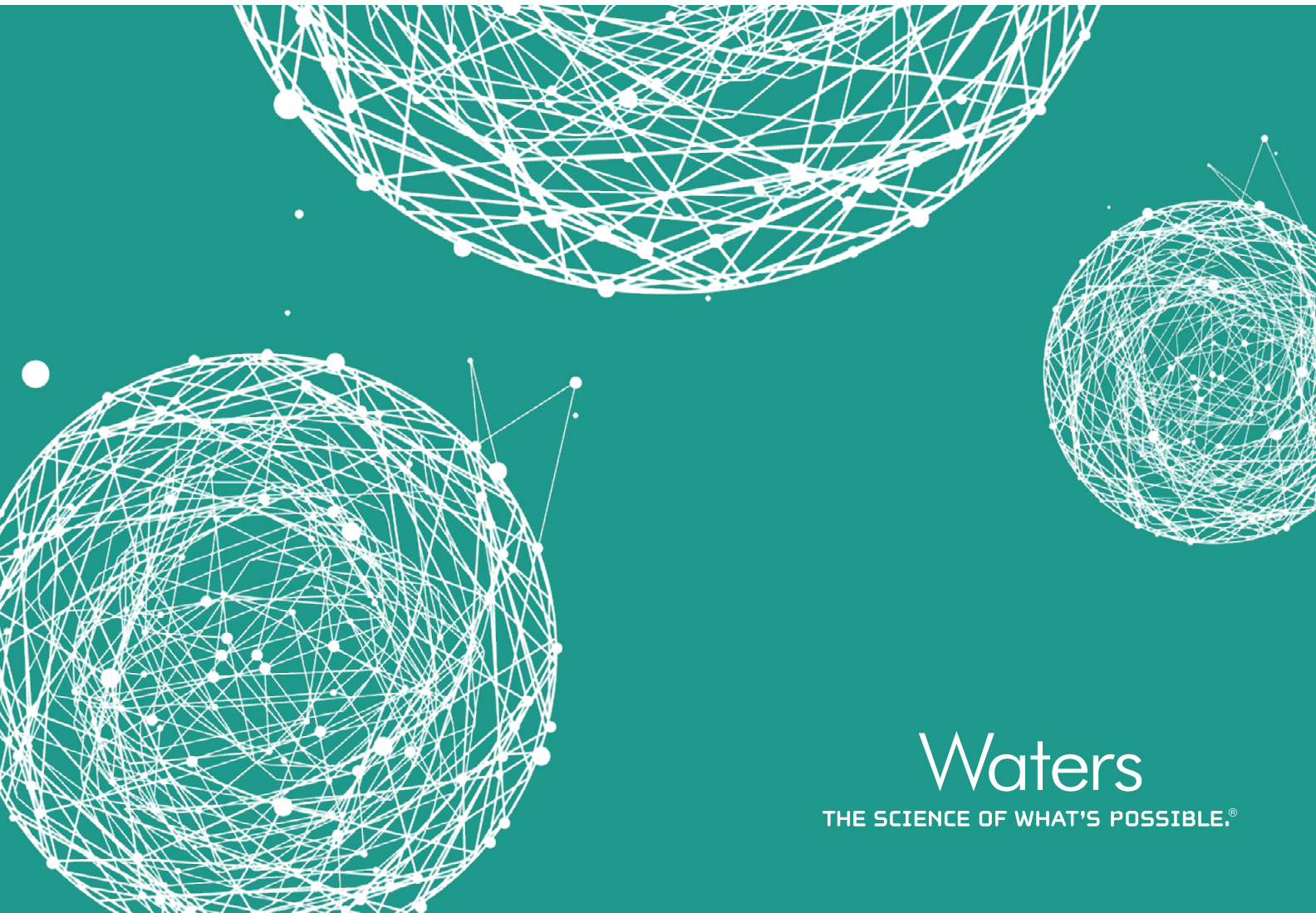
2017/18

Biomolecule Purification, Characterization, and Analyses

[INNOVATIVE HPLC, UHPLC, and UPLC CHEMISTRY CONSUMABLES FOR BIOSEPARATIONS]



Waters

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Biomolecule Purification, Characterization, and Analyses

Innovative Technologies from the Leader 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.

As a leading analytical supplier of instrumentation, software, service and support, and chemistry products, Waters® is uniquely positioned to provide researchers the tools, technologies, and integrated solutions desired to tackle the formidable challenges involving various biomolecules. 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. We continue to develop new, innovative columns and sample preparation consumables that support the HPLC, UHPLC, UPLC,® and LC-MS analyses of peptides, oligonucleotides, proteins, amino acids, and glycans.

Waters comprehensive chemistry and consumables family includes:

- Peptide columns for nano, capillary, analytical, and preparative peptide 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 and standards, and Waters Glycan Columns for the analysis of released glycans
- ACQUITY UPLC® Glycoprotein BEH Amide, 300Å Column for the analyses of intact glycoproteins, