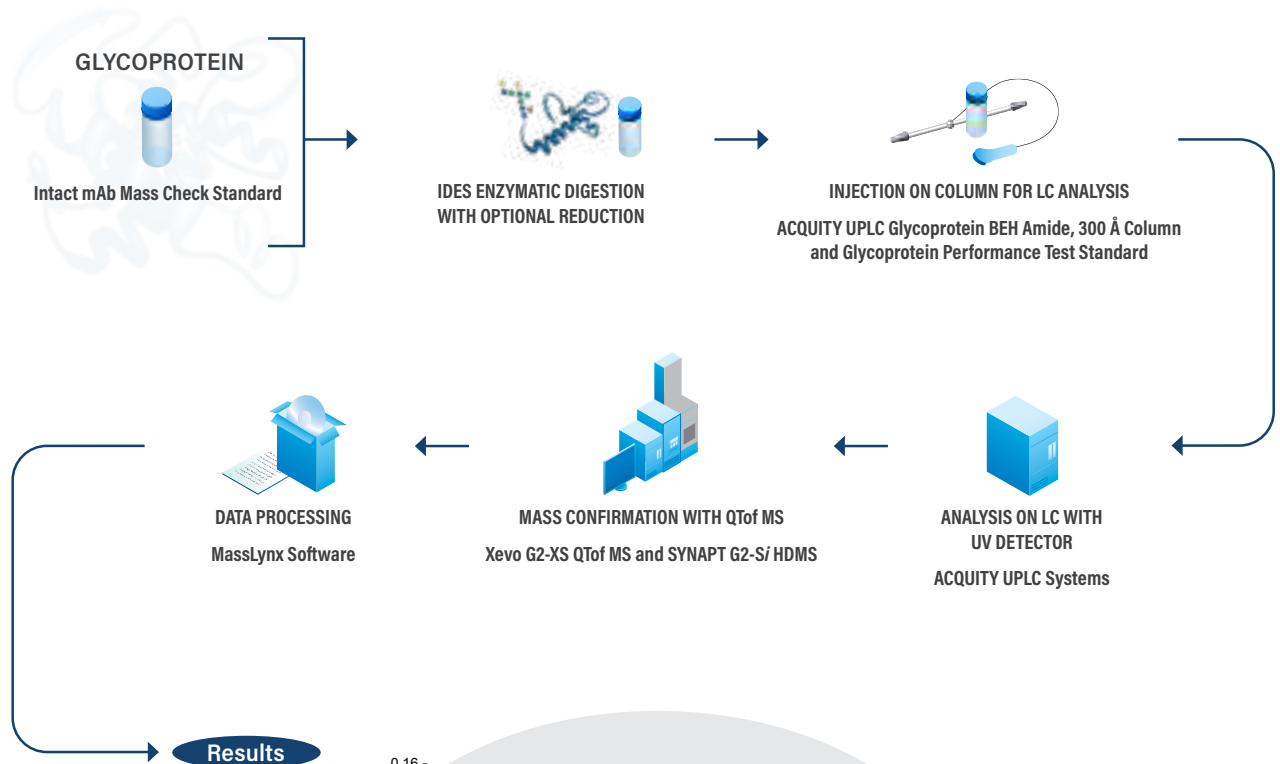
GLYCOPROTEIN SUBUNIT ANALYSIS

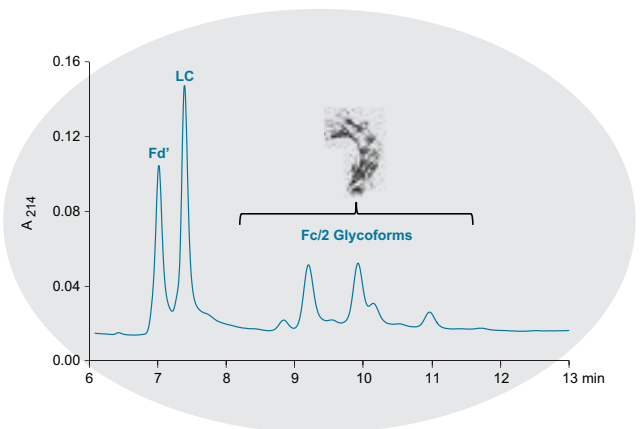


Reversed-phase chromatography is a well-established and commonly used technique to analyze intact protein of protein subunits generated from digestions with enzymes such as FabRICATOR (IdeS protease) that generates a site cleavage at the hinge region of a monoclonal antibody generating Fc and F(ab')2 fragments (genovis.com).

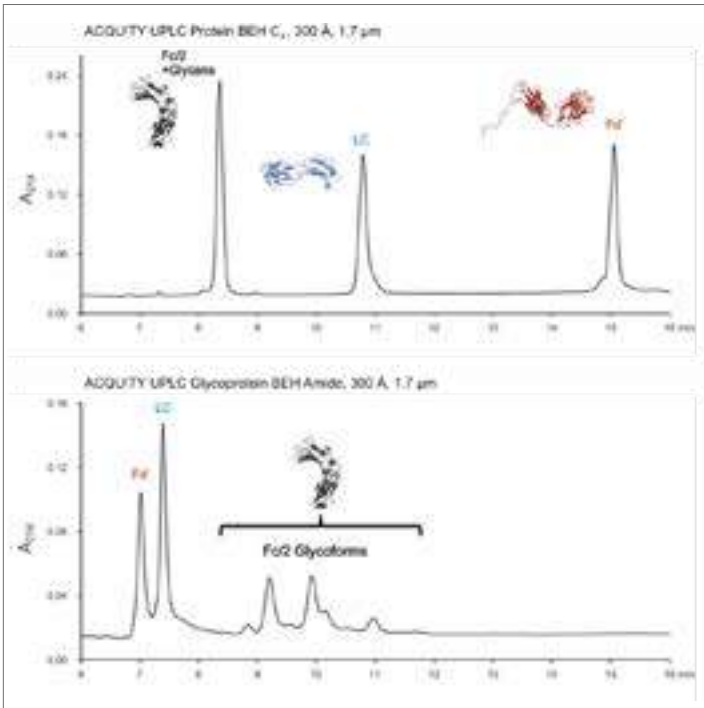
- Provides orthogonal and complementary results, compared to C_4-based reversed-phase separations for glycoprotein subunits

Subunit Analysis Workflow





HILIC Amide Offers an Orthogonal, Complementary, and Information-Rich Approach to IgG Subunit Analyses



LC Conditions

System:	ACQUITY UPLC H-Class Bio, Xevo G2 QTof, 500–4000 *m/z*, 2 Hz
Sample:	1 µg of IdeS digested mAb
Injection:	0.67 µL
Mobile phase A:	HPLC-grade water with 0.1% TFA,
Mobile phase B:	HPLC acetonitrile with 0.1% TFA
Flow rate:	0.2 mL/min

Reversed-Phase C_4 (top)

Column:	ACQUITY UPLC Protein BEH C_4, 300 Å, 1.7 µm, 2.1 × 100 mm (p/n: 186004496)
Temp.:	80 °C
Gradient:	5%-33% acetonitrile in 1 min, then 33 to 40% acetonitrile in 20 min

HILIC Amide (bottom)

Column:	ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm, 2.1 × 100 mm Column Kit (p/n: 176003701) that contains Glycoprotein Performance Test Standard (p/n: 186008010)
Temp.:	80 °C
Gradient:	20% to 30% water in 1 min, then 30% to 37% water in 20 min

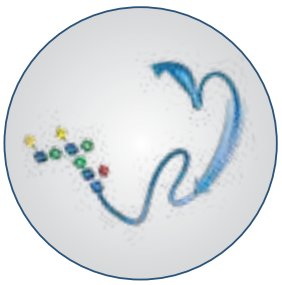
Trastuzumab subunit separations. Top: 1 µg of reduced IdeS digest separated using an ACQUITY UPLC Protein BEH C_4, 300 Å, 1.7 µm Column (0.7 µL aqueous injection). Bottom: 1 µg of reduced IdeS digest separated using an ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm Column (0.7 µL aqueous injection).

Ordering Information

ACQUITY UPLC Glycoprotein BEH Amide Columns, Kits, and Standards

Description	P/N
ACQUITY UPLC Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 50 mm, 1/pk with Standard	176003700
ACQUITY UPLC Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 100 mm, 1/pk with Standard	176003701
ACQUITY UPLC Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 150 mm, 1/pk with Standard	176003702
ACQUITY UPLC Glycoprotein BEH Amide VanGuard Pre-Column, 300 Å, 1.7 µm, 2.1 × 5 mm, 3/pk with Standard	176003699
ACQUITY UPLC Glycoprotein BEH Amide Method Validation Kit, 300 Å, 1.7 µm, 2.1 × 100 mm, 3/pk with Standard	176003703
Glycoprotein Performance Test Standard	186008010
Intact mAb Mass Check Standard	186006552
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 50 mm, 1/pk	186009547
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 100 mm, 1/pk	186009548
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 150 mm, 1/pk	186009549
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 50 mm, 1/pk with Glycoprotein Performance Test Standard	176004866
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 100 mm, 1/pk with Glycoprotein Performance Test Standard	176004867
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 150 mm, 1/pk with Glycoprotein Performance Test Standard	176004868

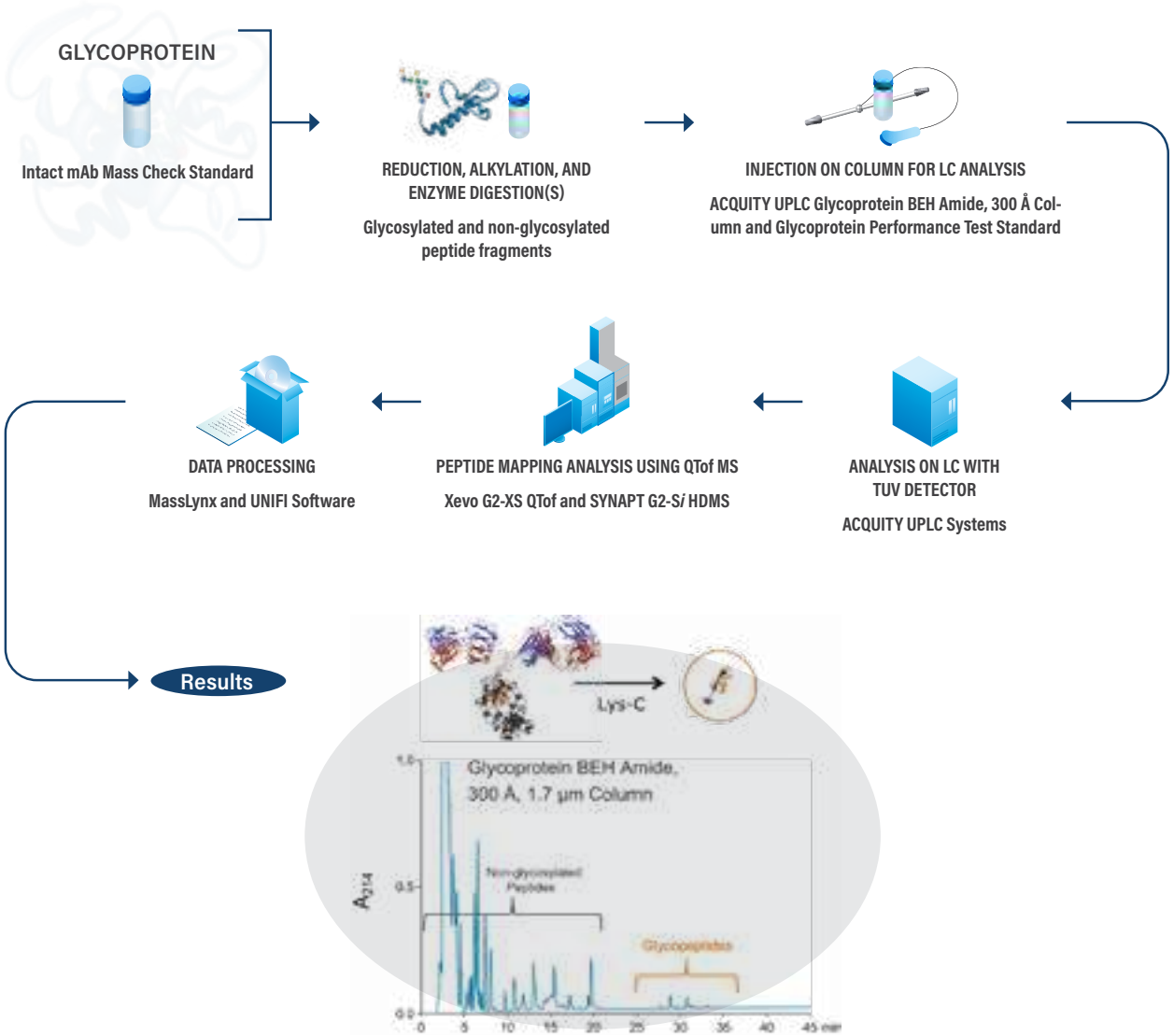
GLYCOPEPTIDE ANALYSIS



While reversed-phase, UPLC-based separations can resolve glycosylated peptides into their glycoforms, the complete resolution of glycopeptide micro-heterogeneity (same peptide sequence, various glycoforms) remains difficult. This is because retention in RP-LC is mainly due to peptide hydrophobicity, and is less affected by the presence of hydrophilic glycans. The separation is further complicated by the presence of non-glycosylated peptides in the sample that often elute in the vicinity of the glycopeptides of interest. HILIC-based glycopeptide separation provides the following benefits:

- Effectively generate data related to glycan heterogeneity and site occupancy of a trypsin digest N-linked glycoprotein
- Useful for the characterization of O-linked glycans because of the lack of specific and efficient enzymes for their release and characterization of O-linked glycoproteins

Glycopeptide Mapping Workflow



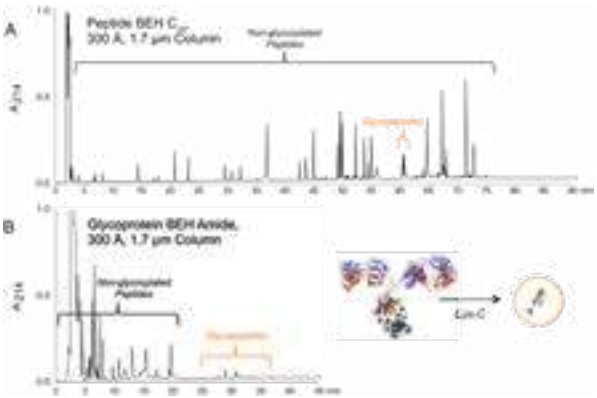
Reversed-Phase vs. HILIC-Based Analyses of a Lys-C Digest of Trastuzumab

LC Conditions

System:	ACQUITY UPLC H-Class Bio
Sample temp.:	10 °C
Vials:	Polypropylene 12 × 32 mm Screw Neck, 300 µL volume (p/n: 186002640)

Reversed-Phase LC

Column:	ACQUITY UPLC Peptide BEH C_{18}, 300 Å, 1.7 µm, 2.1 × 150 mm (p/n: 186003687)
Column temp.:	60 °C
Injection vol.:	24.2 µL (aqueous digest)



Flow rate:	0.2 mL/min
Mobile phase A:	0.1% (v/v) TFA, water
Mobile phase B:	0.1% (v/v) TFA, acetonitrile

Gradient:	Time	%A	%B	Curve
	0.0	98.0	2.0	6
	96.0	50.0	50.0	6

HILIC LC

Column:	ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm, 2.1 × 150 mm Column Kit (p/n: 176003702) that contains Glycoprotein Performance Test Standard (p/n: 186008010)
Column temp.:	30 °C
Injection volume:	100–250 µL (aqueous digests were diluted with 4 parts acetonitrile and 0.1 part dimethylsulfoxide to obtain a miscible, HILIC-compatible diluent
Flow rate:	0.2 mL/min
Mobile phase A:	0.1% (v/v) TFA, water
Mobile phase B:	0.1% (v/v) TFA, acetonitrile

Gradient:	Time	%A	%B	Curve
	0.0	20.0	80.0	6
	60.0	50.0	50.0	6

MS Conditions

System:	SYNAPT G2-S HDMS	Source temp.:	120 °C
Ionization mode:	ESI+	Desolvation temp.:	350 °C
Analyzer mode:	Resolution (~20 K)	Desolvation gas flow:	800 L/hr
Capillary voltage:	3.0 kV	Acquisition:	50–2500 *m/z*, 0.1 sec scan rate
Cone voltage:	25 V	Data management:	MassLynx Software v4.1/UNIFI v1.7

A traditional reversed-phase separation of the Lys-C digest using an ACQUITY UPLC Peptide BEH C_{18}, 300 Å, 1.7 µm, 2.1 × 150 mm Column (top) vs. a HILIC separation of the Lys-C digest using an ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm, 2.1 × 150 mm Column (bottom). In each analysis, 9.2 µg of the Lys-C digest was separated using the same gradient slope and injecting sample from a diluent comprised of either approximately 0.2% TFA in 80:20 acetonitrile/water (HILIC) or 100% water (reversed phase).

For more information, reference application note 720005409EN.

Two Parallel Strategies for Glycoprotein Analyses: Glycopeptide Mapping vs. Released Glycan Analysis

LC Conditions (left)

System:	ACQUITY UPLC H-Class Bio
Sample temp.:	10 °C
Vials:	Polypropylene 12 × 32 mm Screw Neck, 300 µL volume (p/n: 186002640)
Column:	ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm, 2.1 × 150 mm Column Kit (p/n: 176003702) that contains Glycoprotein Performance Test Standard (p/n: 186008010)
Column temp.:	60 °C
Injection:	Analyses were performed of 9.2 µg of Lys-C/tryptic digest in a sample diluent of approximately 0.2% TFA in 80:20 acetonitrile/water
Flow rate:	0.2 mL/min
Mobile phase A:	0.1% (v/v) TFA, water
Mobile phase B:	0.1% (v/v) TFA, acetonitrile

Gradient:	Time	%A	%B	Curve
	0.0	20.0	80.0	6
	60.0	50.0	50.0	6

MS Conditions

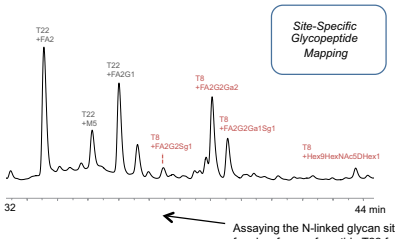
System:	SYNAPT G2-S*i* HDMS
Ionization mode:	ESI+
Analyzer mode:	Resolution (~20 K)
Capillary voltage:	3.0 kV
Cone voltage:	25 V
Source temp.:	120 °C
Desolvation temp.:	350 °C
Desolvation gas flow:	800 L/hr
Acquisition:	50–2500 *m/z*, 0.1 sec scan rate
Data management:	MassLynx Software (v4.1)/ UNIFI v1.7

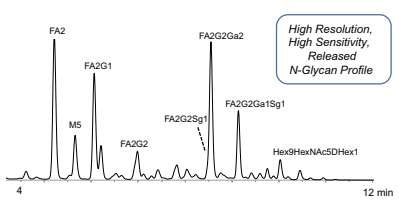
LC Conditions (right)

System:	ACQUITY UPLC H-Class Bio
Sample temp.:	10 °C
Vials:	Polypropylene 12 × 32 mm Screw Neck, 300 µL volume (p/n: 186002640)
Column:	ACQUITY UPLC Glycan BEH Amide 130 Å, 1.7 µm, 2.1 × 50 mm (p/n: 186004740)
Column temp.:	60 °C
Injection volume:	10 µL (DMF/acetonitrile diluted sample)
Mobile phase A:	50 mM ammonium formate, pH 4.4 LC-MS grade; from a 100x concentrate (p/n: 186007081)
Mobile phase B:	Acetonitrile (LC-MS grade)

Gradient:	Time (min)	Flow Rate (mL/min)	%A	%B	Curve
	0.0	0.4	25	75	6
	11.7	0.4	46	54	6
	12.2	0.2	100	0	6

Fluorescence detection: Ex 265/Em 425 nm (*Rapi*Fluor-MS) (5 Hz scan rate [50 mm column], Gain = 1)





Assaying the N-linked glycan sites of cetuximab and their microheterogeneity using a combined Lys-C/tryptic digest. Assignments for glycoforms of peptide T22 from the Fc domain of cetuximab are shown in dark gray, while assignments to the glycoforms of peptide T8 from the Fab domain are shown in red. Analyses were performed on 9.2 µg of Lys-C/tryptic digest using an ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 150 mm Column and a sample diluent of approximately 0.2% TFA in 80:20 acetonitrile/water. For more information, reference application note 720005409EN.

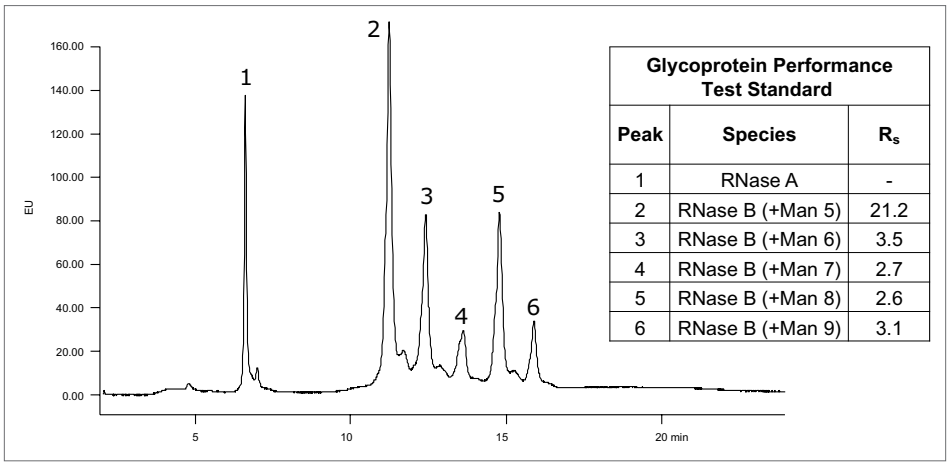
HILIC Profiling of cetuximab glycosylation. HILIC-fluorescence chromatograms of RapiFluor-MS labeled N-glycans from cetuximab obtained using an ACQUITY UPLC Glycan BEH Amide, 300 Å, 1.7 µm, 2.1 × 50 mm Column. Mass spectral data supporting the assignments of the RapiFluor-MS labeled N-glycans are provided.

For more information, reference application note 720005385EN.

GLYCOPROTEIN PERFORMANCE TEST STANDARD

Benchmarking, Method Development, and Troubleshooting

Glycoprotein Performance Test Standard is a mix of ribonuclease B from bovine pancreas at 90 µg/vial with ribonuclease A from bovine pancreas at 10 µg/vial used to quality control the ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm Column, and is recommended to be used on a regular basis for benchmarking and monitoring column and system performance and lifetime.



Glycoprotein Performance Test Standard		
Peak	**Species**	**R_s**
1	RNase A	-
2	RNase B (+Man 5)	21.2
3	RNase B (+Man 6)	3.5
4	RNase B (+Man 7)	2.7
5	RNase B (+Man 8)	2.6
6	RNase B (+Man 9)	3.1

Separation of the Glycoprotein Performance Test Standard (RNase A + RNase B glycoforms) using an ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm, 2.1 × 150 mm Column. Fluorescence detection at Ex 280 nm and Em 320 nm and a column temperature of 45 ˚C were employed in this example.

Ordering Information

ACQUITY UPLC Glycoprotein BEH Amide Columns, Kits, and Standards

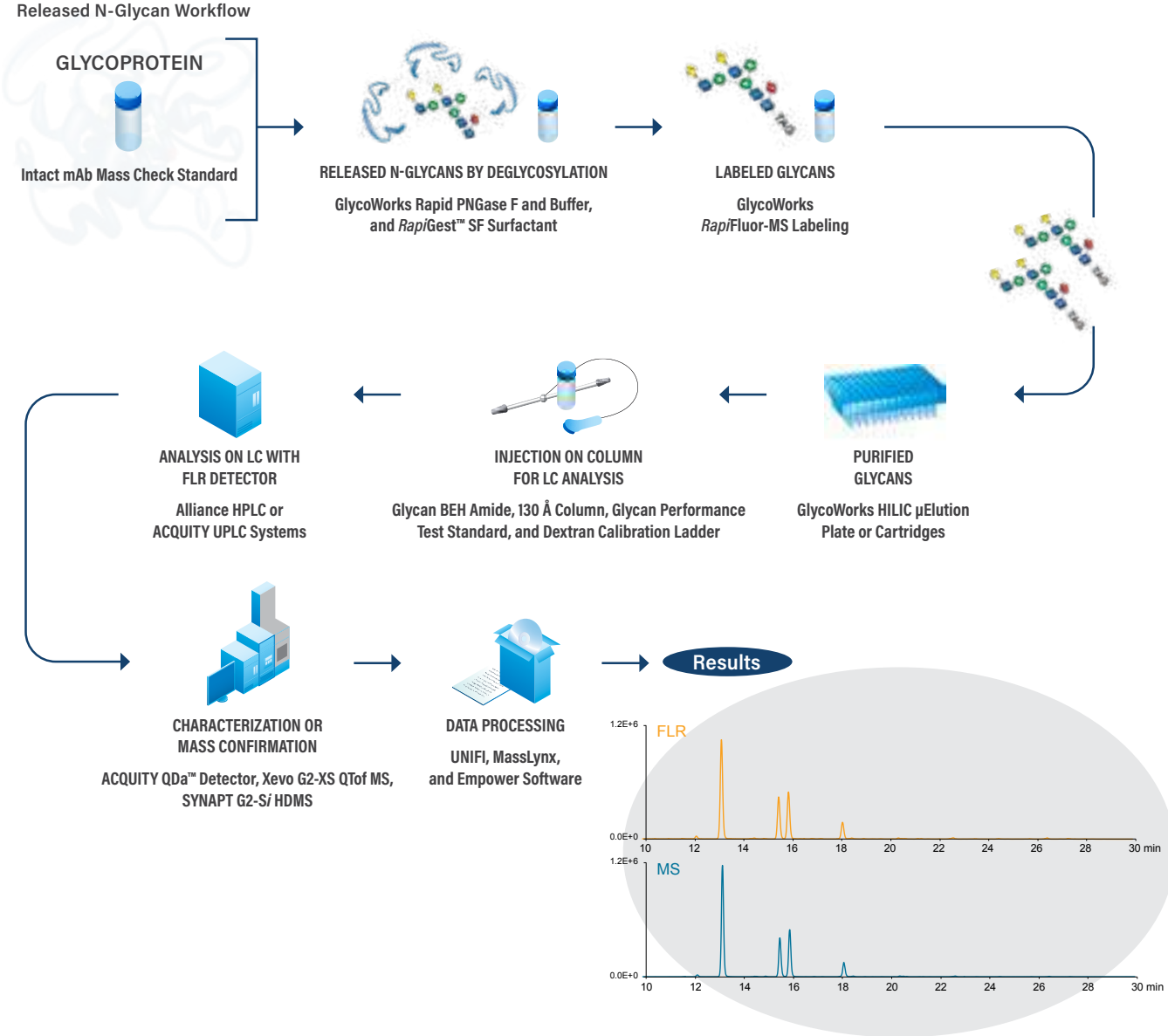
Description	P/N
ACQUITY UPLC Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 50 mm, 1/pk with Standard	176003700
ACQUITY UPLC Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 100 mm, 1/pk with Standard	176003701
ACQUITY UPLC Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 150 mm, 1/pk with Standard	176003702
ACQUITY UPLC Glycoprotein BEH Amide VanGuard Pre-Column, 300 Å, 1.7 µm, 2.1 × 5 mm, 3/pk with Standard	176003699
ACQUITY UPLC Glycoprotein BEH Amide Method Validation Kit, 300 Å, 1.7 µm, 2.1 × 100 mm, 3/pk with Standard	176003703
Glycoprotein Performance Test Standard	186008010
Intact mAb Mass Check Standard	186006552
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 50 mm, 1/pk	186009547
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 100 mm, 1/pk	186009548
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 150 mm, 1/pk	186009549
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 50 mm, 1/pk with Glycoprotein Performance Test Standard	176004866
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 100 mm, 1/pk with Glycoprotein Performance Test Standard	176004867
ACQUITY Premier Glycoprotein BEH Amide Column, 300 Å, 1.7 µm, 2.1 × 150 mm, 1/pk with Glycoprotein Performance Test Standard	176004868

RELEASED N-GLYCAN ANALYSIS

Waters GlycoWorks Sample Preparation Kits and Standards, along with the ACQUITY UPLC and HPLC Glycan Columns, were designed cohesively to provide a seamless and efficient workflow from bench to analysis.

- Fast and simplified sample preparation with the GlycoWorks *Rapi*Fluor-MS N-Glycan Kit
- Automation-enabled sample preparations with verified scripts
- Alternative selectivity with either HILIC or Mixed-mode separations
- MaxPeak Premier column format reduces sample adsorption onto metal surfaces and delivers the representative performance from the first injection
- Glycan standards for benchmarking chromatographic performance, calibration and quantification, and complex profiling

Released N-Glycan Workflow



GlycoWorks *Rapi*Fluor-MS N-Glycan Kits

Reduce complicated, time consuming sample preparation

- Increased fluorescence quantification and supreme mass spectral response
- One label that provides valuable information from characterization to routine monitoring
- Simple to follow protocols with detailed tips and tricks provided for adaptation
- The ability to easily train non-glycan experts
- An experimentally derived library to help with data analysis

waters.com/glycans

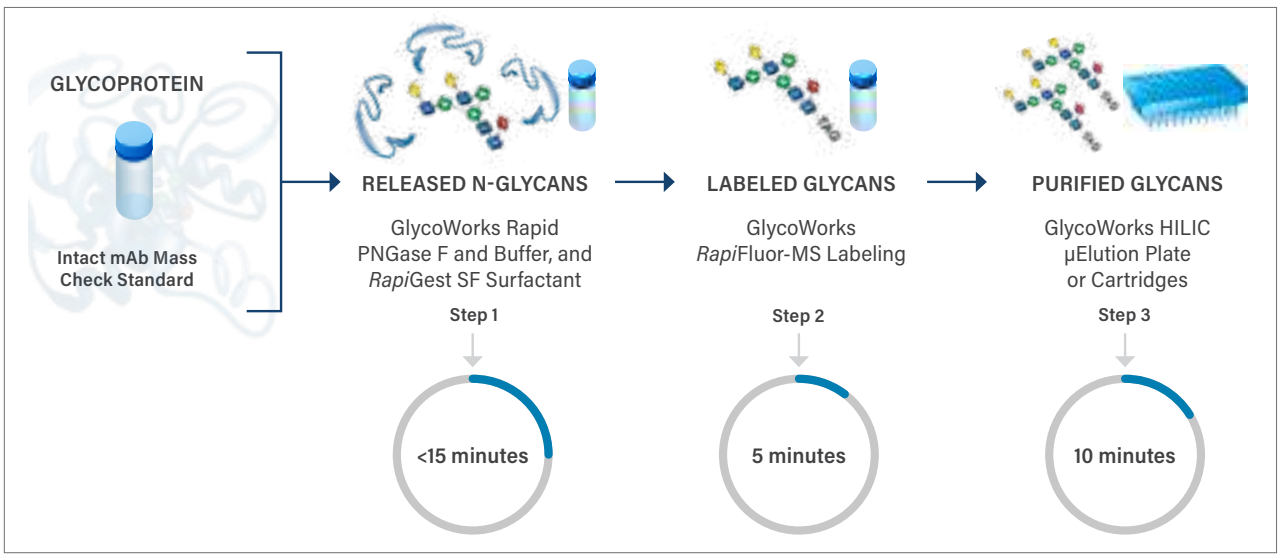
See page 361 for more information.

GLYCOWORKS *RAPI*FLUOR-MS RELEASED N-GLYCANS SAMPLE PREPARATION

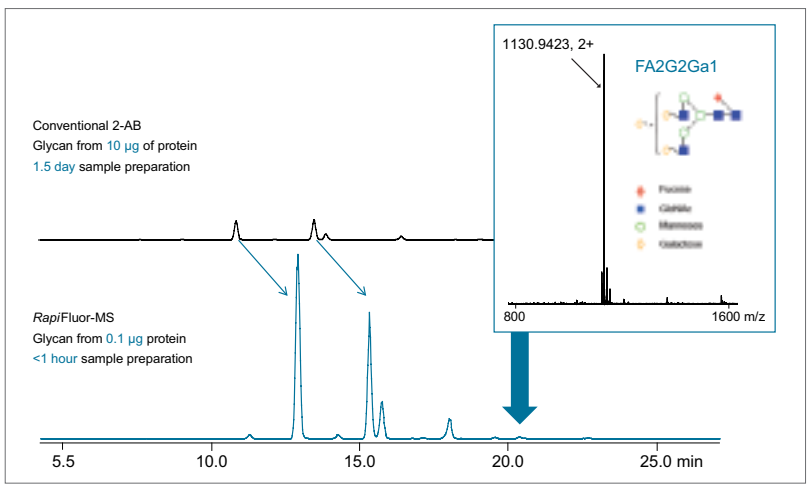
Waters GlycoWorks Consumables offer a more convenient, comprehensive, and effective sample-preparation solution for glycan analysis.

- The GlycoWorks *Rapi*Fluor-MS N-Glycan Kit ensures easy, quick preparation of released-labeled, N-glycan samples
- Streamline standard protocols (720005470EN, 720005343EN) for mAbs and a variety of glycoproteins; Optimized reducing protocols (720006992EN, 720006991EN) for complex proteins with multiple disulfide bonds
- Greatly improved FLR and MS signal intensities help easily identify low-abundance N-linked glycans
- Complete modules for processing 96 samples with flexibility of processing between 8, 24, and 48 samples at a time depending on laboratory demands with automation scripts available
- Support easy training of analysts and the transferring of methods throughout an organization

Three Steps, as little as 30 minutes



Glycan Characterization by UPLC FLR with Xevo G2-XS QTof Mass Spectrometer



Un-ionized form of acids and bases give most retention. Retention of neutral analytes not affected by pH.

Learn more about Waters latest Glycan Solutions.

Visit **waters.com/glycans**

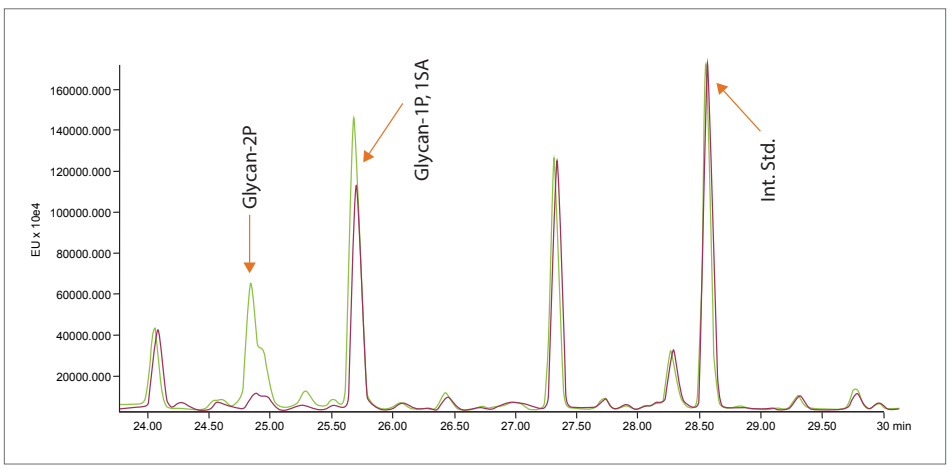
AUTOMATION OF RELEASED N-GLYCAN ANALYSIS

Waters GlycoWorks Consumables offer a more convenient, comprehensive, and effective sample-preparation solution for Released N-glycan analysis. The sample preparation procedures involve multiple steps including enzymatic deglycosylation, labeling, and SPE clean up. Due to this complexity, Waters has developed an application-specific configuration of the Andrew+ Pipetting Robot for released N-glycan analysis. Additionally, verified scripts for the GlycoWorks *Rapi*Fluor-MS method are available for our partner platforms. We provide you with the information needed, no matter the platform, to ensure that you can start achieving your automation results faster.

*GlycoWorks **Rapi**Fluor MS N-Glycan Kit*

PHOSPHOGLYCAN SPE BUFFER IMPROVES RECOVERY

The loss of labeled acidic glycans, especially phosphorylated glycan species, during SPE purification has been considered a challenge to accurately monitor the glycosylation of biotherapeutics. The GlycoWorks Phosphoglycan SPE Elution Buffer, optimized with citrate additive, facilitates the elution and recovery of phosphorylated glycans and achieves maximum yield.



Improved recovery of phosphorylated glycans using citrate containing SPE eluent

GlycoWorks *Rapi*Fluor-MS Released N-Glycan Sample Preparation Kits

Description	P/N
GlycoWorks *Rapi*Fluor-MS N-Glycan Starter Kit—96 Sample Kit contains: GlycoWorks Deglycosylation Module, GlycoWorks Labeling Module, GlycoWorks Cleanup Module, GlycoWorks Sample Collection Module, ACQUITY UPLC Glycan BEH Amide, 1.7 µm, 2.1 × 150 Column, Ammonium Formate Solution – Glycan Analysis, Glycan *Rapi*Fluor-MS performance Test std, Intact mAb Mass Check Standard	176003635
GlycoWorks *Rapi*Fluor-MS N-Glycan Kit—96 Sample Kit contains: GlycoWorks Deglycosylation Module, GlycoWorks Labeling Module, GlycoWorks Cleanup Module, GlycoWorks Sample Collection Module, Intact mAb Mass Check Standard	176003606
GlycoWorks *Rapi*Fluor-MS N-Glycan Starter Kit—24 sample Kit contains: GlycoWorks Deglycosylation Module, GlycoWorks Labeling Module, GlycoWorks Cleanup Module, GlycoWorks Sample Collection Module, ACQUITY UPLC Glycan BEH Amide, 1.7 µm, 2.1 × 150 mm Column, Ammonium Formate Solution – Glycan Analysis, Glycan *Rapi*Fluor-MS performance Test std, Intact mAb Mass Check Standard	176003712
GlycoWorks *Rapi*Fluor-MS N-Glycan Kit—24 sample Kit contains: GlycoWorks Deglycosylation Module, GlycoWorks Labeling Module, GlycoWorks Cleanup Module, GlycoWorks Sample Collection Module, Intact mAb Mass Check Standard	176003713
GlycoWorks *Rapi*Fluor-MS N-Glycan Refill Kit—24 sample Kit contains one of each: GlycoWorks Deglycosylation Module and the GlycoWorks Labeling Module	176003714
GlycoWorks Rapid Deglycosylation 1 × 24 Kit contains: one vial of GlycoWorks Rapid PNGaseF Enzyme and Buffer; and, one vial of 10-mg *Rapi*Gest SF Surfactant	186008939
GlycoWorks Rapid Deglycosylation 3 × 8	186008841
GlycoWorks Rapid Deglycosylation Kit 2 × 48	186004579
GlycoWorks Rapid Deglycosylation kit 4 × 24	186008840
GlycoWorks *Rapi*Fluor -MS Labeling Kits—24 Sample	186008091
GlycoWorks *Rapi*Fluor -MS Labeling Kits—96 Sample	186007989
GlycoWorks SPE Reagents	186007992
GlycoWorks Phosphoglycan SPE Reagents HILIC	186010209
GlycoWorks Phosphoglycan SPE Elution Buffer, 4/pk	186009763
GlycoWorks HILIC uElution Plate	186002780
GlycoWorks Sample Collection Module	186007988

GlycoWorks *Rapi*Fluor-MS N-Glycan Automation Kits

Description	P/N
GlycoWorks *Rapi*Fluor-MS N-Glycan Script Starter Kit – Automation Kit contains: GlycoWorks Automation Script Pack-CD; Intact mAb Mass Check Standard (unlabeled); *Rapi*Fluor-MS Intact mAb Mass Check Standard (deglycosylated, labeled, and purified); GlycoWorks Rapid Deglycosylation Kit – 2 × 48; GlycoWorks *Rapi*Fluor-MS Labeling Module – Automation; GlycoWorks HILIC µElution Plate; GlycoWorks SPE Reagents – Automation; GlycoWorks Sample Collection Module – Automation; ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 150 mm Column; Mobile phase concentrate: ammonium formate	176004151
GlycoWorks *Rapi*Fluor-MS N-Glycan Starter Kit – Automation Kit contains: Intact mAb Mass Check Standard (unlabeled); *Rapi*Fluor-MS Intact mAb Mass Check Standard (deglycosylated, labeled, and purified); GlycoWorks Rapid Deglycosylation Kit – 2 × 48; GlycoWorks *Rapi*Fluor-MS Labeling Module – Automation; GlycoWorks HILIC µElution Plate; GlycoWorks SPE Reagents – Automation; GlycoWorks Sample Collection Module – Automation; ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 150 mm Column; Mobile phase concentrate: ammonium formate	176004152
GlycoWorks *Rapi*Fluor-MS N-Glycan Kit - Automation Kit contains: GlycoWorks Rapid Deglycosylation Kit – 2 × 48, GlycoWorks *Rapi*Fluor-MS Labeling Module – Automation, GlycoWorks HILIC µElution Plate, GlycoWorks SPE Reagents – Automation and GlycoWorks Sample Collection Module – Automation	176004153
GlycoWorks *Rapi*Fluor-MS N-Glycan Basic Kit - Automation Kit contains: GlycoWorks Rapid Deglycosylation Kit – 2 × 48, GlycoWorks *Rapi*Fluor-MS Labeling Module – Automation, GlycoWorks HILIC µElution Plate, and GlycoWorks SPE Reagents – Automation	176004154
Andrew+ 24 Sample GlycoWorks Application Kit contains: GlycoWorks Rapid Deglyco Module 24-sample, GlycoWorks *Rapi*Fluor-MS Labeling—24 sample, GlycoWorks HILIC uElution Plate, GlycoWorks SPE Reagents – Automation, Intact mAb Mass Check Standard	176003349
Andrew+ 96 Sample GlycoWorks Application Kit contains: GlycoWorks Rapid Deglyco Module 96-sample, GlycoWorks *Rapi*Fluor-MS Labeling—96 sample, GlycoWorks HILIC uElution Plate, GlycoWorks SPE Reagents – Automation, Intact mAb Mass Check Standard	17600335
Andrew+ 96 Sample GlycoWorks Application Kit contains: GlycoWorks Rapid Deglyco Module 2 × 48, GlycoWorks *Rapi*Fluor-MS Labeling – Automation, GlycoWorks HILIC µElution Plate, GlycoWorks SPE Reagents – Automation, Intact mAb Mass Check Standard	176003351

GLYCAN PERFORMANCE TEST STANDARDS AND DEXTRAN CALIBRATION LADDERS

Benchmarking, Method Development, and Troubleshooting

Waters purified glycan library standards are used as qualitative/quantitative standards for LC/FLR and LC/MS. These standards come pre-labeled, lyophilized for long term storage in Waters Certified Vials for ease of solubilization and injection.

Chromatographic Performance
To ensure that the system and chromatographic method is working, it is highly recommended to use a pre-labeled standard to access observed retention time by monitoring the major peaks for performance of the method.

Calibration and Quantitation
When using LC optical detection, it is important to have standards to assist in profiling glycans under HILIC conditions to ensure reproducible chromatographic assignment providing confidence in data generation.

Complex Profiling
These performance test standards are helpful when looking for specific glycans monitored in manufacturing and are useful to check retention time of major peaks in LC/FLR, accurate mass or to assess sample preparation efficiency.

Ordering Information

*Rapi*Fluor-MS Released N-Glycan Standards and Accessories

Description	P/N
*Rapi*Fluor-MS Dextran Calibration Ladder 50 µg/vial	186007982
*Rapi*Fluor-MS Glycan Performance Test Standard 400 pmol total/vial	186007983
*Rapi*Fluor-MS High Mannose Standard	186008317
*Rapi*Fluor-MS Intact mAb Standard	186008843
*Rapi*Fluor-MS Quantitative Glycan Standard	186008791
*Rapi*Fluor-MS Sialylated Glycan Performance Test Standard	186008660
Intact mAb Mass Check Standard*	186006552
2-AB Glycan Performance Test Standard	186006349
2-AB Dextran Calibration Ladder	186006841

* Controls Standard included in kit.
** Essential for kit use.

Description	P/N
*Rapi*Gest SF 3 mg vial	186008090
*Rapi*Gest SF 10 mg vial	186002123
96-Well Plate Extraction Manifold	186001831
Vacuum Manifold Shims,** 3/set	186007986
Positive Pressure Manifold Spacer for the GlycoWorks *Rapi*Fluor-MS N-Glycan Kit,* 1/pk	186007987
Vacuum Pump 220 v/240 v 50 Hz	725000604
Positive Pressure Manifold	186006961
Modular Heat Block for 1 mL tubes/96 wells	186007985
GlycoWorks Rapid Buffer—5 mL	186008100

GLYCAN COLUMNS

Choose the most appropriate LC-based technology to address your specific glycan analysis needs and laboratory instrumentation

HILIC for Released Glycans
ACQUITY UPLC and XBridge Glycan BEH Amide 130 Å Columns are best suited for the analysis of released, N-labeled glycans using pre-column labeling with 2-AB, 2-AA, or Waters innovative and enabling *Rapi*Fluor-MS reagent. ACQUITY Premier Glycan BEH Amide Columns with MaxPeak High Performance Surfaces (HPS) Technology, that reduces sample loss caused by non-specific adsorption on metal surfaces, deliver the representative performance of Glycan BEH Amide chemistry from the first injection.

Mixed Mode for Released Glycans
Glycan BEH C_{18} AX Columns are packed with a mixed-mode, reversed-phase/anion-exchange chemistry that provides charge-based separations and extra resolution for acidic glycans. With the mixed-mode separation, glycans are grouped by different charged states, and retention is increased with analyte net charge. Additional resolution of isomeric glycan species within the same charged group can be achieved due to the RP properties of the stationary phase. The ACQUITY Premier Glycan BEH C_{18} AX Columns are integrated with an easy-to-use, MS-grade mobile-phase concentrate and the *Rapi*Fluor-MS Sialylated Glycan Performance Test Standard, providing a complete charge-based separation solution.

Application Example 1

LC Conditions

System:	ACQUITY UPLC H-Class Bio
Data Acquisition:	MassLynx v4.1
Column:	ACQUITY Premier Glycan BEH C_{18} AX, 1.7 µm, 2.1 × 150 mm (p/n: 186009760)
Sample Temp.:	8 °C
Sample Injection Volume:	1 µL
FLR Wavelengths:	265 Ex/425 Em (RFMS-labeled glycans) 3 30 Ex/420 Em (2-AB-labeled glycans)
Column Temp.:	60 °C
Seal Wash:	330% ACN/70% 18.2 MΩ water v/v (seal wash interval set to 5 min)
Mobile Phase A[1]:	18.2 MΩ water
Mobile Phase B[2]:	10% IonHance Glycan C_{18} AX 1 M ammonium formate concentrate in 40%/60% water/acetonitrile (v/v)
Active Preheater:	Enabled
Scan Rate:	10 points/sec
Filter Time Constant:	Normal
Autozero on Inject Start:	Yes
Autozero on Wavelength:	Maintain baseline

1. The water only mobile phase can be susceptible to bacterial growth. It is recommended to replace the mobile phase frequently (every 3 days) and periodically flush solvent line with 90/10 acetonitrile/water mixture.

2. Mobile Phase B is prepared by diluting 100 mL of IonHance Glycan C_{18} AX 1 M Ammonium Formate concentrate with 320 mL of MilliQ water and 580 mL of acetonitrile. It is recommended to replace this mobile phase B frequently (every 3 days) to avoid any potential performance change caused by acetonitrile evaporation.

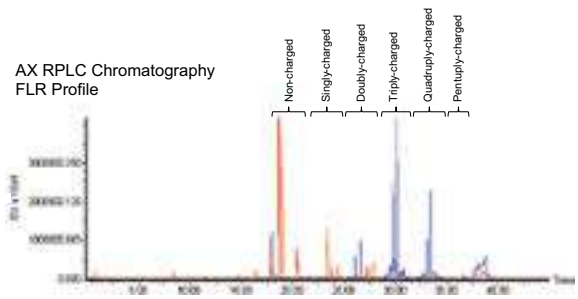
MS Conditions

System:	Xevo G2-XS QTof
Ionization Mode:	ESI, Positive
Acquisition Range:	700–3000 Da
Capillary Voltage:	2.2 kV
Source Offset:	50 V
Collision Energy:	Off
Cone Voltage:	75 V
Desolvation Gas:	600 L/hr
Source Temp.:	120 °C
Desolvation Temp.:	500 °C
Scan Rate:	2 Hz

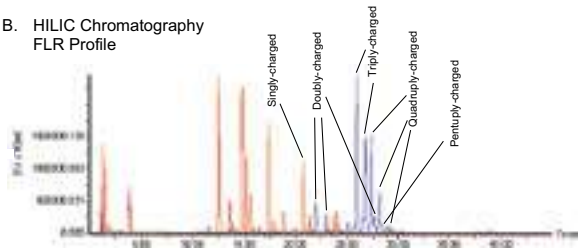
Gradient - *Rapi*Fluor-MS Labeled Glycans

Time (min)	Flow (mL/min)	%A	%B	Curve
0.0	0.4	100	0	Initial
36.0	0.4	78	22	6
36.3	0.4	0	100	6
37.3	0.4	0	100	6
38.0	0.4	100	0	6
45.0	0.4	100	0	6

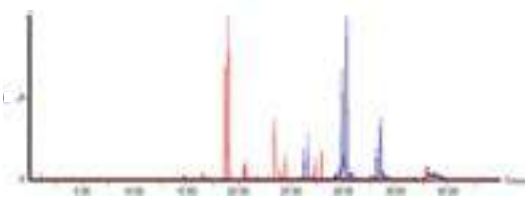
A. AX RPLC Chromatography FLR Profile



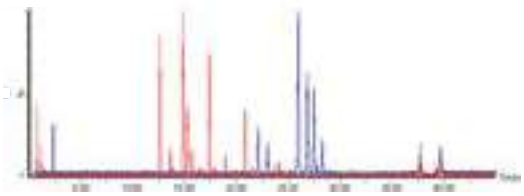
B. HILIC Chromatography FLR Profile



C. AX RPLC Chromatography MS BPI Profile



D. HILIC Chromatography MS BPI Profile



FLR Chromatograms of RFMS-labeled glycans from human IgG (red) and Fetuin (blue) using different LC chromatography. Stainless hardware with High Performance Surface were used to pack all columns. Figure 1 A, C: FLR profiling (A) and MS BPI profiling (C) using an anion exchange reversed phase liquid chromatography (AX RPLC) mixed mode separation with BEH C_{18} AX stationary phase. Figure 1 B, D: FLR profiling (B) and MS BPI profiling (D) using HILIC separation achieved with amide bonded BEH stationary phase.

For more information, reference application note 720007038EN.

Application Example 2

LC Conditions
Standard Configuration

System:	ACQUITY UPLC I-Class PLUS
Detection:	ACQUITY FLR Detector (λexcitation=265 nm, λemission=425 nm, 2 Hz)
Columns:	ACQUITY Glycan BEH Amide Column, 1.7 µm, 130 Å, 2.1 × 150 mm (p/n: 186004742)
Vials:	QuanRecovery with MaxPeak HPS 300 µL Vials (p/n: 186009186)
Column Temp.:	60 °C
Sample Temp.:	6 °C
Injection Amount:	1 µL
Seal Wash:	20% acetonitrile in water
Mobile Phase A:	H_2O with 50 mM NH_4HCO_2
Mobile Phase B:	Acetonitrile

MS Conditions

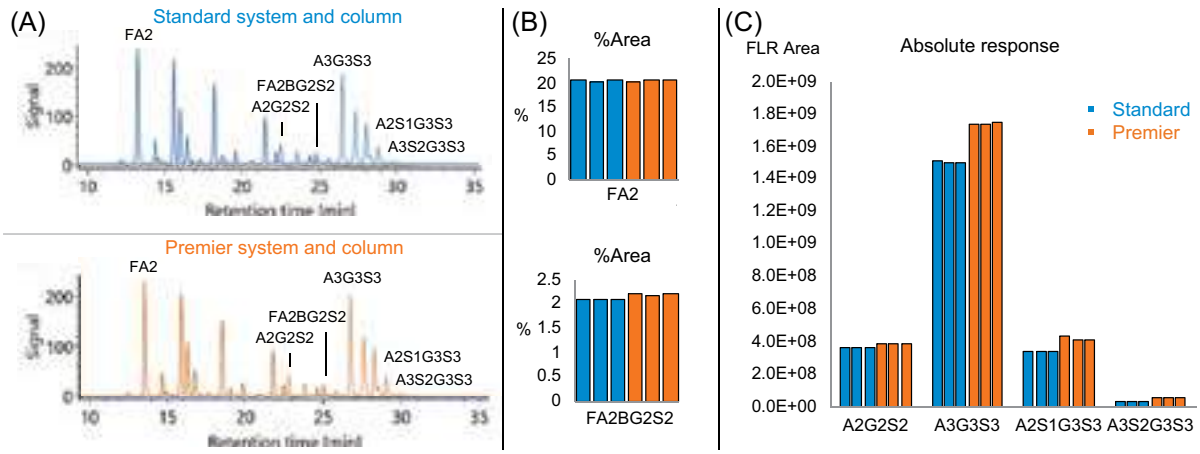
System:	ACQUITY RDa Mass Detector
Ionization Mode:	ESI, Positive
Acquisition Range:	50–2,000 *m/z*
Capillary Voltage:	1.5 kV
Source Offset:	50 V
Cone Voltage:	45 V
Fragmentation CV:	70–90 V

LC Conditions
AQUITY Premier Solution

System:	ACQUITY Premier (BSM) UPLC
Detection:	ACQUITY Premier FLR Detector (λexcitation=265 nm, λemission=425 nm, 2 Hz)
Columns:	ACQUITY Premier Glycan BEH Amide Column, 1.7 µm, 130 Å, 2.1 × 150 mm (p/n: 186009524)
Vials:	QuanRecovery with MaxPeak HPS 300 µL Vials (p/n: 186009186)
Column Temp.:	60 °C
Sample Temp.:	6 °C
Injection Amount:	1 µL
Seal Wash:	20% acetonitrile in water
Mobile Phase A:	H_2O with 50 mM NH_4HCO_2
Mobile Phase B:	Acetonitrile

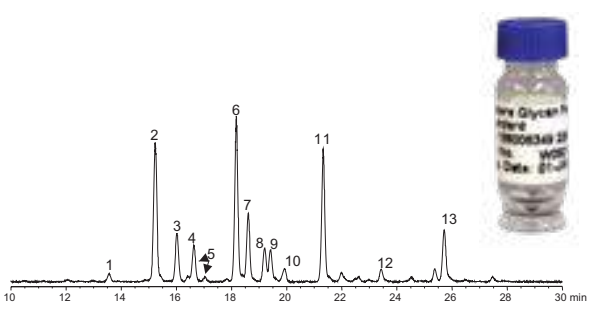
Gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
0.00	0.4	25	75	6
35.00	0.4	46	54	6
36.50	0.2	80	20	6
39.50	0.2	80	20	6
43.10	0.2	25	75	6
47.60	0.4	25	75	6
55.00	0.4	25	75	6



The recovery of sialylated glycans are slightly better using the BioAccord System with ACQUITY Premier LC and Column. (A) overlaid chromatograms of the separation of neutral and sialylated RFMS labeled glycan performance test standards (GPTS) on Standard vs. ACQUITY Premier System and Column. (B) Comparison of relative abundances of a neutral glycan, FA2, vs. a sialylated glycan, FA2BG2S2 in the neutral GPTS. (C) Comparison of the absolute response of the representative glycans in the sialylated GPTS.

For more information, reference application note 720007261EN.



Peak Identification

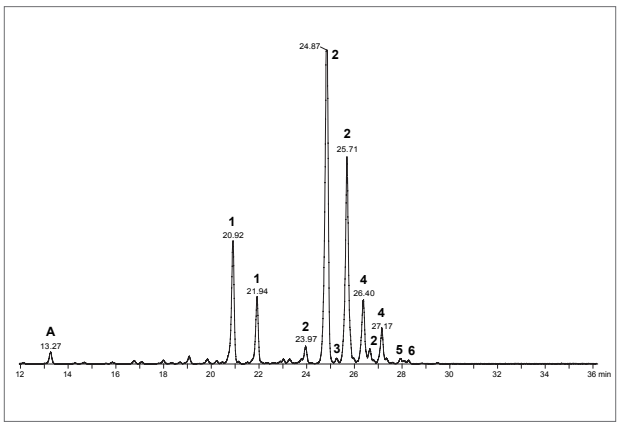
1. G0 (NGA2)	5. G1	9. Man-6	13. G2F+SA (A1F)
2. G0F (NGA2F)	6. G1Fa (NA2G1F)	10. G2 (NA2)	
3. Man-5	7. G1Fb (NA2G1F)	11. G2F (NA2F)	
4. G0F+GN	8. G1F+GN	12. G1F+SA	

ACQUITY UPLC Glycan BEH Amide Columns and Method Validation Kits

Description	P/N
ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 50 mm Column	186004740
ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 100 mm Column	186004741
ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 5 mm VanGuard Column, 3/pk	186004739
ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 100 mm Column Method Validation Kit[1]	186004907
ACQUITY UPLC Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 150 mm Column	186004742

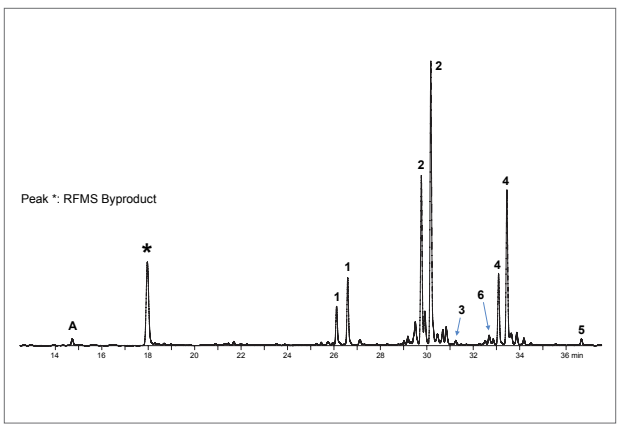
Note: ACQUITY UPLC Glycan BEH Amide, 1.7 µm Columns are designed for use with the ACQUITY UPLC System. The benefits of the small particle packing in ACQUITY UPLC Glycan BEH Amide, 1.7 µm Columns are only realized with the low system volume and low detector dispersion of an ACQUITY UPLC System.
[1]Three columns from three different batches of BEH Amide, 130 Å material.

HILIC Separation



Chromatographic profile of the RapiFluor-MS™ Sialylated Glycan Performance Test Standard.

Mixed-mode Separation



Chromatographic Profile of the RapiFluor-MS Sialylated Glycan Performance Test Standard.

Peak	Name	*Rapi*Flour-MS Labeled Glycan Composition	M, (Da)
1	A2G2S2	C101H159N11O63	2533.9576
2	A3G3S3	C126H199N13O81	3190.1852
3	FA3G3S3	C132H209N13O85	3336.2431
4	A3S1G3S3	C137H216N14O89	3481.2806
5	A3S2G3S3	C148H233N15O97	3772.3761
6	A4G4S4	C151H239N15O99	3846.4128
A	M5	C63H99N7O37	1545.6080

Ordering Information

ACQUITY Premier Glycan BEH Amide columns and Eluent

Description	P/N
ACQUITY Premier Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 50 mm Column	186009522
ACQUITY Premier Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 100 mm Column	186009523
ACQUITY Premier Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 150 mm Column	186009524
ACQUITY Premier Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 50 mm VanGuard FIT Column	186009974
ACQUITY Premier Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 100 mm VanGuard FIT Column	186009975
ACQUITY Premier Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 150 mm VanGuard FIT Column	186009976
ACQUITY Premier Glycan BEH Amide, 130 Å, 1.7 µm, 2.1 × 5 mm (Guard) VanGuard FIT Column	186009977
Ammonium Formate Solution -Glycan Analysis	186007081

ACQUITY Premier Glycan BEH C_{18} AX columns and Eluent

Description	P/N
ACQUITY Premier Glycan BEH C_{18} AX, 1.7 µm, 2.1 × 50 mm Column	186009758
ACQUITY Premier Glycan BEH C_{18} AX, 1.7 µm, 2.1 × 100 mm Column	186009759
ACQUITY Premier Glycan BEH C_{18} AX, 1.7 µm, 2.1 × 150 mm Column	186009760
ACQUITY Premier Glycan BEH C_{18} AX, 1.7 µm, 2.1 × 50 mm VanGuard FIT Column	186009970
ACQUITY Premier Glycan BEH C_{18} AX, 1.7 µm, 2.1 × 100 mm VanGuard FIT Column	186009971
ACQUITY Premier Glycan BEH C_{18} AX, 1.7 µm, 2.1 × 150 mm VanGuard FIT Column	186009972
ACQUITY Premier Glycan BEH C_{18} AX, 1.7 µm, 2.1 × 5 mm (Guard) VanGuard FIT Column	186009973
ACQUITY C_{18} AX Charge Based Glycan Kit (Standard Mobile Phase Concentrate, and 1.7 µm, 2.1 × 50 mm Column)	186004732
ACQUITY C_{18} AX Charge Based Glycan Kit (Standard Mobile Phase Concentrate, and 1.7 µm, 2.1 × 100 mm Column)	186004733
ACQUITY C_{18} AX Charge Based Glycan Kit (Standard Mobile Phase Concentrate, and 1.7 µm, 2.1 × 150 mm Column)	186004734
IonHance Glycan C_{18} AX Ammonium Formate Concentrate	186009762

XBridge Glycan BEH Amide HPLC and UHPLC Columns and Method Validation Kits

Description	P/N
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 2.1 × 5 mm VanGuard Column, 3/pk	186007262
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 2.1 × 50 mm ***XP*** Column	186007263
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 2.1 × 100 mm ***XP*** Column	186007264
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 2.1 × 150 mm ***XP*** Column	186007265
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 2.1 × 150 mm ***XP*** Column Method Validation Kit[1]	186007266
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 3.0 × 30 mm ***XP*** Column	186008038
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 3.0 × 75 mm ***XP*** Column	186008039
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 3.0 × 150 mm ***XP*** Column	186008040
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 4.6 × 20 mm Guard Column, 2/pk[3]	186007267
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 4.6 × 50 mm ***XP*** Column	186007268
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 4.6 × 100 mm ***XP*** Column	186007269
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 4.6 × 150 mm ***XP*** Column	186007270
XBridge Glycan BEH Amide, 130 Å, 2.5 µm, 4.6 × 150 mm ***XP*** Column Method Validation Kit[1]	186007271
XBridge Glycan BEH Amide, 130 Å, 3.5 µm, 2.1 × 10 mm Sentry Guard Cartridge, 2/pk[2]	186007505
XBridge Glycan BEH Amide, 130 Å, 3.5 µm, 2.1 × 50 mm Column	186007502
XBridge Glycan BEH Amide, 130 Å, 3.5 µm, 2.1 × 100 mm Column	186007503
XBridge Glycan BEH Amide, 130 Å, 3.5 µm, 2.1 × 150 mm Column	186007504
XBridge Glycan BEH Amide, 130 Å, 3.5 µm, 4.6 × 20 mm Sentry Guard Cartridge, 2/pk[3]	186007272
XBridge Glycan BEH Amide, 130 Å, 3.5 µm, 4.6 × 50 mm Column	186007273
XBridge Glycan BEH Amide, 130 Å, 3.5 µm, 4.6 × 100 mm Column	186007274
XBridge Glycan BEH Amide, 130 Å, 3.5 µm, 4.6 × 150 mm Column	186007275
XBridge Glycan BEH Amide, 130 Å, 3.5 µm, 4.6 × 150 mm Column Method Validation Kit[1]	186007277
XBridge Glycan BEH Amide, 130 Å, 3.5 µm, 4.6 × 250 mm Column	186007276
XBridge Premier Glycan BEH Amide, 130 Å, 2.5 µm, 2.1 × 50 mm Column	186009941
XBridge Premier Glycan BEH Amide, 130 Å, 2.5 µm, 2.1 × 100 mm Column	186009942
XBridge Premier Glycan BEH Amide, 130 Å, 2.5 µm, 2.1 × 150 mm Column	186009943
XBridge Premier Glycan BEH Amide, 130 Å, 2.5 µm, 4.6 × 50 mm Column	186009944
XBridge Premier Glycan BEH Amide, 130 Å, 2.5 µm, 4.6 × 100 mm Column	186009945
XBridge Premier Glycan BEH Amide, 130 Å, 2.5 µm, 4.6 × 150 mm Column	186009946
XBridge Premier Glycan BEH C_{18} AX, 2.5 µm, 2.1 × 50 mm Column	186009947
XBridge Premier Glycan BEH C_{18} AX, 2.5 µm, 2.1 × 100 mm Column	186009948
XBridge Premier Glycan BEH C_{18} AX, 2.5 µm, 2.1 × 150 mm Column	186009949
XBridge Premier Glycan BEH C_{18} AX, 2.5 µm, 4.6 × 50 mm Column	186009950
XBridge Premier Glycan BEH C_{18} AX, 2.5 µm, 4.6 × 100 mm Column	186009951
XBridge Premier Glycan BEH C_{18} AX, 2.5 µm, 4.6 × 150 mm Column	186009952

[1] Three columns from three different batches of BEH Amide, 130 Å material.
[2] Requires 2.1 × 10 mm Universal Sentry Guard Holder, p/n: WAT097958.
[3] Requires 4.6 × 20 mm Universal Sentry Guard Holder, p/n: WAT046910.

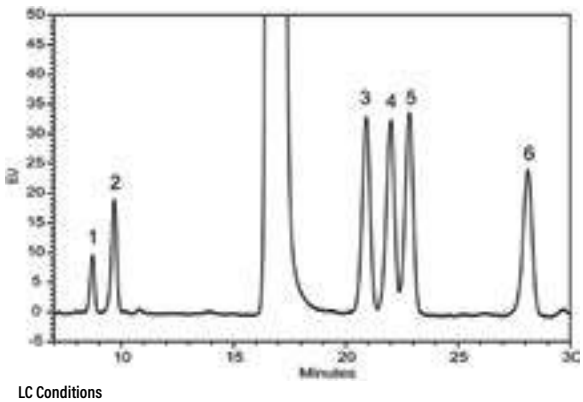
Reductive Amination Glycan Sample Preparation Kits and Standards

Description	P/N
GlycoWorks Reductive Amination High-Throughput Prep Kit Kit contains: GlycoWorks HILIC µElution 96-Well Plate, *Rapi*Gest SF 1 mg Vial, GlycoWorks Reagent Kit, Manifold Waste Tray	176003090
GlycoWorks HILIC µElution 96-Well Plate	186002780
*Rapi*Gest SF 1 mg Vial	186001860
GlycoWorks Reagent Kit	186007034
Manifold Waste Tray	600001282
GlycoWorks Reductive Amination Single-Use Prep Kit Kit contains: GlycoWorks HILIC 1 cc Cartridge (20/pk), *Rapi*Gest SF 1 mg Vial, GlycoWorks Reagent Kit	176003119
GlycoWorks HILIC 1 cc Cartridge (20/pk)	186007080
*Rapi*Gest SF 1 mg Vial	186001860
GlycoWorks Reagent Kit	186007034
2-AB Glycan Performance Test Standard The Glycan Performance Test Standard is a 2-AB labeled human IgG-like standard that is QC verified to contain the components needed to benchmark and evaluate ACQUITY UPLC Glycan BEH , 1.7 µm Columns	186006349
2-AB Dextran Calibration Ladder The 2-AB labeled, Dextran Calibration Ladder is used to calibrate the HILIC column from retention time to GU values. This calibration ladder provides good peak shape and reliable identification from 2 to 30 glucose units.	186006841
GlycoWorks HILIC 1 cc Cartridge, 20/pk	186007080
GlycoWorks HILIC 1 cc Flangeless Cartridge	186007239
GlycoWorks HILIC µElution Plate	186002780
GlycoWorks Reagent Kit	186007034
GlycoWorks SPE Reagents	186007992
Ammonium Formate Solution – Glycan Analysis	186007081

Monosaccharide Analyses

Apart from charged sialic acid species, the primary monosaccharides found in N-linked and O-linked glycans are the neutral monosaccharides N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc), galactose (Gal), glucose (Glc), mannose (Man), and fucose (Fuc). Analyses of non-charged monosaccharides frequently begins by acid hydrolysis of the glycan by incubation with trifluoroacetic acid or hydrochloric acid. Usually, a three-hour incubation at 100 °C with 2 M trifluoroacetic acid releases all of the monosaccharides; however, during hydrolysis, the N-acetyl groups on GlcNAc and GalNAc are hydrolyzed to glucosamine (GlcN) and galactosamine (GalN). Following hydrolysis, the released monosaccharides are derivatized using 2-aminobenzoic acid (2-AA), as detailed in the Waters application note "Future Proofing the Biopharmaceutical QC Laboratory: Chromatographic Scaling of HPLC Monosaccharide Analyses Using the ACQUITY UPLC H-Class Bio System" (720005255EN). As the application note explains, this method can reliably generate sensitive, high resolution, and quantitative monosaccharide analyses independent of a laboratory's available LC instrumentation.

HPLC-Based Analyses of 2-AA Labeled Monosaccharides from Acid Hydrolyzed Bovine Fetuin



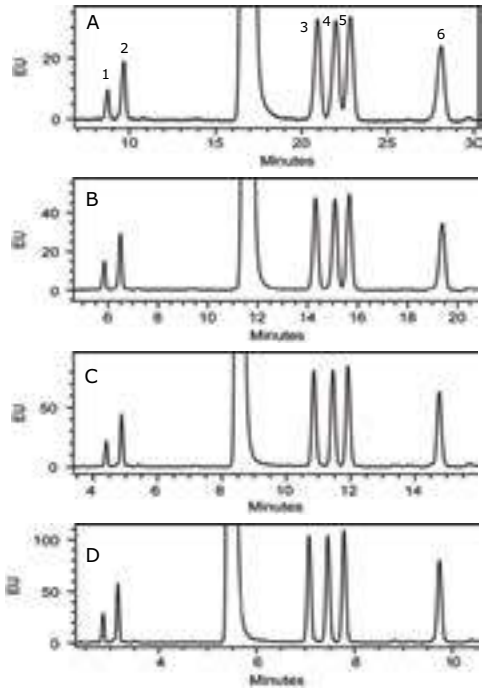
LC Conditions

System:	ACQUITY UPLC H-Class
Sample:	Acid Hydrolyzed Bovine Fetuin followed by 2-AA derivatization
Injection:	4.8 µL
Column:	XBridge BEH C_{18}, 130 Å, 5 µm, 4.6 × 100 mm (p/n: 186003115)
Mobile phase A:	0.2% N-butylamine, 0.5% phosphoric acid, and 1% THF in water
Mobile phase B:	Acetonitrile with 0.1% trifluoroacetic acid
Flow rate:	0.48 mL/min
Gradient:	See table
Excitation wavelength:	360 nm
Emission wavelength:	425 nm
Column temp.:	30 °C

Measurement	Particle Size (µm)			
	5	3.5	2.5	1.7
Column I.D. (mm)	4.6	4.6	2.1	2.1
Column Length (mm)	100	100	100	100
Average W1/2h	0.349	0.189	0.125	0.076
T_0	20.00	9.99	14.02	6.80
P_c	58	75	81	90
GlcNAc	8.71	5.84	4.40	2.85
GalNAc	9.69	6.49	4.87	3.15
Gal	20.92	14.32	10.87	7.08
Man	22.01	15.09	11.46	7.46
Glc	22.83	15.66	11.92	7.78
Fuc	28.12	19.39	14.76	9.74
GlcNAc	3.65	3.83	3.69	3.67
GalNAc	8.26	8.52	8.23	8.31
Gal	22.17	22.48	22.45	22.06
Man	23.04	23.07	23.05	22.72
Glc	23.76	23.89	23.88	23.61
Fuc	19.11	18.21	18.71	19.62
Rs				
GlcNAc, GalNAc	1.12	1.13	1.13	1.15
GalNAc, Gal	2.26	2.38	2.47	2.67
Gal, Man	1.05	1.06	1.06	1.06
Man, Glc	1.04	1.04	1.04	1.05
Glc, Fuc	1.24	1.25	1.26	1.28
GlcNAc, GalNAc	2.52	3.10	3.16	3.32
GalNAc, Gal	21.05	27.12	30.69	33.25
Gal, Man	1.62	2.10	2.48	2.62
Man, Glc	1.20	1.53	1.90	2.23
Glc, Fuc	7.34	9.59	11.41	12.78

HPLC analysis of monosaccharides. A separation performed with a Waters XBridge BEH C_{18}, 130 Å, 5 µm Column as detailed in Waters Applications Note: 720005255EN. Monosaccharides are identified as follows: (1) N-acetylglucosamine (GlcNAc), (2) N-acetylgalactosamine (GalNAc), (3) Galactose (Gal), (4) Mannose (Man), (5) Glucose (Glc), and (6) Fucose (Fuc).

Effect of Particle Size on the Analyses of 2-AA Labeled Monosaccharides from Acid Hydrolyzed Bovine Fetuin



LC Conditions

System:	ACQUITY UPLC H-Class
Sample:	Acid Hydrolyzed Bovine Fetuin followed by 2-AA derivitization
Injection:	4.8 µL on 4.6 mm I.D. column, 2.1 µL on 2.1 mm I.D. columns
Columns:	XBridge BEH C_{18}, 130 Å, 5 µm, 4.6 × 100 mm (p/n: 186003115) XBridge BEH C_{18}, 130 Å, 3.5 µm, 2.1 × 100 mm (p/n: 186003033) XBridge BEH C_{18}, 130 Å, 2.5 µm ***XP***, 2.1 × 100 mm (p/n: 186006031) ACQUITY UPLC BEH C_{18}, 130 Å, 1.7 µm, 2.1 × 100 mm (p/n: 186002352)
Mobile phase A:	0.2% N-butylamine,0.5% phosphoric acid, and 1% THF in water
Mobile phase B:	Acetonitrile with 0.1% trifluoroacetic acid
Gradient:	See table
Flow rate:	See table
Excitation wavelength:	360 nm
Emission wavelength:	425 nm
Column temp.:	30 °C

Method Details (flow rate and time)

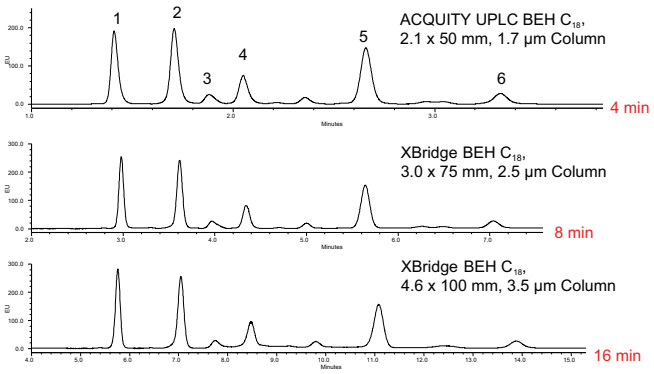
		5 µm		3.5 µm		2.5 µm		1.7 µm	
Step	%B[1]	Flow (mL min-1)	Time (min)	Flow (mL min-1)	Time (min)	Flow (mL min-1)	Time (min)	Flow (mL min-1)	Time (min)
1	7	0.480	0.00	0.685	0.00	0.200	0.00	0.294	0.00
2	7	0.480	7.78	0.685	5.45	0.200	3.89	0.294	2.64
3	17	0.480	27.78	0.685	19.47	0.200	13.88	0.294	9.44
4	100	0.480	28.89	0.685	20.24	0.200	14.43	0.294	9.82
5	100	0.480	40.00	0.685	28.03	0.200	19.99	0.294	13.60
6	7	0.480	41.11	0.685	28.81	0.200	20.54	0.294	13.97
7	7	0.480	50.00	0.685	35.04	0.200	24.98	0.294	17.00

Geometric scaling of a monosaccharide separation on XBridge BEH C_{18}, 130 Å, (A) 5 µm particle, (B) 3.5 µm particle, (C) 2.5 µm particle, and (D) 1.7 µm particle noting higher throughput and improved component Rs via use of 1.7 µm particle technology.

Sialic Acid Analyses

A diverse range of sialic acids are found in nature, but the two major sialic acids species found on N- and O-linked glycans contained in biopharmaceuticals are N-acetyl-neuraminic acid (Neu5Ac) and N-glycolyl-neuraminic acid (Neu5Gc). Since sialylation can enhance serum half-life as well as affect biological activity, it is important to accurately monitor both the quantitative levels and types of sialic acids during all stages of the product life cycle. Many LC-based methods begin with the release of the targeted sialic acids under milder acid hydrolysis conditions (e.g., 2 M acetic acid for two hours at 80 °C). The released sialic acids can be then derivatized with 1, 2-diamino-4, 5-methylenedioxybenzene-2HCl (DMB) dye. Of particular importance is the fact that DMB- labeled sialic acids are light sensitive and liable to degradation and should be analyzed within 24 hours of labeling. This can become a significant problem if a large number of samples need to be analyzed using traditional HPLC-based techniques that can take more than 30 minutes per sample analysis.

UPLC vs. HPLC-Based Analyses of DMB-Labeled, Sialic Acid Test Mix



1. Neu5Gc, 2. Neu5Ac, 3. Neu5,7Ac2, 4. Neu5,Gc9Ac, 5. Neu5,9Ac2, 6. Neu5,7,(8)9Ac3

LC Conditions

System:	ACQUITY UPLC		
Mobile phase:	Acetonitrile:methanol:water (9:7:84)		
Detection:	Fluorescence : λex = 373, λem = 448		
Sample:	Prozyme GLYKO sialic acid reference panel		
Wash solvents:	Acetonitrile:methanol:water (9:7:84)		
Columns:	ACQUITY UPLC, BEH C_{18}, 1.7 µm	XBridge BEH C_{18}, 2.5 µm	XBridge C_{18}, 3.5 µm
Configuration:	2.1 × 50 mm	3.0 × 75 mm	4.6 × 100 mm
Flow rate:	0.18	0.26	0.43
Loop size:	2 µL	2 µL	10 µL
Injection vol.:	0.7 µL	2.0 µL	6.7 µL
Run time:	6.0 min	12.5 min	24.0 min

Note: The higher throughput and improved component resolution associate with the 1.7 µm particle technology.

Geometric scaling of DMB-labeled sialic acid standards on XBridge BEH C_{18}, 130 Å, 3.5 µm particle (bottom), 2.5 µm particle (middle), and ACQUITY UPLC BEH C_{18}, 130 Å, 1.7 µm particle (top).

Ordering Information

ACQUITY UPLC BEH C_{18}, 130 Å and XBridge BEH C_{18}, 130 Å HPLC and UHPLC Columns

	Particle Size: 1.7 µm	
ACQUITY UPLC BEH C_{18}, 130 Å	**Dimension**	**P/N (1/pk)**
	2.1 × 50 mm	186002350
	2.1 × 100 mm	186002352
	2.1 × 150 mm	186004742
XBridge BEH C_{18}, 130 Å, *XP*	**Particle Size: 2.5 µm**	
	2.1 × 100 mm	186006031
	3 × 100 mm	186006035
	3 × 150 mm	186006710
XBridge BEH C_{18}, 130 Å	**Particle Size: 3.5 µm**	
	2.1 × 100 mm	186003033
	Particle Size: 5 µm	
	4.6 × 100 mm	186003115

Is NSA Sabotaging Your Lab's Productivity?

The Hidden Impacts of Non-Specific Adsorption (NSA)

- Did you know that NSA can negatively impact your chromatographic peak shapes, sensitivity and reproducibility?
- Did you believe the only solutions include the use of additives or lengthy passivation procedures?

MAXPEAK™ PREMIER

Waters MaxPeak Premier Columns dramatically reduce NSA with MaxPeak High Performance Surfaces, innovative technologies to reduce analyte loss due to analyte/surface interactions.

Learn more on pages 93-104 or by visiting waters.com

Nucleic Acid Separations

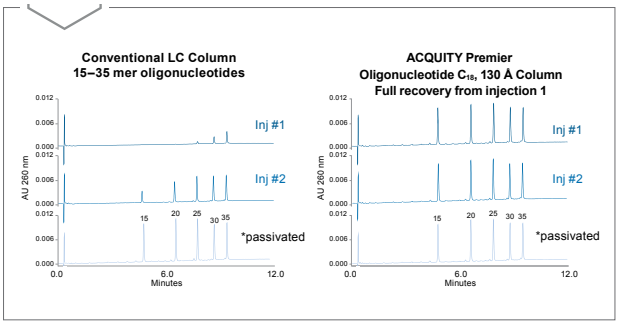
For more than 30 years, Waters has pioneered LC-based separations for nucleic acids from HPLC to UPLC. The ACQUITY and XBridge Premier Oligonucleotide BEH C_{18} Columns utilize MaxPeak™ High Performance Surfaces which is our latest innovation designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.

Waters Oligonucleotide Columns whether it is in Premier hardware or stainless steel hardware contain second-generation hybrid silica BEH Technology particles functionalized with C_{18}. The separation of detritylated synthetic oligonucleotide samples is based on the well-established method of ion-pair, reversed-phase chromatography.

The availability of 1.7 µm UPLC particles or 2.5 µm HPLC particles in various column dimensions provides flexibility to meet various lab-scale isolation or analysis needs, and delivers exceptional sample resolution and superior column life. In addition, Waters manufacturing and quality control testing procedures help ensure consistent batch-to-batch and column-to-column performance regardless of application demands.

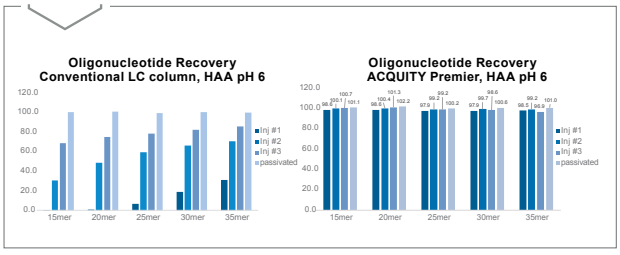
- Synthetic oligonucleotide separations efficiencies equivalent to or exceeding those of PAGE, CGE, or ion-exchange HPLC methods
- The ability to distinguish/separate failure sequences from detritylated full-length products
- Column scalability for laboratory-scale isolation needs
- Exceptional column life for reduced cost per analysis
- QC tested with MassPREP Oligonucleotide Standard (p/n: 186004135) to help ensure performance consistency

MAXPEAK™ PREMIER **Out-of-the-box Performance**



*Injection of 2 µL of standard diluted in water, 10 pmol of each oligonucleotide injected on column. *"Passivation" with 500 pmol injection of 35 mer, followed by "post passivation" injection of 10 pmol of standard..*

MAXPEAK™ PREMIER **High Recovery and Repeatability**



*Injection of 2 µL of standard diluted in water, 10 pmol of each oligonucleotide injected on column. *"Passivation" with 500 pmol injection of 35 mer, followed by "post passivation" injection of 10 pmol of standard.*

Have confidence that the methods you develop today will have the same repeatable results tomorrow. Quality is at the heart of everything we do, whether it is column particles, reliable quality manufacturing, customer support, or supply chain.

EXCEPTIONAL RESOLUTION OF OLIGONUCLEOTIDE MIXTURES

ACQUITY UPLC Oligonucleotide BEH C_{18}, 1.7 µm (designed for use with an ACQUITY UPLC System) and XBridge Oligonucleotide BEH C_{18}, 2.5 µm Columns are well suited for the analysis of detritylated oligonucleotides using ion-pair, reversed-phase chromatography. As indicated (see figure on right), separations are comparable to those obtained by capillary gel electrophoresis (CGE) in terms of component resolution, yet analysis times are significantly decreased using Waters UPLC Technology. The ability to resolve large oligonucleotide sequences (e.g., N from N-1) is possible due to the enhanced resolving power obtained using sub-3-µm, BEH Technology particles. In addition, quantitation with molecular weight characterization of the separated target oligonucleotide product from failure sequences is possible using Waters Oligonucleotide Columns with hyphenated-mass spectrometry methods and MS-friendly eluents.

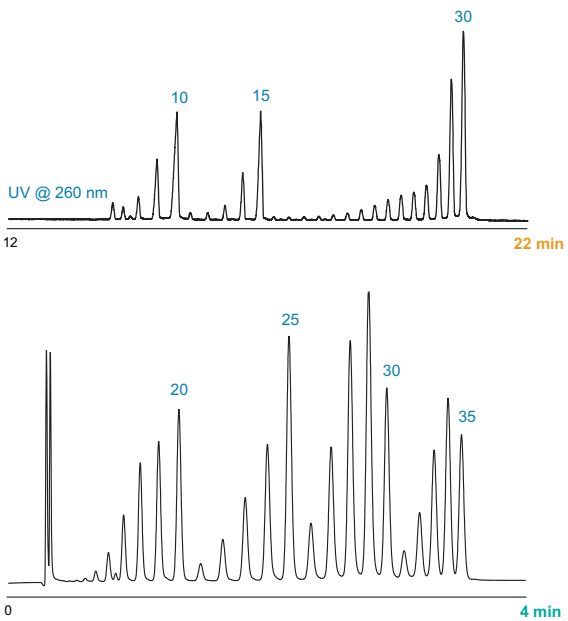
Separation of Detritylated Oligodeoxythymidine Ladders by Capillary Gel Electrophoresis (CGE) vs. Ion-Pair, Reversed-Phase Chromatography

CGE Conditions

System:	Capillary Gel Electrophoresis
Column:	PEG sieving matrix (BioCap 75 µm × 27.5 [to detector]/ 34.5 cm [total length])
Injection:	45 injection at 5 kV
Running:	15 kV
Column temp.:	30 °C

LC Conditions

System:	ACQUITY UPLC
Column:	ACQUITY UPLC Oligonucleotide BEH C_{18}, 1.7 µm, 2.1 × 50 mm (p/n: 186003949)
Mobile phase A:	15 mM TEA, 400 mM hexafluoroisopropanol, pH 7.9
Mobile phase B:	50% A, 50% methanol
Flow rate:	0.4 mL/min
Column temp.:	60 °C
Gradient:	40 to 48% B in 4 min (20–24% methanol)
UV detection:	260 nm



Ordering Information

ACQUITY Premier Columns for Oligonucleotide Analysis

	Particle Size: 1.7 µm	
Oligonucleotide BEH C_{18}, 130 Å, 1.7 µm	**Dimension**	**P/N**
	2.1 × 50 mm	186009484
	2.1 × 100 mm	186009485
	2.1 × 150 mm	186009486
Peptide BEH C_{18}, 300 Å, 1.7 µm*	2.1 × 50 mm	186009493
	2.1 × 100 mm	186009494
	2.1 × 150 mm	186009495

*Quality control tested for peptides; large pore size that is well suited for oligonucleotide separations.

XBridge™ Premier Columns for Oligonucleotide Analysis

	Particle Size: 2.5 µm	
Oligonucleotide BEH C_{18}, 130 Å, 2.5 µm	**Dimension**	**P/N**
	2.1 × 50 mm	186009836
	2.1 × 100 mm	186009837
	2.1 × 150 mm	186009838
	4.6 × 50 mm	186009901
	4.6 × 100 mm	186009902
	4.6 × 150 mm	186009903
Peptide BEH C_{18}, 300 Å, 2.5 µm*	2.1 × 50 mm	186009892
	2.1 × 100 mm	186009893
	2.1 × 150 mm	186009894
	4.6 × 50 mm	186009895
	4.6 × 100 mm	186009896
	4.6 × 150 mm	186009897

*Quality control tested for peptides; large pore size that is well suited for oligonucleotide separations.

ACQUITY UPLC Oligonucleotide BEH C_{18} Columns and Method Validation Kits

	Particle Size: 1.7 µm	
BEH C_{18}, 130 Å*	**Dimension**	**P/N**
	2.1 × 50 mm	186003949
	2.1 × 100 mm	186003950
	2.1 × 150 mm	186005516
BEH C_{18}, 130 Å Method Validation Kit**	2.1 × 100 mm	186004898

* For use on Waters ACQUITY UPLC Systems.
**Three Columns from three different batches of material.

XBridge Oligonucleotide BEH C_{18} HPLC and UHPLC Columns and Method Validation Kits

	Particle Size: 2.5 µm	
BEH C_{18}, 130 Å	**Dimension**	**P/N**
	2.1 × 50 mm	186003952
	4.6 × 50 mm	186003953
BEH C_{18}, 130 Å OBD Prep	10 × 50 mm	186008212
	19 × 50 mm	186008962
	30 × 50 mm	186008963
	50 × 50 mm	186008964
BEH C_{18}, 130 Å Method Validation Kit**	4.6 × 50 mm	186004906

**Three Columns from three different batches of material.

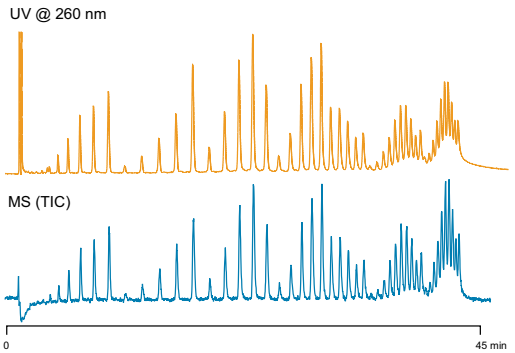
"The Waters ACQUITY™ Premier Peptide BEH C_{18} 300 Å Column shows an excellent degree of specificity and selectivity in denaturing and non-denaturing analysis of synthetic oligonucleotides, due to the absence of non-specific binding properties of this new column hardware in combination with great stationary phase performance. The Waters ACQUITY Premier Column is a highly valuable addition to our test package for the future development of synthetic oligonucleotides analytical methods."

ORGANIZATION: Janssen

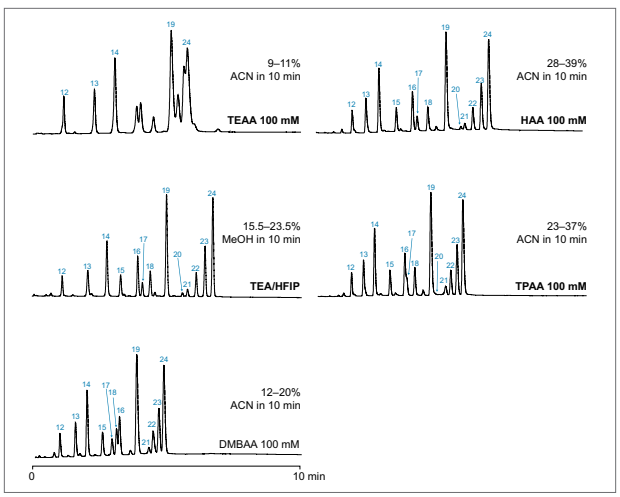
Separation of a 15–60 mer Detritylated Oligodeoxythymidine Ladder

LC Conditions

System:	ACQUITY UPLC with PDA and Q-Tof micro MS	Flow rate:	0.1 mL/min
Column:	ACQUITY UPLC Oligonucleotide BEH C_{18}, , 130 Å, 1.7 µm, 2.1 × 50 mm (p/n: 186003949)	Column temp.:	60 °C
Mobile phase A:	15 mM TEA–400 mM hexafluoroisopropanol	Gradient:	19 to 26.5.5% B in 60 min
Mobile phase B:	Methanol	UV detection:	260 nm 10 scans per second MS: 1 scan per second, 0.1 sec interscan



Impact of Different Ion-Pairing Agents on Varying Oligonucleotide Sequence Separations



Improved oligonucleotides separations can be achieved using alternative IP agents compared to use of traditional TEAA.

UPLC-MS Analysis of Interfering RNA Oligonucleotides

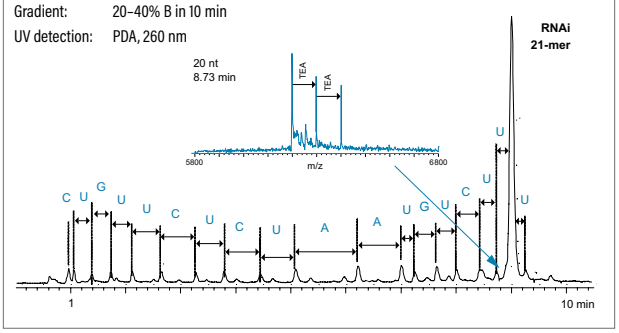
Discovery of the RNA interference (RNAi) mechanism now broadly used for silencing of target gene expression has prompted a need for the analysis of small interfering RNAs (siRNA) molecules. To satisfy the need for a robust, fast, and sensitive analysis of 20–25 nucleotides of small interfering RNA (siRNA), a UPLC-MS method has been developed utilizing UPLC Oligonucleotide Columns and SYNAPT HDMS™ Mass Spectrometer.

The acquisition of the accurate masses allowed for an assignment of the peaks of 5′-truncated oligomers (failed sequences generated during oligonucleotide synthesis), as well as some other impurities. The mass of each peak in the MS chromatogram was deconvoluted using MaxEnt 1 Software. The tentative 5′-end failure products are assigned in the below figure. Nearly the entire sequence of the parent oligonucleotide was elucidated. MS analysis also revealed a presence of an extra uridine mononucleotide added to the target 21-mer RNAi sequence.

LC-MS Analysis of RNA (21 mer)

LC Conditions

System:	ACQUITY UPLC	TIC MS:	SYNAPT MS
Column:	ACQUITY UPLC Oligonucleotide BEH C_{18}, 130 Å, 1.7 µm, 2.1 × 50 mm (p/n: 186003949)	Capillary:	2000 V
Column temp.:	60 °C	Sample cone:	35 V
Sample injected:	2.5 µL (100 pmol of 21 nt RNAi)	Extraction cone:	3 V
Flow rate:	0.2 mL/min	Desolvation temp.:	200 °C
Mobile phase A:	15 mM TEA. 400 mM roisopropanol	Source temp.:	120 °C
Mobile phase B:	50% A, 50% methanol	Cone gas flow:	50 L/hr
Gradient:	20–40% B in 10 min	Desolvation gas flow:	600 L/hr
UV detection:	PDA, 260 nm		



Outstanding Column Life

Waters Oligonucleotide Columns packed with BEH Technology particles have shown remarkable column longevity, under these demanding separation conditions, while maintaining outstanding separation performance. By comparison, significantly reduced column life results when traditional silica-based columns are used under these same demanding separation conditions.

Scalable DNA and RNAi Separations with Good Product Recovery

XBridge Oligonucleotide BEH C_{18}, 130 Å Columns are the preferred offering for detritylated oligonucleotide purifications due to the availability of column sizes designed to meet lab-scale isolation requirements. The choice of XBridge Oligonucleotide C_{18} Column dimension and operating flow rate depends primarily on the scale of the synthesis reaction mixture. For example, a 4.6 × 50 mm column containing XBridge Oligonucleotide BEH C_{18}, 130 Å, 2.5 µm material is an excellent selection when oligonucleotide mass loads are less than or equal to 0.2 µmol. Selection of the appropriate column size for the amount of oligonucleotide sample loaded is recommended to maximize component resolution and recovery of the target product from non-desired failure sequences.

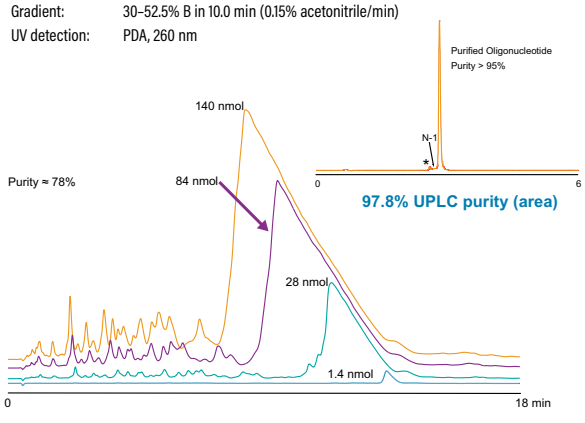
For researchers involved in gene silencing it is often necessary to work with RNA of high purity. Crude synthetic oligonucleotides used for gene knockout are typically purified. The figure below illustrates a lab-scale purification of 21 mer RNA at various column loads. Using an Oligonucleotide column chemistry and an Alliance System, large quantities of crude single stranded RNA can be successfully purified yielding material of high purity, approximately 95%, with an estimated yield of 55% based on collected peak area to the total peak area of the sample.

In addition, XBridge Oligonucleotide Columns are well suited for the analysis and purification of siRNA. As shown in the figure below, siRNA is well resolved from single stranded RNA and truncated duplexes.

Purification of Single Stranded RNA

LC Conditions

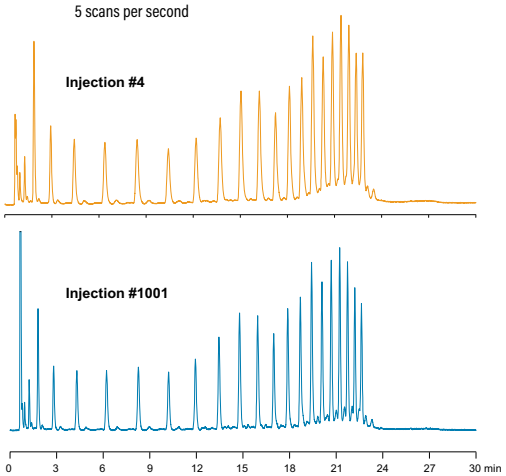
System:	Alliance HPLC
Column:	XBridge Oligonucleotide BEH C_{18}, 130 Å, 2.5 µm, 4.6 × 50 mm (p/n: 186003953)
Column temp.:	60 °C
Flow rate:	1.0 mL/min
Mobile phase A:	0.1 M triethylammonium acetate, pH 7.5
Mobile phase B:	20% acetonitrile in A
Gradient:	30–52.5% B in 10.0 min (0.15% acetonitrile/min)
UV detection:	PDA, 260 nm



Separation of 5–25 mer Detritylated Oligodeoxythymidine Ladder

LC Conditions

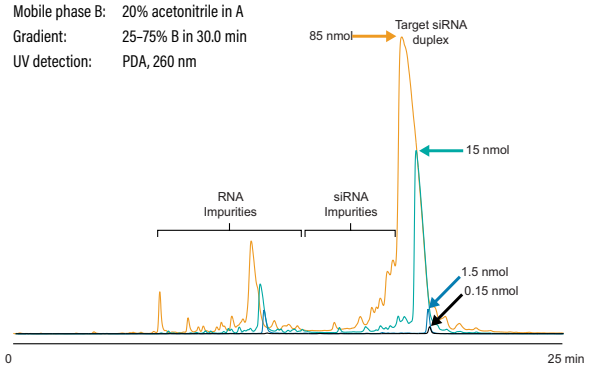
System:	Alliance HPLC with PDA
Column:	XBridge Oligonucleotide BEH C_{18}, 130 Å, 2.5 µm, 2.1 × 50 mm (p/n: 186003952)
Mobile phase A:	10% methanol/90% (385 mM Hexafluoroisopropanol + 14.3 mM TEA)
Mobile phase B:	25% methanol/75% (385 mM Hexafluoroisopropanol + 14.3 mM TEA)
Column temp.:	60 °C
Gradient:	0–100% B in 30 min (10–25% methanol)
Flow rate:	1.0 mL/min
UV detection:	260 nm
	5 scans per second



Purification of siRNA Duplex from Impurities

LC Conditions

System:	Alliance HPLC
Column:	XBridge Oligonucleotide BEH C_{18}, 130 Å, 2.5 µm, 4.6 × 50 mm (p/n: 186003953)
Column temp.:	20 °C
Flow rate:	1.0 mL/min
Mobile phase A:	0.1 M triethylammonium acetate, pH 7.0
Mobile phase B:	20% acetonitrile in A
Gradient:	25–75% B in 30.0 min
UV detection:	PDA, 260 nm



Dimension	Approx Mass Load**	Yield***	Flow Rate
2.1 × 50 mm	0.04 µmol	0.2 mg	0.2 mL/min
4.6 × 50 mm	0.20 µmol	1.0 mg	1.0 mL/min
10 × 50 mm	1.00 µmol	4.5 mg	4.5 mL/min
19 × 50 mm*	4.00 µmol	16.0 mg	16.0 mL/min
30 × 50 mm*	9.00 µmol	40.0 mg	40.0 mL/min
50 × 50 mm*	25.00 µmol	110.0 mg	110.0 mL/min

* Oligonucleotide custom column.
** Values are only approximates and vary depending on oligonucleotide length, base composition, and "heart-cutting" fraction collection method used.
*** Estimated for average oligonucleotide MW and synthesis yield.

COLUMNS FOR LARGE DNA/RNA SPECIES

In general, molecular biology methods for manipulation of DNA rely on restriction enzymes, polymerase-chain reaction (PCR), and sequencing techniques. Using these methods, genomic DNA is typically converted into shorter double stranded (ds)DNA sequences, typically 100–1000 base pairs (bp) in length. The shorter dsDNA molecules are often analyzed or isolated by methods such as slab gel or capillary electrophoresis. Use of Waters ACQUITY UPLC and ACQUITY Premier Peptide BEH C_{18}, 300 Å Reversed-Phase, Protein-Pak Hi Res Q or Gen-Pak FAX Anion-Exchange Columns offer alternatives to more traditional electrophoretic methods and are particularly well suited for various analytical and small-scale purification applications

siRNA duplex analysis was performed on ACQUITY Premier Peptide BEH C_{18}, 300 Å Column as shown on right with more details available in the following application: "Analysis of siRNA Drugs at Denaturing UPLC Conditions Using MaxPeak Premier Column Technology" Waters Application Note 720007362.

Ordering Information

ACQUITY Premier Peptide BEH C_{18}, 300 Å Columns for DNA/RNA Fragments

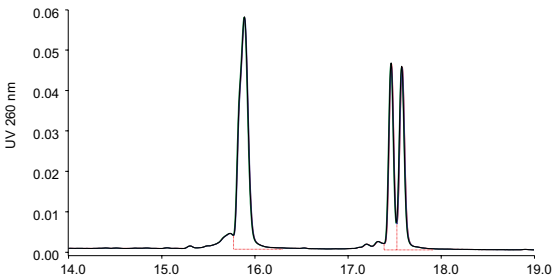
Peptide BEH C_{18}, 300 Å, 1.7 µm	Particle Size: 1.7 µm	
	Dimension	P/N
	2.1 × 50 mm	186009493
	2.1 × 100 mm	186009494
	2.1 × 150 mm	186009495

siRNA Duplex Analysis on ACQUITY Premier Peptide BEH C_{18} 300 Å Column

Method **Conditions**

Column: ACQUITY Premier Peptide BEH C_{18}, 300 Å, 1.7 µm, 2.1 × 150 mm (p/n: 186009495)
Mobile phase 1: 0.07% (v/v) TEA and 0.60% v/v HFIP solution in Milli-Q water (5 mM TEA, 60 mM HFIP aqueous solution)
Mobile phase 2: 70/30% v/v Methanol/acetonitrile
Mobile phase 3: 85/15% (v/v) Mixture of mobile phase 1 and 2
Mobile phase 4: 30/70% (v/v) Mixture of mobile phase 1 and 2
Column temp.: 75 °C
UV detection: 260 nm PDA, titanium 5 mm detector cell
Injection volume: 3 µL

Time (min)	Flow (mL/min)	A (% vol)	C (% vol)	D (% vol)	Curve
0	0.30	100	0	0	Initial
20	0.30	0	100	0	6
25	0.30	0	0	100	6
26	0.30	0	0	100	6
28	0.30	100	0	0	6
34	0.30	100	0	0	6



Four consecutive injections of 3 µL of Duplex C (0.40 mg/mL) using the ACQUITY Premier Peptide BEH C_{18}, 300 Å Column.

ANION-EXCHANGE HPLC OF NUCLEIC ACIDS

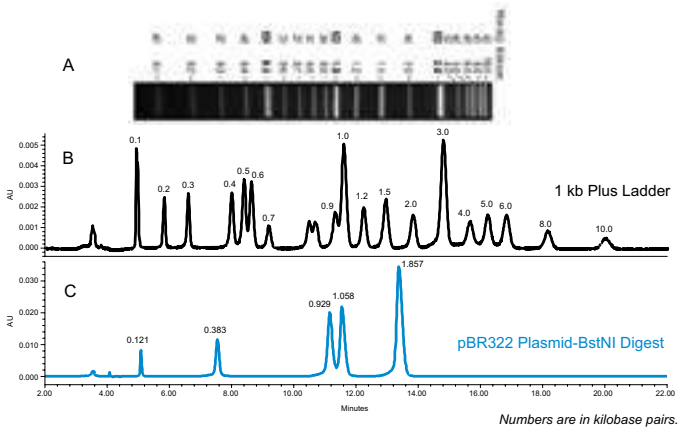
Protein-Pak Hi Res Q Anion Exchange Columns

Waters Protein-Pak Hi Res Q columns although commercially developed for protein applications, more recently over the last few years have been used in nucleic acid applications for separations of empty/full capsids of adeno-associated viruses (AAVs), plasmid isoforms, mRNA and dsDNA fragment separation and size assessment of up to 10 kilobase pairs (kbp). The non-porous particle is functionalized with a quaternary ammonium ligand which is a strong anion exchanger that provides excellent separations for negatively charged species such as nucleic acids. The chromatogram below is a 1 kb plus DNA Ladder and a pBR322 Plasmid-BstNI Digest separated on the Protein-Pak Hi Res Q column and more details can be found in the application: "Separation and Size Assessment of dsDNA Fragments by Anion-Exchange Chromatography" Waters Application Note 720007321.

Separation and Size Assessment of dsDNA Fragments by Anion Exchange Chromatography

LC Conditions

System:	ACQUITY UPLC H-Class Bio System
Column:	Protein-Pak Hi Res Q, 5 µm, 4.6 × 100 mm (p/n: 186004931)
Column temp.:	30 °C
Sample temp.:	10 °C
Injection volume:	0.3 µm
Flow rate:	0.4 mL/min
Vials:	Polypropylene 12 × 32 mm Screw Neck Vial, with Cap an Pre-slit PTFE/ Silicone Septum, 300 µL Volume, 100/pk (p/n: 186002639)
Mobile phase A:	100 mM Tris-HCl
Mobile phase B:	100 mM Tris Base
Mobile phase C:	13 M Tetramethylammonium chloride (TMAC)
Mobile phase D:	Water
Buffer conc. to deliver:	20 mM
UV detection:	AQUITY UPLC TUV Detector with 5 mm titanium flow cell, wavelength: 260 nm



Numbers are in kilobase pairs.

Time (min)	%A	%B	%C	%D
0	17.8	2.2	0.0	80.0
1	17.8	2.2	0.0	80.0
2	17.8	2.2	54	26
4	17.8	2.2	54	26
24	17.8	2.2	59.3	20.7
26	17.8	2.2	80.0	0.0
26.1	17.8	2.2	0.0	80.0
42	17.8	2.2	0.0	80.0

Ordering Information

Protein-Pak Hi Res Q, 5 µm Column

Description	Dimension	P/N
Protein-Pak Hi Res Q, 5 µm	4.6 × 100 mm	186004931

Gen-Pak FAX Anion-Exchange Columns

Waters Gen-Pak FAX Columns offer the highest resolution available for anion-exchange HPLC of nucleic acids. The Gen-Pak FAX Column contains a weak anion exchanger based on DEAE functionalized non-porous resin. It contains 2.5 µm particles and is well suited for analytical and micro-preparative applications.

Ordering Information

Gen-Pak FAX HPLC Column

Description	Dimension	P/N
Gen-Pak FAX Column	4.6 × 100 mm	WAT015490
Gen-Pak FAX Replacement Inlet Filter	—	WAT015715

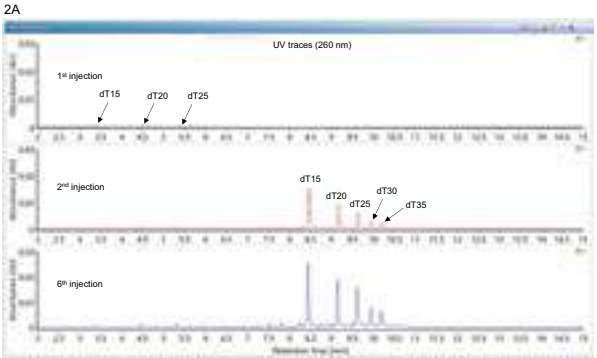
HYDROPHILIC INTERACTION CHROMATOGRAPHY (HILIC) OF NUCLEIC ACIDS

ACQUITY Premier BEH Amide Columns

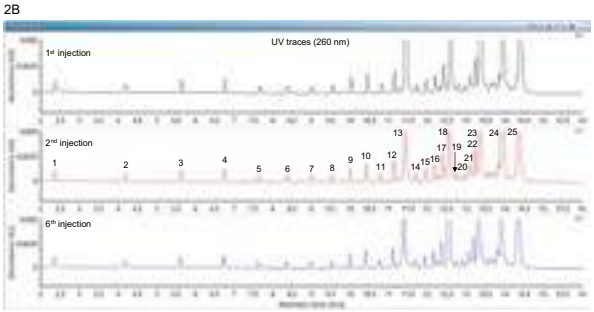
HILIC separation of oligonucleotides offers three main advantages in terms of mobile phase considerations when compared with the traditional ion-pair reversed phase (IP-RP) separations: at least 10x reduction in mobile phase cost, significantly reduced toxicity and at least 10x improvement in mobile phase stability (up to two weeks) for LC-MS operation. The ACQUITY Premier BEH Amide Column with MaxPeak High Performance Surfaces resulted in no column conditioning as compared with the traditional ACQUITY BEH Amide Column in stainless-steel hardware, saving time in column passivation right out of the box as shown below in the chromatograms. For more details, refer to application: "HILIC as an Alternative Separation Mode for Intact Mass Confirmation of Oligonucleotides on the BioAccord System" Waters Application Note 720007395.

HILIC as an Alternative Separation Mode for Intact Mass Confirmation of Oligonucleotides on the BioAccord System

2A



2B



LC Conditions

System:	BioAccord System incorporating the ACQUITY UPLC I-Class PLUS System, TUV Detector, and the ACQUITY RDa Detector
Column:	ACQUITY Premier BEH Amide 1.7 µm, 130 Å, 2.1 × 50 mm, (p/n: 186009504)
Column temp.:	60 °C
Sample temp.:	6 °C
Injection volume:	1 µL
Flow rate:	300 µL/min
Vials:	QuanRecovery MaxPeak Vials (p/n: 186009186)
Mobile phase A:	10 mM Ammonium acetate, 75% acetonitrile in DI water
Mobile phase B:	10 mM Ammonium acetate, 25% acetonitrile in DI water

Time (min)	Flow rate (mL/min)	Solvent A composition (%)	Solvent B composition (%)	Curve profile
0.00	0.3	100	0	Initial
1.00	0.3	100	0	
16.00	0.3	50	50	6
20.00	0.3	50	50	6
21.00	0.3	10	90	6
22.00	0.3	10	90	6
23.00	0.3	100	0	6
30.00	0.3	100	0	6

TUV chromatograms showing the first consecutive injections of the OST mixture performed on 2.1 × 50 mm columns: (A) conventional stainless-steel ACQUITY UPLC BEH Amide Column (p/n: 186000480[0]); (B) ACQUITY Premier BEH Amide Column (p/n: 186009504). The conventional column required extensive conditioning before a stable UV signal could be produced, while the ACQUITY Premier Column does not require any conditioning. Reproducible chromatographic separations can be obtained even for low-level impurities on the ACQUITY Premier Column without any need for column conditioning/passivation. The oligonucleotides separated in Figure 2B correspond to the following failure sequences of deoxythiamidates: Peak 1-dT3, 2-dT4, 3-dT5, 4-dT6, 5-dT7, 6-dT8, 7-dT9, 8-dT10, 9-dT11, 10-dT12, 11-dT13, 12-dT14, 13-dT15 (major component), 14-dT16, 15-dT17, 16-dT18, 17-dT19, 18-dT20 (major component), peaks 19–22 correspond to dT21–24 according to their elution order, and peaks 23–25 belong to the major components dT25, dT30, and dT35.

Ordering Information

ACQUITY and XBridge Premier BEH Amide Columns for HILIC Separations of Nucleic Acids

ACQUITY Premier BEH Amide, 130 Å	**Particle Size: 1.7 µm**	
	Dimension	**P/N (1/pk)**
	2.1 × 50 mm	186009504
	2.1 × 100 mm	186009505
	2.1 × 150 mm	186009506
ACQUITY Premier BEH Amide, 130 Å, VanGuard FIT	**Particle Size: 1.7 µm**	
	2.1 × 50 mm	186009507
	2.1 × 100 mm	186009508
	2.1 × 150 mm	186009509
XBridge Premier BEH Amide, 130 Å	**Particle Size: 2.5 µm**	
	2.1 × 50 mm	186009928
	2.1 × 100 mm	186009929
	2.1 × 150 mm	186009930
	4.6 × 50 mm	186009935
	4.6 × 100 mm	186009936
	4.6 × 150 mm	186009937
XBridge Premier BEH Amide, 130 Å, VanGuard FIT	**Particle Size: 2.5 µm**	
	2.1 × 50 mm	186009931
	2.1 × 100 mm	186009932
	2.1 × 150 mm	186009933
	4.6 × 50 mm	186009938
	4.6 × 100 mm	186009939
	4.6 × 150 mm	186009940
VanGuard FIT Catridges	**Particle Size: 1.7 µm**	
	2.1 × 5 mm	186009510
	Particle Size: 2.5 µm	
	2.1 × 5 mm	186009927
	3.9× 5 mm	186009934

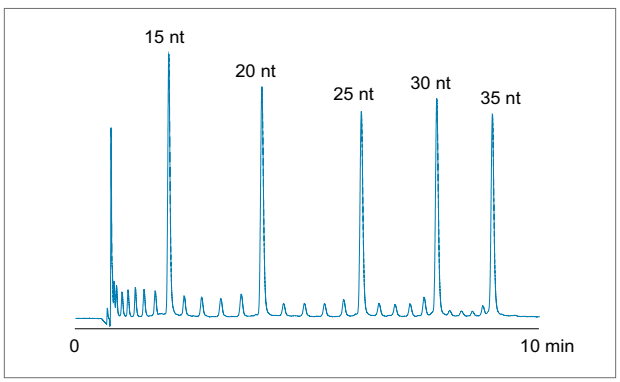
MassPREP OLIGONUCLEOTIDE STANDARD

Benchmarking, Method Development, and Troubleshooting

- Contains a carefully defined mixture of synthesized oligodeoxythymidine fragments
- Useful in testing and confirming HPLC/UPLC, LC-MS, and column performance for oligonucleotide applications
- Each is QC tested and shipped with a certificate of analysis

The pre-packaged MassPREP Oligonucleotide Standard is designed for verification of HPLC/UPLC instrument and column performance for analysis of synthetic oligonucleotides. Approximate equimolar amounts of 15, 20, 25, 30, and 35 nucleotide (nt) long oligodeoxythymidines are lyophilized and packaged in 1.5 mL LC vials. These vials are vacuum-sealed in foil pouches to reduce degradation that can occur by excessive exposure to light and air. Approximately 1 nmole of each oligonucleotide is present in the vial.

Separation of MassPREP Oligonucleotide Standard on ACQUITY UPLC Oligonucleotide C_{18}, 130 Å, 1.7 µm Column



Waters ACQUITY UPLC analysis of MassPREP Oligonucleotide Standard on an ACQUITY UPLC Oligonucleotide C_{18}, 130 Å, 1.7 µm Column. The main components are labeled. Small peaks eluting between labeled oligonucleotides are N-1, N-2, etc. failure sequences generated during the oligonucleotide syntheses. The ACQUITY UPLC System is equipped with 50 µL standard mixer and PDA detector (260 nm).

Ordering Information

MassPREP Oligonucleotide Standard

Description	Qty.	P/N
MassPREP Oligonucleotide Standard	1/pk	186004135

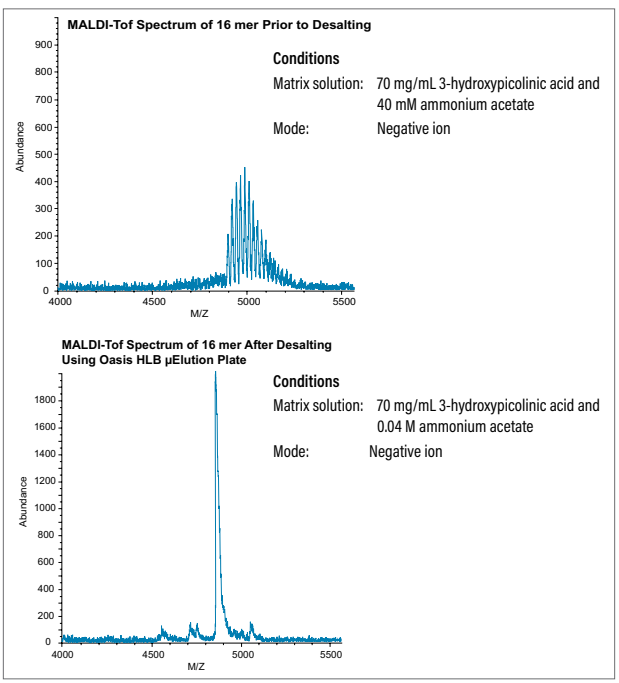
OLIGONUCLEOTIDE DESALTING BY SOLID-PHASE EXTRACTION

Oasis µElution Plates

- Removes salt prior to MS analysis
- Low elution volumes
- High sensitivity
- Sample concentrating
- High throughput

Desalting of synthetic oligonucleotides is essential for MS analysis (QC, genotyping applications and SNP analysis). Waters Oasis µElution Plate is an excellent choice for high-throughput analysis with minimal amount of sample. The Oasis µElution Plate combines patented plate design, proven chemistries, and generic protocols enabling elution volumes as low as 25 µL. You can perform SPE cleanup and concentration of very small sample volumes. The Oasis Hydrophilic-Lipophilic-Balanced (HLB) Sample Extraction Products incorporate a patented copolymer made from a balanced ratio of two monomers; the lipophilic divinylbenzene and the hydrophilic N-vinylpyrolodone that is ideally suited for this application.

Effective Use of Oasis HLB for Oligonucleotide Desalting Prior to MALDI-Tof MS

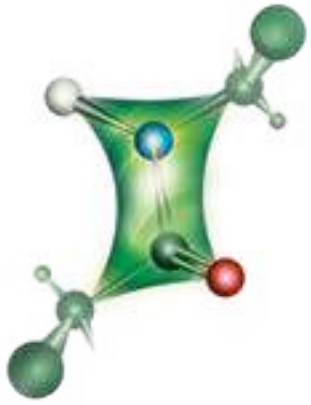


Ordering Information

Oasis HLB µElution Plate (for Oligonucleotides)

Description	P/N
Oasis HLB µElution Plate (for Oligonucleotides)	186001828BA

Peptide Analysis



Separating, quantifying, and identifying peptides in biotherapeutic characterization and proteomics applications is challenging.

To address these challenges, reversed-phase chromatography using ion-pairing reagents such as TFA and formic acid can deliver highly resolved separations of complex peptide mixtures, (e.g., tryptic protein digests or lengthy synthetic peptide sequences) whose sequences may differ by a single amino acid. In general, the hydrophobicity of the peptide determines the elution order, with the least hydrophobic peptides eluting first.

Factors such as particle composition (silica vs hybrids), pore size (130 Å vs 300 Å), ligand density, as well as separation conditions (e.g., gradient duration, separation temperature, flow rate) all play an important role in obtaining a separation that meets application requirements.

As with other bioseparations, when using smaller particle sizes, UPLC/UHPLC column offerings provide superior component resolution and in less time compared to HPLC-based columns for this demanding application.

A WIDE RANGE OF CHEMISTRIES FOR REVERSED-PHASE PEPTIDE SEPARATIONS

A peptide column needs to adapt to a wide range of peptides: hydrophobic, hydrophilic, small, and large. See the options below to choose the right column for your analysis.

Hybrid Particles

Silica Particles

BEH Technology™

BEH (Ethylene-Bridged Hybrid)

Trifunctional C_{18} ligand, fully end-capped, and bonded to the Ethylene-Bridged Hybrid (BEH) particles.

- Ideally suited for separation of a wide range of peptides: large and small, acidic and basic, hydrophilic and hydrophobic
- Stable across a wide pH range (pH 1–11) so neutral or alkaline pH eluents can be used to alter peptide separation selectivities
- High temperature stability (up to 80 °C) expands method development capabilities
- Outstanding peak capacity and superior peak shape in trifluoroacetic acid (TFA) or formic acid (FA) ion pair eluents when compared to use of 100% silica based C_{18} columns
- Two pore sizes (130 Å and 300 Å) provide different separation selectivities for a wide range of peptides and small proteins

CSH™ Technology

CSH (Charged Surface Hybrid)

Trifunctional C_{18} ligand, fully end-capped, bonded to Charged Surface Hybrid (CSH) particles.

- Outstanding peak capacities with formic acid for LC-MS based applications
- Excellent performance with TFA for optical based applications
- Accepts greater peptide mass loads than many competitive technologies for detection of low-level impurities
- Offers unique selectivity when compared to Waters Peptide BEH C_{18} Columns
- Optimal for separations from pH 1–5
- The 130 Å pore size is best suited for compounds less than 10,000 Daltons

HSS HIGH STRENGTH SILICA

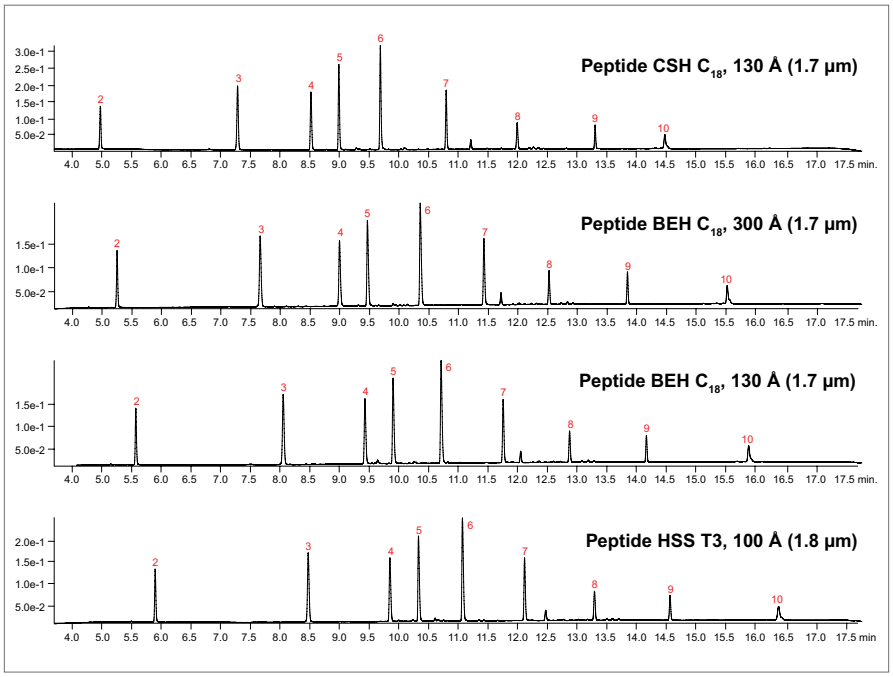
HSS (High Strength Silica)

Trifunctional C_{18} ligand, fully end-capped, bonded to High Strength Silica (HSS) particles.

- Viable option when either the hybrid-based, Peptide BEH C_{18} or Peptide CSH C_{18} do not meet a specific peptide application need
- Ideal choice for the separation of small, hydrophilic peptides since retentivity is greater than that obtained using Waters hybrid-based peptide separation columns

Three Outstanding Peptide Column Chemistries that Address Varied Peptide Separations

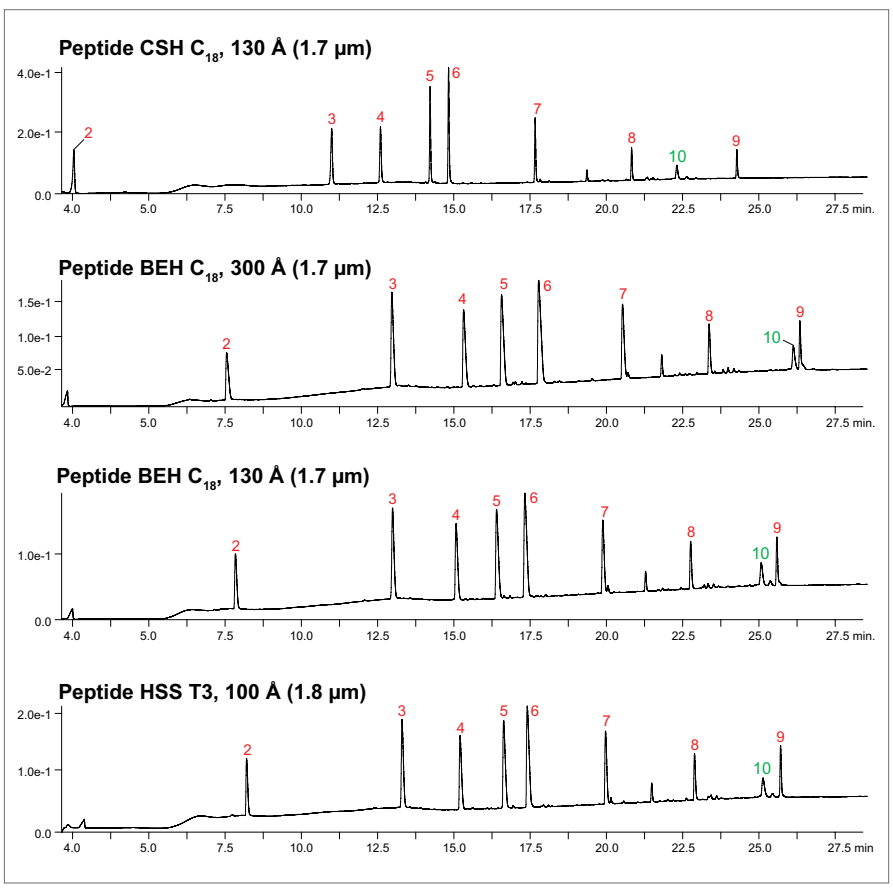
Separation of Peptide Standards Using 0.1% TFA Ion Pairing on Waters Peptide Separation Columns



Peptides contained in Waters MassPREP Peptide Standard Mixture, p/n: 186002337, were separated on 2.1 × 150 mm columns containing Waters Peptide CSH C_{18} 130 Å (1.7 µm), Peptide BEH C_{18} 300 Å (1.7 µm), Peptide BEH C_{18} 130 Å (1.7 µm), or Peptide HSS T3 100 Å (1.8 µm) UPLC-based particles on a Waters ACQUITY UPLC H-Class Bio System using a gradient of increasing acetonitrile concentration with 0.1% TFA ion-pairing. Flow at 0.4 mL/min.

The MassPREP Peptide Standard Mixture contains allantoin (a void volume marker) and nine carefully selected peptides with a broad range of polarities and isoelectric points. 1 = Allantoin 158 Da (not shown in figure since elutes at column void volume), 2 = RASG-1: 1, 000 Da, 3 = Angiotensin frag.1–7: 898 Da 4 = Bradykinin: 1060 Da, 5 = Angiotensin II: 1046 Da, 6 = Angiotensin I: 1296 Da, 7 = Renin: 1758 Da, 8 = Enolase T35: 1872 Da, 9 = Enolase T37: 2827 Da, 10 = Melittin: 2846)

Separation of Peptide Standards Using 0.1% FA Ion Pairing on Waters Peptide Separation Columns



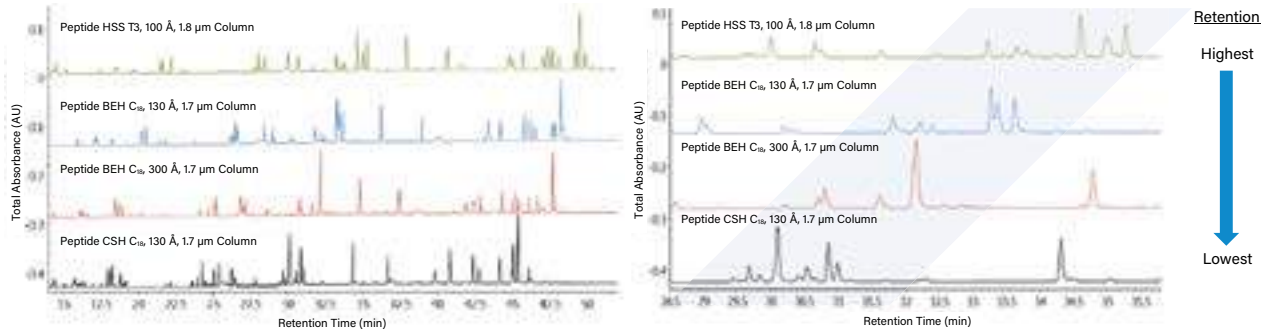
Peptides contained in Waters MassPREP Peptide Standard Mixture, p/n: 186002337, were separated on 2.1 × 150 mm columns containing Waters Peptide CSH C_{18} 130 Å (1.7 µm), Peptide BEH C_{18} 300 Å (1.7 µm), Peptide BEH C_{18} 130 Å (1.7 µm), or Peptide HSS T3, 100 Å (1.8 µm) UPLC-based particles on a Waters ACQUITY UPLC H-Class Bio System using a gradient of increasing acetonitrile concentration with 0.1% FA ion-pairing. Flow at 0.2 mL/min.

Sample as above.

Note: Different peptide separation selectivities and comparative retention time differences among the tested columns.

Elution order of peaks 9 and 10 switch when run in 0.1 FA vs. 0.1% TFA.

Separation of Tryptic Digest of Reduced Alkylated NIST mAb on Waters Peptide Separation Columns



LC system:	ACQUITY UPLC H-Class Bio	Eluent A:	Water with 0.1% TFA
Sample:	Tryptic Digest of NIST mAb reference material	Eluent B:	Acetonitrile with 0.1% TFA
		Gradient:	0.5% - 50% B in 60 min, linear
Columns:	2.1 × 150 mm	Flow rate:	0.20 mL/min
		Column temp.:	60 °C

Waters UPLC (shown) and HPLC-based Peptide Separation Columns deliver different peptide selectivities and high peak capacities for the separation of complex peptide mixtures. In addition, each batch of material is specifically QC tested and qualified with a tryptic digest of cytochrome c to help ensure column-to-column consistency when used in validated methods.

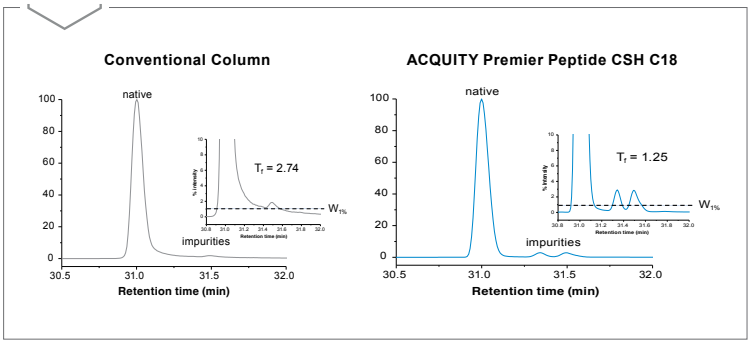
MAXPEAK PREMIER PEPTIDE COLUMNS

In addition, Waters offers versions of each of the three peptide chemistries as MaxPeak Premier columns. Waters MaxPeak Premier Columns represent the latest innovative technologies and provide the highest level of chromatographic performance, flexibility, and assurance to enhance the capabilities of scientific laboratories around the world. Available in 1.7 µm (ACQUITY Premier Columns) and 2.5 µm (XBridge Premier Columns, XSelect Premier Columns) particle sizes.

Utilizing MaxPeak High Performance Surfaces (HPS) technology in the column hardware design, MaxPeak Premier Columns provide significant improvements in reproducibility, peak shape, and recovery by minimizing analyte/surface interactions.

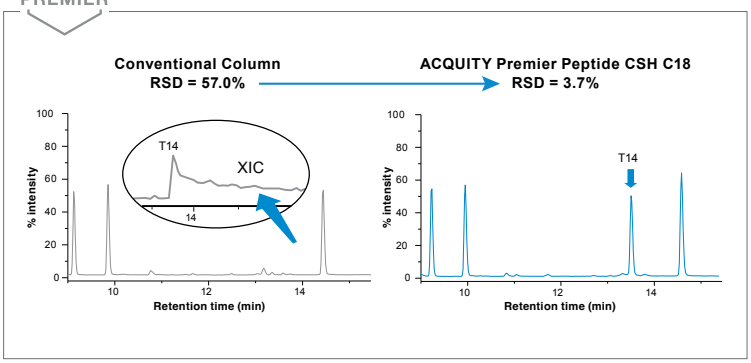
MaxPeak Premier columns also reduce the need for time-consuming passivation procedures or complex additive use that is typically required to achieve optimal performance with traditional stainless-steel columns.

MAXPEAK™ PREMIER **Reduced Tailing**



54% reduction in tailing.

MAXPEAK™ PREMIER **Increased Sensitivity and Repeatability**



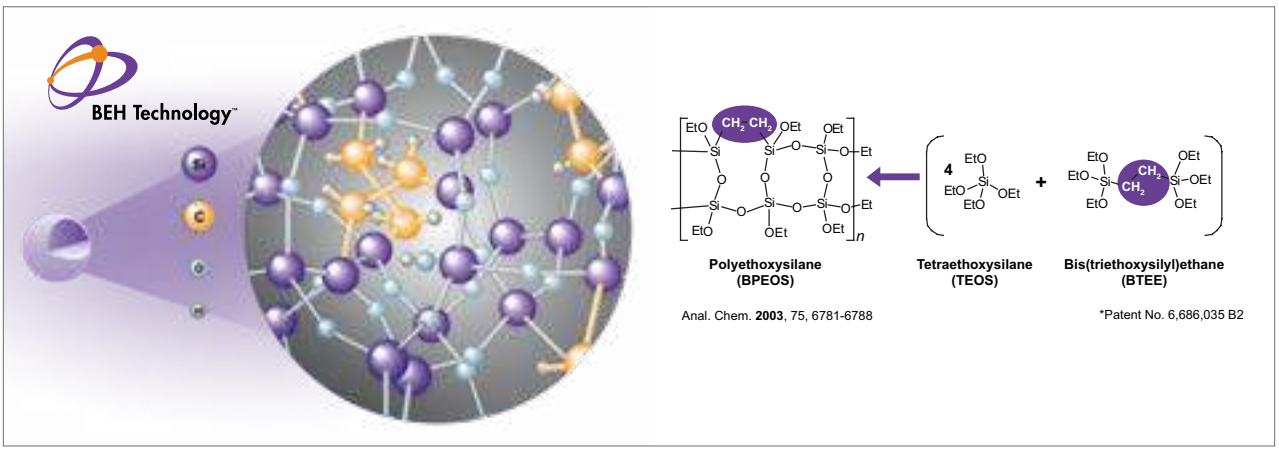
35-fold increase in sensitivity.

PEPTIDE BEH C_{18}, 130 Å AND 300 Å COLUMNS

Hybrid-Based Particles for Reversed-Phase Peptide Separations

In 1999, Waters first demonstrated how organic/inorganic Hybrid Particle Technology columns successfully addressed limitations (e.g., pH stability) that exist using 100% silica-based, reversed-phase columns for biocompound separations. In 2009, we advanced LC-based peptide separation capabilities by commercializing our Peptide BEH C_{18}, 130 Å, and BEH C_{18}, 300 Å HPLC- and UPLC-based columns both based on the second-generation BEH particles. In addition, we added an additional quality control test using a tryptic digest of cytochrome *c* to help ensure consistent column-to-column performance. To date, hundreds of referenced journal citations provide data that support the effective use of this column chemistry for a variety of separations in various diverse application areas.

The BEH Particle: First Key Chemistry Enabler of Waters UPLC Technology



Ethylene Bridged Hybrid (BEH) Technology synthesis creates particles that ensure extreme column performance and long column lifetime under harsh operating conditions.

CSH Technology Particles for Peptide Separations

Waters innovative Peptide CSH C_{18}, 130 Å offerings expands on the already successful and well-recognized Peptide BEH C_{18}, 130 Å and BEH C_{18}, 300 Å columns. Based on comparative peptide separations, Peptide CSH C_{18}, 130 Å Columns exhibit improved load ability, greater peak capacities, and unique selectivity compared to Peptide BEH C_{18}, 130 Å. Its performance is also significantly less dependent on TFA ion pairing, making it ideal for MS applications where high sensitivity is desired. The use of the well-controlled, charged surface hybrid properties of Peptide CSH C_{18}, 130 Å holds significant promise for facilitating either challenging LC and/or LC-MS peptide separations.

The CSH Particle: Expands Upon BEH Technology

Charged Surface Hybrid (CSH) Technology improves selectivity and offers the highest possible performance for basic compounds in the acidic, low-ionic strength mobile phases commonly used in LC-MS laboratories.

PEPTIDE CSH C_{18}, 130 Å COLUMNS

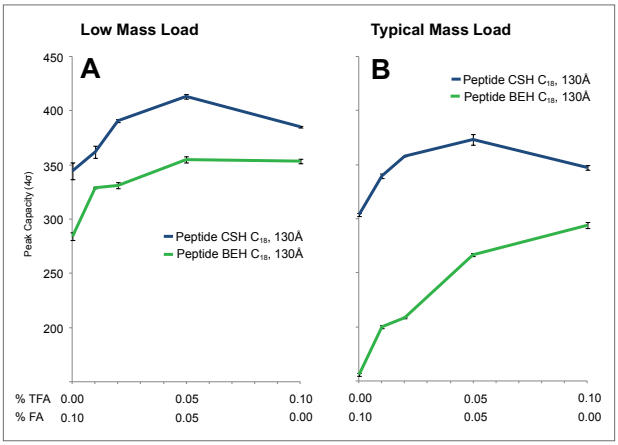
Charged Surface Hybrid Particles Deliver Superior Peptide Separations in LC and LC-MS Applications

Waters patented synthesis process for its Charged Surface Hybrid (CSH) Technology particles imparts a low-level, positive charge to the surface of each particle. For that reason, when using our Peptide CSH C_{18}, 130 Å Columns, you must ensure a mobile-phase pH of less than 5 to enable peptide/CSH surface-charge interactions. CSH Technology allows the columns to be successfully used with standard eluents containing trifluoroacetic acid or a weaker acid modifier, such as formic acid. You do not need to compromise between selecting a reversed-phase eluent that delivers sharp, symmetrically separated peaks (e.g., 0.1% trifluoroacetic acid) and one that minimizes reduction of MS signal (e.g., 0.1% formic acid). Additionally, the ability of the CSH C_{18}, 130 Å Column chemistry to accept greater peptide mass loads than many other columns enhances the ability to detect potentially important low-level constituents of the major components of interest.

Excellent Mass Loading of Complex Peptide Samples

One of the inherent performance advantages of our CSH Technology is improved sample-mass loadability, the quantity of analyte that you can load onto a column before peak shape deteriorates. At typical mass loads, Peptide CSH C_{18}, 130 Å delivers a remarkably better performance than many existing C_{18} offerings. When loading 10× less sample, the difference in performance was less pronounced. Improved peptide-mass loadability is an excellent column asset for challenging separations, particularly for those that involve mixtures that comprise species present at vastly different concentrations.

Comparative Averaged Peptide Peak Capacities on Peptide CSH C_{18}, 130 Å vs. Peptide BEH C_{18}, 130 Å Based Columns (2.1 × 150 mm) at Two Peptide Mass Loads and Differing Concentrations of Formic Acid and Trifluoroacetic Acid



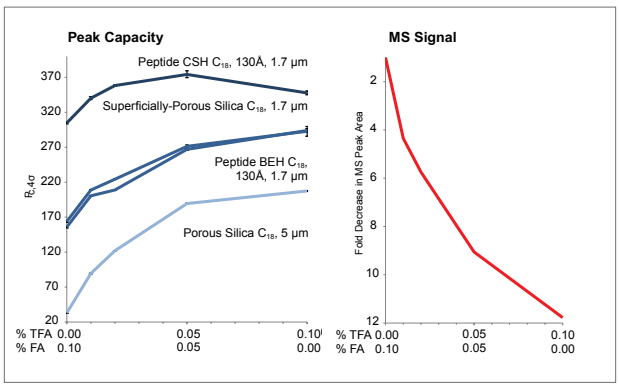
Effect of column mass load on separated peptide peak capacity in formic acid, trifluoroacetic acid, and eluent blends of formic acid and trifluoroacetic acid. (A) approximate sample load of 0.06 µg peptide mixture. (B) approx. 0.6 µg peptide mixture. Values were derived from two replicates. Waters MassPREP Peptide Standard Mixture (p/n: 186002337) was used in the study.

A need persists for columns compatible with LC instrumentation. We recommend the use of low-dispersion LC instrumentation to extract full performance from a well-packed column containing 1.7 µm particles. Waters' e***X***tended ***P***erformance (***XP***) Columns packed with 2.5 µm ***XP*** particles improves the productivity of existing HPLC instrumentation. You can scale high peak capacity peptide separations performed using a Peptide CSH C_{18}, 130 Å, 1.7 µm Column to a Peptide CSH C_{18}, 130 Å, 2.5 µm ***XP*** Column simply by altering flow rate and gradient time. You can readily employ CSH Technology for high peak capacity peptide separations using either HPLC, UHPLC, or UPLC instrumentation.

Superior Performance in Eluents Containing Formic Acid or Trifluoroacetic Acid

Waters' Peptide CSH C_{18}, 130 Å particles contain a low and carefully defined concentration of positive charges that yield comparatively excellent peak shape for peptide separations that rely on mobile phases that contain formic acid or trifluoroacetic acid . The fact that the performance of a Peptide CSH C_{18}, 130 Å Column exhibits little dependence on strong ion-pairing agents makes it ideal for LC or LC-MS applications.

Comparative Averaged Peptide Peak Capacities on Selected Reversed-Phase Columns with Differing Concentrations of Formic Acid and Trifluoroacetic Acid



Effect of trifluoroacetic acid on peak capacity and MS signal. (A) Peak capacity as a function of acid modifier. Values were derived from two replicates. (B) Fold decrease in MS peak area as a function of acid modifier. Waters MassPREP Peptide Standard Mixture (p/n: 186002337) was used in study.

PEPTIDE HSS T3 COLUMNS

High pore volume HPLC particles do not possess the mechanical stability necessary to withstand the high pressures inherent in UPLC separations. Waters' material scientists addressed this challenge by developing a silica particle designed for high mechanical stability with the appropriate morphology to provide long UPLC column lifetimes and high UPLC efficiencies at high pressures. The 1.8 µm High Strength Silica (HSS) particle is the first and only 100% silica particle designed, tested, and intended for use in applications up to 15,000 psi (1034 bar).

The HSS particle technology is available in ACQUITY UPLC Peptide HSS T3, 100 Å, 1.8 µm as well as XSelect Peptide HSS T3, 100 Å, ***XP*** 2.5 µm and 5 µm for UHPLC and HPLC-based separations for seamless transfer between UPLC and HPLC/UHPLC instrument platforms.

Simplifying Column Choice for Peptide Purifications

Our peptide columns are versatile. Often, a single C_{18}-based chemistry can separate a wide range of peptides, requiring little time and expense to obtain satisfactory results. We offer peptide packings in many particle sizes and column dimensions. (See the "Peptide Preparative Column Selection Guide" below.)

Increased Assurance with Waters Peptide Columns

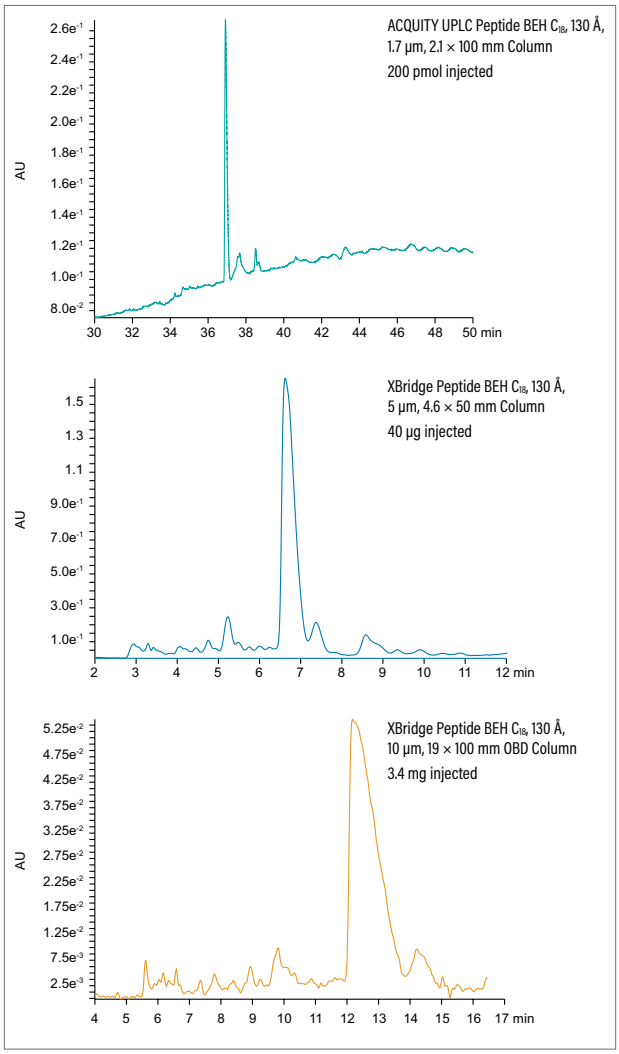
Waters rigorously tests each batch of our synthesized Peptide BEH C_{18}, 130 Å; Peptide BEH C_{18}, 300 Å; Peptide CSH C_{18}, 130 Å; and Peptide HSS T3, 100 Å particles used in our manufactured columns. To pass, each batch of material must satisfactorily separate a complex protein digest using a gradient separation with well-defined pass/fail criteria. In addition, each and every manufactured column is tested and must exceed established packed column efficiency values before accepted for customer purchase. In combination, these tests (results available in Certificate of Analysis documentation) help ensure consistent batch-to-batch and column-to-column performance.

Certificate of analysis information includes a labeled chromatogram of the gradient separation of a tryptic digest of bovine cytochrome *c* (p/n: 186006371) using eluents that contain 0.1% formic acid. You can purchase the same protein digest test mixture to ensure the proper performance of your Peptide CSH C_{18}, 130 Å Column.

Peptide Packing Material in OBD Columns for Maximum Chemical and Physical Stability

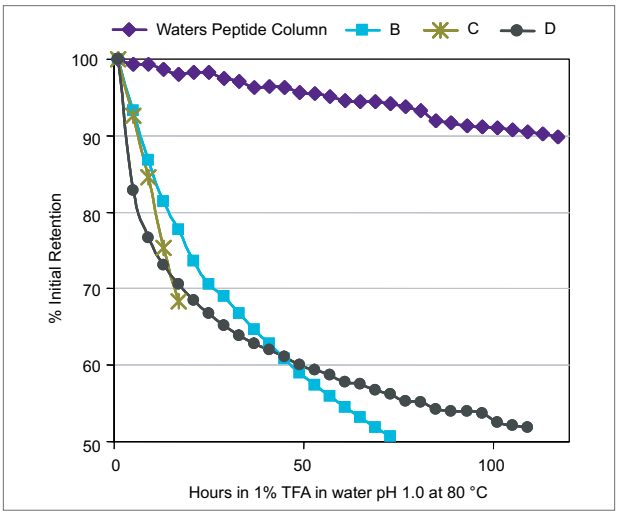
When columns fail, they do so both physically and chemically. For columns used with low-pH mobile phases, the usual cause of abbreviated column life is hydrolysis of the bonded phase, which manifests itself as significant changes in peptide retention. Our BEH Technology Columns incorporate proprietary procedures for bonding and end-capping that yield stable bonded phases. In low-pH stability tests, BEH C_{18} columns showed only minimal retention loss. Our patented Optimum Bed Density (OBD) Technology, developed to create packed beds that are the most stable of any available, regardless of manufacturer, ensures the physical stability of these columns. Visit waters.com/OBD for details about OBD Technology.

Separation of 13 Residue Peptides at Various Sample Loads



Offered in many particle sizes and column configurations, our peptide columns are well-suited for various laboratory-scale purification needs.

Long-Term Stability



We tested several peptide columns to observe how they performed when injections were repeated, comparing them with the performance columns B, C, and D made by other manufactures. (Retention was monitored to determine column lifetime.)

Peptide Preparative Column Selection Guide

OBD Prep Columns, 5 and 10 µm 130 Å and 300 Å				
I.D. (mm)	Length (mm)	µmol of a Single Peptide	Weight of a Single Peptide (mg)	Typical Flow Rate (mL/min)
10	50	0.25–5	0.5–10	4.5–9
10	100	0.25–5	0.5–10	4.5–9
10	150	0.25–5	0.5–10	4.5–9
10	250	0.25–5	0.5–10	4.5–9
19	50	1–18	2.0–36	16–32
19	100	1–18	2.0–36	16–32
19	150	1–18	2.0–36	16–32
19	250	1–18	2.0–36	16–32

OBD Prep Columns, 10 µm 130 Å and 300 Å				
I.D. (mm)	Length (mm)	µmol of a Single Peptide	Weight of a Single Peptide (mg)	Typical Flow Rate (mL/min)
30	50	2.5–25	5–100	40–80
30	100	2.5–25	5–100	40–80
30	150	2.5–25	5–100	40–80
30	250	2.5–25	5–100	40–80

Ordering Information

ACQUITY Premier Peptide Columns

	Particle Size: 1.7 µm	
BEH C_{18}, 130 Å	**Dimension**	**P/N**
	2.1 × 50 mm	186009481
	2.1 × 100 mm	186009482
	2.1 × 150 mm	186009483
CSH C_{18}, 300 Å	**Particle Size: 1.7 µm**	
	2.1 × 50 mm	186009487
	2.1 × 100 mm	186009488
	2.1 × 150 mm	186009489
HSS T3, 100 Å	**Particle Size: 1.8 µm**	
	2.1 × 50 mm	186009490
	2.1 × 100 mm	186009491
	2.1 × 150 mm	186009492
BEH C_{18}, 300 Å	**Particle Size: 1.7 µm**	
	2.1 × 50 mm	186009493
	2.1 × 100 mm	186009494
	2.1 × 150 mm	186009495

ACQUITY UPLC Peptide BEH C_{18} Guards and Columns

	Particle Size: 1.7 µm	
BEH C_{18}, 130 Å	**Dimension**	**P/N**
	2.1 × 5 mm	186003975*
	2.1 × 50 mm	186003554
	2.1 × 100 mm	186003555
	2.1 × 150 mm	186003556
BEH C_{18}, 300 Å	**Particle Size: 1.7 µm**	
	1.0 × 50 mm	186005592
	1.0 × 100 mm	186005593
	1.0 × 150 mm	186005594
	2.1 × 5 mm	186004629*
	2.1 × 50 mm	186003685
	2.1 × 100 mm	186003686
	2.1 × 150 mm	186003687

*VanGuard Pre-column, 3/pk.

ACQUITY UPLC Peptide BEH C_{18} Method Validation Kits*

	Particle Size: 1.7 µm	
BEH C_{18}, 130 Å	**Dimension**	**P/N**
	2.1 × 100 mm	186004896
	2.1 × 150 mm	186006516
BEH C_{18}, 300 Å	**Particle Size: 1.7 µm**	
	2.1 × 100 mm	186004897
	2.1 × 150 mm	186006517

*Each Method Validation Kit contains 3 columns, each from a different batch.

XBridge Premier Peptide Columns

	Particle Size: 2.5 µm	
BEH C_{18}, 130 Å	**Dimension**	**P/N**
	2.1 × 50 mm	186009733
	2.1 × 100 mm	186009734
	2.1 × 150 mm	186009735
	4.6 × 50 mm	186009898
	4.6 × 100 mm	186009899
	4.6 × 150 mm	186009900
BEH C_{18}, 300 Å	**Particle Size: 2.5 µm**	
	2.1 × 50 mm	186009892
	2.1 × 100 mm	186009893
	2.1 × 150 mm	186009894
	4.6 × 50 mm	186009895
	4.6 × 100 mm	186009896
	4.6 × 150 mm	186009897

XSelect Premier Peptide Columns

	Particle Size: 2.5 µm	
CSH C_{18}, 130 Å	2.1 × 50 mm	186009904
	2.1 × 100 mm	186009905
	2.1 × 150 mm	186009906
	4.6 × 50 mm	186009907
	4.6 × 100 mm	186009908
	4.6 × 150 mm	186009909
HSS T3,100 Å	**Particle Size: 2.5 µm**	
	2.1 × 50 mm	186009839
	2.1 × 100 mm	186009840
	2.1 × 150 mm	186009841
	4.6 × 50 mm	186009910
	4.6 × 100 mm	186009911
	4.6 × 150 mm	186009912

XBridge Peptide BEH C_{18} Method Validation Kits*

BEH C_{18}, 130 Å	Particle Size: 2.5 µm		Particle Size: 3.5 µm		Particle Size: 5 µm	
	Dimension	P/N	Dimension	P/N	Dimension	P/N
	2.1 × 150 mm	186009002	4.6 × 100 mm	186004904	4.6 × 100 mm	186005463
	3 × 150 mm	186009003				
	4.6 × 150 mm	186009004				

BEH C_{18}, 300 Å	Particle Size: 2.5 µm		Particle Size: 3.5 µm		Particle Size: 5 µm	
	2.1 × 150 mm	186009079	4. × 100 mm	186004905	4.6 × 100 mm	186005464
	3 × 150 mm	186009080				
	4.6 × 150 mm	186009081				

*Each Method Validation Kit contains 3 columns, each from a different batch.

XBridge Peptide BEH C_{18} VanGuard Cartridges, 3/pk

BEH C_{18}, 130 Å	Particle Size: 2.5 µm	
	Dimension	P/N
	2.1 × 5 mm	186008988
	3.9 × 5 mm	186008989

BEH C_{18}, 300 Å	Particle Size: 2.5 µm	
	2.1 × 5 mm	186009077
	3.9 × 5 mm	186009078

XBridge Peptide BEH C_{18} Guards and Columns

BEH C_{18}, 130 Å

Particle Size: 2.5 µm		Particle Size: 3.5 µm		Particle Size: 5 µm		Particle Size: 10 µm	
Dimension	P/N	Dimension	P/N	Dimension	P/N	Dimension	P/N
2.1 × 50 mm	186008979	1.0 × 50 mm	186003560	1.0 × 50 mm	186003571	4.6 × 50 mm	186003648
2.1 × 100 mm	186008980	1.0 × 100 mm	186003561	1.0 × 100 mm	186003572	4.6 × 100 mm	186003649
2.1 × 150 mm	186008981	1.0 × 150 mm	186003562	1.0 × 150 mm	186003573	4.6 × 150 mm	186003650
3 × 50 mm	186008982	2.1 × 50 mm	186003563	2.1 × 50 mm	186003574	4.6 × 250 mm	186003651
3 × 100 mm	186008983	2.1 × 100 mm	186003564	2.1 × 100 mm	186003575	10 × 10 mm	186004465*,1
3 × 150 mm	186008984	2.1 × 150 mm	186003565	2.1 × 150 mm	186003576	10 × 50 mm	186008194
4.6 × 50 mm	186008985	2.1 × 250 mm	186003566	2.1 × 250 mm	186003577	10 × 100 mm	186008195
4.6 × 100 mm	186008986	4.6 × 50 mm	186003567	4.6 × 50 mm	186003578	10 × 150 mm	186008196
4.6 × 150 mm	186008987	4.6 × 100 mm	186003568	4.6 × 100 mm	186003579	10 × 250 mm	186008197
		4.6 × 150 mm	186003569	4.6 × 150 mm	186003580	19 × 10 mm	186004464*,2
		4.6 × 250 mm	186003570	4.6 × 250 mm	186003581	19 × 50 mm	186003656
				10 × 10 mm	186004469*,1	19 × 150 mm	186003657
				10 × 50 mm	186008186	19 × 250 mm	186003658
				10 × 100 mm	186008187	30 × 10 mm	186006880*,3
				10 × 150 mm	186008188	30 × 50 mm	186003659
				10 × 250 mm	186008189	30 × 100 mm	186003660
				19 × 10 mm	186004468*,2	30 × 150 mm	186003661
				19 × 50 mm	186003586	30 × 250 mm	186003662
				19 × 100 mm	186003587		
				19 × 150 mm	186003945		

BEH C_{18}, 300 Å

Particle Size: 2.5 µm		Particle Size: 3.5 µm		Particle Size: 5 µm		Particle Size: 10 µm	
2.1 × 5 mm	186009077	1.0 × 50 mm	186003604	1.0 × 50 mm	186003615	4.6 × 50 mm	186003663
2.1 × 50 mm	186009068	1.0 × 100 mm	186003605	1.0 × 100 mm	186003616	4.6 × 100 mm	186003664
2.1 × 100 mm	186009069	1.0 × 150 mm	186003606	1.0 × 150 mm	186003617	4.6 × 150 mm	186003665
2.1 × 150 mm	186009070	2.1 × 50 mm	186003607	2.1 × 50 mm	186003618	4.6 × 250 mm	186003666
3.0 × 50 mm	186009071	2.1 × 100 mm	186003608	2.1 × 100 mm	186003619	10 × 10 mm	186004467*,1
3.0 × 100 mm	186009072	2.1 × 150 mm	186003609	2.1 × 150 mm	186003620	10 × 50 mm	186008198
3.0 × 150 mm	186009073	2.1 × 250 mm	186003610	2.1 × 250 mm	186003621	10 × 100 mm	186008199
4.6 × 50 mm	186009074	4.6 × 50 mm	186003611	4.6 × 50 mm	186003622	10 × 150 mm	186008200
4.6 × 100 mm	186009075	4.6 × 100 mm	186003612	4.6 × 100 mm	186003623	10 × 250 mm	186008201
4.6 × 150 mm	186009076	4.6 × 150 mm	186003613	4.6 × 150 mm	186003624	19 × 10 mm	186004466*,2
		4.6 × 250 mm	186003614	4.6 × 250 mm	186003625	19 × 50 mm	186003671
				10 × 10 mm	186004471*,1	19 × 150 mm	186003672
				10 × 50 mm	186008190	19 × 250 mm	186003673
				10 × 100 mm	186008191	30 × 50 mm	186003674
				10 × 150 mm	186008192	30 × 100 mm	186003675
				10 × 250 mm	186008193	30 × 150 mm	186003676
				19 × 10 mm	186004470*,2	30 × 250 mm	186003677
				19 × 50 mm	186003630	30 × 10 mm	186006882*,3
				19 × 100 mm	186003631		
				19 × 150 mm	186003946		

*Guard Cartridge.
[1] Requires 10 × 10 mm Prep Guard Holder, p/n: 289000779.
[2] Requires 19 × 10 mm Prep Guard Holder, p/n: 186000709.
[3] Requires 30 × 10 mm Prep Guard Holder, p/n: 186006912.

ACQUITY UPLC Peptide CSH C_{18} Columns and Kits

CSH C_{18}, 130 Å	Particle Size: 1.7 µm		
	Dimension	Column P/N	Kit P/N[1]
	1.0 × 50 mm	186006933	176003061
	1.0 × 100 mm	186006934	176003062
	1.0 × 150 mm	186006935	176003063
	2.1 × 50 mm	186006936	176003064
	2.1 × 100 mm	186006937	176003065
	2.1 × 150 mm	186006938	176003066

[1] Kit contains Peptide CSH C_{18}, 130 Å Column plus one vial of Cytochrome *c* Digestion Standard, p/n: 186006371.

ACQUITY UPLC Peptide CSH C_{18} VanGuard Pre-Column, 3/pk

CSH C_{18}, 130 Å	Particle Size: 1.7 µm		
	Dimension	Column P/N	Kit P/N[1]
	2.1 × 5 mm	186006939	176003067

[1] Kit contains Peptide CSH C_{18}, 130 Å Column plus one vial of Cytochrome *c* Digestion Standard, p/n: 186006371.

ACQUITY UPLC Peptide CSH C_{18} Method Validation Kits*

CSH C_{18}, 130 Å	Particle Size: 1.7 µm		
	Dimension	Column P/N	Kit P/N[1]
	2.1 × 150 mm	186006940	176003068

*Kit contains 3 columns, each from a different batch.
[1] Kit contains Peptide CSH C_{18}, 130 Å Column plus one vial of Cytochrome *c* Digestion Standard, p/n: 186006371.

XSelect Peptide CSH C_{18} Guards, Columns, and Kits

CSH, C_{18}, 130 Å	Particle Size: 2.5 µm			Particle Size: 3.5 µm			Particle Size: 5 µm	
	Dimension	Column P/N	Kit P/N[1]	Dimension	Column P/N	Kit P/N[1]	Dimension	Column P/N (1/pk)
	2.1 × 50 mm ***XP***	186006941	176003069	2.1 × 10 mm[2,4]	186006954	176003081	4.6 × 50 mm	186007076
	2.1 × 100 mm ***XP***	186006942	176003070	2.1 × 50 mm	186006950	176003077	4.6 × 100 mm	186007077
	2.1 × 150 mm ***XP***	186006943	176003071	2.1 × 100 mm	186006951	176003078	4.6 × 150 mm	186007078
	4.6 × 50 mm ***XP***	186006946	176003074	2.1 × 150 mm	186006952	176003079	10 × 10 mm*	186007015
	4.6 × 100 mm ***XP***	186006947	176003075	4.6 × 20 mm[3,4]	186006958	176003085	10 × 50 mm*	186008264
	4.6 × 150 mm ***XP***	186007038	176003093	4.6 × 50 mm	186006955	176003082	10 × 100 mm*	186008265
				4.6 × 100 mm	186006956	176003083	10 × 150 mm*	186008266
				4.6 × 150 mm	186006957	176003084	10 × 250 mm*	186008267
							19 × 10 mm*	186007019**
							19 × 50 mm*	186007022
							19 × 100 mm*	186007020
							19 × 150 mm*	186007021
							19 × 250 mm*	186007031
							30 × 50 mm*	186007026
							30 × 100 mm*	186007025
							30 × 150 mm*	186007023
							30 × 250 mm*	186007024
							50 × 50 mm*	186007030
							50 × 100 mm*	186007027
							50 × 150 mm*	186007028
							50 × 250 mm*	186007029

* OBD Column.
** Requires 19 × 10 mm Cartridge Holder, p/n: 186000709.
[1] Kit contains Peptide CSH C_{18}, 130 Å Column plus one vial of Cytochrome *c* Digestion Standard, p/n: 186006371.
[2] Requires 2.1 × 10 mm Universal Sentry Guard Holder, p/n: WAT097958.
[3] Requires 4.6 × 20 mm Universal Sentry Guard Holder, p/n: WAT046910.
[4] 2/pk.

XSelect Peptide CSH C_{18} Columns and Method Validation Kits*

CSH C_{18}, 130 Å	Particle Size: 2.5 µm			Particle Size: 3.5 µm		
	Dimension	Column P/N	Kit P/N[1]	Dimension	Column P/N	Kit P/N[1]
	2.1 × 100 mm	186006945	176003073	2.1 × 100 mm	186006953	176003080
	4.6 × 100 mm	186006966	176003076	4.6 × 100 mm	186006959	176003086

*Each Method Validation Kit contains three columns, each from a different batch.
[1] Kit includes three Peptide CSH C_{18}, 130 Å, Columns, each from a different batch; and three vials of Cytochrome *c* Digestion Standard, p/n: 186006371.

XSelect Peptide CSH C_{18} VanGuard Cartridges,* 3/pk

CSH, C_{18}, 130 Å	Particle Size: 2.5 µm		
	Dimension	Column P/N	Kit P/N[1]
	2.1 × 5 mm	186006944	176003072

*Requires VanGuard Cartridge Universal Holder, p/n: 186007949.
[1] Kit includes three Peptide CSH C_{18}, 130 Å Guard Columns and one vial of Cytochrome *c* Digestion Standard, p/n: 186006371.

Purification and Isolation Cartridge Holders and Replacement O-rings

Description	Qty.	P/N
10 × 10 mm Cartridge Holder	1/pk	289000779
19 × 10 mm Cartridge Holder	1/pk	186000709
Replacement O-ring 7.8 mm	2/pk	700001019
Replacement O-ring 10 mm	2/pk	700001436

ACQUITY UPLC Peptide HSS T3 Columns and Kits

HSS T3, 100 Å	Particle Size: 1.8 µm		
	Dimension	Column P/N	Kit P/N[1]
	1.0 × 50 mm	186008751	176003992
	1.0 × 100 mm	186008752	176003993
	1.0 × 150 mm	186008753	176003994
	2.1 × 50 mm	186008754	176003995
	2.1 × 100 mm	186008755	176003996
	2.1 × 150 mm	186008756	176003997

[1] Kit includes Peptide HSS T3 Column plus one vial of Cytochrome *c* Digestion Standard, p/n: 186006371.

ACQUITY UPLC Peptide HSS T3 VanGuard Pre-Column, 3/pk

HSS T3, 100 Å	Particle Size: 1.8 µm	
	Dimension	P/N
	2.1 × 5 mm	186008757

ACQUITY UPLC Peptide HSS T3 Method Validation Kits*

HSS T3, 100 Å	Particle Size: 1.8 µm	
	Dimension	P/N
	2.1 × 150 mm	186008782

*Each Method Validation Kit contains 3 columns, each from a different batch.

XSelect Peptide HSS T3 Columns

HSS T3, 100 Å	Particle Size: 2.5 µm			Particle Size: 5 µm		
	Dimension	Column P/N	Kit P/N[1]	Dimension	Column P/N	Kit P/N[1]
	2.1 × 50 mm	186008758	176003998	2.1 × 100 mm	186008775	176004017
	2.1 × 100 mm	186008759	176003999	2.1 × 150 mm	186008776	176004018
	2.1 × 150 mm	186008760	176004006	4.6 × 100 mm	186008779	176004020
	4.6 × 50 mm	186008762	176004007	4.6 × 150 mm	186008780	176004021
	4.6 × 100 mm	186008763	176004008			
	4.6 × 150 mm	186008764	176004009			

[1] Kit includes Peptide HSS T3 Column plus one vial of Cytochrome *c* Digestion Standard, p/n: 186006371.

XSelect Peptide HSS T3 VanGuard Cartridges, 3/pk*

HSS T3, 100 Å	Particle Size: 2.5 µm		Particle Size: 5 µm	
	Dimension	P/N	Dimension	P/N
	2.1 × 5 mm	186008761	2.1 × 5 mm	186008777
	3.9 × 5 mm	186008765	3.9 × 5 mm	186008781

*Requires a VanGuard Cartridge Universal Holder, p/n: 186007949.

XSelect Peptide HSS T3 Method Validation Kits*

HSS T3, 100 Å	Particle Size: 2.5 µm		Particle Size: 5 µm	
	Dimension	P/N	Dimension	P/N
	2.1 × 150 mm	186008783	2.1 × 150 mm	186008787
	4.6 × 150 mm	186008784	4.6 × 150 mm	186008788

*Each Method Validation Kit contains 3 columns, each from a different batch.

THERAPEUTIC PEPTIDE METHOD DEVELOPMENT KIT

The Therapeutic Peptide Method Development Kit was developed to simplify the process of sample preparation and LC method development for the analysis of therapeutic peptides in plasma. The kit contains an Oasis Peptide µElution Method Development Plate, a Peptide BEH C_{18}, 300 Å reversed-phase column, and the detailed screening protocol which was used to generate the data shown in this publication.

In addition, a comprehensive method development training seminar has been created which describes all aspects of the method development process from the MS conditions to the final validation of a method for the extraction of the therapeutic peptide desmopressin from human plasma.

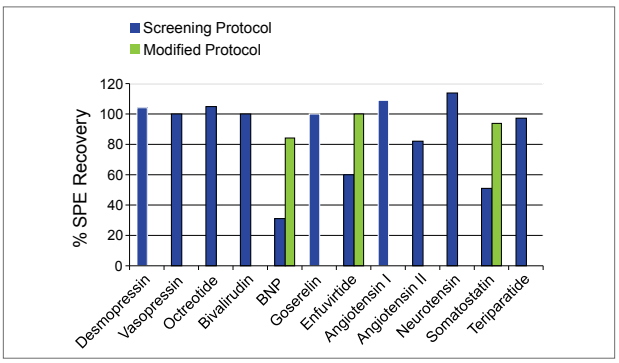
Although big progress has been made in sample pretreatment over the last years, there are still considerable limitations when it comes to overcoming complexity and dynamic range problems associated with peptide analyses from biological matrices. We focus on techniques which can be employed prior to liquid chromatography coupled to mass spectrometry for peptide detection and identification.

The peptide columns are specifically QC tested with a cytochrome *c* tryptic digest that helps ensure batch-to-batch consistency in validated methods ideally suited for separating a wide range of large and small, acidic and basic, hydrophilic and hydrophobic peptides.

The complexity of samples still far exceeds the capacity of currently available analytical systems, and specific sample preparation remains a crucial part of the analysis in a whole.

For more information, visit waters.com/pepkit or contact your local Waters sales office.

High Recovery of Peptides



The innovative Oasis µElution Plate allows for up to a 15x sample concentration, increasing the possibility of reaching the required sensitivity levels for bioanalytical assays. The low (25 µL) elution volume eliminates the need for evaporation and reconstitution significantly reducing the potential analyte loss due to absorption to the walls of the collection plate and/or chemical instability.

Ordering Information

Therapeutic Peptide Method Development Kits

Description	Qty/Box	P/N
UPLC Therapeutic Peptide Method Development Kit		176001835
Oasis µElution Method Development Plate	1	186004713
ACQUITY UPLC Peptide BEH C_{18}, 300 Å, 1.7 µm, 2.1 × 50 mm Column	1	186003685
96-Well 1 mL Collection Plate and Cap Mat	3	600001043
HPLC Peptide Therapeutic Peptide Method Development Kit		176001836
Oasis µElution Method Development Plate	1	186004713
XBridge Peptide BEH C_{18}, 300 Å, 3.5 µm, 2.1 × 50 mm Column	1	186003607
96-Well 1 mL Collection Plate and Cap Mat	3	600001043

Additional Products (Not Included in Kits)		
Oasis MAX 96-Well µElution Plate	1	186001829
Oasis WCX 96-Well µElution Plate	1	186002499
96-Well 1 mL Collection Plate	50	186002481
Cap Mats for 1 mL Collection Plate	50	186002483
Disposable Reservoir Tray	25	WAT058942
Extraction Manifold for 96-Well Plates	1	186001831
Vacuum Box Gasket Kit (contains foam top gaskets and orange O-rings)	2	186003522
SPE Vacuum Pump 115 V, 60 Hz	1	725000417
SPE Vacuum Pump 240 V, 50 Hz	1	725000604

CATION-EXCHANGE PEPTIDE AND POLYPEPTIDE SEPARATIONS

For most analytical and preparative peptide separations, cation-exchange chromatography is used mainly when alternative selectivity is required. In some large-scale purifications, cation exchange can take on a more central role. In these cases, cation exchange is frequently used as the first step in the separation, followed by a secondary purification step using reversed-phase methods.

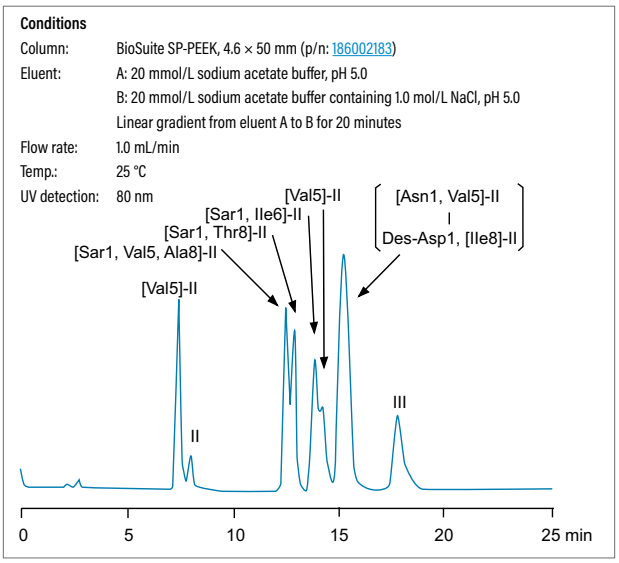
Waters offers BioSuite packings for cation-exchange separations. These packings are useful both for analytical and preparative work. They are based on rigid, hydrophilic polymethacrylate particles with large 1,000 Å pores. The naturally hydrophilic polymer reduces non-specific adsorption, resulting in better recovery of peptide/polypeptide mass and bioactivity. These packings are stable in the pH range of 2–12.

Protein-Pak SP HR 8 and 15 µm packing material is available in pre-packed glass columns.

BIOSUITE CATION-EXCHANGE HPLC COLUMNS

BioSuite SP NP, SP-PEEK, and SP cation-exchange chemistries (CXC) consists of the "strong" sulfopropyl ligand bonded to a pH stable (i.e., pH 2–12), methacrylic ester-based polymeric resin. The availability of different pore and particle size materials provides chromatographers with the flexibility required to isolate and or characterize peptides based upon minor charge differences. Non-porous (NP) and porous IEX Columns are also available to meet various separations requirements. Speed and superior chromatographic resolution are possible using the non-porous IEX offerings, while porous BioSuite offerings are available for applications requiring greater peptide binding capacity. In addition, BioSuite SP material is offered in PEEK hardware as well as in 21.5 mm I.D. stainless steel "lab-scale" preparative column dimensions.

Separation of Angiotensins on BioSuite SP-PEEK Cation-Exchange HPLC Column



Waters BioSuite SP-PEEK Cation-Exchange Column is well suited for the HPLC or UHPLC analyses of a complex peptide mixture using a gradient of increasing salt concentration.

Ordering Information

BioSuite Cation-Exchange HPLC Columns

Description	Matrix	Pore Size	Exclusion Limit (Daltons) against Polyethylene Glycol	Inner Diameter	Length	Column Volume (mL)	# Approx Protein Binding Capacity Per Pre-Packed Column	P/N
BioSuite SP-PEEK, 7 µm CXC	Polymer	1300 Å	>4,000,000	4.6 mm	50 mm	0.83	58 mg*	186002182
BioSuite SP, 2.5 µm NP CXC	Polymer	N/A	500	4.6 mm	35 mm	0.58	2.9 mg**	186002183
BioSuite SP, 10 µm CXC	Polymer	1000 Å	1,000,000	7.5 mm	75 mm	3.31	132 mg**	186002184
BioSuite SP, 13 µm CXC	Polymer	1000 Å	1,000,000	21.5 mm	150 mm	54.45	2178 mg**	186002185

For best resolution of complex samples, do not exceed 20% of the column's protein binding capacity.
* Data generated with gamma globulin.
** Data generated with hemoglobin.

BioResolve SCX mAb Columns

BioResolve SCX mAb Columns for the LC analysis of mAb charge variants as well as other biopharmaceutical therapeutics.

waters.com/bioresolve

BIOSUITE HPLC AND UHPLC PEPTIDE ANALYSIS COLUMNS

- Two HPLC and UHPLC column chemistries that provide alternative chemistries for peptide separations
- Designed for maximum resolution of complex digests
- Available in various configurations for LC or LC-MS applications
- Excellent batch-to-batch reproducibility for consistent results
- Uniquely QC tested specifically for peptide mapping using Waters MassPREP Cytochrome *c* Digestion Standard

BioSuite Peptide Analysis Series

BioSuite PA Series consists of two Waters reversed-phase column chemistries specifically optimized for peptide mapping from simple to complicated digests.

BioSuite C_{18}, 3 µm PA-A

BioSuite C_{18}, 3 µm PA-A is a 100 Å, difunctional bonded, low ligand density, silica-based column.

- Specifically designed for excellent retention of polar peptides
- Ideal choice for LC-MS applications using formic acid (FA) that minimizes ion-suppression
- Excellent performance for traditional HPLC separations using low TFA concentrations (e.g., 0.025% TFA)

BioSuite C_{18}, 3.5 µm PA-B

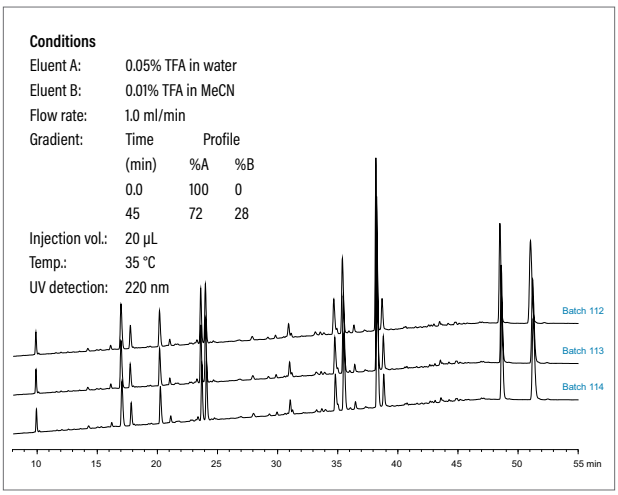
BioSuite C_{18}, 3.5 µm PA-B is a 300 Å, high-ligand density, monofunctional, silica-based column.

- Outstanding performance when separating complex digests containing hydrophilic, hydrophobic, and basic peptides
- Superior peak shape and capacity for peptide separations using TFA containing eluents (e.g., 0.1% TFA)
- Good choice for the separation of larger peptide fragments generated by some endoproteases (e.g., Lys-C)

Consistent Results Due to Superior Batch-to-Batch Reproducibility

Waters' batch-release protocol includes a tryptic map of cytochrome *c* (using Waters MassPREP Cytochrome *c* Digestion Standard [p/n: 186006371]) which is used to test for reproducibility to retention times and resolution. The three test chromatograms below show the results of the protein digest test for different batches of PA-B material.

Cytochrome *c* Tryptic Map QC Test



Waters BioSuite C_{18} PA-A and PA-B Columns are QC tested with tryptic digest of cytochrome c (p/n: 186006371) to help ensure batch-to-batch and column-to-column performance consistency.

Ordering Information

BioSuite Peptide Analysis HPLC and UHPLC Columns

BioSuite C_{18}	Inner Diameter	Length	3 µm PA-A	3.5 µm PA-B
			P/N	P/N
	2.1 mm	50 mm	186002425	186002433
	2.1 mm	100 mm	186002426	186002434
	2.1 mm	150 mm	186002427	186002435
	2.1 mm	250 mm	186002428	186002436
	4.6 mm	50 mm	186002429	186002437
	4.6 mm	100 mm	186002430	186002438
	4.6 mm	150 mm	186002431	186002439
	4.6 mm	250 mm	186002432	186002440

SYMMETRY HPLC AND UHPLC COLUMNS

Waters Symmetry reversed-phase, silica-based particles are synthesized using ultrapure organic reagents, resulting in high purity material with very low silanol activity. When combined with the high surface coverage of the bonded phase, outstanding peptide separations and recoveries are possible.

- Superior manufacturing control for consistent batch-to-batch and column-to-column results
- 100 Å and 300 Å pore size offerings for small or larger size peptides
- SymmetryShield Column chemistry offers complementary selectivity to Symmetry Column offerings
- SymmetryPrep Columns provide direct scale up while maintaining resolution

Symmetry300 Columns: The First Columns Specifically Engineered for the Discovery and Development of New Biopharmaceuticals

Symmetry300 Columns are 300 Å reversed-phase columns specifically designed to provide maximum batch-to-batch and column-to-column performance consistency and recovery of protein and peptide applications.

Symmetry300 Columns are offered in two particle sizes (3.5 µm and 5 µm) and in two chemistries (C_4 for large peptides and proteins, and C_{18} for smaller peptides) to address various needs.

Ordering Information

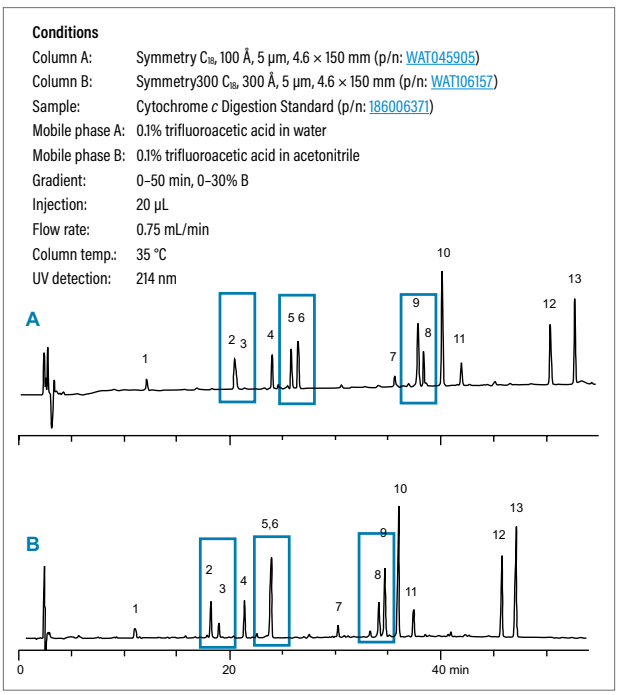
Symmetry300 HPLC and UHPLC Columns

	Particle Size: 3.5 µm		Particle Size: 5 µm	
C_{18}	**Dimension**	**P/N**	**Dimension**	**P/N**
	1.0 × 150 mm	186000185	2.1 × 150 mm	WAT106172
	2.1 × 50 mm	186000187	3.9 × 150 mm	WAT106154
	2.1 × 100 mm	186000188	4.6 × 50 mm	WAT106209
	2.1 × 150 mm	186000200	4.6 × 150 mm	WAT106157
	4.6 × 50 mm	186000201	4.6 × 250 mm	WAT106151
	4.6 × 75 mm	186000189	19 × 10 mm	186001847
	4.6 × 100 mm	186000190	19 × 50 mm	186001848
	4.6 × 150 mm	186000197	19 × 100 mm	186001849
C_4	2.1 × 150 mm	186000276	2.1 × 150 mm	186000285
	3.9 × 150 mm	186000277	3.9 × 150 mm	186000286
	4.6 × 50 mm	186000278	4.6 × 50 mm	186000287
	4.6 × 150 mm	186000279	4.6 × 150 mm	186000288
	4.6 × 250 mm	186000280	4.6 × 250 mm	186000289
	19 × 10 mm	186000281		
	19 × 50 mm	186000282		
	19 × 100 mm	186000283		

High Recoveries of Peptides and Proteins

The heart of the column is high purity-based deactivated silica. Waters dedicated chromatography chemistry manufacturing plant operates under the stringent standards of cGMP and ISO 9001. The silica used in the manufacture of our Symmetry300 Columns is synthesized using ultrapure organic reagents that yields high purity particles with very low silanol activity. These particles when combined with innovative ligand (i.e., C_4 and C_{18}) bonding techniques helps produce reversed-phase columns with minimal non-desired secondary interactions between bound ligand and biomolecules.

Pore Size Effects on Peptide Selectivity: Comparative Results on Symmetry 100 Å vs. Symmetry300 Columns



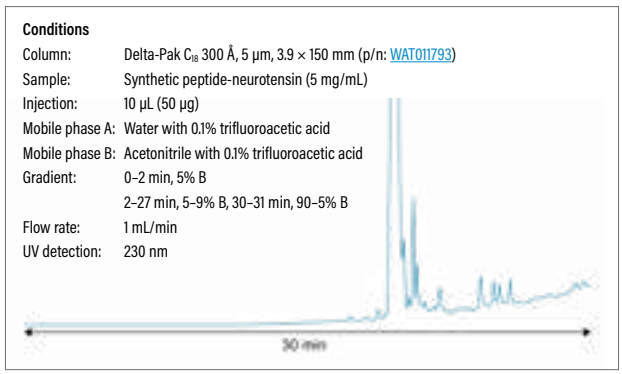
Waters' Symmetry-based C_{18} Column consists of a 100% porous silica particle containing a C_{18} ligand and endcapping to minimize undesired secondary interactions between the peptide analytes and column chemistry. As indicated by the gradient separation of a cytochrome c tryptic digest, different separation selectivities are obtained on the 100 Å column vs. the 300 Å pore size materials, with Symmetry300 C_{18} being preferred for separation on compounds greater than approximately 10,000 Dalton.

The key to a successful separation is the selection of a column that gives the highest chemistry resolution with maximum peak capacity and recovery.

DELTA-PAK HPLC AND UHPLC COLUMNS

Delta-Pak packings, ideal for the separation of peptides, proteins, and natural products, are based on a highly stable, bonded, endcapped 5 or 15 µm spherical silica. Delta-Pak is available in two different pore size materials (100 Å and 300 Å) with a C_{18} or C_4 bonded phase.

Synthetic Peptide Separation on Delta-Pak C_{18} HPLC Column



Waters Delta-Pak C_{18}, 300 Å Columns (available in 5 and 15 µm particle sizes) are well suited for the analysis and lab-scale isolation of synthetic peptide mixtures.

Ordering Information

Delta-Pak Analytical HPLC and UHPLC Columns and Guards

	Particle Size: 5 µm		
Delta-Pak C_{18}, 100 Å	**Dimension**	**Type**	**P/N**
	3.9 × 20 mm	Guard, 2/pk	WAT046880[1]
	3.9 × 20 mm	Guard, 10/pk	WAT036870[1]
	3.9 × 150 mm	Column	WAT011795
Delta-Pak C_{18}, 300 Å	2.1 × 150 mm	Column	WAT023650
	3.9 × 20 mm	Guard, 2/pk	WAT046890[1]
	3.9 × 150 mm	Cartridge, 10/pk	WAT036875[2]
	3.9 × 150 mm	Column	WAT011793
Delta-Pak C_4, 100 Å	3.9 × 20 mm	Guard, 2/pk	WAT046875[1]
	3.9 × 150 mm	Column	WAT011796
Delta-Pak C_4, 300 Å	3.9 × 20 mm	Guard, 2/pk	WAT046885[1]
	3.9 × 150 mm	Cartridge, 10/pk	WAT036865[2]
	3.9 × 150 mm	Column	WAT011794
Guard-Pak Holder			WAT088141
Guard-Pak In-line Filters, 5/pk			WAT032472

[1] Requires 3.0 × 20 mm/4.6 × 20 mm Universal Sentry Guard Holder, p/n: WAT046910.
[2] Requires Guard-Pak Holder, p/n: WAT088141.

Delta-Pak Radial Compression Preparative HPLC and UHPLC Column Segments and PrepPak Cartridges*

	Particle Size: 15 µm		
Delta-Pak C_{18}, 100 Å	**Dimension**	**Type**	**P/N**
	8 × 100 mm	Column	WAT025846
	25 × 10 mm	Guard, 2/pk	WAT038520
	25 × 100 mm	Column	WAT038506
	40 × 10 mm	Guard, 2/pk	WAT037842
	40 × 100 mm	Column	WAT037688
Delta-Pak C_{18}, 300 Å	8 × 100 mm	Column	WAT025845
	25 × 10 mm	Guard, 2/pk	WAT038522
	25 × 100 mm	Column	WAT038507
	40 × 10 mm	Guard, 2/pk	WAT037845
	40 × 100 mm	Column	WAT037692
Delta-Pak C_4, 100 Å	8 × 100 mm	Column	WAT025848
	25 × 10 mm	Guard, 2/pk	WAT038524
	25 × 100 mm	Column	WAT038508
	40 × 10 mm	Guard, 2/pk	WAT037696
Delta-Pak C_4, 300 Å	25 × 10 mm	Guard, 2/pk	WAT038526
	25 × 100 mm	Column	WAT038509
	40 × 10 mm	Guard, 2/pk	WAT037851
	40 × 100 mm	Column	WAT037700

*All column segments and cartridges require the appropriate holder/module.

Delta-Pak Preparative HPLC and UHPLC Guard Columns

	Particle Size: 15 µm		
Delta-Pak C_{18}, 100 Å	**Dimension**	**Type**	**P/N**
	3.9 × 300 mm	Column	WAT011797
	7.8 × 300 mm	Column	WAT011798
	19 × 300 mm	Column	WAT011799
	30 × 300 mm	Column	WAT011800
	50 × 300 mm	Column	WAT011801
Delta-Pak C_{18}, 300 Å	3.9 × 300 mm	Column	WAT011802
	7.8 × 300 mm	Column	WAT011803
	19 × 300 mm	Column	WAT011804
	30 × 300 mm	Column	WAT011805
Delta-Pak C_4, 100 Å	3.9 × 300 mm	Column	WAT011807
	7.8 × 300 mm	Column	WAT011808
	19 × 300 mm	Column	WAT011809
	30 × 300 mm	Column	WAT011810
Delta-Pak C_4, 300 Å	3.9 × 300 mm	Column	WAT011812
	7.8 × 300 mm	Column	WAT011813
	19 × 300 mm	Column	WAT011814
	30 × 300 mm	Column	WAT011815

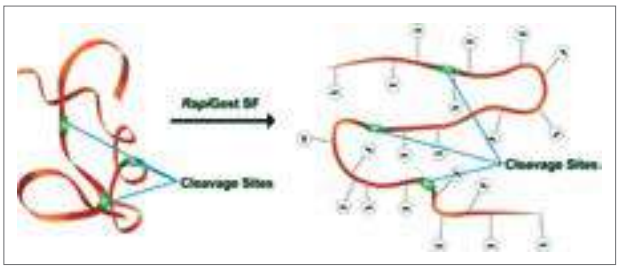
PEPTIDE STANDARDS AND REAGENTS

*Rapi*Gest SF Protein Digestion Surfactant

*Rapi*Gest SF (surfactant) radically enhances protein enzymatic digestions in terms of speed and percent recovery. *Rapi*Gest SF is a patented anionic surfactant that accelerates the production of peptides generated by proteases, such as trypsin, Asp-N, Glu-C, and Lys-C. Many hydrophobic proteins are resistant to proteolysis because their cleavage sites are inaccessible to endoproteases. *Rapi*Gest SF, a mild denaturant, helps solubilize and unfold proteins making them more amenable to cleavage without denaturing or inhibiting common proteolytic enzymes.

- Compatible with liquid chromatography (LC) and MS analysis
- Enables same-day digestions and analysis
- Minimal or no post-digestion sample preparation is required
- Improves efficiency and speed of digestion
- Simplifies digestion protocols and improves throughput of analysis
- Does not inhibit enzyme activity, unlike conventional denaturants
- Allows use of lesser amounts of expensive endoproteases because it is not disruptive of endoprotease activity

How *Rapi*Gest SF Works



Ordering Information

*Rapi*Gest SF Surfactant

Description	P/N
*Rapi*Gest SF 1 mg vial	186001860
*Rapi*Gest SF 1 mg vial (5/pk)	186001861
*Rapi*Gest SF 3 mg vial	186008090
*Rapi*Gest SF 10 mg vial	186002123
*Rapi*Gest SF 50 mg vial	186002122

CYTOCHROME *c* DIGESTION STANDARD

Benchmarking, Method Development, and Troubleshooting

The Cytochrome *c* Digestion Standard was prepared by digesting Bovine Heart Cytochrome *c* (Uniprot #P62894) with sequencing grade trypsin. This standard is recommended for benchmarking system performance and is also used for column QC.

Ordering Information

Cytochrome *c* Digestion Standard

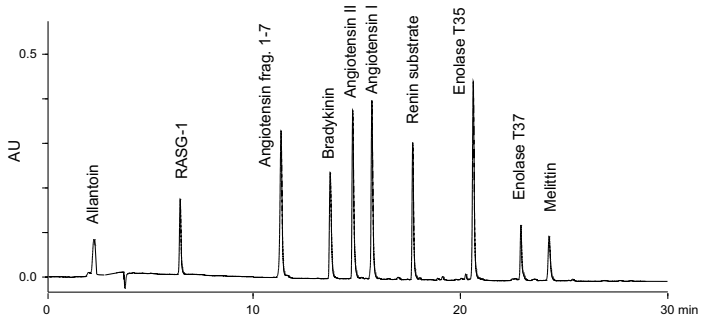
Description	P/N
Cytochrome *c* Digestion Standard	186006371

MassPREP PEPTIDE STANDARD

Benchmarking, Method Development, and Troubleshooting

The MassPREP Peptide Standard Mixture contains a void volume (VO) column marker and nine carefully selected peptides with a broad range of polarities and isoelectric points. The MassPREP Standard is useful to test LC columns and systems dedicated to peptide separations.

Baseline HPLC Resolution of Nine Peptides Contained in MassPREP Standard Mixture



Component Name	Molecular Weight* (g/mol)	pK_a	Peptide Sequence
Allantoin (V0 marker)	158.0440	-	-
RASG-1	1000.4938	9.34	RGDSPASSKP
Angiotensin frag.1–7	898.4661	7.35	DRVYIHP
Bradykinin	1059.5613	12.00	RPPGFSPFR
Angiotensin II	1045.5345	7.35	DRVYIHPF
Angiotensin I	1295.6775	7.51	DRVYIHPFHL
Renin substrate	1757.9253	7.61	DRVYIHPFHLLVYS
Enolase T35	1871.9604	7.34	WLTGPQLADLYHSLMK
Enolase T37	2827.2806	3.97	YPIVSIEDPFAEDDWEAWSHFFK
Melittin	2845.7381	12.06	GIGAVLKVLTTGLPALISWIKRKRQQ

Peptide Analysis on a BioSuite C_{18}, 3 µm, 2.1 × 150 mm Column; Eluent A: 0.02% TFA in water, Eluent B: 0.016% TFA in acetonitrile. Gradient: 0–50% B in 30 minutes. Flow: 0.20 mL/min. Temp: 40 °C.
* Monoisotopic molecular weight.

Waters offers a variety of carefully formulated and QC-tested peptide standards to help chromatographers confirm the performance of their column and LC system prior to analyses of potentially highly valued samples.

Ordering Information

MassPREP Peptide Standards

Description	Volume	P/N
MassPREP Peptide Mixture One vial with approximately 1 nmol of each: Allantoin (Vo Marker); RASG-1, angiotensin frag. 1–7, bradykinin; angiotensin II; angiotensin I, renin substrate, enolase T35, enolase T37, melittin. The peptide standard is useful to test LC columns and systems dedicated to peptide separations.	Solid	186002337
MassPREP Peptide Mixture, 5/pk Each vial contains approximately 1 nmol of each: Allantoin (Vo Marker); RASG-1, angiotensin frag. 1–7, bradykinin, angiotensin II, angiotensin I, renin substrate, enolase T35, enolase T37, melittin. The peptide standard is useful to test LC columns and systems dedicated to peptide separations.	Solid	186002338

MassPREP Protein Digestion Standards

The MassPREP Protein Digestion Standards are prepared under strict quality control procedures and contain no undigested standard proteins, trypsin, or other hydrophilic components. Test results from each batch of digestion standards are provided on an available Certificate of Analysis report.

Ordering Information

MassPREP Digestion Standards

Description	Volume	P/N
Yeast enolase	Solid	186002325
Phosphorylase b	Solid	186002326
Bovine hemoglobin	Solid	186002327
Yeast alcohol dehydrogenase (ADH)	Solid	186002328
Bovine serum albumin (BSA)	Solid	186002329
Cytochrome *c*		186006371
MassPREP Digestion Standard Kit contains (1) of 186002325, 186002326, 186002327, 186002328, 186002329		186002330

NIST Digestion Standards

A line of standards based off the NIST Reference Material 8671 (NIST mAb), a humanized IgG1k expressed from a murine cell line.

Ordering Information

NIST Digestion Standards

Description	P/N
mAb Tryptic Digestion Standard	186009126
mAb Subunit Standard	186008927

Note: mAb Charge Variant Standard (p/n: 186009065) is also available and it is based on the same NIST mAb Reference Material 8671.

Quantitative Peptide Standards

Sets of standards specifically designed, formulated, and quality controlled for quantitative peptide analysis.

- Quantitative peptide retention standard
- Hi3 Phos B and *E. coli* standards
- SILAC Hi3 Phos B and *E. coli* standards

Ordering Information

Quantitative Peptide Analysis Standards

Description	P/N
Hi3 Phosphorylase B Standard The Hi3 Phos B standard is primarily intended for use with the Hi3 quantification method for MS^E proteomics data processed with ProteinLynx Global SERVER for samples of microbial origin. It may also be used in the evaluation and benchmarking of proteomic LC-MS systems comprised of nanoACQUITY UPLC and SYNAPT and Xevo time-of-flight mass spectrometers. The Hi3 Phos B standard is intended for samples of microbial origin. It is a quantitative standard comprised of the top six ionizing peptides in the rabbit phosphorylase B protein. Recommended at -20 °C.	186006011
Hi3 *E. coli* Standard The Hi3 *E. coli* standard is primarily intended for use with the Hi3 quantification method for MS^E proteomics data processed with ProteinLynx Global Server for samples of microbial origin. It may also be used in the evaluation and benchmarking of proteomic LC-MS systems comprised of nanoACQUITY UPLC and SYNAPT and Xevo time-of-flight mass spectrometers. The Hi3 *E. coli* standard is intended for samples of animal origin. It is a quantitative standard comprised of the top six ionizing peptides in the *E. coli* ClpB protein.	186006012
SILAC Hi3 Phos B Standard The SILAC Hi3 Phos B standard is formulated from the same specialized set of the top six ionizing peptides of the rabbit phosphorylase B protein that is contained in the non-labeled counterpart: Hi3 Phos B standard (p/n: 186006011). The main difference is that this standard is produced to have a heavy labeled reference on the lysine (K) or arginine (R) end of the peptide.	186007083
SILAC Hi3 *E. coli* Standard The SILAC Hi3 *E. coli* standard is formulated from the same specialized set of the top six ionizing peptides of the *E. coli* ClpB protein that is contained in the non-labeled counterpart: Hi3 *E. coli* standard (p/n: 186006012). The main difference is that this standard is produced to have a heavy labeled reference on the lysine (K) or arginine (R) end of the peptide.	186007084
Quantitative Peptide Retention Standard The Quantitative Peptide Retention Standard is a quantitative standard that is useful during the calibration, development, and troubleshooting of chromatographic separations ensuring confidence in results. This standard is rigorously QC tested for purity and quantitative formulation and is specifically designed with the following features: ■ Peak retention for chromatographic reproducibility ■ UV absorptivity for signal reproducibility ■ Low- to high-mass range for MS ■ Water solubility ■ Tryptic-like peptides for peptide mapping studies	186006555

MassPREP Phosphopeptide Standards

The MassPREP Phosphopeptide Standards give you greater control over sample preparation, with the option to use pure peptides or to define phosphopeptides to unmodified peptide ratios.

Ordering Information

MassPREP Phosphopeptide Standards

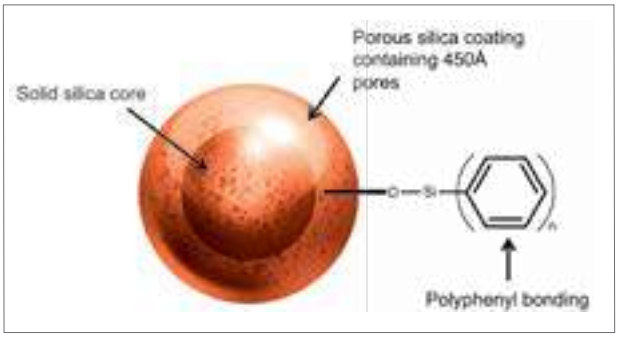
Description	Volume	P/N
MassPREP Phosphopeptide Standard Enolase	Solid	186003285
Four yeast enolase derived phosphorylated peptides: T18 1P, T19 1P, T43 1P, T43 2P. Used to optimize phosphopeptide detection in LC-MS, LC/UV, and MALDI-MS.		
MassPREP Enolase Digest with Phosphopeptides Mix	Solid	186003286
Yeast enolase spiked with four yeast enolase derived phosphorylated peptides: T18 1P, T19 1P, T43 1P, T43 2P. A more complex mixture used to optimize and troubleshoot phosphopeptide detection in LC-MS, LC/UV, and MALDI-MS.		
MassPREP Phosphopeptide Sample Kit—Enolase		186003287
Kit allows one to mix and optimize a complex standard per specific applications. Kit contains two vials:		
MassPREP enolase digestion standard	Solid	186002325
MassPREP phosphopeptide standard enolase	Solid	186003285
MassPREP Enhancer (5 vials)	Solid	186003863
Five 500 mg MassPREP Enhancer. A component in the MassPREP Phosphopeptide Enrichment Kit.		186003864
MassPREP Phosphopeptide Enrichment Kit		186003864
MassPREP phosphopeptide enrichment µElution plate	Solid	186003820
MassPREP enhancer	Solid	186003863
MassPREP enolase digest with phosphopeptides mix		186003286

Protein Analysis

The development and successful commercialization of protein-based biopharmaceuticals and diagnostic reagents frequently depends on the ability to adequately characterize these complex biomolecules. Waters' columns and methods can help solve your protein separation and characterization challenges. Waters technology utilizes:

- Reversed-phase
- Hydrophilic-interaction for ADCs
- HILIC for large molecules
- SEC for aggregate, monomer, and fragment analysis
- Ion-exchange for charge variant analysis

These orthogonal separation techniques help provide the critical characterization data and isolated material required to produce the next-generation drugs.



A schematic representing the particle and bonded phase of a BioResolve RP mAb Polyphenyl, 450 Å, 2.7 µm Column.

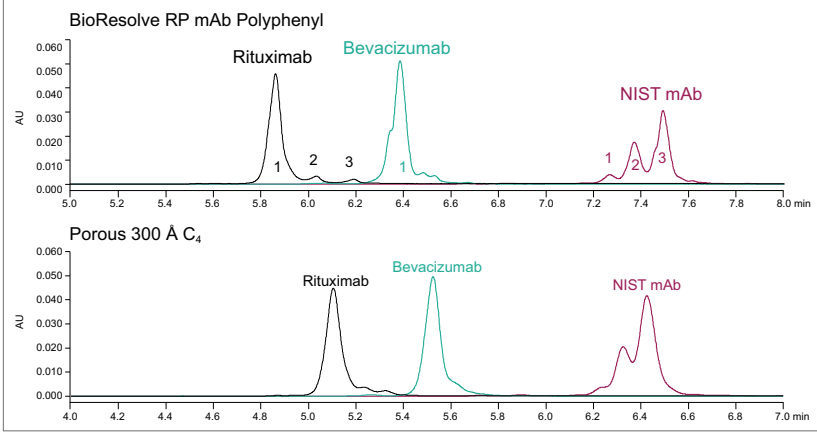
INTACT PROTEIN AND mAb SUBUNIT ANALYSIS

BioResolve RP mAb Polyphenyl Columns

Advances in the LC-MS analysis of biotherapeutic proteins have enabled the analysis at the intact protein and protein subunit level compared to use of peptide mapping protocols. The BioResolve™ RP mAb Columns and VanGuard Cartridges were purposely designed for high quality LC or LC-MS analyses of intact monoclonal antibodies (mAbs), mAb subunits, and antibody drug conjugates (ADCs) using reversed-phase chromatography. This capability was made possible using silica-based, solid core particles containing a well-defined, 450 Å pore coating and polyphenyl ligand bonding.

- Improved resolution for increased quantitation accuracy
- Less injection-to-injection carryover for increased confidence
- Lessened dependence on temperature for minimizing protein degradation
- Amenability to HPLC, UHPLC, and UPLC for use across different laboratories
- LC-MS compatibility and lessened ion pairing dependence for higher quality MS data
- Batch-to-batch consistency ensured by QC testing with the mAb Subunit Standard

Improved Separation Selectivity, Increased Quantitation Accuracy and Enhanced MS Data

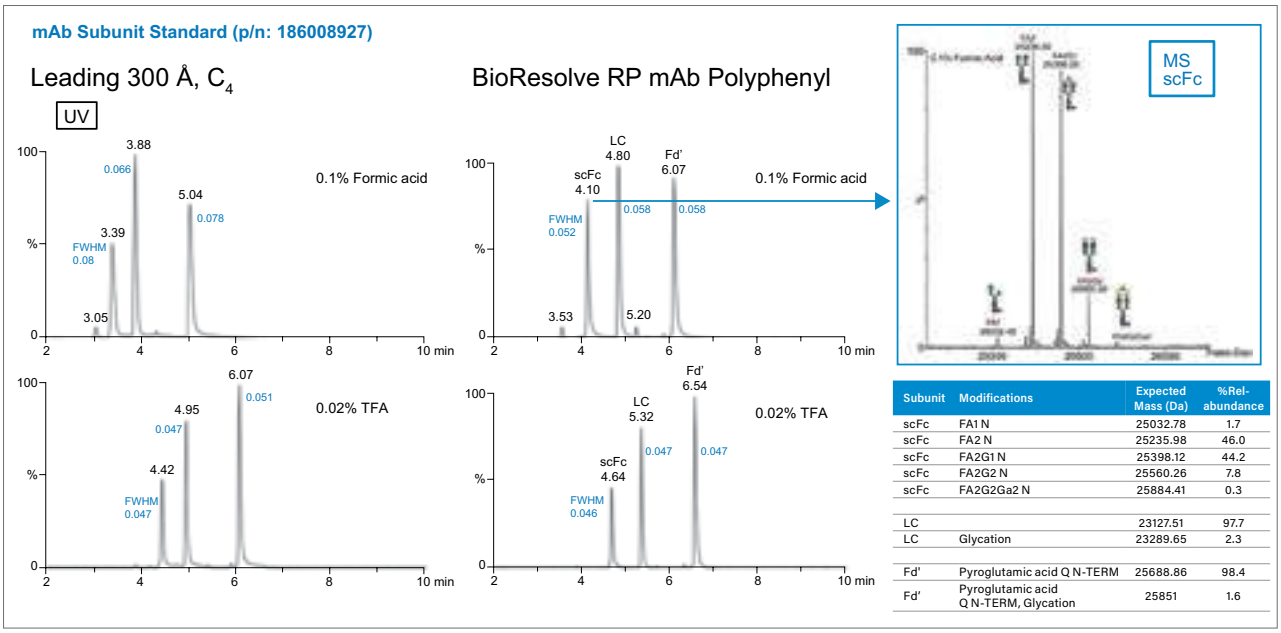


mAb	Peak	Mass (Da)	
Rituximab	1	147,243	Rituximab
	2	147,246	*
	3	147,244	*
Bevacizumab	1	149,202	Bevacizumab
	2	149,191	Sequence variance
	3		*
NIST mAb	1	148,234 (low intensity)	Oxidation
	2	148,220	Oxidation
	3	148,204	NIST mAb
	4	148,205 (low intensity)	*

*Potential deamidation or conformational variant.

Overlay of reversed-phase gradient separation of three different antibodies. The BioResolve RP mAb Polyphenyl Column provides the highest resolution as compared to a leading C_4, 300 Å column in these LC-MS analyses. Masses and potential minor peak identifications are shown in the table. Note: The tentative identifications shown were determined solely on the mass differences against the main peak. Additional testing (e.g., MS- MS) is required to confirm identifies.

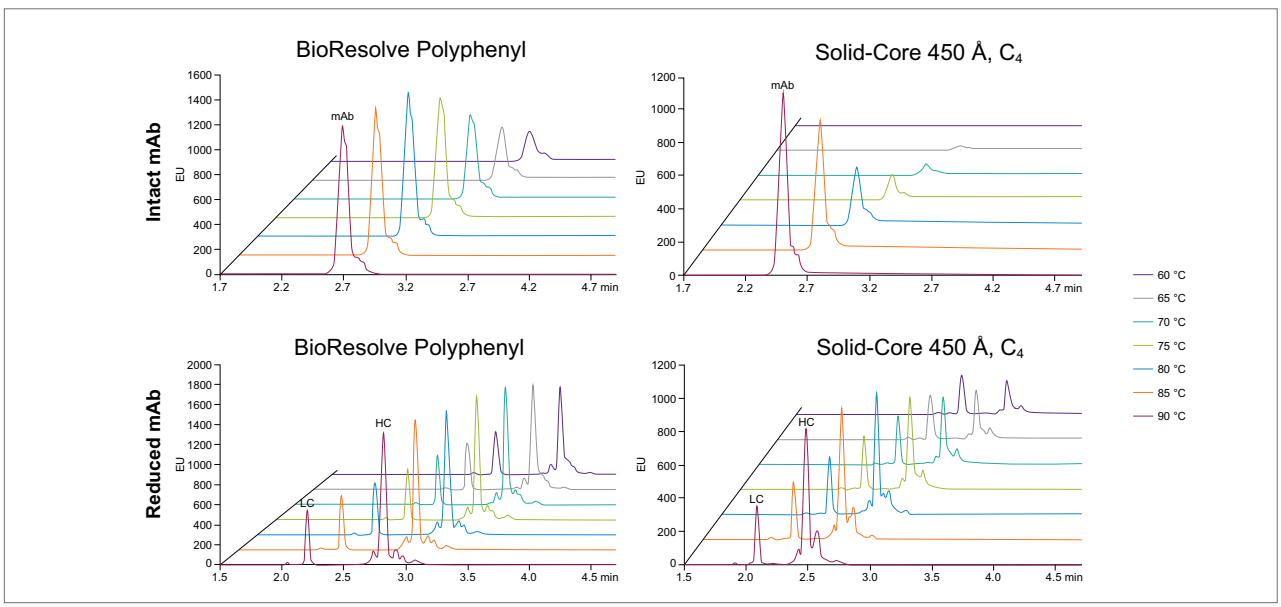
High-Quality MS Data without Adverse Peak Tailing



Subunit	Modifications	Expected Mass (Da)	%Rel-abundance
scFc	FA1 N	25032.78	1.7
scFc	FA2 N	25235.98	46.0
scFc	FA2G1 N	25398.12	44.2
scFc	FA2G2 N	25560.26	7.8
scFc	FA2G2Ga2 N	25884.41	0.3
LC		23127.51	97.7
LC	Glycation	23289.65	2.3
Fd'	Pyroglutamic acid Q N-TERM	25688.86	98.4
Fd'	Pyroglutamic acid Q N-TERM, Glycation	25851	1.6

The ability to obtain acceptable reversed-phase separations in MS-compatible eluents (e.g., 0.1% FA or 0.02% TFA) is an important performance criteria when selecting an appropriate column for these applications. Different than several tested columns (complete data not shown), acceptable LC-MS gradient separations can be achieved with the BioResolve RP mAb Polyphenyl Column using various mobile phases.

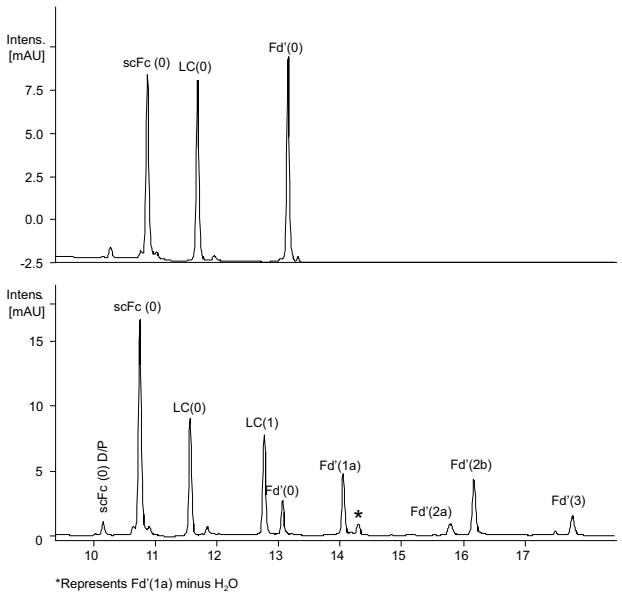
Native mAb (top) vs. Reduced Panitumumab (bottom) Recoveries at Different Gradient Separation Temperatures



The ability to recover proteins from reversed-phase gradient separations can be affected by the separation temperature. While higher temperatures are frequently required to obtain acceptable recoveries, these same on-column high temperatures can cause sample degradation and potential misinformation. Compared to several tested columns (complete data not shown), acceptable gradient separations are possible using lower temperatures on the BioResolve RP mAb Polyphenyl Column.

Bobály, B.; Lauber, M.; Beck, A.; Guillarme, D.; Fekete, S. Utility of a high coverage phenyl-bonding and wide-pore superficially porous particle for the analysis of monoclonal antibodies and related products. J. Chromatogr. A, submitted.

Outstanding Component Resolution and Recovery from IdeS Digested Unconjugated mAbs versus Conjugated (ADC) Species



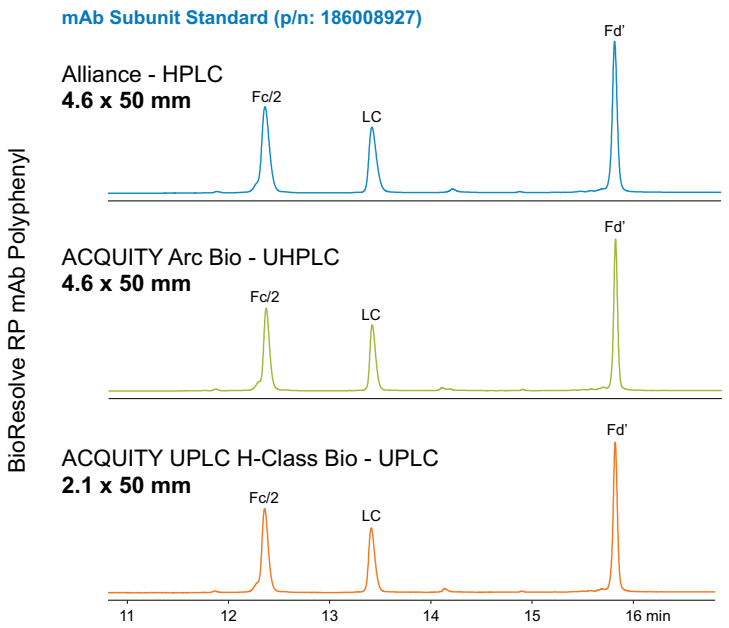
Relative Peak Area of Subunits for mAb and ADC

Isoforms	mAb Relative % Peak Area	mAb Total % Subunit	ADC Relative % Peak Area	ADC % Subunit
Fd' (0)	35.9		5.8	
Fd' (3)	n/a		4.1	
Fd' (2b)	n/a		11.4	
Fd' (2a)	n/a	35.9	3.5	37.4
Fd' (1a) minus water*	n/a		2.8	
Fd' (1a)	n/a		9.8	
L Chain (0)	31.0	31.0	12.6	29.1
L Chain (1)	n/a		16.6	
scFc (0)	33.1	33.1	33.5	33.5

A comparison of an unconjugated mAb versus an antibody drug conjugate showing full recovery of the Fd', LC, and Fc subunits/domains (with and without conjugation). Similar peak areas are recovered from scFc, LC, and Fd' in the ADC vs. the mAb.

Smith, J.; Friese, O.; Rouse, J.; Lauber, M.; Nguyen, J.; Jayaraman, P. High Resolution Chromatography – Mass Spectrometry with a Novel Phenyl RPLC Column for Heightened Characterization of Hydrophobic Monoclonal Antibodies and Antibody Drug Conjugates. WCBP, Washington, DC, January 30-February 1, 2018.

Separations on HPLC and UPLC Systems Using BioResolve RP mAb Polyphenyl, 450 Å, 2.7 µm Columns



Peak Area (%): Comparable profiles

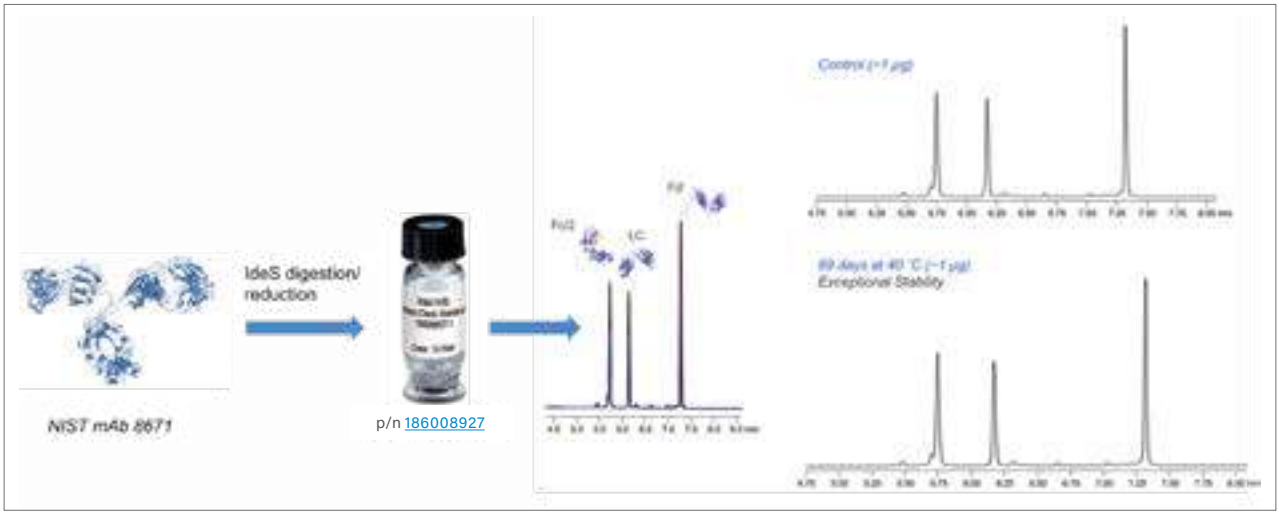
	Fc/2 (%)	LC (%)	Fd'
Alliance	34.52	24.43	41.05
ACQUITY Arc Bio	34.23	24.47	41.30
ACQUITY UPLC H-Class Bio	34.50	24.31	41.19

It is possible to use a column containing the exact same material while developing a method during discovery, working through product development, and implementing QC manufacturing controls. This capability can be attributed to the solid-core particle design and innovative polyphenyl ligand bonding of the BioResolve RP mAb Polyphenyl Column. Method transfer concerns can therefore be minimized.

Benchmarking, Method Development, and Troubleshooting

Waters mAb Subunit Standard can be used in the benchmarking of LC and LC-MS techniques, proficiency testing among different instruments and laboratories, and system suitability. This standard is a filtered and stabilized formulation of reduced, IdeS-digested NIST Reference Material 8671 (NIST mAb), a humanized IgG1κ expressed from a murine cell line.

- 25 µg of reduced, IdeS-digested NIST Reference Material 8671
- Desalted, stabilized with excipients, and lyophilized
- Excellent stability
- Used to QC each batch on BioResolve RP mAb Polyphenyl Column



APPLICATION AREA: Nanobodies

"We purchased this column to characterize our nanobodies which have a molecular weight of around 14 KDa and it worked really well. Even without expecting it when analyzing them by UPLC-MS with the BioResolve Column we were able to distinguish two separate peaks corresponding to the wild type nanobody and an N-terminal pyroglutamat form of it which only differs on 17 units of mass. With that we can say that this column has a really good resolution and is able to distinguish between two close species which may be really useful when working with antibody's modifications."

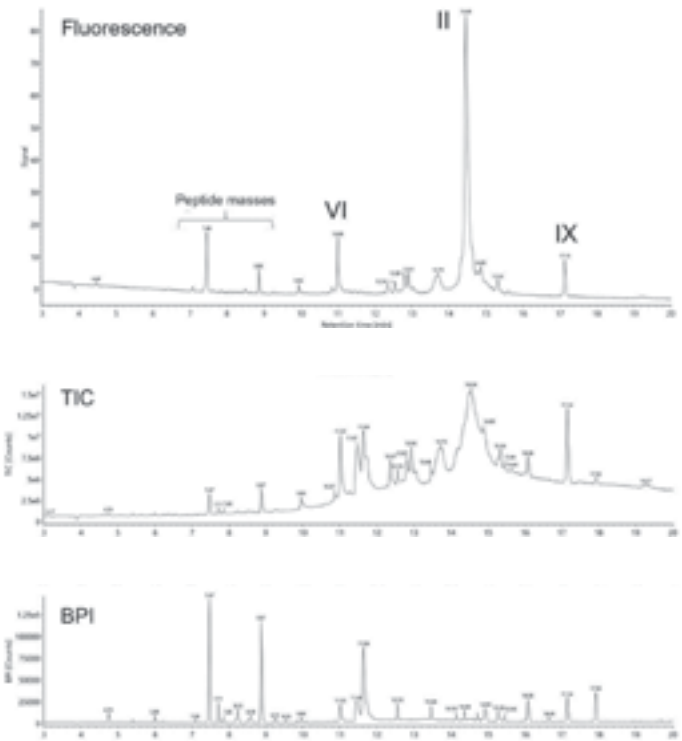
REVIEWER: Sonia Ciudad Fernández

ORGANIZATION: IECB

Analysis of Adenoviral Vector Proteins using BioResolve RP mAb Polyphenyl

Adenovirus (AdV) is being used as a viral vector for vaccines and gene therapies alike. It is comprised of a relatively complex proteome. The use of IonHance Difluoroacetic Acid (DFA) ion pairing and the BioResolve RP mAb Polyphenyl column quickly separates the AdV proteins while obtaining high sensitivity mass spectra. This is a reversed phase method that facilitates investigating protein copy ratios and measurement of intact protein masses. As such, it should present an excellent starting point for the characterization of current and future AdV based vaccines and gene therapies (refer to Waters Application Note 720007403).

Analysis of Adenoviral Vector Proteins by RPLC, Native Fluorescence, and Online MS



LC Conditions

System:	ACQUITY UPLC H-Class PLUS Bio System
Detector:	ACQUITY UPLC FLR
Ex wavelength:	280 nm
Em wavelength:	360 nm
Scan rate:	20 Hz
Column:	BioResolve RP mAb Polyphenyl 450 Å, 2.7 µm, 2.1 × 150 mm (p/n: 186008946)
Column temp.:	80 °C
Sample temp.:	8 °C
Injection:	10 µL of 10 × 10^{12} pfu/mL
Flow rate:	0.4 mL/min
Mobile phase A:	0.1% DFA (v/v) (IonHance DFA, p/n: 186009201) in 18.2 MΩ water
Mobile phase B:	0.1% DFA (v/v) in LC-MS grade acetonitrile

Time (min)	mL/min	A	B	Curve
Initial	0.4	90	10	*
20.00	0.4	50	50	6
20.50	0.4	10	90	6
21.00	0.4	90	10	6
30.00	0.4	90	10	6
Run time = 30 minutes, MS Divert for first 2 minutes				

LC-MS chromatograms of adenoviral vector proteins from HuAdV5 GFP as obtained with 2.1 × 150 mm BioResolve RP mAb Polyphenyl 450 Å 2.7 µm Column and difluoroacetic acid (DFA) modified water/acetonitrile mobile phases. Separations were performed with an ACQUITY UPLC H-Class PLUS Bio System, 10 µL injections of 10 × 1012 pfu/mL sample, a flow rate of 0.4 mL/min, and a column temperature of 80 °C. Fluorescence detection was performed along with intact mass analysis by Tof mass spectrometry.

Ordering Information

BioResolve RP mAb Polyphenyl Columns, Cartridges, Method Validation Kits*, and Standards

BioResolve RP mAb Polyphenyl Column, 450 Å	Particle Size: 2.7 µm		
	Dimension	P/N (1/pk)	P/N (1/pk with Intact mAb and mAb Subunit Stds)
	1.0 × 50 mm	186009015	–
	1.0 × 100 mm	186009016	–
	1.0 × 150 mm	186009017	–
	2.1 × 50 mm	186008944	176004156
	2.1 × 100 mm	186008945	176004157
	2.1 × 150 mm	186008946	176004158
	3.0 × 50 mm	186008948	–
	3.0 × 100 mm	186008949	–
	3.0 × 150 mm	186008950	–
	4.6 × 50 mm	186008953	176004167
	4.6 × 100 mm	186008954	176004168
	4.6 × 150 mm	186008955	176004169

BioResolve RP mAb Polyphenyl VanGuard Cartridge, 450 Å	Dimension	P/N (3/pk)	P/N (3/pk with VanGuard Holder)
	2.1 × 5 mm	186008943	176004212
	3.9 × 5 mm	186008947	176004161

BioResolve RP mAb Polyphenyl Method Validation Kit, 450 Å	Dimension	P/N (3/pk)	P/N (3/pk with Intact mAb and mAb Subunit Stds)
	1.0 × 100 mm	186009018	–
	1.0 × 150 mm	186009019	–
	2.1 × 100 mm	186008956	176004159
	2.1 × 150 mm	186008957	176004160
	3.0 × 100 mm	186008958	–
	3.0 × 150 mm	186008959	–
	4.6 × 100 mm	186008960	176004170
	4.6 × 150 mm	186008961	176004171

*Each Method Validation Kit contains three columns, each from a different batch.

Standards

Description	P/N
Humanized mAb Standard, 1 vial	186009125
Intact mAb Mass Check Standard, 1 vial	186006552
mAb Subunit Standard, 1 vial	186008927

VanGuard Cartridge Universal Holder

Description	P/N
VanGuard Cartridge Universal Holder, 1/pk	186007949

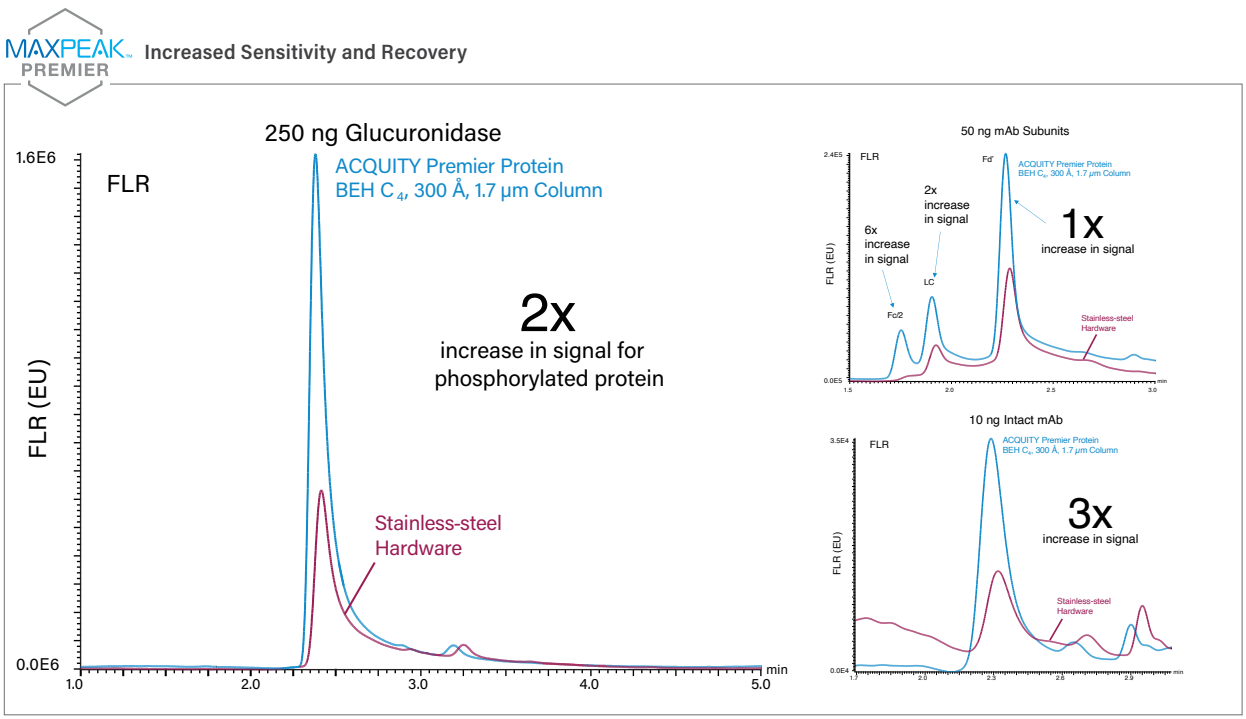
MaxPeak Premier and Protein BEH C_4, 300 Å Columns

The analysis and characterization of protein samples requires the detection of small chemical differences between large molecules. Most often these analyses have employed an array of analytical techniques, each sensitive to a different property of the protein. Reversed-phase HPLC has not been fully exploited in these tests because the separation of proteins often yields relatively broad and asymmetrical peaks with poor recovery and significant carryover. Waters reversed-phase, ethylene-bridged hybrid (BEH Technology) Protein Separation Technology Columns are specifically designed for the high-resolution analysis of proteins.

The latest innovation to Waters reversed-phase protein columns is our MaxPeak Premier Class of Columns, which deliver the chromatographic performance expected from our particle technologies while increasing reproducibility, peak shape, and recovery for metal-sensitive analytes. The ACQUITY and XBridge Premier Protein BEH C_4 300 A columns contain the same stationary phase as the traditional stainless steel version but utilize MaxPeak High Performace Surface technology hardware which has shown to help minimize ionic interactions of phosphorylated proteins and increase sensitivity for low-level intact and subunit analysis of mAbs.

Waters family of Protein BEH C_4, 300 Å Columns for protein separations:

- Separates proteins of various sizes, hydrophobicities, and isoelectric points
- Tolerates extreme pH and temperature
- HPLC/UHPLC (3.5 µm) and UPLC (1.7 µm) column to address instrumentation and application needs
- Preparative columns available in 5- and 10-µm particle offerings
- Quality-control tested with MassPREP Protein Standard Mix (p/n: 186004900)



MaxPeak™ Premier Columns utilize MaxPeak High Performance Surfaces that are designed to increase analyte recovery, sensitivity, and reproducibility by minimizing analyte/surface interactions that can lead to sample losses.

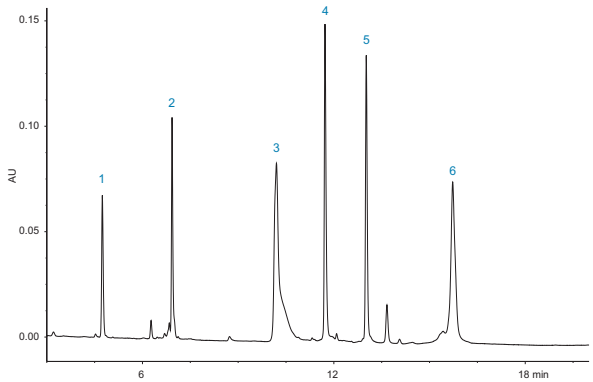
C_4, 300 Å Columns Developed for Protein Chromatography

Conditions

Column:	XBridge Protein BEH C_4, 300 Å, 3.5 µm, 2.1 × 50 mm (p/n: 186004498)
Sample:	MassPREP Protein Standard, 0.1% TFA in 5% acetonitrile (p/n: 186004900)
Mobile phase A:	0.1% trifluoroacetic acid in water
Mobile phase B:	0.075% trifluoroacetic acid in 71.4% acetonitrile
Flow rate:	0.2 mL/min
Gradient:	28–100% B in 25 min
Column temp.:	40 °C
Injection:	5 µL
UV detection:	220 nm

Compounds

1. Ribonuclease A	0.04 mg/mL
2. Cytochrome *c*	0.06 mg/mL
3. Bovine serum albumin	0.20 mg/mL
4. Myoglobin	0.13 mg/mL
5. Enolase	0.22 mg/mL
6. Phosphorylase b	0.59 mg/mL



Protein BEH C_4, 300 Å columns can be used with proteins that have a wide range of properties. This protein mix was chosen to represent a range of isoelectric points, molecular weights, and hydrophobicities.

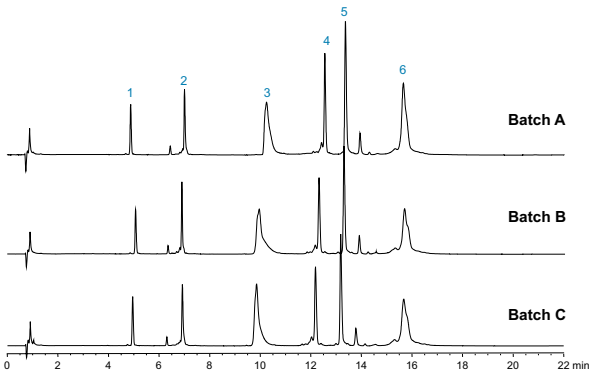
Batch-to-Batch Reproducibility

Conditions

Column:	ACQUITY UPLC Protein BEH C_4, 300 Å, 1.7 µm, 2.1 × 50 mm (p/n: 186004495)
Sample:	MassPREP Protein Standard, 0.1% TFA in 5% acetonitrile (p/n: 186004900)
Eluent A:	0.1% trifluoroacetic acid in 100% HPLC grade water
Eluent B:	0.075% trifluoroacetic acid in 71.4% acetonitrile
Flow rate:	0.2 mL/min
Gradient:	28–100% B in 25 min
Column temp.:	40 °C
UV detection:	220 nm

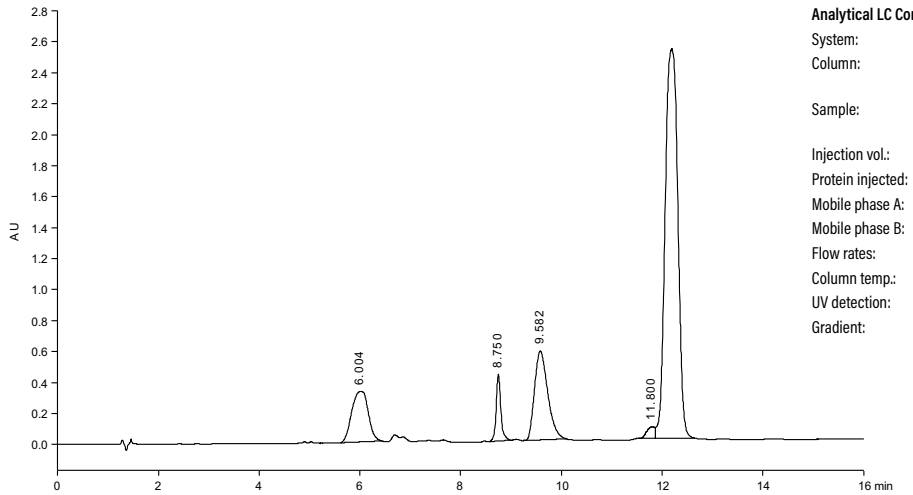
Compounds

1. Ribonuclease
2. Cytochrome *c*
3. BSA
4. Myoglobin
5. Enolase
6. Phosphorylase b



Waters MassPREP Protein Standard Mix is used to critically QC test the ACQUITY UPLC Protein BEH C_4, 300 Å Columns to help ensure consistent batch-to-batch and column-to-column performance.

Optimized Analytical Scale Separation on XBridge Protein BEH C_4, 300 Å, 3.5 µm, 4.6 × 150 mm Column



Analytical LC Conditions

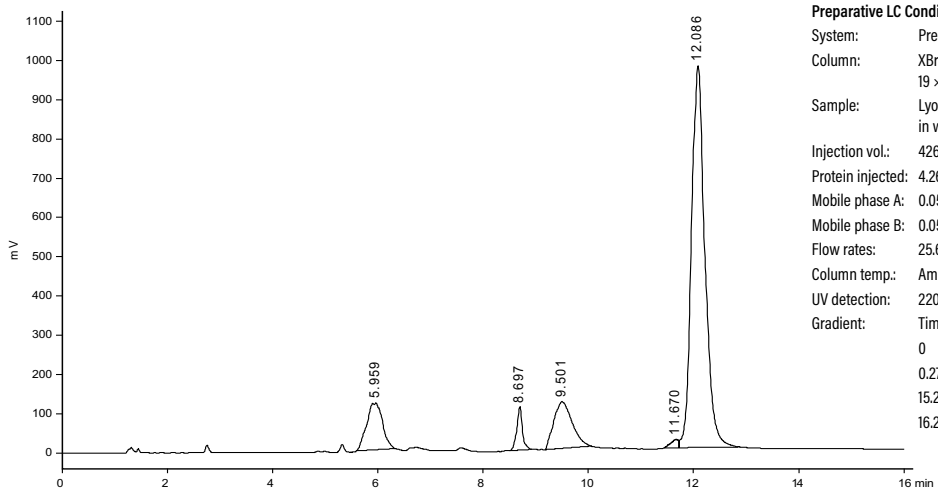
System:	Alliance
Column:	XBridge Protein BEH C_4, 300 Å, 3.5 µm, 4.6 × 150 mm (p/n: 186004504)
Sample:	Lyophilized chicken egg white, 10 mg/mL in 0.05% TFA in water
Injection vol.:	25 µL
Protein injected:	0.25 mg
Mobile phase A:	0.05% TFA in water
Mobile phase B:	0.05% TFA in acetonitrile
Flow rates:	1.5 mL/min
Column temp.:	Ambient
UV detection:	220 nm

Gradient:

Time	%A	%B
0	90	10
15	40	60
16	90	10

Analytical scale separation of 250 µg chicken egg white proteins on XBridge Protein BEH C_4, 300 Å, 3.5 µm, 4.6 × 150 mm Column.

Successful Scaled Preparative Separation on XBridge Protein BEH C_4, OBD Prep, 300 Å, 5 µm, 19 × 150 mm Column



Preparative LC Conditions

System:	Prep 150 LC
Column:	XBridge Protein BEH C_4 OBD Prep, 300 Å, 5 µm, 19 × 150 mm (p/n: 186007313)
Sample:	Lyophilized chicken egg white, 10 mg/mL in 0.05% TFA in water
Injection vol.:	426 µL
Protein injected:	4.26 mg
Mobile phase A:	0.05% TFA in water
Mobile phase B:	0.05% TFA in acetonitrile
Flow rates:	25.6 mL/min
Column temp.:	Ambient
UV detection:	220 nm

Gradient:

Time	%A	%B
0	90	10
0.27	90	10
15.27	40	60
16.27	90	10

Effective method development and scaling of the 250 µm analytical scale separation to the preparative BEH C_4, 300 Å, 5 µm, 19 × 150 mm column results in chromatography showing an almost identical separation pattern.

MassPREP Protein Standard Mix

Benchmarking, Method Development, and Troubleshooting

The MassPREP Protein Standard Mix consists of carefully chosen proteins encompassing a wide range of properties. These mixtures contain proteins that vary in isoelectric points, molecular weights, and hydrophobicities. These characteristics provide the user with an attractive intact protein validation mixture that can be used for a variety of applications. In particular, it is used as a benchmarking standard for ACQUITY UPLC Protein BEH C_4, 300 Å Columns.

MassPREP Protein Standard Mix		
Protein Sample	**Molecular Weight (MW)**	**Isoelectric Point (pI)**
Ribonuclease A, bovine pancreas	13.7 k	9.6
Cytochrome *c*, horse heart, 96%	12.4 k	10.25
Albumin, bovine serum, 96–99%	66.4 k	5.8
Myoglobin, horse heart >90%	16.7 k	6.53
Enolase from baker's yeast (S. cerevisiae)	46.7 k	6.53
Phosphorylase b, rabbit muscle	97.0 k	7.18

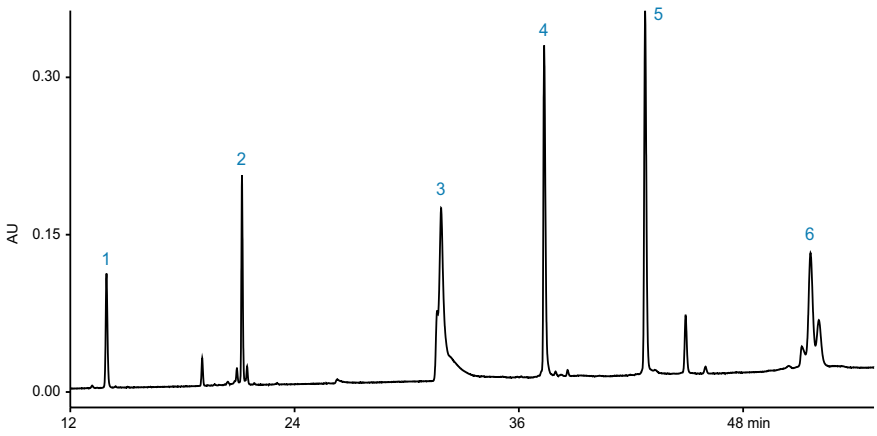
MassPREP Protein Standard Mix on an ACQUITY UPLC Protein BEH C_4, 1.7 µm, 2.1 × 150 mm Column

Conditions

Column:	ACQUITY UPLC Protein BEH C_4, 300 Å, 1.7 µm, 2.1 × 150 mm (p/n: 186004497)
Mobile phase A:	0.1% TFA in water
Mobile phase B:	0.1% TFA in 100% acetonitrile
Flow rate:	0.2 mL/min
Gradient:	20–55% B in 60 min
Column temp.:	40 °C
Injection:	3.3 µL
UV detection:	220 nm
Sample:	MassPREP Protein Standard mixture prepared in 0.1% TFA in 5% acetonitrile

Compounds

1. Ribonuclease A (0.04 mg/mL)
2. Cytochrome *c* (0.06 mg/mL)
3. Bovine serum albumin (0.20 mg/mL)
4. Myoglobin (0.13 mg/mL)
5. Enolase (0.22 mg/mL)
6. Phosphorylase b (0.59 mg/mL)



Use of Waters' carefully formulated and QC tested MassPREP Protein Standard Mix can help chromatographers confirm adequate performance of their reversed-phase column and LC system prior to the analyses of potentially highly valued samples.

MassPREP Protein Standard Mixture Certificate of Analysis

Waters' Analytical Standards and Reagents come with a Certificate of Analysis that contains relevant, lot-specific information. Many times a chromatogram is attached using data acquired the same way a customer would use the standard.

Ordering Information

Protein Standards

Description	P/N
MassPREP Protein Standard Mix	186004900
Intact mAb Mass Check Standard	186006552

ACQUITY UPLC Protein BEH C_4, 300 Å Columns and Method Validation Kits

ACQUITY Premier Protein BEH C_4, 300 Å

Particle Size: 1.7 µm		
Dimension	P/N	w/Standard
2.1 × 50 mm	186010326	176005107
2.1 × 100 mm	186010327	176005108
2.1 × 150 mm	186010328	176005109

ACQUITY Protein BEH C_4, 300 Å

Particle Size: 1.7 µm		
Dimension	P/N	w/Standard
1.0 × 50 mm	186005589	–
1.0 × 100 mm	186005590	–
1.0 × 150 mm	186005591	–
2.1 × 50 mm	186004495	–
2.1 × 100 mm	186004496	–
2.1 × 150 mm	186004497	–

ACQUITY Protein BEH C_4, 300 Å VanGuard Pre-Column, 3/pk

Particle Size: 1.7 µm	
Dimension	P/N
2.1 × 5 mm	186004623

ACQUITY Protein BEH C_4, 300 Å Method Validation Kit*

Particle Size: 1.7 µm	
Dimension	P/N
2.1 × 100 mm	186004899
2.1 × 150 mm	186006549

XBridge Protein BEH HPLC and UHPLC Columns and Method Validation Kits

XBridge Premier Protein BEH C_4, 300 Å

Particle Size: 2.5 µm		
Dimension	P/N	w/Standard
2.1 × 50 mm	186010329	176005110
2.1 × 100 mm	186010330	176005111
2.1 × 150 mm	186010331	176005112
4.6 × 50 mm	186010332	176005113
4.6 × 100 mm	186010333	176005114
4.6 × 150 mm	186010334	176005115

XBridge Protein BEH C_4, 300 Å VanGuard Pre-column, 3/pk*

Particle Size: 2.5 µm	
Dimension	P/N
2.1 × 5 mm	186009131
3.9 × 5 mm	186009140

XBridge Protein BEH C_4, 300 Å Method Validation Kit*

Particle Size: 2.5 µm	
Dimension	P/N
2.1 × 5 mm	186009131
3 × 150 mm	186009135
4.6 × 150 mm	186009139

XBridge Protein BEH C_4, 300 Å

Particle Size: 2.5 µm		Particle Size: 3.5 µm		Particle Size: 5 µm		Particle Size: 10 µm	
Dimension	P/N	Dimension	P/N	Dimension	P/N	Dimension	P/N
2.1 × 50 mm	186009127	2.1 × 10 mm Guard Cartridge	186007230[1]	10 × 10 mm Guard Cartridge	186007305[3]	10 × 10 mm Guard Cartridge	186007325[3]
2.1 × 100 mm	186009128	2.1 × 50 mm	186004498	10 × 50 mm	186008272	10 × 50 mm	186008276
2.1 × 150 mm	186009129	2.1 × 100 mm	186004499	10 × 100 mm	186008273	10 × 100 mm	186008277
3 × 50 mm	186009132	2.1 × 150 mm	186004500	10 × 150 mm	186008274	10 × 150 mm	186008278
3 × 100 mm	186009133	2.1 × 250 mm	186004501	10 × 250 mm	186008275	10 × 250 mm	186008279
3 × 150 mm	186009134	4.6 × 20 mm Guard Cartridge	186007235[2]	19 × 10 mm Guard Cartridge	186007310[4]	19 × 10 mm Guard Cartridge	186007330[4]
4.6 × 50 mm	186009136	4.6 × 50 mm	186004502	19 × 50 mm	186007311	19 × 50 mm	186007331
4.6 × 100 mm	186009137	4.6 × 100 mm (MVK)*	186005465	19 × 100 mm	186007312	19 × 100 mm	186007332
4.6 × 150 mm	186009138	4.6 × 100 mm	186004503	19 × 150 mm	186007313	19 × 150 mm	186007333
		4.6 × 150 mm	186004504	19 × 250 mm	186007314	19 × 250 mm	186007334
		4.6 × 250 mm	186004505	30 × 10 mm Guard Cartridge	186007315[5]	30 × 10 mm Guard Cartridge	186007335[5]
				30 × 50 mm	186007316	30 × 50 mm	186007336
				30 × 75 mm	186007317	30 × 75 mm	186007337
				30 × 100 mm	186007318	30 × 100 mm	186007338
				30 × 150 mm	186007319	30 × 150 mm	186007339
				30 × 250 mm	186007320		

*Three columns from three different batches of material.
** Requires VanGuard Cartridge Universal Holder, p/n: 186007949
[1]Requires 2.1 × 10 mm Universal Sentry Guard Holder, p/n WAT097958.
[2]Requires 4.6 × 20 mm Universal Sentry Guard Holder, p/n WAT046910.
[3]Requires 10 × 10 mm Cartridge Holder, p/n 289000779.
[4]Requires 19 × 10 mm Cartridge Holder, p/n 186000709.
[5]Requires 30 × 10 mm Prep Guard Holder, p/n 186006912.

Protein-Pak Hi Res HIC Columns and HIC Protein Standard

Protein-Pak Hi Res HIC (Hydrophobic Interaction Chromatography) columns contain non-porous, polymethacrylate-based particles (2.5 µm) functionalized with a butyl-ligand coating and are well suited for the characterization of proteins and biotherapeutics including monoclonal antibodies (mAb) and antibody drug conjugates (ADC).

While reversed-phase chromatography is a frequently used bioanalytical technique, HIC offers attractive orthogonal separation advantages. In reversed-phase LC, proteins are retained by hydrophobic interaction with alkyl groups (e.g., C_{18}) on the packing material. However, the butyl-ligand density on Waters Protein-Pak Hi Res HIC Column is comparatively less resulting in fewer protein-ligand hydrophobic interactions. Consequently, HIC-based elution is possible using gradients of decreasing salt concentration at physiological pH values. Use of denaturing organic solvent eluents (e.g., acetonitrile in 0.1% TFA) thus allowing biotherapeutics (e.g., acid labile, cysteine-linked ADCs) to be analyzed in non-denaturing conditions.

In addition, Waters has developed HIC Protein Standard Test Mix designed for user verification of HPLC/UPLC instrument and Protein-Pak Hi Res HIC Column performance prior to sample analyses. This intact protein validation mix, when used on a regular basis, helps monitor system and column performance and is also highly valuable in method development and/or troubleshooting. The standard contains a carefully chosen set of six proteins that provide good chromatographic representation using a gradient of decreasing salt concentration.

- Ideally suited for hydrophobic-based separations for protein characterization using non-denaturing conditions
- Use of non-porous particles help deliver fast, efficient separations to address high-throughput needs
- Shipped with Waters HIC Protein Test Standard to help test for acceptable instrument and HIC column performance
- Successfully used for the analysis of cysteine-based, antibody drug conjugates

Ordering Information

Protein-Pak Hi Res HIC Columns and HIC Protein Standards

Description	Dimension	P/N
Protein-Pak Hi Res HIC, 2.5 µm Column and HIC Protein Standard	4.6 × 35 mm	176003575
Protein-Pak Hi Res HIC, 2.5 µm Column and HIC Protein Standard	4.6 × 100 mm	176003576
HIC Protein Test Standard	—	186007953

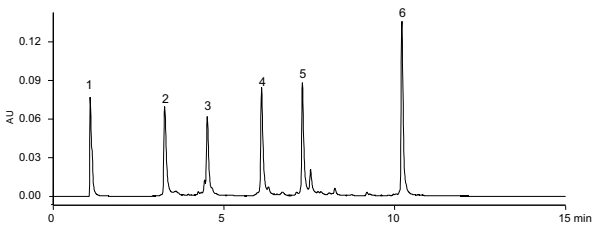
Protein-Pak Hi Res HIC Column and HIC Protein Standard

Conditions

Sample:	HIC Protein Standard Test Mix (p/n: 186007953) dissolved in 100 µL 50% A/50% B 2 µL injected
Column:	Protein-Pak Hi Res HIC, 2.5 µm, 4.6 × 100 mm (p/n: 176003576)
Flow rate:	0.6 mL/min
Mobile phase A:	2 M $(NH_4)_2SO_4$ in 50 mM NaH_2PO_4/NaH_2PO_4, pH 6.9
Mobile phase B:	50 mM NaH_2PO_4/NaH_2PO_4, pH 6.9
Gradient:	0–100% B in 15 min
UV detection:	220 nm
Temp.:	30 °C

Compounds

1. Cytochrome *c*
2. Myoglobin
3. Ribonuclease A
4. Lysozyme
5. Enolase
6. Alpha-chymotrypsinogen A

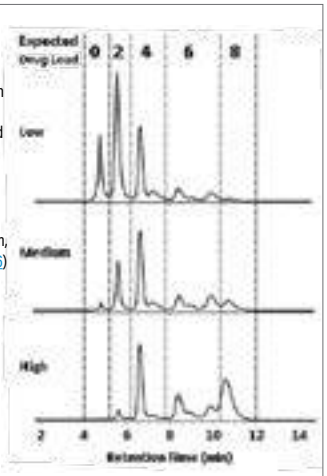


Using a gradient of decreasing salt concentration and on-denaturing eluents, Waters Protein-Pak Hi Res HIC Column is well suited for the separation of proteins of various molecular weights and hydrophobic interactions.

Separation of ADC Samples on Protein-Pak Hi Res HIC Column

Conditions

Sample:	Cysteine-conjugated ADCs were obtained by a collaborator at a concentration of 10 mg/mL in formulation buffer. Samples were prepared for analysis at a concentration of 2 mg/mL in 1 M ammonium sulfate ($(NH_4)_2SO_4$) 10 µL injected
Column:	Protein-Pak Hi Res HIC, 2.5 µm, 4.6 × 100 mm (p/n: 176003576)
Flow rate:	0.7 mL/min
Mobile phase A:	2.5 M $(NH_4)_2SO_4$ 125 mM phosphate buffer, pH 6.7 and 5% isopropanol
Mobile phase B:	125 mM phosphate buffer, pH 6.7 and 5% isopropanol
Gradient:	0–50% B in 10 min. Hold at 50% B for 5 min.
UV detection:	280 nm
Temp.:	30 °C



Monitoring drug load variability. Three batches of cysteine-linked ADCs were synthesized, each with a different level of drug conjugation (low, medium, high) and separated using hydrophobic interaction chromatography. The drug load distribution shifted from low-to-high corresponding to an increase in the load of the hydrophobic drug.

BioSuite Hydrophobic-Interaction Chromatography (HIC) HPLC Columns

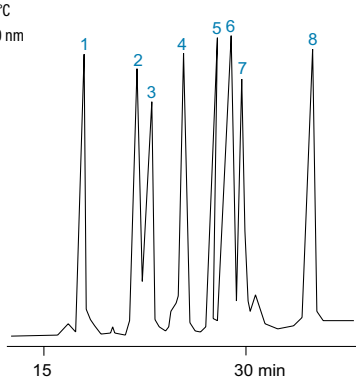
The separation of proteins and peptides based upon hydrophobic characteristics is a powerful chromatographic technique. However, some proteins denature at elevated organic solvent concentrations making reversed-phase chromatography (RPC) difficult. BioSuite Phenyl Hydrophobic-interaction Chromatography (HIC) provides a viable separation alternative to RPC. HIC is characterized by the adsorption of compounds to a weakly hydrophobic surface at high salt concentrations, followed by elution with a decreasing salt gradient. HIC combines the non-denaturing characteristics of salt precipitation with the precision of HPLC to yield excellent separation of biologically active material. BioSuite Phenyl, 1000 Å, 10 µm HIC column media consists of a phenyl group bonded to a methacrylic ester-based polymeric resin.

The large 1000 Å pore size accommodates proteins up to 5,000,000 Daltons. A 21.5 × 150 mm column is also available for "lab scale" isolations.

Hydrophobic Proteins are Well Resolved by Reversed-Phase Chromatography on BioSuite pPhenyl RP Column

Conditions

Sample:	Ribonuclease (Peak 1), bovine insulin, (Peak 2), cytochrome *c* (Peak 3), lysozyme (Peak 4), transferrin (Peak 5), bovine serum albumin (Peak 6), myoglobin (Peak 7), ovalbumin (Peak 8)
Column:	BioSuite Phenyl, 1000 Å, 10 µm, 4.6 × 75 mm (p/n: 186002157)
Eluent A:	HPLC-grade water with 0.05% trifluoroacetic acid
Eluent B:	Acetonitrile with 0.05% trifluoroacetic acid
Flow rate:	1.0 mL/min
Gradient:	Linear gradient from 5 to 80% B in 60 min
Column temp.:	25 °C
UV detection:	220 nm

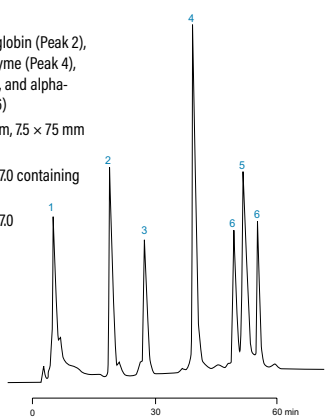


The BioSuite pPhenyl, 1000 Å RPC Columns have a higher ligand density compared to the BioSuite Phenyl, 1000 Å HIC Columns and are not recommended for hydrophobic-interaction separations.

Hydrophobic-Interaction Chromatography on BioSuite Phenyl HIC Column is an Excellent Alternative to Reversed-Phase Methods

Conditions

Sample:	Cytochrome *c* (Peak 1), myoglobin (Peak 2), ribonuclease (Peak 3), lysozyme (Peak 4), alphachymotrypsin (Peak 5), and alpha-chymotrypsinogen A (Peak 6)
Column:	BioSuite Phenyl, 1000 Å, 10 µm, 7.5 × 75 mm (p/n: 186002159)
Eluent A:	0.1 M sodium phosphate, pH 7.0 containing 2.0 M ammonium sulfate
Eluent B:	0.1 M sodium phosphate, pH 7.0
Flow rate:	1.0 mL/min
Gradient:	Linear gradient from 0 to 100% B in 60 min
Column temp.:	25 °C
UV detection:	280 nm

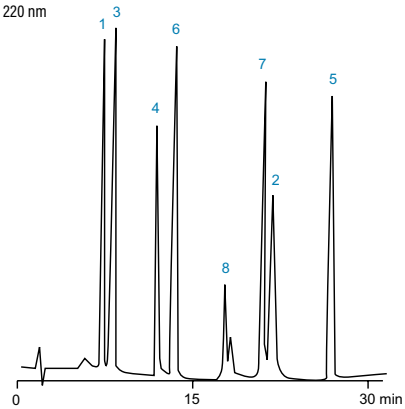


The BioSuite Phenyl, 1000 Å HIC Columns have a lower ligand density compared to the BioSuite pPhenyl, 1000 Å RPC Columns and are not recommended for reversed-phase separations.

Reversed-Phase Chromatography at Elevated pH on BioSuite pC_{18} RP Column Possible on Polymer Based Material

Conditions

Sample:	Met-enkephalin (Peak 1), bradykinin (Peak 2), leu-enkephalin (Peak 3), neurotensin (Peak 4), bombesin (Peak 5), angiotensin I (Peak 6), somatostatin (Peak 7), insulin (Peak 8)
Column:	BioSuite C_{18}, 500 Å, 7 µm, 4.6 × 150 mm (p/n: 186002154)
Eluent A:	0.2 M ammonium hydroxide, pH 10.8
Eluent B:	0.2 M ammonium hydroxide, pH 10.8 (20%)/acetonitrile (80%)
Flow rate:	1.0 mL/min
Gradient:	Linear gradient from 0 to 80% B in 50 min
Column temp.:	25 °C
UV detection:	220 nm



Use of "pH stable" methacrylate-based particles contained in Waters BioSuite pC_{18} Reversed-Phase Columns allow scientists to change separation selectivity by using a pH not possible with 100% silica-based C_{18} columns.

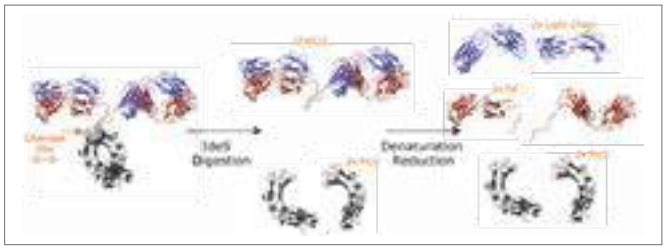
Ordering Information

Hydrophobic-Interaction HPLC and UHPLC Column

Description	Dimension	P/N
Shodex PH-814 Column, 8 µm	8 × 75 mm	WAT035520

ACQUITY UPLC Glycoprotein BEH Amide, 300 Å Columns

HILIC for Large Molecules

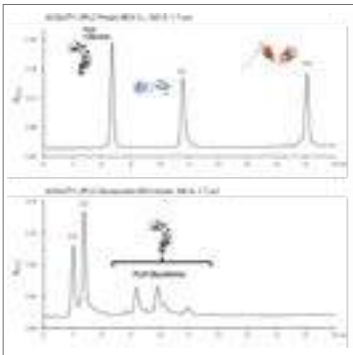


IdeS digestion and reduction scheme for preparing IgG LC, Fd', and Fc/2 subunits.

In what is commonly referred to as a middle-up or middle-down analysis, native mAbs can be proteolyzed into subunits to facilitate characterization. One increasingly popular way to produce subunit digests of mAbs is via the IdeS protease (Immunoglobulin Degrading Enzyme of S. pyogenes). IdeS cleaves with high fidelity at a conserved sequence motif in the hinge region of humanized mAbs to cleanly produce, upon reduction, three 25 kDa mAb fragments that are amenable to mass spectrometry and useful for localizing different attributes of therapeutic mAbs (below).

IdeS digestion combined with reversed-phase (RP) chromatography on Waters ACQUITY UPLC Protein BEH C_4, 300 Å Column has been successfully used as a simple identity test for mAbs and fusion proteins, because IdeS produced subunits from different drug products will exhibit diagnostic RP retention times. However, it should be kept in mind that many IgG modifications more strongly elicit changes in the hydrophilicity of a mAb along with its capacity for hydrogen bonding.

Compared to the reversed-phase separation of glycoprotein subunits, HILIC-based chromatography on Waters ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm Columns offers additional information related to a mAb digest as shown in the figures below.



Gradient used for reversed-phase (RP) separations of trastuzumab subunits:

Time	%A	%B	Curve
0.0	95.0	5.0	6
1.0	66.7	33.3	6
21.0	59.7	40.3	6
22.0	20.0	80.0	6
24.0	20.0	80.0	6
25.0	95.0	5.0	6
35.0	95.0	5.0	6

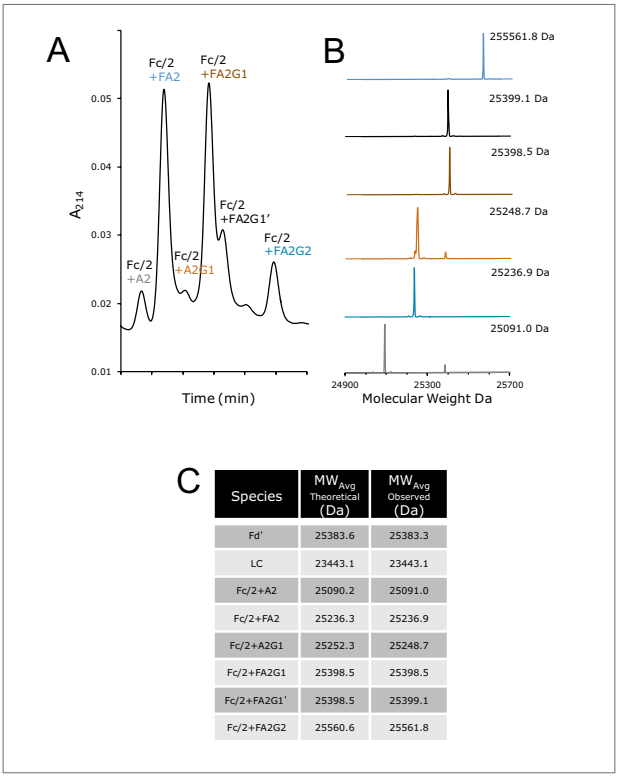
Gradient used for HILIC separations of IgG subunits:

Time	%A	%B	Curve
0	20.0	80.0	6
1	30.0	70.0	6
21	37.0	63.0	6
22	100.0	0.0	6
24	100.0	0.0	6
25	20.0	80.0	6
35	20.0	80.0	6

LC Conditions

System:	ACQUITY UPLC H-Class Bio
Columns	Top: ACQUITY UPLC Protein BEH C_4, 300 Å, 1.7 µm, 2.1 × 150 mm (p/n: 186004497) Bottom: ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm, 2.1 × 150 mm Column Kit (p/n: 176003702) that contains Glycoprotein Performance Test Standard (p/n: 186008010)
Sample temp.:	5 °C
Column temp.:	45 °C
Flow rate:	0.2 mL/min
Mobile phase A:	0.1% (v/v) TFA, water
Mobile phase B:	0.1% (v/v) TFA, acetonitrile
Injection vol.:	≤1.2 µL (aqueous diluents)

Trastuzumab subunit separations. (A) 1 µg of reduced, IdeS digested separated using an ACQUITY UPLC Protein BEH C_4, 300 Å, 1.7 µm Column (0.7 µL aqueous injection). (B) 1 µg of reduced, IdeS digested separated using an ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm Column (0.7 µL aqueous injection).



C

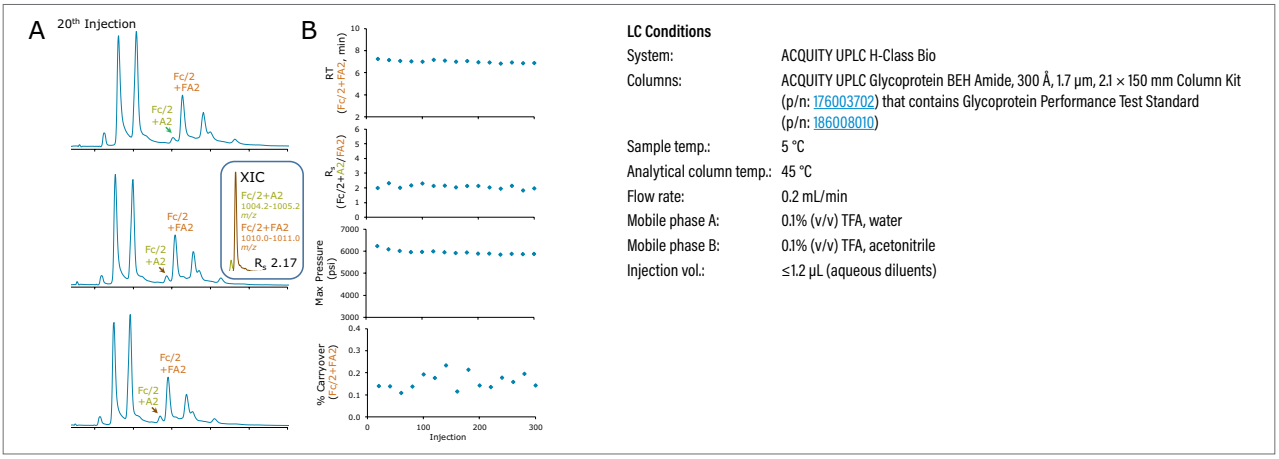
Species	MW_{Avg} Theoretical (Da)	MW_{Avg} Observed (Da)
Fd'	25383.6	25383.3
LC	23443.1	23443.1
Fc/2+A2	25090.2	25091.0
Fc/2+FA2	25236.3	25236.9
Fc/2+A2G1	25252.3	25248.7
Fc/2+FA2G1	25398.5	25398.5
Fc/2+FA2G1'	25398.5	25399.1
Fc/2+FA2G2	25560.6	25561.8

Profiling trastuzumab Fc/2 subunit glycoforms. (A) Retention window corresponding to the glycoform separation space. (B) Deconvoluted ESI mass spectra for the HILIC chromatographic peaks. Chromatographic peaks are labeled with the same color as their corresponding mass spectra. (C) Molecular weights for the observed trastuzumab subunits.

Lifetime Testing of ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm Columns for Profiling IgG Subunit Glycoforms

The ability of Waters BEH Amide, 300 Å, 1.7 µm Column to robustly deliver separations over time is shown below by data collected from a series 300 sequential injections of a reduced, IdeS digested trastuzumab sample.

This was a potentially challenging use scenario given that the reduced, IdeS digested mAb sample contains both high concentrations of guanidine denaturant and TCEP reducing agent. Total ion chromatograms corresponding to the 20th, 180th, and 300th injections of this experiment are displayed. In these analyses, particular attention was paid to the half-height resolution of the Fc/2+A2 and Fc/2+FA2 species, which was assessed every 20th separation using extracted ion chromatograms (XICs). In this testing, several additional chromatographic parameters were also monitored, including the retention time of the Fc/2+FA2 species, the maximum system pressure observed during the chromatographic run, and the percent (%) carryover of the most abundant glycoform, the Fc/2+FA2 species. Plots of these parameters underscore the consistency of the subunit separation across the lifetime of the column.

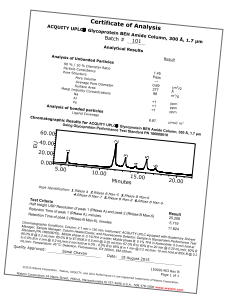


LC Conditions

System:	ACQUITY UPLC H-Class Bio
Columns:	ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm, 2.1 × 150 mm Column Kit (p/n: 176003702) that contains Glycoprotein Performance Test Standard (p/n: 186008010)
Sample temp.:	5 °C
Analytical column temp.:	45 °C
Flow rate:	0.2 mL/min
Mobile phase A:	0.1% (v/v) TFA, water
Mobile phase B:	0.1% (v/v) TFA, acetonitrile
Injection vol.:	≤1.2 µL (aqueous diluents)

Lifetime testing of an ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm, 2.1 × 150 mm Column for sequential injections of reduced, IdeS digested trastuzumab. (A) Total ion chromatograms (TICs) from the 20th, 180th, and 300th injections. Example extracted ion chromatograms (XICs) for Fc/2+A2 and Fc/2+FA2 that were used to measure resolution. (B) Chromatographic parameters observed across the 300 injection lifetime test. Each panel shows results for each 20th injection, including retention time (RT) of the FA2 glycoform, Rs between A2 and FA2 glycoforms, maximum pressure across the run, and % carryover as measured by a repeat gradient and XICs.

ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm Column Consistency



To help ensure batch-to-batch and column-to-column consistency in validated methods, each batch of material selected for use in the ACQUITY UPLC Glycoprotein BEH Amide, 300 Å, 1.7 µm Column offering is specifically QC tested with Waters Glycoprotein Performance Test Standard (p/n 186008010). This same standard is shipped (at no additional cost) with each column to help benchmark method development and/or troubleshoot use of this column and instrumentation.

ACQUITY Premier Glycoprotein BEH Amide Columns

Utilizing the MaxPeak High Performance Surfaces (HPS) Technology, the ACQUITY Premier Glycoprotein BEH Amide columns reduce sample loss caused by non-specific adsorption onto metal surfaces, deliver the representative performance known of the BEH Amide chemistry from the first injection.

Ordering Information

ACQUITY UPLC Glycoprotein BEH Amide, 300 Å Columns and Standards

	Particle Size: 1.7 µm		
BEH Amide, 300 Å	**Dimension**	**Qty.**	**P/N**
	2.1 × 5 mm	3/pk with standard	176003699
	2.1 × 50 mm	1/pk with standard	176003700
	2.1 × 100 mm	1/pk with standard	176003701
	2.1 × 150 mm	1/pk with standard	176003702
	2.1 × 100 (MVK)	3/pk with standard	176003703
ACQUITY Premier Glycoprotein BEH Amide, 300 Å	2.1 × 50 mm	—	176009547
	2.1 × 100 mm	—	176009548
	2.1 × 150 mm	—	176009549
ACQUITY Premier Glycoprotein BEH Amide, 300 Å	2.1 × 50 mm	1/pk with standard	176004866
	2.1 × 100 mm	1/pk with standard	176004867
	2.1 × 150 mm	1/pk with standard	176004868
Glycoprotein Performance Test Standard			186008010

PROTEIN SEC COLUMNS

Size-Exclusion Chromatography

Size-exclusion chromatography (SEC), also known as gel filtration, separates proteins based on their sizes (hydrodynamic radii versus absolute molecular weight) in solution with larger sized species eluting before smaller proteins. The primary driving mechanism for this isocratic based technique is based on the pore size and volume of the SEC particles used in the packed column. As the molecular weight of the proteins of interest increases, SEC columns containing comparatively larger pore size particles are selected.

Why choose BEH-based SEC column technology?

- Less undesired secondary interactions with BEH-Diol and BEH-PEO SEC particles for increased confidence in results and data reproducibility
- Unlike silica-based particles, BEH based particles have enhanced > pH 8.0 stability for method flexibility
- Outstanding column lifetime that includes SEC columns containing 1.7 µm, 2.5 µm, or 3.5 µm particles
- Scalable chemistries for method transfer from drug discovery to manufactured product testing
- Pore size options include 125 Å, 200 Å, 250 Å, and 450 Å depending on application needs
- Comprehensive series BEH-based SEC Care and Use manuals assist users in developing reliable methods
- Synthesized batches are QC tested with relevant samples (e.g., mAb size Variant Std) to help ensure consistency
- Synergistic combination of MaxPeak™ Premier hardware with BEH-PEO particles for use on Platform Methods

BEH Technology for SEC-based Separations

In 1999, Waters launched the family of XTerra™ HPLC columns featuring patented, first-generation hybrid particle technology (HPT). HPT enabled XTerra Columns to become one of the most successful column productsin the history of Waters. In HPT, the best properties of inorganic (silica) and organic (polymeric) packings are combined to produce a material that has superior mechanical strength, efficiency, high-pH stability, and peak shape for basic compounds.

The first-generation methyl-hybrid particles of XTerra Columns did not possess the mechanical strength or efficiency necessary to fully realize the potential speed, sensitivity, and resolution capabilities of UPLC Technology. Therefore, a new pressure-tolerant particle needed to be created. This second-generation hybrid material utilizes an ethylene-bridged hybrid (BEH) structure. Compared to the

first-generation methyl-hybrid particle of XTerra Columns, the BEH particle of ACQUITY™ UPLC BEH Columns exhibits improved efficiency, strength and pH range. BEH Technology is a key enabler of the speed, sensitivity, and resolution of both small and large molecule UPLC separations. Waters SEC columns containing diol-coated, BEH particles were developed to primarily minimize non-desired secondary ionic interaction between the biomolecules and separation media. In 2022, was introduced PEO-coated, BEH particles packed in Waters MaxPeak Premier technology to help address both non-desired secondary ionic and hydrophobic interactions.

SEC AGGREGATE ANALYSIS

Size-exclusion chromatography (SEC) is the analytical "gold standard" for the separation and accurate quantitation of aggregates contained in biotherapeutic peptides and proteins (e.g., mAbs).

The principle of SEC chromatography involves the ability of an appropriately selected column to separate molecules based on differences in the molecules' "size in solution" that loosely correlates to their molecular weight.

A partial list of customer-desired benefits using Waters BEH-based SEC columns include:

- Available guards and columns containing 125 Å, 200 Å, 250 Å, or 450 Å pores
- Purposely designed columns containing different particle sizes for UPLC, UHPLC, or HPLC-based applications
- Less ionic interactions using stable diol-coating for higher confidence in obtained data.
- Less non-desired ionic interactions using MaxPeak Premier HPS Guards and Columns
- State-of-the-art column packing technologies for outstanding column life and pH stability from 2–10 for enhanced method development flexibility for challenging samples
- Quality control tested with relevant proteins and peptides to help ensure consistent batch-to-batch and column-to-column performance

The following four factors provide guidance for selecting an appropriate SEC column that matches your application and laboratory needs.

1) Molecular weight vs pore size selection

Column pore size and sample molecular weight (MW) go in-hand when selecting an SEC column. The pore size of the column media, generally expressed in angstroms (Å), determines both how quickly a sample will travel through the column and how well the sample will be retained in relation to the sample's molecular weight. The inclusion of "in relation to your samples molecular weight" is an important distinction to make here. Without it, it might be assumed that smaller pore size equals better results, however, that is not the case. For example, if the pore size is too small, based on the sample's MW, larger molecules will not move as freely, reducing retention and column efficiency.

Therefore, the MW of the substance being tested would influence, if not determine, what column pore size to choose. A sample with a molecular weight between 1000–8000 Da would be best suited for a 125 Å column. This selection will provide better retention characteristics in separating small compounds compared to a similar column with a pore size of 200 or 450 Å. If the sample's molecular weight is between 10,000–450,000 Da, then a column pore size of 200 or 450 Å should be chosen. Any sample with a MW over 450,000 Da should be analyzed with a 450 Å column.

2) LC system dispersion

LC system dispersion can also significantly affect SEC column choice. In SEC, analytes elute within a single column volume during the isocratic separation. This makes it important to consider the total LC system volume, including the injector, tubing, and detector volumes of the obtained separation. In general, the lower the total LC system dispersion volume relative to the column volume, the narrower the peaks.

Examples of system dispersion specifications for column recommendations:

LC system dispersions <20 µL (UPLC) = 1.7 µm column

LC system dispersions >20 – <35 µL (UHPLC) = 2.5 µm column

LC system dispersions >35 µL (HPLC) = 2.5 or 3.5 µm column

3) Resolving multiple species that are less than two-fold different in molecular weight

The ability to adequately resolve compounds that differ by two-fold in molecular weight (e.g., 300 K, mAb IgG dimer from 150 K monomer) can be relatively easy to accomplish when using an appropriate SEC column. However, a far more challenging scenario involves the species separation that differs by less than 2x molecular weight (e.g., 150 K, mAb IgG monomer from 100 K "Clip"). In addition, the ability to obtain reliable quantitation is challenged when the minor components exist at <0.5% compared to the major peak of interest.

4) Speed of separation

The final factor to consider when selecting an appropriate SEC column is the desired speed for the separation. Generally, there is a trade-off between resolution and speed when implementing size-exclusion chromatography. However, a balance can be achieved by selecting the appropriate column. When an SEC column containing comparatively smaller particles (e.g., 1.7 µm) is used on an appropriate LC system, quicker results are obtained which differs from separations performed on larger particle-sized (2.5 or 3.5 µm) SEC columns. For example, an SEC 1.7 µm, 4.6 × 300 mm column can provide excellent resolution in under nine minutes. Meanwhile a separation on an SEC 2.5 µm, 7.8 × 300 mm column will generally take approximately 12 minutes; and, on an SEC 3.5 µm, 7.8 × 300 mm column it will take 18 minutes.

An appropriate SEC column selection, that is based on the separation needs and the LC system being used, can generate reproducible separations and accurate component quantitation for various protein and peptide samples. To get the best resolution, reproducibility, and speed, keep in mind the four factors outlined above and how they relate to your specific samples. This will help ensure you select the best possible column for your application.

Four-Step Guide for Successful SEC Column Selection

What is the molecular weight of what you are trying to separate?			
NEED:	**MW 1–8K Da**	**MW 10–450K / 650K Da**	**MW 100–1500K Da**
Recommended column specifications	125 Å	200 A / 250 A	450 Å

What type of LC system dispersion* are you using?			
NEED:	**<20 µL (UPLC)**	**>20–<35 µL (UHPLC)**	**>35 µL (HPLC)**
Recommended column specifications	1.7 µm or 2.5 µm	2.5 µm	2.5 µm or 3.5 µm

Do you need to resolve something that is less than 2-fold difference in MW?**			
NEED:	**2.5 µm**	**2.5 µm**	**2.5 µm or 3.5 µm**
REC. Recommended column specifications SPEC:	4.6 × 300 mm or 7.8 × 300 mm	7.8 × 300 mm	7.8 × 300 mm

Do you need maximum speed on a MW greater than two-fold?			
NEED:	**<9 min**	**<12 min**	**<18 min**
REC. Recommended column specifications SPEC:	1.7 µm 4.6 × 150 mm	2.5 µm 4.6 × 150 mm	2.5 µm 7.8 × 150 mm

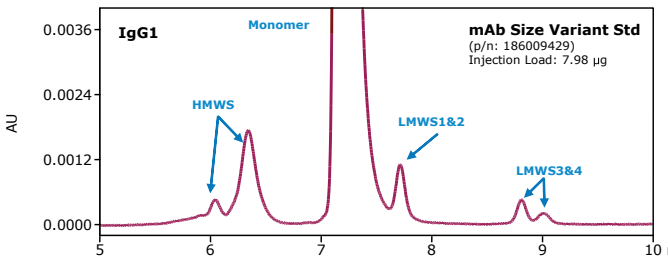
**For guidance on measuring system dispersion, download the SEC Optimization Guide (720006067EN) on waters.com.*
*** To understand the "why" behind these recommendations, read the Application Note (720006336EN) on waters.com.*

ACQUITY AND XBRIDGE PREMIER PROTEIN SEC OFFERINGS FOR SEC PLATFORM METHOD APPLICATIONS

ACQUITY™ and XBridge™ PREMIER Protein SEC 250 Å columns help scientists obtain reliable protein separations as has been made possible through the use of Waters MaxPeak High Performance Surfaces and novel BEH-PEO SEC particle technologies. Advancements in SEC column hardware and particle technology work to minimize secondary ionic or hydrophobic interactions between proteins and the column to allow chromatographers to use a "generic" or "platform-type" method.

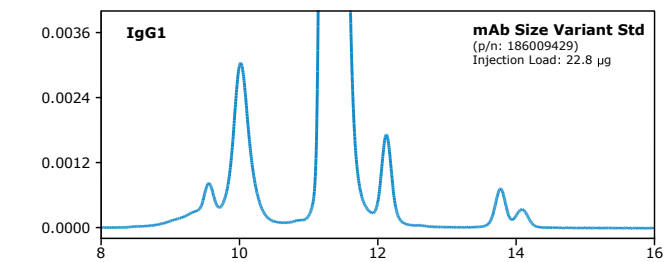
Waters XBridge PREMIER Protein SEC 250 Å 2.5 µm guard column is also available, which can provide effective trapping of insoluble particulates and excipients sometimes present in samples and eluents, thereby extending the analytical column's lifetime.

Scalable SEC Column Offerings using 2x PBS Mobile Phase



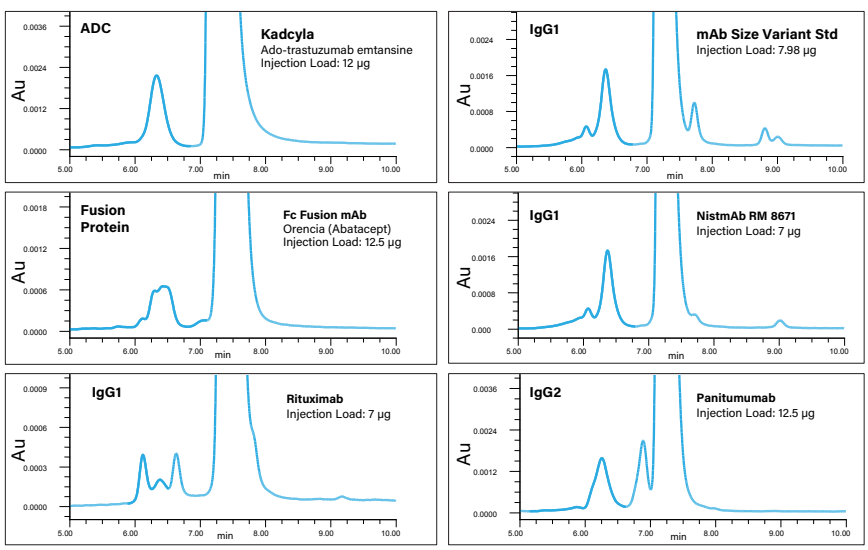
Conditions

Column:	ACQUITY™ Premier Protein SEC 250 Å,1.7 µm
Dimension:	4.6 × 300 mm
Flow rate:	300 µL/min
Temp.:	35 °C
Wavelength:	280 nm
Mobile phase:	2x PBS (20 mM phosphate, 276 mM NaCl, 5.4 mM KCl pH 7.4)
Instrument:	ACQUITY UPLC H-Class Bio
System dispersion:	<10 µL



Column:	XBridge™ Premier Protein SEC 250 Å, 2.5 µm
Dimension:	7.8 × 300 mm
Flow rate:	575 µL/min
Temp.:	35 °C
Wavelength:	280 nm
Mobile phase:	2x PBS (20 mM phosphate, 276 mM NaCl, 5.4 mM KCl pH 7.4)
Instrument:	ACQUITY UPLC H-Class Bio
Systemdispersion:	30 µL

Classes of therapeutic proteins separated on ACQUITY™ Premier Protein SEC 250 Å, 1.7 µm Column

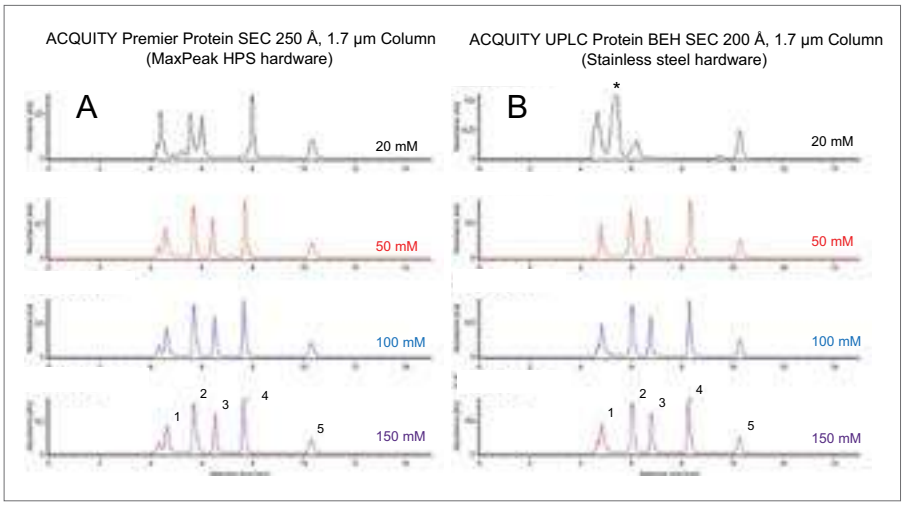


Conditions

Column:	ACQUITY Premier Protein SEC 250 Å, 1.7 µm
Dimension:	4.6 × 300 mm
Flow rate:	0.3 mL/min
Temp.:	35 °C
Wavelength:	280 nm
Mobile phase:	2xPBS (20 mM phosphate, 276 mM NaCl, 5.4 mM KCl pH 7.4)
Instrument:	ACQUITY UPLC™ H-Class Bio
LC system dispersion:	<10 µL

MS Compatibility

Native SEC separations can now be readily coupled to MS to facilitate deep characterization of protein therapeutics and the study of non-covalent protein complexes. The inertness of the ACQUITY PREMIER Protein SEC columns improves upon the chromatography that has to date been obtainable with ammonium acetate mobile phases. This can be seen in the form of improved sample recoveries, the preservation of non-covalent protein complexes, and lower limits of detection. Figure W provides a comparison study wherein it was found that a PREMIER Protein SEC 250 Å column was able to yield protein recoveries in ammonium acetate mobile phase at a level significantly lower than that of an alternative state-of-the-art column technology.



Ammonium Acetate Native SEC Chromatography with 4.6 × 150 mm, 1.7 µm Columns. A sample of BEH200 Protein Standard Mix was used for analysis with an (A) ACQUITY Premier Protein SEC 250 Å or (B) ACQUITY UPLC BEH SEC 200 Å Column. Mobile phases with varying ammonium acetate concentrations were evaluated. Peak 1: Thyroglobulin (pre-peak is thyroglobulin dimer); Peak 2: IgG; Peak 3: Bovine Serum Albumin; Peak 4: Myoglobin; Peak 5: Uracil. The asterisk (*) denotes a co-elution of IgG and BSA.

Ordering Information

MaxPeak Premier SEC 1.7 and 2.5 µm

Pore Size	MW Range	Particle Size	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N	P/N
			4.6 mm ID × Column Length								
			30 mm Guard	150 mm No Standard	300 mm No Standard	150 mm w/Standard	300 mm w/Standard	150 mm w/Guard	300 mm w/Guard	150 mm Guard w/Std	300 mm Guard w/Std
250 Å	10 K–650 K	1.7 µm	—	186009963	186009964	176005071	176005072	176004783	176004784	176004794	176004795
250 Å	10 K–650 K	2.5 µm	186009969	186009959[5]	186009960	176005067	176005068	176004779	176004780	176004790	176004791

Pore Size	MW Range	Particle Size	7.8 mm ID × Column Length								
			30 mm Guard	150 mm No Standard	300 mm No Standard	150 mm w/Standard	300 mm w/Standard	150 mm Guard w/Std	300 mm Guard w/Std	150 mm Guard w/Std	300 mm Guard w/Std
250 Å	10 K–650 K	1.7 µm	—	—	—	—	—	—	—	—	—
250 Å	10 K–650 K	2.5 µm	—	186009961	186009962	176005069	176005070	176004781	176004782	176004792	176004793

mAb Size Variant Standard, 160 g *	186009429
XBridge™ Premier Protein SEC 250 Å, 2.5 µm, 4.6 × 150 mm Column MVK	176004801
XBridge™ Premier Protein SEC 250 Å, 2.5 µm, 4.6 × 300 mm Column MVK	176004802
XBridge™ Premier Protein SEC 250 Å, 2.5 µm, 7.8 × 150 mm Column MVK	176004803
XBridge™ Premier Protein SEC 250 Å, 2.5 µm, 7.8 × 300 mm Column MVK	176004804
ACQUITY™ Premier Protein SEC 250 Å, 1.7 µm, 4.6 × 150 mm Column MVK	176004805
ACQUITY™ Premier Protein SEC 250 Å, 1.7 µm, 4.6 × 300 mm Column MVK	176004806

Straight Connection Tubing and End-fittings	WAT022681
U-Bend Connection Tubing and End-fittings	WAT084080

** Method Validation Kit (MVK) contains three columns from three dierent batches.

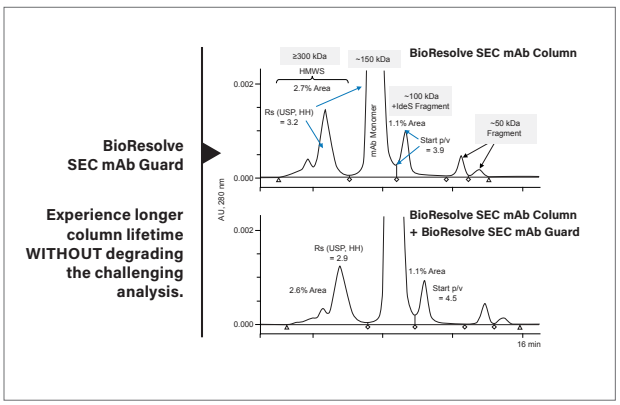
BIORESOLVE SEC MAB OFFERINGS FOR mAb AGGREGATE, MONOMER, AND FRAGMENT ANALYSES

Waters™ BioResolve™ SEC mAb Columns and BioResolve SEC mAb Guards are specifically manufactured and quality control tested to deliver reproducible, accurate quantitation of monoclonal antibodies (mAbs), associated high molecular weight aggregates (≥300,000 Da) and lower molecular weight fragments (≤100,000 Da). A range of available column sizes provides flexibility for performance optimization on a variety of chromatographic platforms, ranging from higher dispersion HPLC to lower dispersion UPLC™ systems. A guard column is also available which can provide effective trapping of insoluble particulates and excipients sometimes present in samples and/or eluents, thereby extending analytical column lifetime.

The BioResolve SEC mAb Columns and Guards:

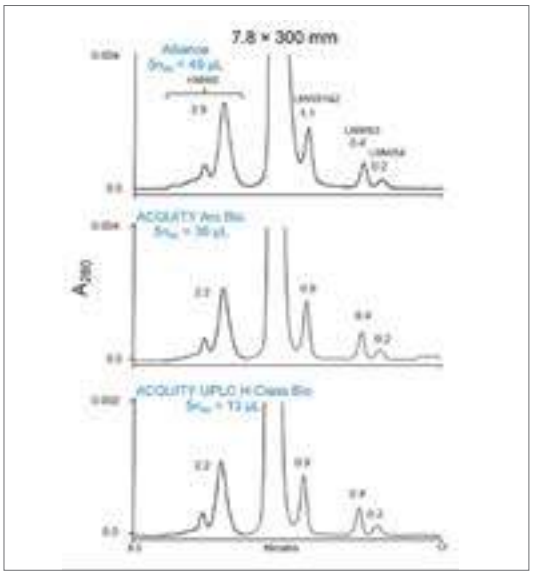
- Provide out-of-the-box performance
- Tested with Waters NIST-based, mAb Size Variant to help ensure consistent column-to-column and batch-to-batch performance, regardless of LC platform

Use of BioResolve SEC mAb Guard can help extend SEC column life without compromising needed component resolution



Separation of Waters mAb Size Variant Standard on BioResolve SEC mAb,

More Than Just a Column



Separation of Waters mAb Size Variant Standard on BioResolve SEC mAb, 7.8 × 300 mm and 7.8 × 150 mm Columns on LC systems with 49 µL (Alliance), 30 µL (ACQUITY Arc), and 10 or 13 µL (ACQUITY UPLC H-Class) system dispersions. Percent areas are reported for each chromatogram. Conditions provided in the experimental section.

Ordering Information

BioResolve SEC mAb Columns, Guards, and Method Validation Kits

Pore Size	MW Range	Particle Size	P/N	P/N	P/N	P/N	P/N	P/N	
			4.6 mm ID × Column Length						
			30 mm Guard	150 mm No Standard	300 mm No Standard	150 mm w/Standard	300 mm w/Standard	150 mm Guard w/Std	300 mm Guard w/Standard
200 Å	10 K–450 K	2.5 µm	186009443	186009435	186009437	176004592	176004593	176004596	176004597

Pore Size	MW Range	Particle Size	7.8 mm ID × Column Length						
			30 mm Guard	150 mm No Standard	300 mm No Standard	150 mm w/Standard	300 mm w/Standard	150 mm Guard w/Std	300 mm Guard w/Std
200 Å	10 K–450 K	2.5 µm	—	186009439	186009441	176004594	176004595	176004598	176004599

BioResolve SEC mAb Method Validation Kit: 200 Å, 2.5 µm, 4.6 × 150 mm Columns**	176004639
BioResolve SEC mAb Method Validation Kit: 200 Å, 2.5 µm, 4.6 × 300 mm Columns**	176004640
BioResolve SEC mAb Method Validation Kit: 200 Å, 2.5 µm, 7.8 × 150 mm Columns**	176004641
BioResolve SEC mAb Method Validation Kit: 200 Å, 2.5 µm, 7.8 × 300 mm Columns**	176004642
mAb Size Variant Standard, 160 g *	186009429

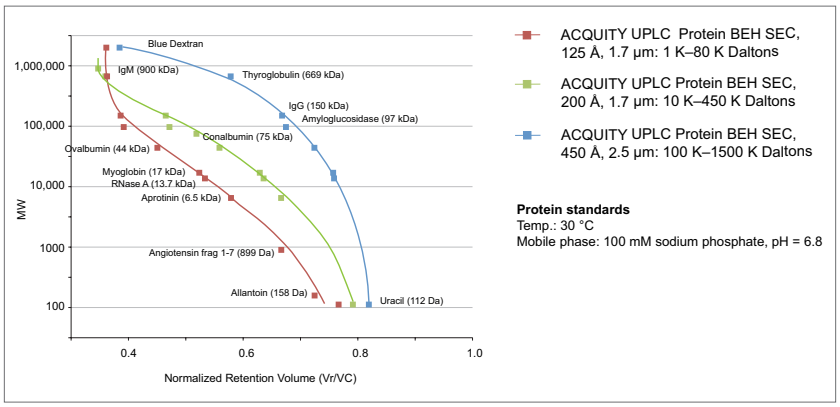
Straight Connection Tubing and End-fittings	WAT022681
U-Bend Connection Tubing and End-fittings	WAT084080

SEC Protein Standards are matched to the pore size of the column.
** Method Validation Kit (MVK) contains three columns from three dierent batches.

ACQUITY PROTEIN SEC OFFERINGS FOR HIGH THROUGHPUT SEC APPLICATIONS

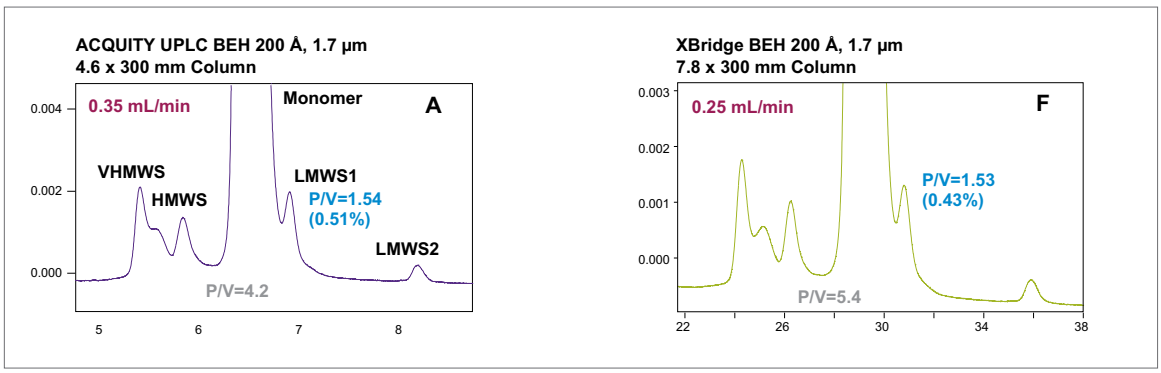
Waters™ ACQUITY™ UPLC™ Protein BEH SEC Guard, Column, and/or Standard Mix that are integral parts of Waters' ACQUITY UPLC SEC System solution. The ACQUITY UPLC Protein BEH SEC 125 Å offering is best suited for the analysis of peptides and proteins in the molecular weight range from 1000–80,000 Daltons that include insulin and its aggregates. The ACQUITY UPLC Protein BEH SEC 200 Å Column was designed to characterize proteins ranging in molecular weight range from 10,000–450,000 Daltons that include monoclonal IgG monomers from aggregates, while our ACQUITY UPLC Protein BEH SEC 450 Å is best suited for the analyses of proteins and conjugates that range from 100,000–1.5 million Daltons

Calibration Curves on ACQUITY UPLC Protein BEH SEC, 125 Å, 200 Å, and 450 Å Columns



All of these SEC chemistries are based on Waters' Ethylene Bridged Hybrid (BEH)-based particle technology and diol-bonded surface that provide a stable chemistry with minimal non-desired secondary interactions for proteins and peptides.

When Speed is Critical for SEC – Harnessing the power of UPLC



ACQUITY UPLC Technology allows scientists the ability to exceed what is obtainable using UHPLC or traditional HPLC separations by improving data quality while increasing sample throughput and productivity.

Ordering Information

ACQUITY UPLC Protein BEH SEC Columns, 1.7 and 2.5 µm

			P/N	P/N	P/N	P/N	P/N	P/N	P/N
Pore Size	MW Range	Particle Size	4.6 mm ID × Column Length						2.1 mm ID × CL
			30 mm Guard*	50 mm No Standard	150 mm No Standard	300 mm No Standard	150 mm w/Standard	300 mm w/Standard	50 mm No Standard
125 Å	1 K–80 K	1.7 µm	186006504	—	186006505	186006506	176003906	176003907	—
200 Å	10 K–450 K	1.7 µm	186005793	186009082	186005225	186005226	176003904	176003905	186008471
450 Å	100 K–1500 k	2.5 µm	186006850	—	186006851	186006852	176002996	176002997	—

Straight Connection Tubing and End-fittings	WAT022681
U-Bend Connection Tubing and End-fittings	WAT084080

SEC Protein Standards are matched to the pore size of the column.

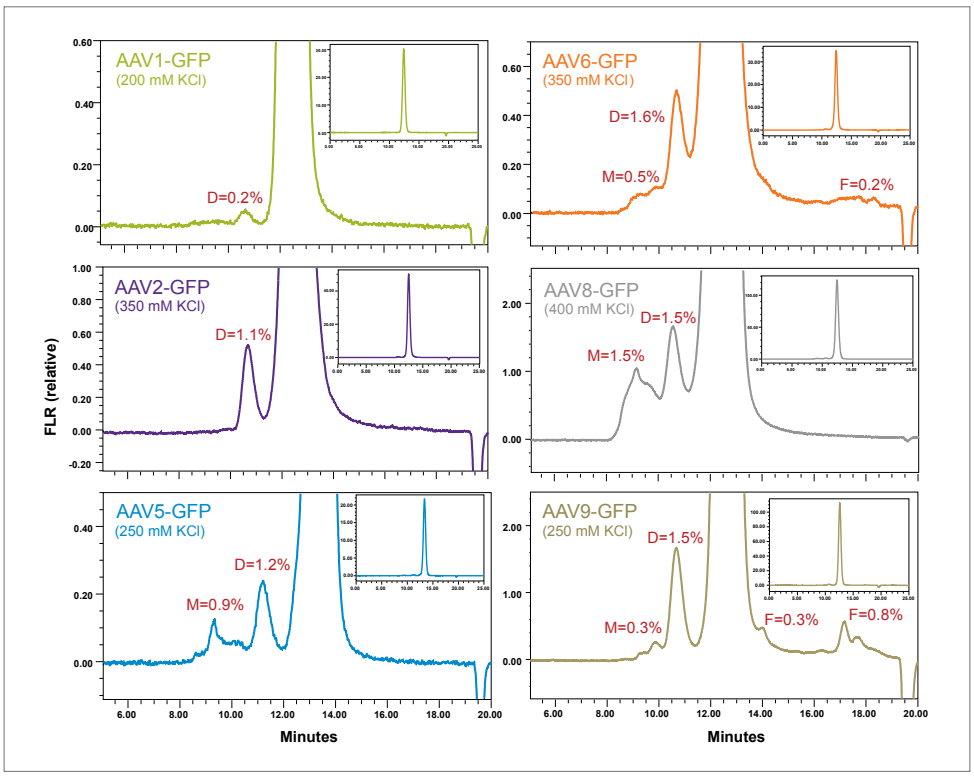
*Size-exclusion chromatography may require modifications to an existing ACQUITY UPLC System. Please reference "Size-Exclusion and Ion-Exchange Chromatography of Proteins using the ACQUITY UPLC System" (p/n: 715002147) or "Size Exclusion and Ion-Exchange Chromatography of Proteins using the ACQUITY UPLC H-Class System" (p/n: 715002909) for specific recommendations.

*To connect two UPLC SEC Columns together in series, we recommend using a Waters Sample Loop (p/n: 430001516).

XBRIDGE BEH SEC OFFERINGS

Waters™ XBridge™ Protein BEH SEC, 125 Å, 200 Å, and 450 Å, 2.5 and 3.5 µm Columns were developed to complement the existing line of UPLC™-based SEC offerings for use where traditional HPLC-based instrumentation and methods are employed for peptide or protein size-exclusion chromatography (SEC). These HPLC/UHPLC-based, SEC chemistries are based on the same Waters Ethylene Bridged Hybrid (BEH)-based particle technology and diol-bonded surface coating as used in our successful line of UPLC-based SEC columns. This process offers chromatographers the option and ability to easily transfer methods based on lab instrumentation and component resolution or sample throughput needs.

Adeno-Associated Virus (AAV) Analyses



XBridge BEH SEC 450 Å, 3.5 µm Columns effectively separate and quantitate AAV monomers from their HMW DII/3/2021 dimers, lower valency multimers, and LMW fragments.

Ordering Information

XBridge Protein BEH SEC Columns, 2.5 µm, UHPLC

Pore Size	MW Range	Particle Size	P/N	P/N	P/N	P/N	P/N	P/N
			4.6 mm ID × Column Length					
			30 mm Guard	150 mm No Standard	300 mm No Standard	30 mm Guard w/ Std	150 mm w/Standard	300 mm w/Standard
125 Å	1 K–80 K	2.5 µm	186009170	186009171	186009172	176004331	176004332	176004333
200 Å	10 K–450 K	2.5 µm	186009174	186009175	186009176	176004334	176004335	176004336
450 Å	100 K–1500 k	2.5 µm	186006850	186009179	186009180	176002995	176002996	176002997

Pore Size	MW Range	Particle Size	7.8 mm ID × Column Length					
			30 mm Guard No Standard	150 mm No Standard	300 mm No Standard	30 mm Guard w/Std	150 mm w/Standard	300 mm w/Standard
125 Å	1 K–80 K	2.5 µm	186009158	186009159	186009160	176004322	176004323	176004324
200 Å	10 K–450 K	2.5 µm	186009162	186009163	186009164	176004325	176004326	176004327

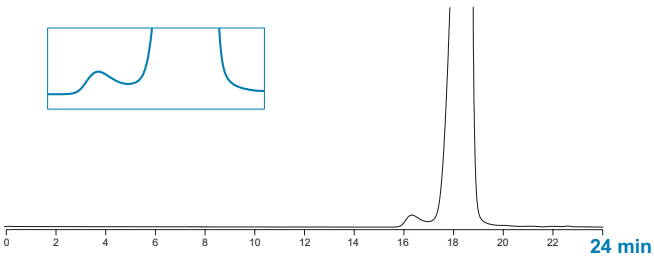
Straight Connection Tubing and End-fittings	WAT022681
U-Bend Connection Tubing and End-fittings	WAT084080

SEC Protein Standards are matched to the pore size of the column.

SEC ANALYSIS OF INSULIN

Size-exclusion chromatography (SEC) is the USP and EP standard method for the analysis of covalent HMW insulin in therapeutic preparations. Compared to use of traditional HPLC-based SEC methods, significant improvement in insulin component resolution, while reducing analysis time and mobile-phase consumption, is obtained using a Waters Protein BEH SEC, 125 Å, 1.7 µm Column with Waters UltraPerformance LC™ (UPLC) Instrumentation (shown below).

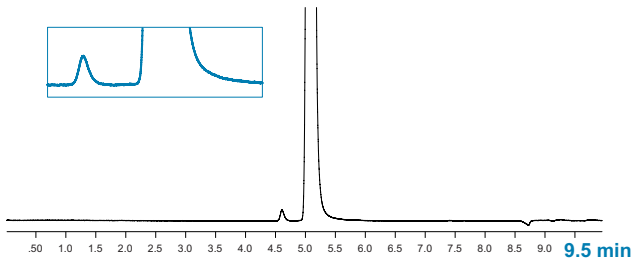
Insulin Analyses by Traditional HPLC-SEC vs. UPLC-SEC



LC Conditions

Column:	Insulin HMWP SEC, 125 Å, 10 µm, 7.8 × 300 mm (p/n: WAT201549)
Sample:	Insulin, human recombinant (Sigma I-2643) (4 mg/mL)
Injection:	100 µL
Eluent:	L-arginine (1 gm/liter)/acetic acid/acetonitrile (65/15/20, v/v/v)
Flow:	0.5 mL/min (isocratic)
UV detection:	276 nm

Alliance HPLC System



LC Conditions

Column:	ACQUITY UPLC Protein BEH SEC, 125 Å, 1.7 µm, 4.6 × 300 mm (p/n: 186006506)
Sample:	Insulin, human recombinant (Sigma I-2643) (4 mg/mL)
Injection:	5 µL
Eluent:	L-arginine (1 gm/liter)/acetic acid/acetonitrile (65/15/20, v/v/v)
Flow:	0.4 mL/min (isocratic)
UV detection:	276 nm

ACQUITY UPLC System

Compared to use of traditional HPLC-based SEC technology for the analysis of earlier eluting insulin aggregates from desired monomer species, Waters ACQUITY UPLC BEH SEC Technology delivers benefits of improved component resolution and in less time.

mAB SIZE VARIANT STANDARD

Waters mAb Size Variant Standard (p/n: 186009429) contains the NIST humanized monoclonal antibody (Reference Material 8671) and non-reduced IdeS digested NIST mAb fragments F(ab')2 (~100,000 Da) and (Fc/2)2 (~50,000 Da). By aliquoting small, standard amounts of IdeS fragments, Waters mAb size variant standard can be effectively used to test column and LC System ability to separate mAb aggregates, monomer, and fragments/clips via SEC.

Ordering Information

mAb Size Variant Standard

Description	P/N
mAb Size Variant Standard	186009429

PROTEIN STANDARDS

Each standard contains proteins selected for ACQUITY UPLC and XBridge Protein BEH SEC Columns. Use these standards for purposes of quality control, to test an HPLC or UPLC column, and to monitor column performance over time.

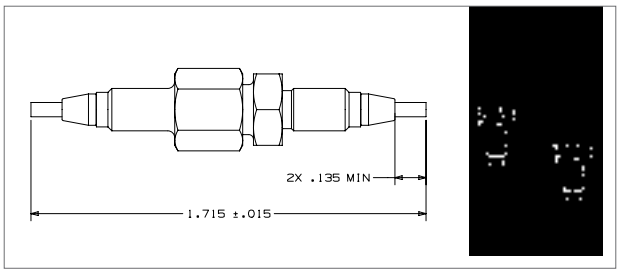
Ordering Information

BEH SEC Column Protein Standards

Description	P/N
BEH125 SEC Protein Standard Mix	186006519
A mix of four proteins: thyroglobulin, ovalbumin, ribonuclease A and uracil	
BEH200 SEC Protein Standard Mix	186006518
A mix of five proteins: thyroglobulin, IgG, BSA, myoglobin, uracil	
BEH450 SEC Protein Standard Mix	186006842
A mix of five proteins: thyroglobulin, IgG, BSA, myoglobin, uracil	

SEC COLUMN CONNECTORS AND CONNECTOR KITS

Connectors to attach BEH SEC columns in series and/or BEH SEC guards to BEH SEC columns.



Ordering Information

UPLC Column Connectors

Description	P/N
ACQUITY APC CM-S Column Connector, U, .004" I.D.*	700009535
ACQUITY APC CM-S Column Connector, Offset U, .004" I.D.*	700009534
ACQUITY APC CM-S Column Connector Tube, Long, .004" I.D.	700009560
ACQUITY APC CM-S Inline Column Connection, .005" I.D.	700009524
0.005 × 1.75 UPLC SEC Connection Tubing, 2/pk	186006613

* Ferrules are not staked on tubing upon receipt. The two-piece ferrule is permanently seated upon installation once the fitting is tightened into the column.

HPLC Column Connectors

Description	P/N
Column Joining Tube Assembly*	WAT084080
Rigid Connector Package*	WAT022681

*The ferrules are permanently seated to Waters' depth setting upon receipt.

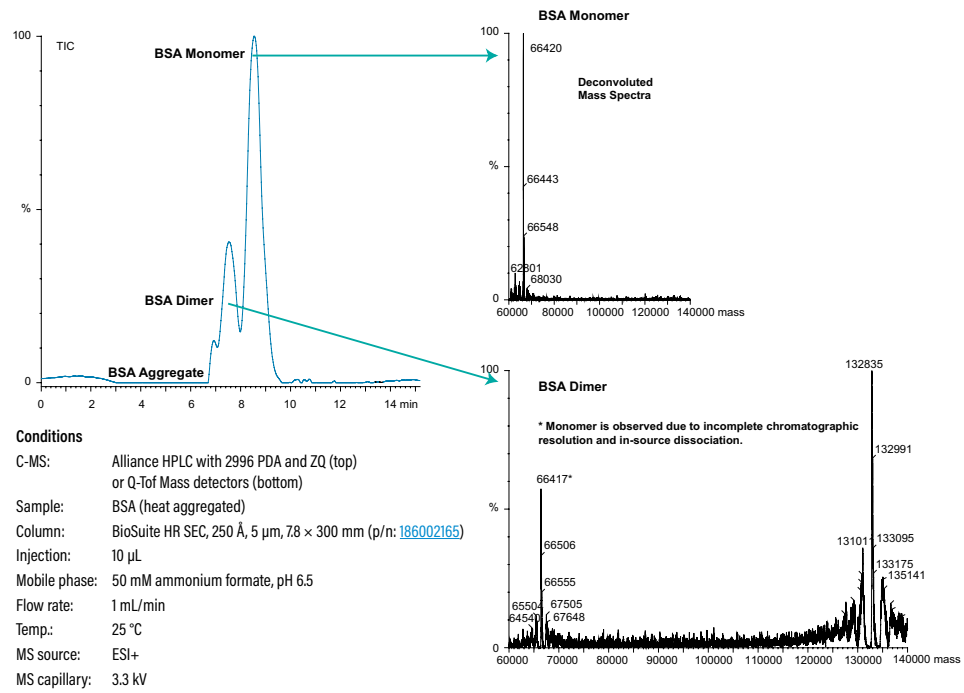
Connector Kits

Description	P/N
ACQUITY CM-S 4-Column Bank Connection Kit Kit contains: Two ACQUITY APC CM-S Inline Column Connector, .005" I.D. (p/n: 700009524) Two ACQUITY APC CM-S Column Connector, U, .004" I.D. (p/n: 700009535) One ACQUITY APC CM-S Column Connector, Offset U, .004" I.D. (p/n: 700009534)	205001172
ACQUITY CM-S 3-Column Bank Connection Kit Kit contains: One ACQUITY APC CM-S Inline Column Connector, .005" I.D. (p/n: 700009524) Two ACQUITY APC CM-S Column Connector, U, .004" I.D. (p/n: 700009535)	205001171
ACQUITY CM-S 2-Column Bank Connection Kit Kit contains: One ACQUITY APC CM-S Inline Column Connector, .005" I.D. (p/n: 700009524) One ACQUITY APC CM-S Column Connector, U, .004" I.D. (p/n: 700009535)	205001169

BioSuite Size-Exclusion (SEC) HPLC Columns

BioSuite ultra-high resolution (UHR), high resolution (HR), and standard size-exclusion column packings use a rigid yet "wettable" silica-based media that is stable from pH 2.5–7.5. As indicated in the calibration curve tables, the exclusion limits of BioSuite SEC packings are determined by the particle and pore size of the silica-based material. Particle size of the SEC packing media as well as column length are important parameters that determine separation efficiency. BioSuite 4 µm particle size, UHR Columns provide maximum separation efficiency, followed by BioSuite HR Columns and BioSuite Standard SEC Columns. To maximize column life of analytical (i.e., 4.6 mm or 7.8 mm I.D.) or preparative (i.e., 21.5 mm I.D.) SEC Columns, use of BioSuite Guard Columns is recommended.

LC-MS Analysis of BSA Aggregation Using BioSuite HR SEC, 250 Å, 5 µm Column SEC Column

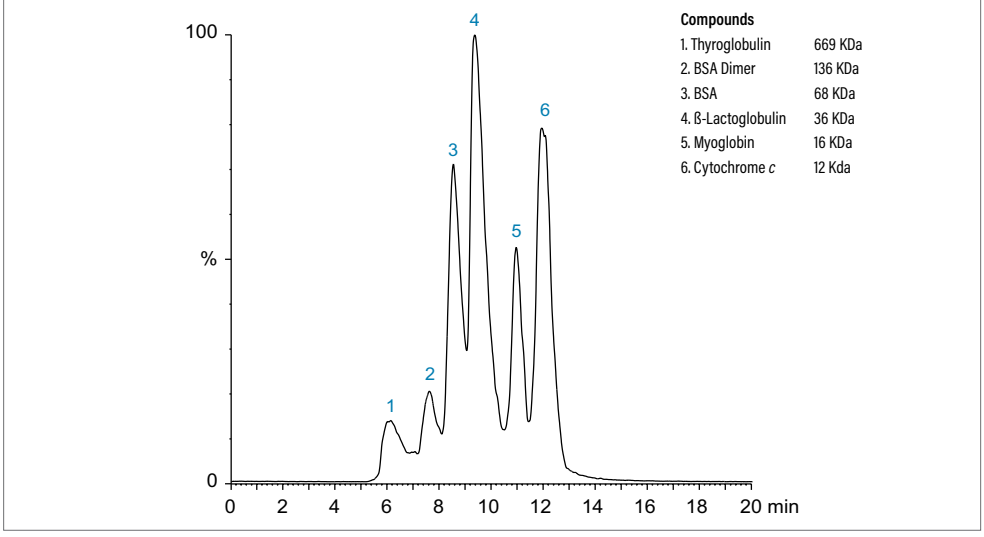


Conditions

C-MS:	Alliance HPLC with 2996 PDA and ZQ (top) or Q-Tof Mass detectors (bottom)
Sample:	BSA (heat aggregated)
Column:	BioSuite HR SEC, 250 Å, 5 µm, 7.8 × 300 mm (p/n: 186002165)
Injection:	10 µL
Mobile phase:	50 mM ammonium formate, pH 6.5
Flow rate:	1 mL/min
Temp.:	25 °C
MS source:	ESI+
MS capillary:	3.3 kV
MS cone:	30 V
Mass range:	400–3000 *m/z*

SEC is an effective technique to separate and quantitate higher molecular weight protein aggregates from lower molecular weight monomers using optical detection. Use of MS-compatible SEC eluents provides an additional dimension of useful data by providing real time mass data of the separated protein components.

LC-MS Analysis of Protein Standards Using BioSuite HR SEC, 250 Å, 5 µm Column (LC-MS conditions as above)

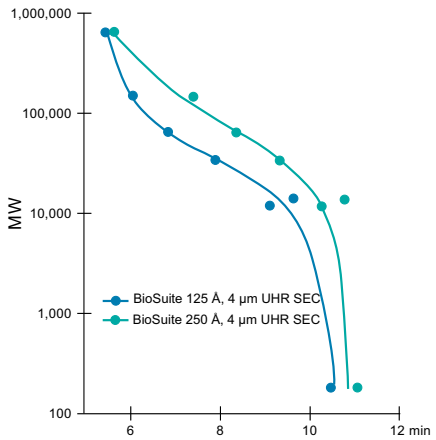


Compounds

1. Thyroglobulin	669 KDa
2. BSA Dimer	136 KDa
3. BSA	68 KDa
4. ß-Lactoglobulin	36 KDa
5. Myoglobin	16 KDa
6. Cytochrome *c*	12 Kda

BioSuite SEC Reference: SEC-MS Analysis of Aggregates in Protein Mixtures. Application Book Supplement of LC/GC Europe. Sept. 2003. (Waters Literature Reference: 720000743EN)

Protein Calibration Curves for BioSuite Ultra-High Resolution (UHR) SEC Columns



Conditions

Sample: Thyroglobulin (670,000 Da), gamma globulin (155,000 Da), bovine serum albumin (66,330 Da), beta lactoglobulin (18,400 Da), lysozyme (14,300 Da), cytochrome *c* (12,400 Da), triglycine (189 Da)

Columns: BioSuite UHR SEC, 250 Å, 4 µm, 4.6 × 300 mm (p/n: 186002162)
BioSuite SEC, 125 Å, 4 µm, 4.6 × 300 mm (p/n: 186002161)

Eluent: 0.15 M sodium phosphate, pH 6.8

Flow rate: 0.35 mL/min

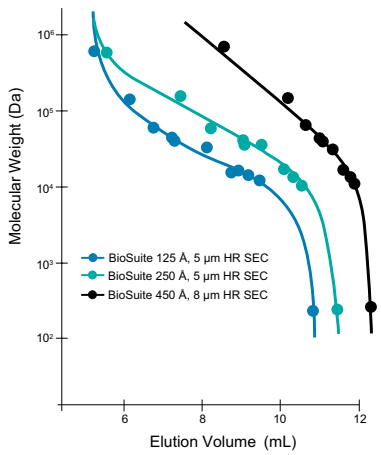
Column temp.: 25 °C

UV detection: 220 nm

Column	Globular Protein MW Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125 Å	5000–150,000	1000–30,000	500–15,000
BioSuite 250 Å	10,000–500,000	2000–70,000	1000–35,000

Note: Operating flow rates for BioSuite Ultra-High Resolution (UHR) SEC Columns (4.6 mm) are from 0.1–0.4 mL/min. Use of an HPLC system (e.g. Waters Alliance HPLC System) capable of operating at these flow rates is essential for optimal UHR SEC Column performance.

Protein Calibration Curves for BioSuite High Resolution (HR) SEC Columns



Conditions

Sample: Thyroglobulin (MW 670,000 Da), IgG (MW 156,000 Da), BSA (66,330 Da), ovalbumin (MW 43,000 Da), peroxidase (40,200 Da), beta lactoglobulin (MW 18,400 Da), myoglobin (MW 16,900 Da), ribonuclease A (MW 13,700 Da), cytochrome *c* (12,400 Da), glycine tetramer (246 Da)

Columns: BioSuite HR SEC, 450 Å, 8 µm, 7.8 × 300 mm (p/n: 186002166)
BioSuite HR SEC, 250 Å, 5 µm, 7.8 × 300 mm (p/n: 186002165)
BioSuite SEC, 125 Å, 5 µm, 7.8 × 300 mm (p/n: 186002164)

Eluent: 0.1 M sodium phosphate, pH 7.0 containing 0.3 M sodium chloride

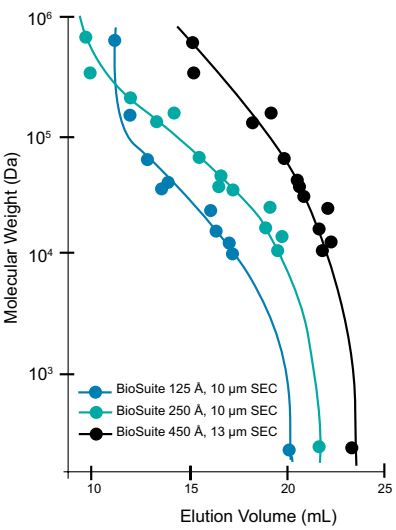
Flow rate: 1.0 mL/min

Column temp.: 25 °C

UV detection: 220 nm

Column	Globular Protein MW Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125 Å	5000–150,000	1000–30,000	500–15,000
BioSuite 250 Å	10,000–500,000	2000–70,000	1000–35,000
BioSuite 450 Å	20,000–1,000,000	4000–500,000	2000–250,000

Protein Calibration Curves for BioSuite Standard SEC Columns



Conditions

Sample: Thyroglobulin (MW 670,000 Da), IgG (MW 156,000 Da), BSA (66,330 Da), ovalbumin (MW 43,000 Da), peroxidase (40,200 Da), beta lactoglobulin (MW18,400 Da), myoglobin (MW 16,900 Da), ribonuclease A (MW 13,700 Da), cytochrome *c* (12,400 Da), glycine tetramer (246 Da)

Columns: BioSuite SEC, 450 Å, 13 µm, 7.5 × 300 mm (p/n: 186002172)
BioSuite SEC, 250 Å, 13 µm, 7.5 × 300 mm (p/n: 186002170)
BioSuite SEC, 125 Å, 10 µm, 7.5 × 300 mm (p/n: 186002168)

Eluent: 0.1 M sodium phosphate, pH 7.0 containing 0.3 M sodium chloride

Flow rate: 1.0 mL/min

Column temp.: 25 °C

UV detection: 220 nm

Column	Globular Protein MW Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125 Å	5000–150,000	1000–30,000	500–15,000
BioSuite 250 Å	10,000–500,000	2000–70,000	1000–35,000
BioSuite 450 Å	20,000–1,000,000	4000–500,000	2000–250,000

BioSuite 4.6 mm, 7.8 mm, and 7.5 mm analytical and 21.5 mm preparative columns are available.

Ordering Information

BioSuite SEC HPLC and UHPLC Columns

Description	Matrix	Diameter Width	Diameter Length	Column Volume	Suggested Volume Load for Maximum Multicomponent Resolution*	Multicomponent Resolution**	P/N
BioSuite 125 Å, 4 µm UHR SEC	Silica	4.6 mm	300 mm	4.98 mL	Less than 8 mg/mL	Less than 40 µL	186002161
BioSuite 250 Å, 4 µm UHR SEC	Silica	4.6 mm	300 mm	4.98 mL	Less than 8 mg/mL	Less than 80 µL	186002162
BioSuite UHR Guard SEC	Silica	4.6 mm	35 mm	—	—	—	186002163
BioSuite 125 Å, 5 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002164
BioSuite 250 Å, 5 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002165
BioSuite 450 Å, 8 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002166
BioSuite HR Guard SEC	Silica	6 mm	40 mm	—	—	—	186002167
BioSuite 125 Å, 10 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002168
BioSuite 125 Å, 13 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mL	186002169
BioSuite 250 Å, 10 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002170
BioSuite 250 Å, 13 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mL	186002171
BioSuite 450 Å, 13 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002172
BioSuite 450 Å, 17 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mL	186002173
BioSuite Guard SEC	Silica	7.5 mm	75 mm	—	—	—	186002174
BioSuite Guard SEC	Silica	21.5 mm	75 mm	—	—	—	186002175

* Using a BSA protein standard in a 50 mM phosphate buffer containing salt (either 0.1 M NaCl or 0.1 M Na_2SO_4) eluent. Useful protein mass loads will vary depending upon separation eluent, complexity of sample, and type of proteins contained in mixture. In general, maximum component resolution is obtained by injecting the smallest possible volume of a dilute protein solution.

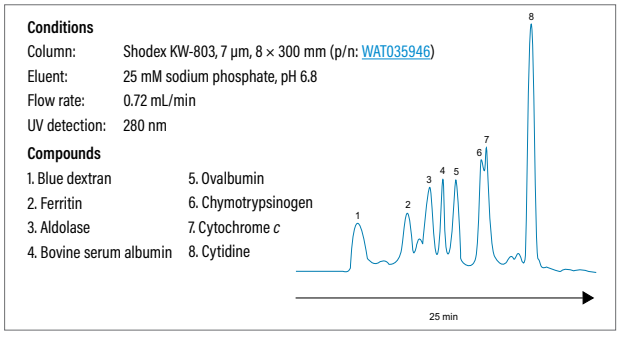
** Operating flow rates for BioSuite Ultra-High Resolution (UHR) SEC Columns (4.6 mm I.D.) are from 0.1–0.4 mL/min. Use of an HPLC system (e.g., Waters Alliance HPLC System) capable of operating at these flows is essential for optimal UHR SEC Column performance.

Protein-Pak and Shodex Size-Exclusion HPLC Columns

Waters offers two families of packings for size-exclusion chromatography. Protein-Pak packings are based on a 10 µm, diol-bonded silica and are available in a selection of pore sizes and column configurations. In addition, Waters offers a series of Shodex 7 µm, high-resolution, gel filtration packings.

The Protein-Pak Size-exclusion Columns can be expected to resolve proteins that differ in molecular weight by a factor of two and to distinguish proteins differing by as little as 15% in molecular weight. The degree of resolution is more dependent on the sample mass and volume than the interaction between the sample and the stationary phase. Ideally, there should be no interaction between the stationary phase and the sample molecules. Secondary interactions are most often ionic and can, therefore, be reduced by increasing the ionic strength of the mobile phase. Typical, salt concentrations range to 0.2–0.5 M NaCl. It may also be useful in some cases to consider adding 10–20% methanol to eliminate hydrophobic and other hydrogen-bonding interactions.

Standard Protein Mix on KW-803 Column



This gel-filtration separation of protein standards demonstrates the ability to separate proteins in a wide range of molecular weights in minutes for high sensitivity analysis or protein isolation up to the milligram scale.

Ordering Information

Protein-Pak SEC HPLC Columns and Guards

Steel Column	Dimension	MW Range	P/N
Protein-Pak 60	7.8 × 300 mm	1000–20,000	WAT085250
Protein-Pak 60	19 × 300 mm	1000–20,000	WAT025830
Protein-Pak 125	7.8 × 300 mm	2000–80,000	WAT084601
Protein-Pak 125	19 × 300 mm	2000–80,000	WAT025831
Protein-Pak 300SW	7.5 × 300 mm	10,000–300,000	WAT080013
Protein-Pak 125 Sentry Guard Column, 3.9 × 20 mm, 2/pk (requires holder)			186000926
Sentry Universal Guard Column Holder			WAT046910

Glass Column	Dimension	MW Range	P/N
Protein-Pak 200SW	8 × 300 mm	500–60,000	WAT011786
Protein-Pak 300SW	8 × 300 mm	10,000–300,000	WAT011787

Shodex Size-Exclusion and Anion-Exchange HPLC and UHPLC Columns

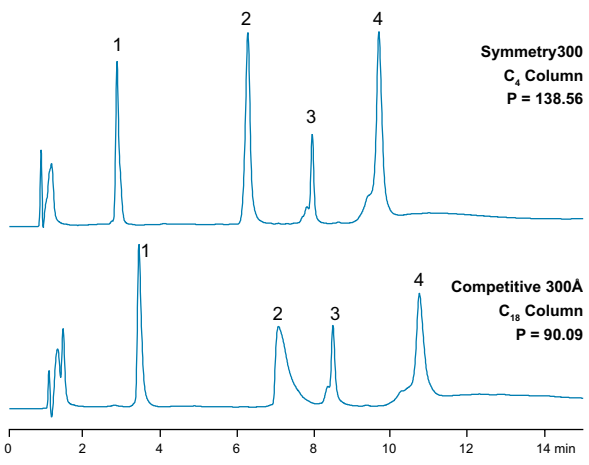
Description	Particle Size	Dimension	MW Range	P/N
Protein KW-802.5	7 µm	8 × 300 mm	100–50,000	WAT035943
Protein KW-803	7 µm	8 × 300 mm	100–150,000	WAT035946
Protein KW-804	7 µm	8 × 300 mm	500–600,000	WAT036613

Symmetry300 C_4 HPLC and UHPLC Columns

Compared to our Protein BEH C_4, 300 Å offerings, Symmetry300 C_4 particles are 100% silica-based and are synthesized using ultrapure organic reagents resulting in high-purity material with very low silanol activity for outstanding peptide and protein separations and recoveries.

- 300 Å pore for peptide and protein applications
- Fully endcapped to minimize undesired secondary interactions
- Alternative separation selectivity compared to Waters BEH C_4, 300 Å hybrid material
- QC tested with peptide samples to help ensure excellent batch-to-batch consistency

Protein: Symmetry300 C_4 vs. Competitors



Conditions

Mobile phase A:	0.1% trifluoroacetic acid in water
Mobile phase B:	0.08% trifluoroacetic acid in acetonitrile
Flow rate:	0.75 mL/min
Gradient:	Time (min) / %A / %B 0.00 / 80 / 20 15.00 / 35 / 65
Injection vol.:	20 µL
Column temp.:	40 °C
UV detection:	220 nm
System:	Alliance 2790, 996 PDA

Compounds

1. RNAse
2. BSA
3. B-Lactoglobulin
4. Ovalbumin

Compared to many competitive 100% silica-based C_{18} columns, Waters proprietary bonding and end-capping technologies help deliver improved peak shape with less undesired tailing.

Ordering Information

Symmetry300 HPLC and UHPLC Columns

C_4	Particle Size: 3.5 µm		Particle Size: 5 µm	
	Dimension	P/N	Dimension	P/N
	2.1 × 150 mm	186000276	2.1 × 150 mm	186000285
	3.9 × 150 mm	186000277	3.9 × 150 mm	186000286
	4.6 × 50 mm	186000278	4.6 × 50 mm	186000287
	4.6 × 150 mm	186000279	4.6 × 150 mm	186000288
	4.6 × 250 mm	186000280	4.6 × 250 mm	186000289
	19 × 10 mm	186000281		
	19 × 50 mm	186000282		
	19 × 100 mm	186000283		

CHARGE VARIANT AND ION-EXCHANGE ANALYSIS

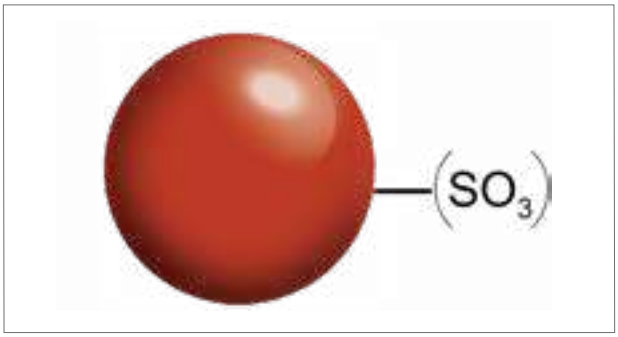
Ion-Exchange (IEX) separations are most commonly performed using gradients of increasing salt, changing pH, or simultaneous salt increases and pH changes with less charged protein species eluting prior to more highly charged molecules. Based on protein type and separation pH, either an anion or cation exchanger is selected for the separation. In addition, gradient duration, buffer composition and pH, flow rate, as well as separation temperature all play an important part in obtaining needed protein separations.

BioResolve SCX mAb Columns

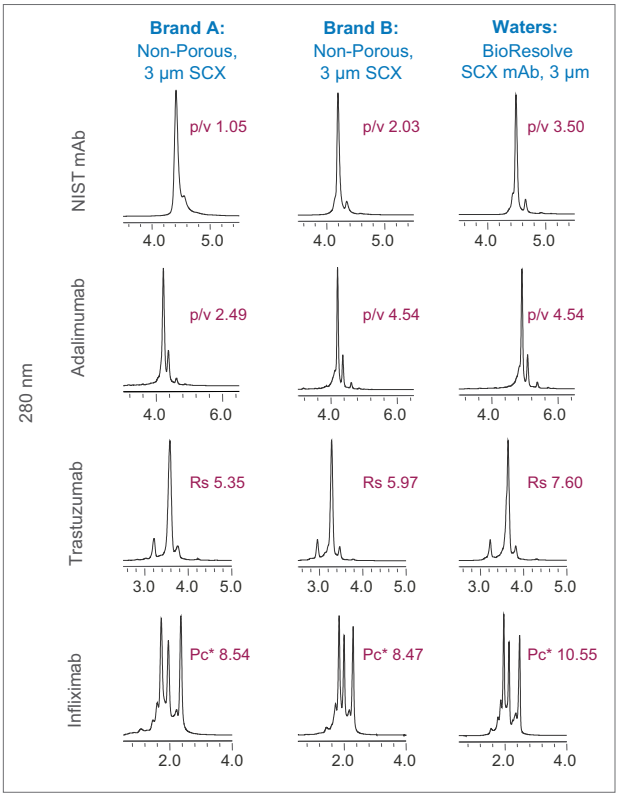
Charge variant profiling is one of several important characterization techniques performed on monoclonal antibody biotherapeutics. To help ensure that reliable results are obtained in these types of analyses, Waters developed corrosion–resistant columns containing BioResolve SCX mAb, non-porous, polymer-based particles grafted with a rigorously-optimized, multi-component network of negatively charged sulfonic acid ligands. This innovative column technology delivers high-resolution, charged-based separations of mAbs in both LC and LC-MS applications using both salt and pH gradient elution.

Benefits include:

- Strong-cation exchanger based on non-porous (NP) polymeric particles that deliver high mechanical strength and chemical tolerance for LC or LC-MS charge based separations
- Developed through extensive prototyping and comprehensive testing with a wide range of mAbs and separations based on both salt and pH-gradient chromatography
- Based on a non-porous, 3 µm particle for optimal diffusion kinetics; high pressure capability; and amenability to HPLC, UHPLC, and UPLC systems
- Quality-control tested with the mAb Charge Variant Standard (derived from NIST mAb Reference Material 8671) to help ensure batch-to-batch column consistency

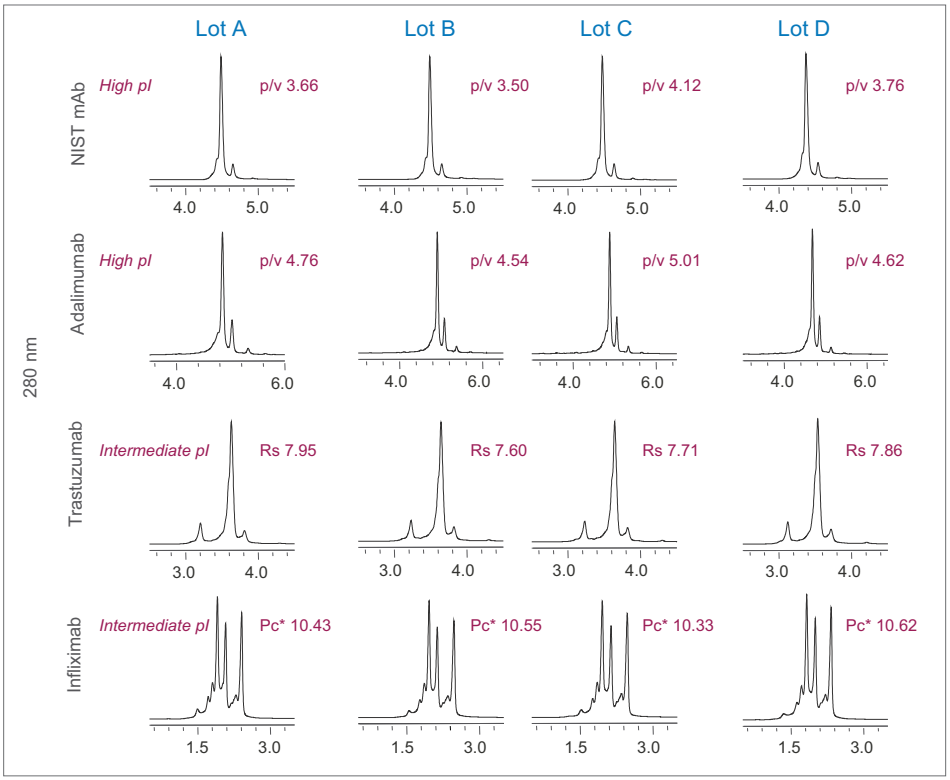


LC Analysis of Monoclonal Antibodies - BioResolve SCX mAb Column vs. Commercially Available, Non-Porous, Cation-Exchange Columns



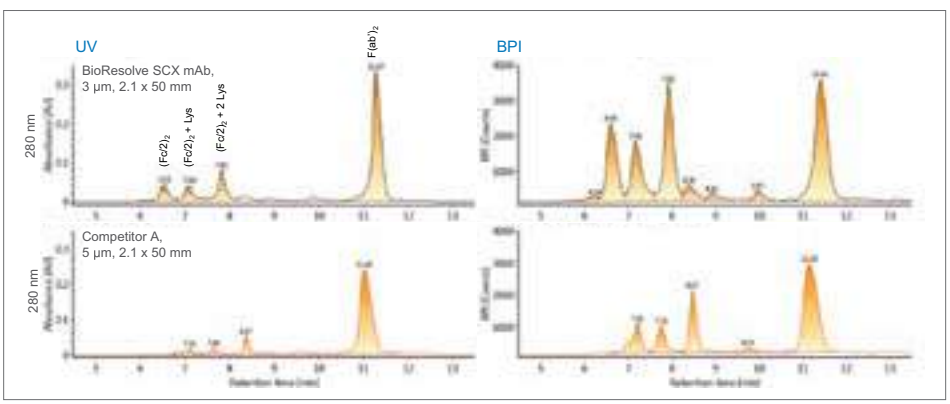
Comparative peak valley (P/V) ratios, component resolution (Rs), and measured peak capacities of four biotherapeutic antibodies separated on Waters vs. commercially available, cation-exchange columns noting higher quality data obtained on a BioResolve SCX mAb, 3 µm Column. All separations were performed at 30 ˚C on an ACQUITY UPLC H-Class Bio System at the same linear velocity (i.e., 0.72 mL/min for 4.6 × 50 mm and 0.54 mL/min for 4 × 50 mm columns) with appropriately scaled injection volumes using a 10 min linear gradient from 10 mm to 200 mM NaCl contained in 20 mM MES, pH 7 buffer.

Outstanding Batch-to-Batch Reproducibility of BioResolve SCX mAb Cation-Exchange Columns in the Analysis of Four mAbs



Comparative peak valley (P/V) ratios, component resolutions (Rs), and measured peak capacities of four monoclonal antibodies on four different manufactured batches of BioResolve SCX mAb, 3 µm, 4.6 × 50 mm Columns. All separations were performed at 30 ˚C on an ACQUITY UPLC H-Class Bio System at 0.72 mL/min using a 10 min linear gradient from 10 mm to 200 mM NaCl contained in 20 mM MES, pH 7 buffer.

LC-MS Analysis of IdeS Digested Infliximab on a BioResolve SCX mAb Column vs. an Alternative Commercially Available, Non-Porous, Cation-Exchange Column



Higher resolution and higher recovery separations using volatile, MS-compatible mobile phases and a BioResolve SCX mAb, 3 µm, 2.1 × 50 mm Column. Separations were performed at 30 ˚C on an ACQUITY UPLC I-Class System at 0.11 mL/min using an 18.3 min linear gradient from 15–50% buffer B (buffer A: 50 mM ammonium formate, pH 3.9 and buffer B: 500 mM ammonium acetate, pH 7.4).

mAb Charge Variant Standard

The mAb Charge Variant Standard is a proficiency and suitability standard used to confirm and monitor column and instrument performance. This standard is formulated as a filtered and stabilized mixture of a void marker (tryptophan), conalbumin from chicken egg white, and NIST Reference Material 8671 (NIST mAb, a humanized IgG1κ expressed from a murine cell line). Every vial contains approximately 0.5 µg of tryptophan, 200 µg of conalbumin, and 100 µg of NIST mAb. Shown on the right is a pH-gradient chromatogram example of the mAb Charge Variant Standard as obtained with BioResolve CX pH Concentrates.

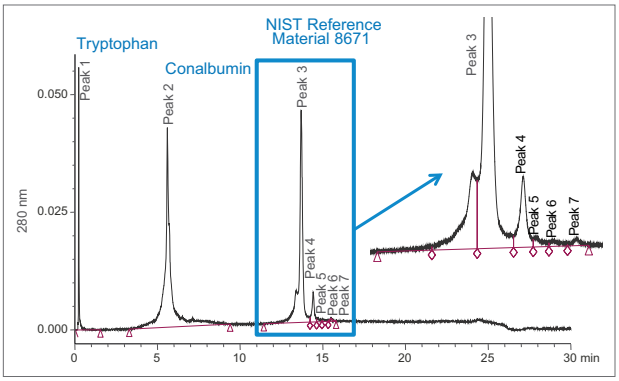
VanGuard FIT Cartridge

The injection of column fouling excipients (e.g., polysorbate) and particles (e.g., insoluble sample or microbes) is known to cause the premature failure of columns. Careful sample and eluent preparation helps address this concern. Yet experienced chromatographers recognize the value of using a guard column, containing the same material as the analytical column, to further help ensure maximum column life. Traditional guard columns help protect the analytical column. However, they are relatively expensive and introduce compromising levels of additional dispersion.

To address these shortcomings, Waters has enhanced the value of the existing VanGuard Technology by introducing the novel VanGuard Fully Integrated Technology (FIT) Cartridge - a, simplified guard column design that maximizes column life without degrading biomolecule component resolution. Based on customer preference, the BioResolve SCX mAb Column can be purchased with or without a VanGuard FIT Cartridge.[1, 2]

[1] The VanGuard FIT Cartridge contains the same BioResolve SCX mAb, 3 µm material as used in an analytical BioResolve SCX mAb Column.
[2] Replacement BioResolve SCX mAb, 3 µm VanGuard FIT Cartridges cannot be used on columns that lack the VanGuard FIT Cartridge option.

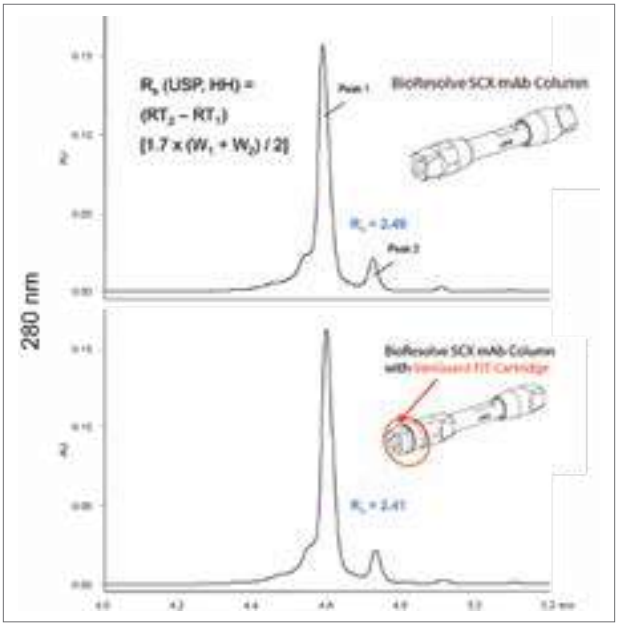
Separation of Waters mAb Charge Variant Standard on a BioResolve SCX mAb, 3 µm Column



Separation of the mAb Charge Variant Standard on a BioResolve SCX mAb, 3 µm, 4.6 × 50 mm Column with a VanGuard FIT Cartridge showing excellent resolution of various mAb charge variant species. Separation was performed on an ACQUITY UPLC H-Class System at 30 °C and at 1.44 mL/min using a 24 min linear gradient from pH 5 to 10.2.

*The interpretation of charge variants was extrapolated from BioDrugs, 2016, 30, 321–338.

No Compromise Column Protection and Extended Lifetimes with VanGuard FIT Enhancement



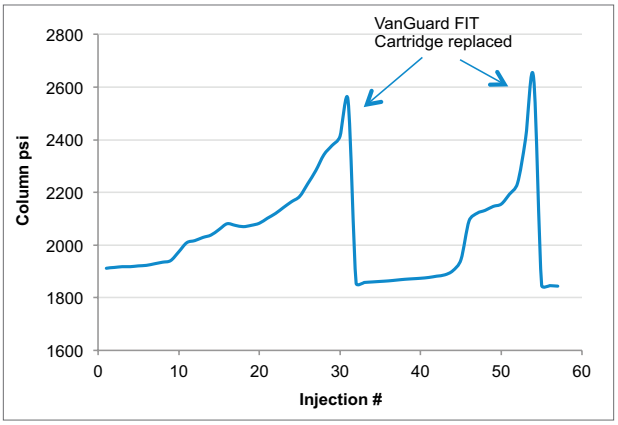
Separation of NIST mAb Reference Material 8671 (12.5 µg injected) on a BioResolve SCX mAb, 3 µm, 4.6 × 50 mm Column with and without an Integrated VanGuard FIT Cartridge. All separations were performed on an ACQUITY UPLC H-Class Bio System at 0.96 mL/min using a 7.5 min linear gradient from 10 mm to 200 mM NaCl contained in 20 mM MES, pH 6 buffer.

When chromatography degrades from unintentional fouling (e.g., injections of particulates originating from a sample, LC system, and/or mobile phase), the VanGuard FIT Cartridge can be easily changed with available replacements to restore column performance and extend the life of the analytical column.

BioResolve CX pH Buffers

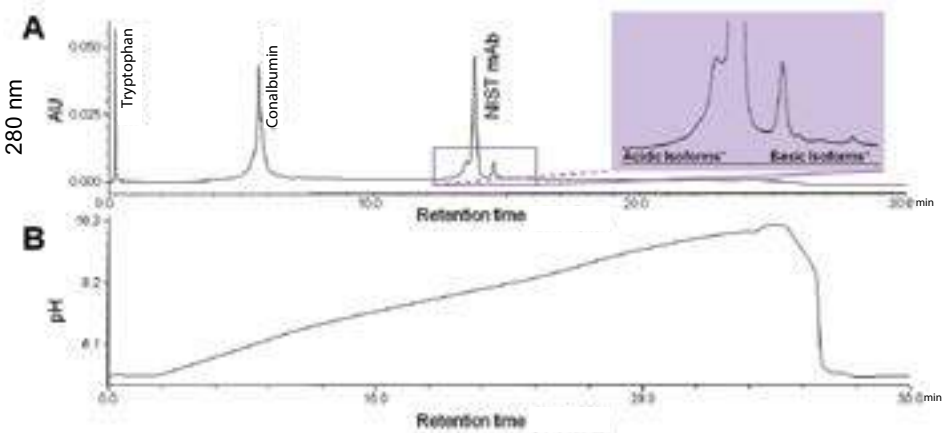
While ion-exchange chromatography using a gradient of increasing salt concentration is commonly used for charge variant profiling of mAb-based therapeutics, it often requires the optimization of methods for each individual sample. By comparison, use of a more universal pH gradient offers the potential of being applicable to many different samples. The BioResolve CX pH Concentrates facilitate obtaining high-resolution separations with BioResolve SCX mAb, 3 µm Columns. Together, the two technologies provide a robust and simple-to-use pH gradient based method for charge variant analysis of different mAb species. Each set of the carefully formulated concentrates was designed so users can quickly prepare mobile phases of controlled pH and ionic strength to yield robust cation-exchange separations. Each concentrate is accurately packaged as a 100 mL volume of a 10x concentrated solution that can be prepared into 1 L of mobile phase by means of a simple 10-fold aqueous dilution. The resulting buffers can be used in a universally applicable binary gradient separation method that runs from pH 5.0 to 10.2.

Extension of BioResolve SCX mAb Column by Replacement of VanGuard Fit Cartridge on Particulate Fouled Column



Repeated 5 µL injections of 20 mM sodium phosphate, pH 6.8 containing 0.1 µm latex particles onto a BioResolve SCX mAb, 3 µm, 4.6 × 50 mm Column with VanGuard FIT. Testing was performed on an ACQUITY UPLC H-Class System at 0.50 mL/min using 20 mM sodium phosphate, pH 6.8 with injections made every 5 min noting pressure increases that were reduced when the existing VanGuard FIT Cartridge was replaced with a new one at injections #30 and #54. Note: 0.1 µm latex particles were selected due to their size being similar to bacterial cells (0.2 to 10 µm) that are a potential source of column fouling if present in eluents that lack bacteriostatic agents.

Separation of mAb Charge Variant Standard on a BioResolve SCX mAb, 3 µm Column Using a Turn-Key pH Gradient Generated Using BioResolve CX pH Concentrates



Representative ion-exchange chromatogram (A) and pH trace (B) for a separation of the mAb Charge Variant Standard (p/n: 186009065) on a BioResolve SCX mAb, 3 µm, 4.6 × 50 mm Column. The data was obtained at 30 ˚C on an ACQUITY UPLC H-Class Bio System using a 24 min linear pH gradient from pH 5.0 to 10.2 at a flow rate of 1.44 mL/min. Note: the pH trace was obtained with GE Healthcare Life Sciences Monitor pH/C-900.

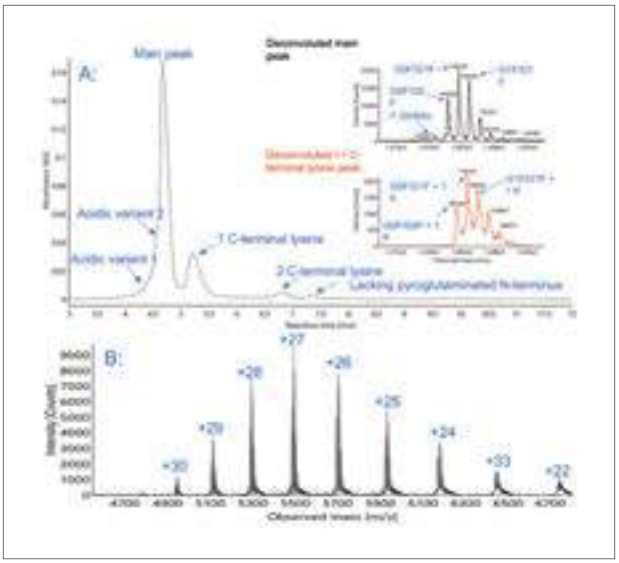
LC-MS ANALYSIS WITH IONHANCE CX-MS PH BUFFERS

Native protein analysis by cation-exchange chromatography coupled to mass spectrometry (CX-MS) is a robust way to characterize microheterogeneities in biopharmaceuticals, particularly monoclonal antibodies (mAbs), from research through final commercialization. To address this need, Waters developed IonHance CX-MS pH Buffers (Concentrates A and B) for use with the BioResolve SCX mAb, 3 µm Columns.

The IonHance CX-MS pH 10x concentrates were purposely designed to deliver robust, charge-based separations and high-quality MS spectral data. They are shipped as 100 mL aliquots in 1 L trace metal certified low-density polyethylene bottles (confirmed <100 ppb levels of sodium, potassium, and calcium). Concentrate A is formulated to yield a pH 5.0 mobile phase and Concentrate B is formulated to generate a higher ionic strength pH 8.5 mobile phase. Both concentrates are prepared with 20% acetonitrile to minimize bacterial growth.

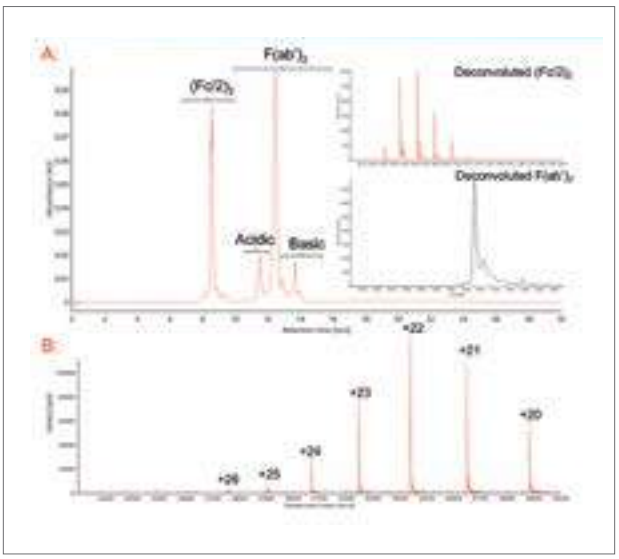
When running IonHance CX-MS buffers, it is recommended to use the Humanized mAb Mass Check Standard (p/n: 186009125) as a system QC and not the mAb Charge Variant Standard (p/n: 186009065). The mAb Charge Variant Standard is a mix of NIST mAb, conalbumin amd tryptophan. The conalbumin peak is not well resolved using the IonHance CX-MS buffers.

LC-MS Analysis of Intact Adalimumab Using a BioResolve SCX mAb Column and IonHance CX-MS pH Buffer



Representative UV chromatogram (280 nm) of adalimumab with deconvolution of base and +1 lysine (K) peak between 147.8–148.8 kDa, as well as mass spectra for base peak with m/z window of 4600–7000. The data were obtained with a BioAccord System comprised of an ACQUITY UPLC I-Class PLUS System coupled to an ACQUITY TUV Detector that was set to 280 nm, fitted with a 2.1 × 50 mm BioResolve SCX mAb Column. Buffer A = 10 mM ammonium acetate, pH 5.00. Buffer B = 75 mM ammonium acetate, pH 8.38.

LC-MS Analysis of Non-Reduced IdeS Digested Trastuzumab



(A) Representative UV chromatogram of IdeS digested Trastuzumab with deconvolution of main (Fc/2)2 peak between 50.1–52.0 kDa and the main F(ab')2 peak between 97.0–98.0 kDa.(B) Mass spectra for primary F(ab')2 peak with m/z window of 3200–5000. The data were obtained with a BioAccord System comprised of an ACQUITY UPLC I-Class PLUS System coupled to an ACQUITY RDa Mass Detector fitted with a BioResolve SCX mAb, 2.1 × 50 mm Column.

Ordering Information

BioResolve SCX mAb Columns, Method Validation Kits, Cartridges, and Standards

Column	Particle Size: 3 µm		
	Dimension	P/N (1/pk) with VanGuard FIT and mAb Charge Variant Standard	P/N (1/pk) with mAb Charge Variant Standard
	2.1 × 50 mm	176004341	176004342
	2.1 × 100 mm	176004343	176004344
	4.6 × 50 mm	176004346	176004347
	4.6 × 100 mm	176004348	176004349

Method Validation Kit*	Particle Size: 3 µm		
	Dimension	P/N (3/pk) with VanGuard FIT and mAb Charge Variant Standard	P/N (3/pk) with mAb Charge Variant Standard
	2.1 × 100 mm	176004345	–
	4.6 × 100 mm	–	176004350

Description	P/N
BioResolve SCX mAb VanGuard FIT Cartridge, 3 µm, 3.9 × 5 mm, 3/pk**	186009062
BioResolve SCX mAb VanGuard FIT Replacement Cartridge, 3 µm, 2.1 × 5 mm, 3/pk**	186009061
mAb Charge Variant Standard	186009065
BioResolve CX pH Concentrate A, pH 5 (100 mL bottle of 10x concentrate)	186009063
BioResolve CX pH Concentrate B, pH 10.2 (100 mL bottle of 10x concentrate)	186009064
BioResolve CX pH Concentrate Kit	176004340
Certified LDPE Container, 1000 mL (2/pk)	186009110

* Method Validation Kit (MVK) contains three columns from three different batches.
**VanGuard FIT Replacement Cartridges can ONLY be used on BioResolve SCX mAb Columns that have VanGuard FIT component.

BioResolve SCX mAb Startup Kits

Description	P/N
BioResolve SCX mAb, 3 µm, 2.1 × 50 mm Column w/ VanGuard FIT Cartridge; mAb Charge Variant Standard; BioResolve SCX pH Concentrates; and two Certified LDPE Containers	176004351
BioResolve SCX mAb, 3 µm, 2.1 × 50 mm Column; mAb Charge Variant Standard; BioResolve SCX pH Concentrates; and two Certified LDPE Containers	176004355
BioResolve SCX mAb, 3 µm, 2.1 × 100 mm Column w/VanGuard FIT Cartridge; mAb Charge Variant Standard; BioResolve SCX pH Concentrates; and two Certified LDPE Containers	176004352
BioResolve SCX mAb, 3 µm, 2.1 × 100 mm Column; mAb Charge Variant Standard; BioResolve SCX pH Concentrates; and two Certified LDPE Containers	176004356
BioResolve SCX mAb, 3 µm, 4.6 × 50 mm Column w/ VanGuard FIT Cartridge; mAb Charge Variant Standard; BioResolve SCX pH Concentrates; and two Certified LDPE Containers	176004353
BioResolve SCX mAb 3 µm, 4.6 × 50 mm Column; mAb Charge Variant Standard; BioResolve SCX pH Concentrates; and two Certified LDPE Containers	176004357
BioResolve SCX mAb, 3 µm, 4.6 × 100 mm Colum w/ VanGuard FIT Cartridge; mAb Charge Variant Standard; BioResolve SCX pH Concentrates; and two Certified LDPE Containers	176004354
BioResolve SCX mAb 3 µm, 4.6 × 100 mm Column; mAb Charge Variant Standard; BioResolve SCX pH Concentrates; and two Certified LDPE Containers	176004358

IonHance CX-MS pH Concentrates

Description	P/N
IonHance CX-MS pH Concentrate A in Certified LDPE Container	186009280
IonHance CX-MS pH Concentrate B in Certified LDPE Container	186009281
IonHance CX-MS pH Concentrates A&B Kit	176004498

IonHance CX-MS pH Concentrate and BioResolve SCX mAb Startup Kits

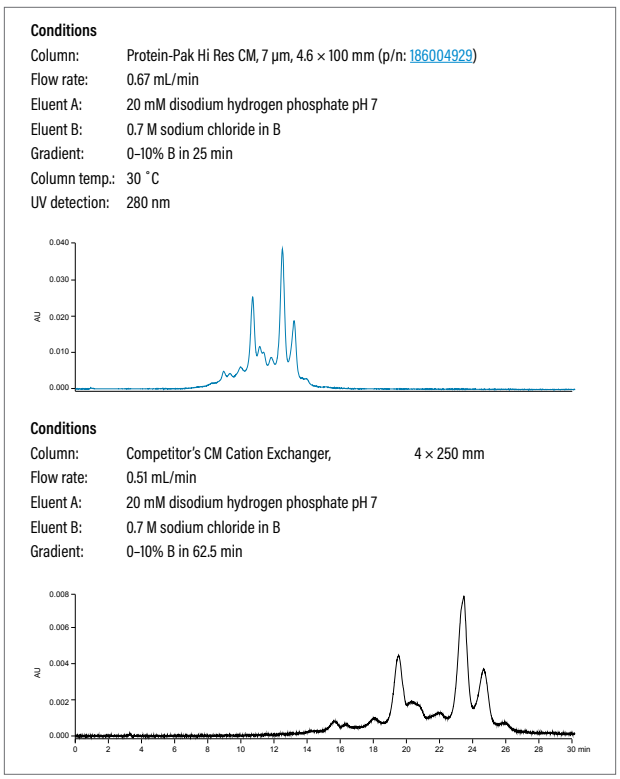
Description	P/N
Kit: IonHance CX-MS pH Concentrates A and B in certified LDPE containers; BioResolve SCX mAb, 3 µm, 2.1 × 50 mm Column with VanGuard FIT; and Humanized mAb Mass Check Standard	176004499
Kit: IonHance CX-MS pH Concentrates A and B in certified LDPE containers; BioResolve SCX mAb, 3 µm, 2.1 × 50 mm Column; and Humanized mAb Mass Check Standard	176004500
Kit: IonHance CX-MS pH Concentrates A and B in certified LDPE containers; BioResolve SCX mAb, 3 µm, 2.1 × 100 mm Column with VanGuard FIT; and Humanized mAb Mass Check Standard	176004501
Kit: IonHance CX-MS pH Concentrates A and B in certified LDPE containers; BioResolve SCX mAb, 3 µm, 2.1 × 100 mm Column; and Humanized mAb Mass Check Standard	176004502

Protein-Pak Hi Res Ion-Exchange (IEX) Columns for ACQUITY UPLC Applications

Protein-Pak Hi Res Ion-Exchange (IEX) Columns were developed to assist in the characterization of recombinant proteins, monoclonal antibodies, and other biological compounds. The non-porous, high compound binding capacity of these particles yields outstanding resolution of charged species in less time compared to use of many traditional porous IEX offerings. In addition, quality control testing with defined protein standards helps ensure consistent batch-to-batch performance.

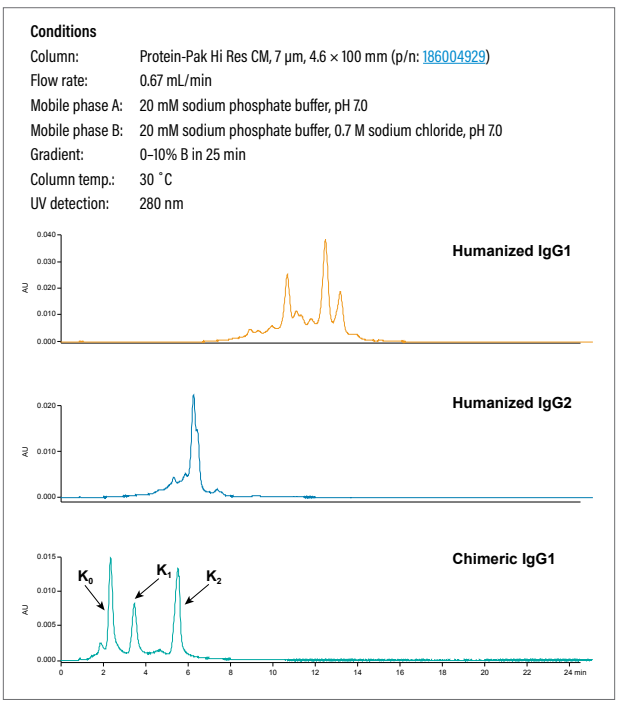
- Designed for the characterization of protein charge variants and other biocompounds
- Two cation-exchangers (carboxymethyl and sulfopropyl) and one anion exchanger (quaternary ammonium) that address selectivity needs
- Non-porous, high-capacity stationary phases deliver fast separations that address high-throughput needs
- QC tested with protein standards to ensure batch-to- batch consistency
- eCord enabled to help monitor column use on ACQUITY UPLC Systems

Resolved Monoclonal Antibody (mAb) Isoform Separation



Cation-exchange chromatography is a useful tool for the characterization and quantitation of mAb or recombinant protein variants. Use of Waters Protein-Pak Hi Res CM Column on an ACQUITY UPLC System increases sample throughput while maintaining resolution between intended product and undesired variants.

Protein-Pak Hi Res CM Analysis of Three mAbs Containing Different Levels of Variants



Sequence, production, storage, and shipping conditions influence the degree of variants contained in a biotherapeutic protein. Waters Protein-Pak Hi Res CM Column can successfully resolve variations that may involve as little as a single amino acid change (K0 = No terminal lysines, K1 = One terminal lysine, and K2 = Two terminal lysines).

Ordering Information

Protein-Pak Hi Res UPLC Columns

Description	Particle Size	Dimension	P/N (1/pk)
Protein-Pak Hi Res CM	7 µm	4.6 × 100 mm	186004929
Protein-Pak Hi Res SP	7 µm	4.6 × 100 mm	186004930
Protein-Pak Hi Res Q	5 µm	4.6 × 100 mm	186004931

Note: Only when Protein-Pak Hi Res IEX Columns are combined with the ACQUITY UPLC System are the full performance benefits realized. See Waters service notes, p/n: 715002147A for ACQUITY UPLC System configuration guidelines for ion-exchange chromatography.

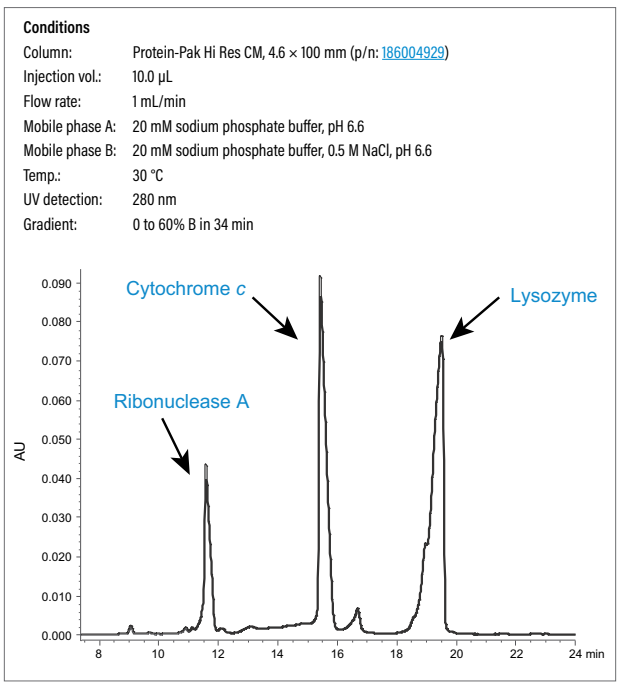
Ion-Exchange Standards

Benchmarking, Method Development, and Troubleshooting

Ion-Exchange Standards are sets of standards that allow the user to benchmark anion- or cation-exchange columns on a regular basis in order to have confidence in results as well as providing a troubleshooting tool for any issues that may arise.

- IEX Anion Test Standard
- IEX Cation Test Standard

Protein-Pak Hi Res CM Column using the IEX Cation Test Standard



Waters offers a variety of carefully formulated and QC-tested anion-exchange and cation-exchange protein standards to help chromatographers confirm adequate performance of their IEX column and LC system prior to the analyses of potentially highly valued samples.

Ordering Information

IEX Standards

Description	P/N (1/pk)
IEX Anion Test Standard	186006869
IEX Cation Test Standard	186006870

Application of Waters UPLC Technology for Biotherapeutic Characterization

ACQUITY UPLC allows analytical chemists to reach far beyond conventional LC separations and has proven itself to be an asset to laboratories around the world. UPLC sets new standards in resolution, sensitivity, and throughput by being the first holistically-designed system that maximizes for rapid, high-resolution analyses. It has fueled hundreds of peer-reviewed papers, helps laboratories conserve resources, and has served the needs of regulatory agencies around the globe. ACQUITY UPLC simultaneously makes your laboratory more sustainable and more efficient.

Manufacturing Consistency for Enhanced Assurance

The ability to obtain the same high-quality separations regardless of column lot is of critical importance to the successful development and commercialization of biotherapeutics. Each batch of Protein-Pak Hi Res IEX material is tested with a relevant mixture of protein standards to help ensure consistent column-to-column performance.

ACQUITY UPLC Technology for biotherapeutic characterization.

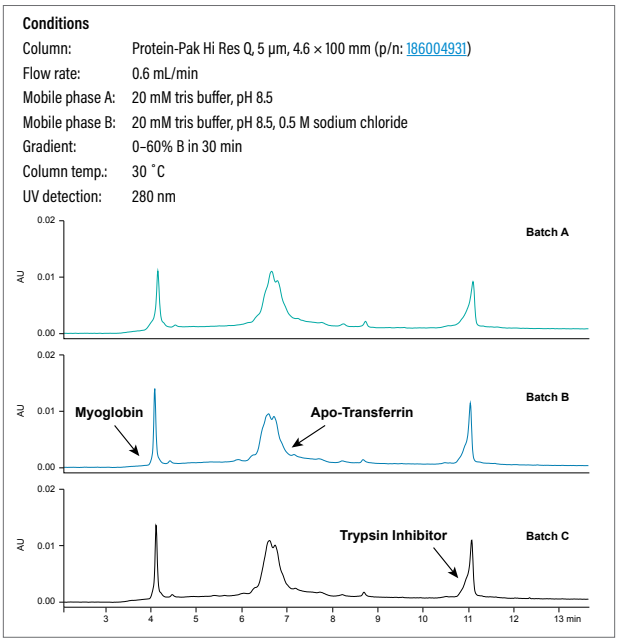
Novel IEX Particles Ideal for Biomolecule Characterizations

Protein-Pak Hi Res IEX Columns contain non-porous, pH tolerant, hydrophilic particles whose surface consists of a multi-layered network of either anion (5 µm) or cation (7 µm) exchange groups. This innovative particle and bonding chemistry produces particles with greater protein loading capacities than found on many traditional mono-disperse, non-porous resins. This translates into columns that can resolve complex mixtures of biomolecules in comparatively short times compared to use of alternative porous or non-porous IEX Column offerings.

Column	Protein-Pak Hi Res Q	Protein-Pak Hi Res CM	Protein-Pak Hi Res SP
Ion Exchange	**Strong Anion**	**Weak Cation**	**Strong Cation**
Functional group	Quaternary ammonium	Carboxymethyl	Sulfopropyl
Matrix	Hydrophilic polymer	Hydrophilic polymer	Hydrophilic polymer
Particle size	5 µm	7 µm	7 µm
Pore size	Non porous	Non porous	Non porous
I.D. × L	4.6 × 100 mm	4.6 × 100 mm	4.6 × 100 mm
Counter ion	Cl-	Na+	Na+
pH range	3–10	3–10	3–10
Temperature	10–60 °C	10–60 °C	10–60 °C
pK_a	10.5	4.9	2.3
Flow rates	0.3–0.6 mL/min	0.5–1.4 mL/min	0.5–1.4 mL/min
Approximate protein binding capacity in mgs per column (i.e., BSA for Hi Res Q column, lysozyme for Hi Res CM and Hi Res SP columns)*	58	33	25

* For optimal resolution of complex samples, do not exceed 20% of the column's protein binding capacity.

Protein-Pak Hi Res IEX Column Batch-to-Batch Reproducibility

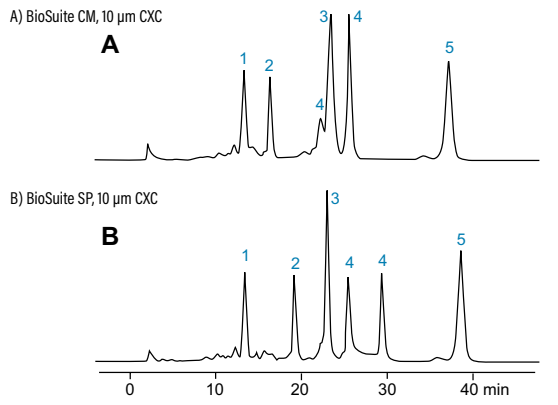


Each batch of Protein-Pak Hi Res SP, CM, and Q Column packing material is chromatography tested using a relevant protein standard mixture to help ensure consistent and predictable performance.

BioSuite Ion-Exchange HPLC Columns

BioSuite Ion-Exchange (IEX) Column offerings include strong and weak cation (CXC) and anion exchangers (AXC) bonded to a pH stable (i.e., pH 2–12), methacrylic ester-based polymeric resin. The availability of four separation chemistries provides chromatographers with the flexibility required to develop methods that separate proteins or peptides based upon minor charge differences. Non-porous (NP) and porous IEX columns are also available. Speed and superior chromatographic resolution are possible using the NP IEX offerings. Waters' porous ion exchangers are available for applications requiring greater protein or peptide binding capacity. In addition, selected BioSuite Ion-Exchange Columns are offered in PEEK hardware as well as in 21.5 mm I.D. preparative column sizes.

Protein Selectivity Differences Observed by Ion-Exchange Chromatography on BioSuite CM (Weak-Cation Exchange) vs. SP (Strong-Cation Exchange) Columns

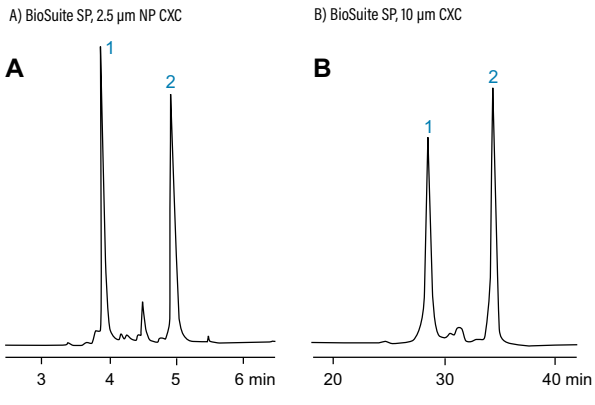


Conditions

Sample:	Trypsinogen (Peak 1), ribonuclease (Peak 2), alpha-chymotrypsinogen A (Peak 3), cytochrome *c* (Peak 4), and lysozyme (Peak 5)
Columns:	A: BioSuite CM, CXC, 10 µm, 7.5 × 75 mm (p/n: 186002186)
	B: BioSuite SP, CXC, 10 µm, 7.5 × 75 mm, (p/n: 186002184)
Eluent A:	20 mM sodium phosphate, pH 7.0
Eluent B:	20 mM sodium phosphate, pH 7.0 containing 0.5 M sodium chloride
Flow rate:	1.0 mL/min
Gradient:	Linear gradient from 0 to 100% B in 60 min
Column temp.:	25 °C
UV detection:	280 nm

BioSuite strong (SP) and weak (CM) cation-exchange columns deliver different separation selectivities useful when developing a method to adequately separate a complex biocompound mixture.

Enhanced Compound Resolution by Ion-Exchange Chromatography on BioSuite SP Non-Porous (NP) vs. Porous CXC Columns



Conditions

Sample:	Alpha-chymotrypsinogen A (Peak 1) and ribonuclease (Peak 2)
Columns:	A: BioSuite, NP CXC, 2.5 µm, 4.6 × 35 mm (p/n:186002183)
	B: BioSuite SP, CXC, 10 µm, 7.5 × 75 mm (p/n:186002184)
Eluent A:	20 mM MES [2(N-morpholino)ethanesulfonic acid], pH 6.0
Eluent B:	20 mM MES, pH 6.0 containing 0.5 M sodium chloride
Flow rate:	A: 1.5 mL/min
	B: 1.0 mL/min
Gradient:	A: Linear gradient from 0 to 100% B in 10 min
	B: Linear gradient from 0 to 100% B in 60 min
Column temp.:	25 °C
UV detection:	280 nm

Use of 2.5 µm, superficially-porous particles, contained in the BioSuite SP NP Columns, can deliver improved peptide component resolution and in less time (left figure) compared to the use of a BioSuite SP, CXC column that contains 10 µm, fully-porous particles (right figure).

Ordering Information

BioSuite pC_{18} and pPhenyl HPLC and UHPLC Columns

Description	Matrix	Dimension	P/N (1/pk)
BioSuite pC_{18}, 2.5 µm NP RPC	Polymer	4.6 × 35 mm	186002152
BioSuite pC_{18}, 500, 7 µm RPC	Polymer	2.0 × 150 mm	186002153
BioSuite pC_{18}, 500, 7 µm RPC	Polymer	4.6 × 150 mm	186002154
BioSuite pC_{18}, 500, 13 µm RPC	Polymer	21.5 × 150 mm	186002155
BioSuite pPhenyl, 1000, 10 µm RPC	Polymer	2.0 × 75 mm	186002156
BioSuite pPhenyl, 1000, 10 µm RPC	Polymer	4.6 × 75 mm	186002157
BioSuite pPhenyl, 1000, 13 µm RPC	Polymer	21.5 × 150 mm	186002158

BioSuite IEX HPLC Columns

Description	Matrix	Pore Size	Exclusion Limit (Daltons) Against Polyethylene Glycol	Dimension	Column Volume (mL)	# Approx Protein Binding Capacity Per Pre-Packed Column	P/N
BioSuite Q-PEEK, 10 µm AXC	Polymer	4000 Å	>5,000,000	4.6 × 50 mm	0.83	58 mg[1]	186002176
BioSuite SP-PEEK, 7 µm CXC	Polymer	1300 Å	>4,000,000	4.6 × 50 mm	0.83	58 mg[2]	186002182
BioSuite DEAE, 2.5 µm NP AXC	Polymer	n/a	500	4.6 × 35 mm	0.58	2.9 mg[1]	186002179
BioSuite SP, 2.5 µm NP CXC	Polymer	n/a	500	4.6 × 35 mm	0.58	2.9 mg[3]	186002183
BioSuite Q, 10 µm AXC	Polymer	1000 Å	1,000,000	7.5 × 75 mm	3.31	331 mg[1]	186002177
BioSuite Q, 13 µm AXC	Polymer	1000 Å	1,000,000	21.5 × 150 mm	54.45	5445 mg[1]	186002178
BioSuite DEAE, 10 µm AXC	Polymer	1000 Å	1,000,000	7.5 × 75 mm	3.31	99 mg[1]	186002180
BioSuite DEAE, 13 µm AXC	Polymer	1000 Å	1,000,000	21.5 × 150 mm	54.45	1633 mg[1]	186002181
BioSuite SP, 10 µm CXC	Polymer	1000 Å	1,000,000	7.5 × 75 mm	3.31	132 mg[3]	186002184
BioSuite SP, 13 µm CXC	Polymer	1000 Å	1,000,000	21.5 × 150 mm	54.45	2178 mg[3]	186002185
BioSuite CM, 10 µm CXC	Polymer	1000 Å	1,000,000	7.5 × 75 mm	3.31	149 mg[3]	186002186
BioSuite CM, 13 µm CXC	Polymer	1000 Å	1,000,000	21.5 × 150 mm	54.45	2450 mg[3]	186002187

[1] Data generated with BSA.
[2] Data generated with gamma globulin.
[3] Data generated with hemoglobin.
Note: For best resolution of complex samples, do not exceed 20% of the column's protein binding capacity.

Protein-Pak PW Series Columns

Waters also offers a line of 10 µm polymer-based ion-exchangers pre-packed in steel or glass columns. The Protein-Pak 5PW Columns are available as DEAE and SP ion exchangers. These columns can be used on HPLC and FPLC systems in both analytical and preparative configurations.

Approximate Protein Binding Capacity per Pre-Packed Column				
	Protein-Pak HR Packing			
Dimension	Q	DEAE	SP	CM
5 × 50 mm	60 mg	40 mg	40 mg	25 mg
5 × 100 mm	130 mg	150 mg	80 mg	45 mg
10 × 100 mm	500 mg	300 mg	300 mg	180 mg

Ordering Information

Protein-Pak PW HPLC Column Series

Description	Dimension	P/N
Polymeric Weak Anion-Exchanger	7.5 × 75 mm	WAT088044
Protein-Pak DEAE 5PW Glass Column	8 × 75 mm	WAT011783
Protein-Pak DEAE 5PW Steel Column	21.5 × 150 mm	WAT010640
Polymeric Strong Cation Exchanger	7.5 × 75 mm	WAT088043
Protein-Pak SP 5PW Glass Column	8 × 75 mm	WAT011784

Protein-Pak High Resolution (HR) Ion-Exchange Glass Columns

Waters Protein-Pak HR packing materials are based on rigid, hydrophilic, polymethacrylate particles with large 1000 Å pores. The naturally hydrophilic polymer reduces non-specific adsorption, resulting in quantitative recovery of protein mass and bioactivity. These packings are compatible with buffers in the pH range 2–12, and will withstand exposure to caustic solutions, such as 0.1–1.0 M sodium hydroxide and acetic solutions, such as 20% acetic acid, for cleaning purposes.

The Protein-Pak HR 8 µm and 15 µm packing materials are available pre-packed in Waters Advanced Purification (AP) Glass Columns in a choice of 5 mm I.D. (mini-column) or 10 mm I.D. by 100 mm in length. The 5 mm I.D. column is also available in a 50 mm length. These columns are compatible with any HPLC and FPLC system with the use of an adapter kit.

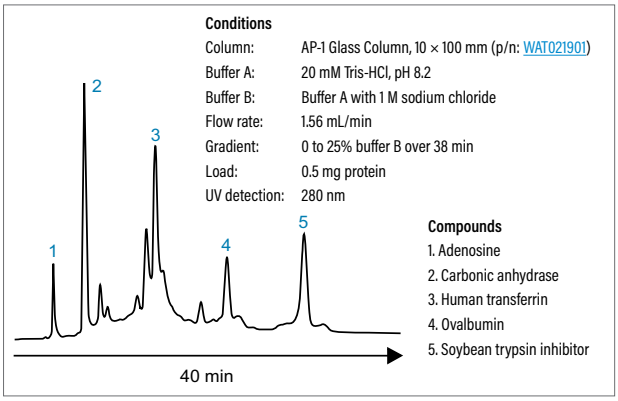
Protein-Pak HR ion exchangers are available with a Q functional group, a strong anion exchanger; DEAE, a weak anion exchanger; SP, a strong cation exchanger; and CM, a weak cation exchanger. The principal difference between a weak and strong ion exchanger does not lie in the protein binding capacity, but in the pH range of operation. Weak ion exchangers tend to have a more restricted useful pH range of operation.

Properties of Protein-Pak HR Columns

	Protein-Pak Q HR1	Protein-Pak DEAE HR2	Protein-Pak CM HR3	Protein-Pak SP HR4
Type of material	Polymer	Polymer	Polymer	Polymer
Protein binding capacity	60 mg/mL	40 mg/mL	25 mg/mL	40 mg/mL
Ion-exchange capacity	200 µeq/mL	250 µeq/mL	175 µeq/mL	225 µeq/mL
Nominal pK	11.7	9.0	5.7	2.2
Typical protein recovery	>95%	>95%	>95%	>95%
Typical recovery of biological activity	>90%	>90%	>90%	>90%
pH stability	2–12	2–12	2–12	2–12

1. For best resolution do not exceed 20% of the protein binding capacity.
2. Bovine serum albumin in 20 mM Tris/Cl pH 8.2 was used to measure protein binding capacity of Protein-Pak Q and DEAE HR.
3. Cytochrome *c* in 25 mM MES pH 5.0 was used to measure protein binding capacity of Protein-Pak SP and CM HR.
4. Same conditions as CM. Protein binding capacity of Protein-Pak SP 40 HR is 20 mg/mL.

Protein Resolution on Protein-Pak DEAE 15HR Anion-Exchange Column



Waters Advanced Purification (AP) Glass Columns, containing Protein-Pak DEAE 15 µm particles, are well suited for the analysis and/or lab-scale purification of various protein mixtures.

Ordering Information

Protein-Pak HR Ion-Exchange Glass Columns

Ion-Exchange Packing	Particle Size	Pore Size	Dimension	Particle Type	P/N
Protein-Pak Q 8HR	8 µm	1000 Å	5 × 50 mm	Polymeric strong anion exchanger	WAT039575
			5 × 100 mm		WAT039630
			10 × 100 mm		WAT035980
Protein-Pak Q 15HR	15 µm	1000 Å	5 × 50 mm	Polymeric strong anion exchanger	WAT039782
			10 × 100 mm		WAT037663
Protein-Pak DEAE 8HR	8 µm	1000 Å	5 × 50 mm	Polymeric weak anion exchanger	WAT039791
			5 × 100 mm		WAT039783
			10 × 100 mm		WAT035650
Protein-Pak DEAE 15HR	15 µm	1000 Å	5 × 50 mm	Polymeric weak anion exchanger	WAT039780
			5 × 100 mm		WAT039786
			10 × 100 mm		WAT038564
Protein-Pak SP 8HR	8 µm	1000 Å	5 × 50 mm	Polymeric strong cation exchanger	WAT039570
			5 × 100 mm		WAT039625
			10 × 100 mm		WAT035655
Protein-Pak SP 15HR	15 µm	1000 Å	10 × 100 mm	Polymeric strong cation exchanger	WAT038567
Protein-Pak CM 8HR	8 µm	1000 Å	5 × 50 mm	Polymeric weak cation exchanger	WAT039790
			5 × 100 mm		WAT039785
			10 × 100 mm		WAT035970
Protein-Pak CM 15HR	15 µm	1000 Å	5 × 50 mm	Polymeric weak cation exchanger	WAT039787

Advanced Purification (AP) Glass Columns

Waters AP series of glass columns are constructed of biocompatible glass and polymeric materials and can be easily used with silica, polymer, or soft gel packings. To optimize flow and ensure uniform sample distribution onto the packed bed, each column incorporates a distributor. A replaceable filter protects the packing from large particulate contaminants. Empty AP Glass Columns are available in a variety of sizes and utilize the same design to ensure predictable methods transfer among them. AP Glass Columns are compatible with both analytical and preparative HPLC and FPLC systems.

Ordering Information

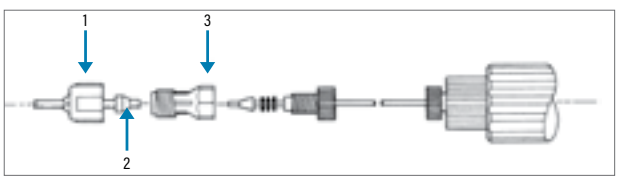
Advanced Purification (AP) Glass Columns

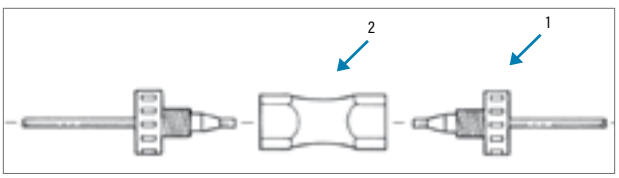
Dimension	Bed Volume (mL)	Flow Rate (mL/min)	Pressure Rating (psi/MPa)	P/N
5 × 50 mm	0.8–1.2	0–4	1500 psi/10 MPa	WAT064-01
5 × 100 mm	1.8–2.2	0–4	1500 psi/10 MPa	WAT064-02
10 × 100 mm	5–8	0–4	1500 psi/10 MPa	WAT021901
10 × 200 mm	13–16	0–4	1500 psi/10 MPa	WAT021902
10 × 300 mm	21–24	0–4	1500 psi/10 MPa	WAT021903
10 × 600 mm	45–48	0–4	1500 psi/10 MPa	WAT021906
20 × 100 mm	22–31	4–16	1000 psi/6.8 MPa	WAT027501
20 × 200 mm	53–62	4–16	1000 psi/6.8 MPa	WAT027502
20 × 300 mm	85–94	4–16	1000 psi/6.8 MPa	WAT027503
20 × 600 mm	179–188	4–16	1000 psi/6.8 MPa	WAT027506
50 × 100 mm	137–196	16–100	500 psi/3.4 MPa	WAT023321
50 × 200 mm	333–392	16–100	500 psi/3.4 MPa	WAT023332
50 × 300 mm	530–589	16–100	500 psi/3.4 MPa	WAT023323
50 × 600 mm	1118–1177	16–100	500 psi/3.4 MPa	WAT023326

Advanced Purification (AP) Glass Column Accessories and Spare Parts

Waters AP Glass Columns feature non-metallic construction and adjustable bed height with easy-to-use coarse and fine adjustments. The AP Glass Columns are available in a variety of dimensions.

Connection of an AP MiniColumn and an AP-1 Column to 1/8" OD Tubing





Ordering Information

AP MiniColumn

Description	Qty.	P/N
1. Collet and Nut Assembly (3/8–24)	10/pk	WAT005138
2. Ferrule 1/8" Tube	10/pk	WAT005136
3. Union 3/8–24 × 'Z' Fitting	5/pk	WAT005137

AP MiniColumn Accessories and Spare Parts

Description	Dimension	P/N
Glass Tube	5 × 50 mm	WAT038802
	5 × 100 mm	WAT038803
Column Jacket	5 × 50 mm	WAT038804
	5 × 100 mm	WAT038805
Filters, 10/pk	–	WAT038806
O-Rings, 13/pk (includes 10 inlet/outlet and 3 funnel)	–	WAT038807
Inlet Connector Assembly	–	WAT038800

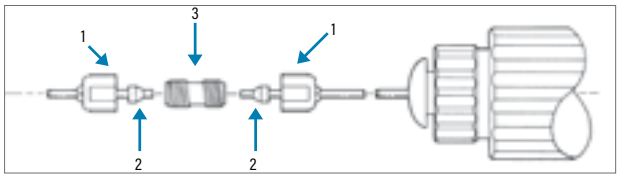
AP-1 Column

Description	Qty.	P/N
1. Compression Screw and Ferrule 'Z' Fitting, Plastic	1/pk	WAT082708
2. Union 'Z' Fitting, Plastic	1/pk	WAT082745

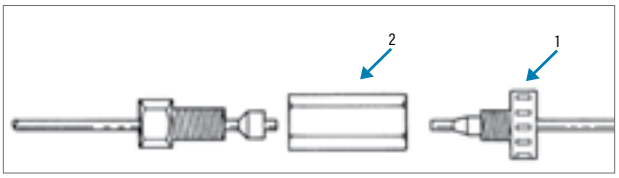
AP-1 Column Accessories and Spare Parts

Description	Dimension	P/N
Glass Tube	10 × 100 mm	WAT021992
	10 × 200 mm	WAT022033
	10 × 300 mm	WAT022034
	10 × 600 mm	WAT022035
Plastic Shield	10 × 100 mm	WAT021927
	10 × 200 mm	WAT021945
	10 × 300 mm	WAT021946
	10 × 600 mm	WAT021947
O-Rings, 5/pk	–	WAT021907
Filters, 10/pk	–	WAT021910
Replacement Tubing (Tefzel) (1/16 in. O.D. × 0.009 in. I.D. × 10 feet) (1.6 mm O.D. × 0.23 mm I.D. × 3 m)	–	WAT021950
Inlet Connector Assembly	–	WAT021904

Connection of an AP-2 and an AP-5 Column to 1/8" O.D. Tubing



Connection of Pharmacia Fitting to 1/16" O.D. Tubing



AP-2 Column

Description	Qty.	P/N
1. Collet and Nut Assembly (3/8–24)	10/pk	WAT005138
2. Ferrule 1/8" Tube	10/pk	WAT005136
3. Union 3/8–24 × 3/8–24	1/pk	WAT082734

AP-2 Column Accessories and Spare Parts

Description	Dimension	P/N
Glass Tube	20 × 100 mm	WAT019891
	20 × 200 mm	WAT019892
	20 × 300 mm	WAT019893
Plastic Shield	20 × 100 mm	WAT027542
	20 × 200 mm	WAT027543
	20 × 300 mm	WAT027544
O-Rings, 5/pk	—	WAT027528
Filters, 2/pk	—	WAT027530
Replacement Tubing (Tefzel) (1/8 in. O.D. × 0.040 in. I.D. × 10 feet) (3.2 mm O.D. × 1.02 mm I.D. × 3 m)	—	WAT023344
Inlet Connector Assembly	—	WAT027525
Distributors/Inserts, 5/pk	—	700004715

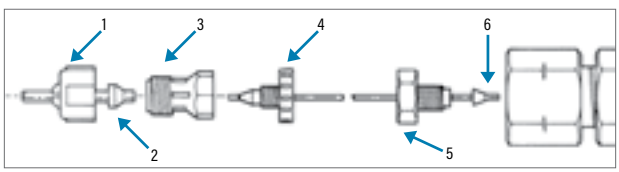
AP-5 Column

Description	Qty.	P/N
1. Compression Screw and Ferrule 'Z' Fitting, Plastic	1/pk	WAT082708
2. Union, Plastic	1/pk	WAT021951

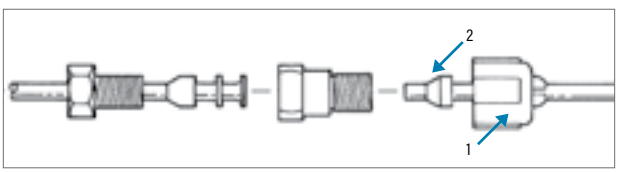
AP-5 Column Accessories and Spare Parts

Description	Dimension	P/N
Glass Tube	50 × 100 mm	WAT019876
	50 × 200 mm	WAT019877
	50 × 300 mm	WAT019878
Plastic Shield	50 × 100 mm	WAT023370
	50 × 200 mm	WAT023371
	50 × 300 mm	WAT023372
	50 × 600 mm	WAT023373
O-Rings, 5/pk	—	WAT023345
Filter, 2/pk	—	WAT023343
Replacement Tubing (Tefzel) 1/8 in. O.D. × 0.040 in. I.D. × 10 feet) (3.2 mm O.D. × 1.02 mm I.D. × 3 m)	—	WAT023344
Inlet Connector Assembly	—	WAT023349
Outlet Connector Assembly	—	WAT023348
Collet and Nut Assembly	—	WAT023346
Ferrule, 10/pk	—	WAT023347
Funnel Assembly	—	WAT023396

Connection of a Protein-Pak Steel Column to 1/16" and 1/8" O.D. Tubing



Connection of 1/8" or 1/16" Flanged Type Fitting to 1/8" O.D. Tubing



Protein-Pak Steel Column

Description	Qty.	P/N
1. Collet and Nut Assembly (3/8–24)	10/pk	WAT005138
2. Ferrule 1/8" Tube	10/pk	WAT005136
3. Union 3/8–24 × 'Z' Fitting	5/pk	WAT005137
4. Compression Screw and Ferrule 'Z' Fitting, Plastic	1/pk	WAT082708
5. Compression Screw 'Z' Fitting, Steel	10/pk	WAT005070
6. Ferrule 1/16" Steel	10/pk	WAT005063

Flanged Type Fitting

Description	Qty.	P/N
1. Collet and Nut Assembly (3/8–24)	10/pk	WAT005138
2. Ferrule 1/8" Tube	10/pk	WAT005136

AccellPlus Ion-Exchange Packings

Solid-Phase Extraction for Protein Sample Preparation

Waters AccellPlus ion-exchange packings are 40 µm, 300 Å polymer-coated, silica-based materials for both lab- and process-scale chromatography. AccellPlus, available as a QMA (strong anion exchanger) or CM (weak cation exchanger), is easy to pack and is excellent for the purification of proteins, enzymes, and immunoglobulins. The rigid silica-based packing material will withstand very high flow rates during cleaning and re-equilibration cycles. Normal flow rates are used during sample loading and elution to obtain the best possible resolution.

AccellPlus bulk material may be packed into our Advanced Purification (AP) Glass Columns.

[1]To estimate packed bed volume for a known amount of AccellPlus: **AccellPlus used (g) × 2 = packed bed volume (mL)**

AccellPlus Sep-Pak Cartridges

Sep-Pak Plus Cartridges packed with AccellPlus ion exchangers provide a rapid, economical means to clean up heavily contaminated samples that would damage a high resolution column. They can also be used to rapidly screen chromatographic conditions. These are also available in a variety of configurations.

Ordering Information

AccellPlus Sep-Pak Cartridges

Description	Ion-Exchange Type	P/N
AccellPlus CM	Weak Cation Exchanger	WAT020550
AccellPlus QMA	Strong Anion Exchanger	WAT020545
AccellPlus QMA Plus	Strong Anion Exchanger	186004540

AccellPlus PrepPak Cartridges (47 × 300 mm)

Economical, convenient preparative separations in the 500 mg to 10 g range. For a complete listing of Waters products for preparative chromatography, visit waters.com

Protein Binding Capacity of AccellPlus	
AccellPlus QMA* 200 mg BSA/g packing	**AccellPlus CM** 175 mg Cytochrome *c*/g packing**
* Bovine serum albumin in 20 mM Tris/Cl pH 7.0 was used to measure protein binding capacity of AccellPlus QMA.	
** Cytochrome *c* in 20 mM sodium phosphate pH 6.3 was used to measure protein binding capacity of AccellPlus CM.	

Note: For best resolution do not exceed 20% of the protein binding capacity.

Ordering Information

AccellPlus PrepPak Cartridges (47 × 300 mm)

Description	Particle Size	Pore Size	P/N
AccellPlus CM*	40 µm	300 Å	WAT036545
PrepPak 1000 Module	—	—	WAT089592

* Requires PrepPak 1000 Module.

AccellPlus Ion-Exchange Bulk Packings

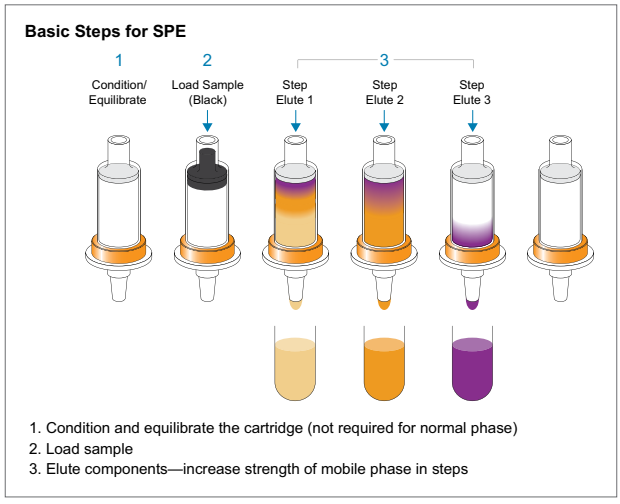
For all preparative isolations based on ionic interactions, particularly proteins, enzymes, and immunoglobulins.

Ion-Exchange Sample Preparation with Sep-Pak Cartridges

To perform ion-exchange sample preparation with Sep-Pak Cartridges, use a gradient of pH or ionic strength with Accell Plus CM, AccellPlus QMA or NH_2 as a sorbent.

- Condition the cartridge with six to ten hold-up volumes of de-ionized water or weak buffer
- Load the sample dissolved in a solution of deionized water or buffer
- Elute unwanted weakly bound components with a weak buffer
- Elute the first component of interest with a stronger buffer (change the pH or ionic strength)
- Elute other components of interest with progressively stronger buffers
- When you recover all of your components, discard the used cartridge in an appropriate manner

General Elution Protocol for Ion-Exchange Chromatography on Sep-Pak Cartridges (NH_2, AccellPlus QMA, AccellPlus CM)



1. Condition and equilibrate the cartridge (not required for normal phase)
2. Load sample
3. Elute components—increase strength of mobile phase in steps

Ordering Information

AccellPlus Ion-Exchange Bulk Packings

Description	Particle Size	Pore Size	Qty.	P/N
AccellPlus QMA	40 µm	300 Å	100 g	WAT010742
Anion Exchanger	—	—	500 g	WAT010741
AccellPlus CM	40 µm	300 Å	100 g	WAT010740
Cation Exchanger	—	—	500 g	WAT010739

BioSuite pC_{18} and pPhenyl Reversed-Phase Chromatography (RPC) HPLC Columns

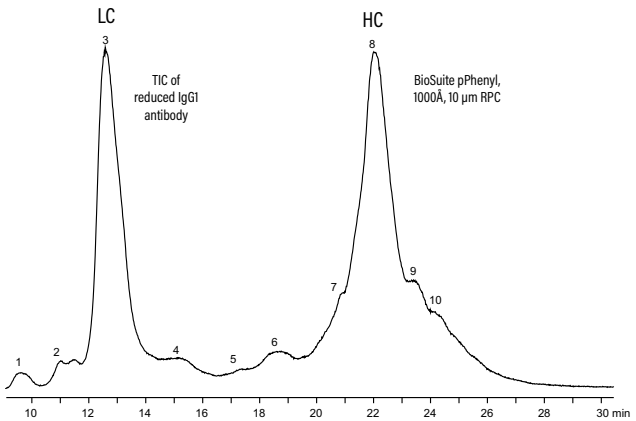
Reversed-phase chromatography (RPC) has become a widely accepted tool for the separation of proteins, peptides, synthetic oligonucleotides, and other biomolecules. For many applications, Symmetry and Symmetry300, Atlantis T3, or BEH 130 Å and BEH 300 Å Chemistries can be successfully used for the isolation and analyses of these biocompounds. However for some applications, the large pore size and high chemical stability of BioSuite phenyl C_{18} and pPhenyl resin-based packings may be preferred. BioSuite RPC Column offerings include a C_{18} (pC_{18}) and a phenyl (pPhenyl) chemistry bonded to a pH stable, methacrylic ester-based polymeric resin. The 500 Å pore size of the pC_{18} base matrix accommodates proteins up to 2,500,000 Daltons while the 1000 Å pore size of the pPhenyl base matrix accommodates proteins up to 5,000,000 Daltons.

The BioSuite pC_{18}, 2.5 µm, NP Column contains a non-porous chemistry that yields superior chromatographic resolution in less time compared to chromatography performed on the porous, pC_{18}, 500 Å, 7 µm RPC selection. Waters' porous, pC_{18}, 500 Å, 7 µm RPC Column is available for applications requiring greater binding capacity. The pC_{18} and pPhenyl RPC chemistries are available in 21.5 × 150 mm columns for "lab-scale" isolations while a 2.0 × 75 mm column is well suited for narrow-bore HPLC and LC-MS applications.

LC-MS Analysis of a Reduced Monoclonal IgG1 Antibody on a BioSuite pPhenyl RPC Column

Conditions

Sample:	Reduced monoclonal IgG1 antibody
Column:	BioSuite Phenyl, 1000 Å, 10 µm, 2.0 × 75 mm (p/n: 186002156)
Eluent A:	Water with 0.1% formic acid
Eluent B:	Acetonitrile with 0.1% formic acid
Flow rate:	0.2 ml/min
Gradient:	10% B for 6 min (waste), then 20–35% B in 30 min
Temp.:	80 °C
UV detection:	Waters LCT Premier ESI-Tof MS



The BioSuite pPhenyl, 1000 Å RPC Columns have a higher ligand density compared to the BioSuite Phenyl, 1000 Å HIC Columns and are not recommended for hydrophobic-interaction separations.

Ordering Information

BioSuite Hydrophobic-Interaction Chromatography HPLC and UHPLC Columns

Description	Matrix	Dimension	P/N
BioSuite Phenyl, 10 µm HIC	Polymer	7.5 × 75 mm	186002159
BioSuite Phenyl, 13 µm HIC	Polymer	21.5 × 150 mm	186002160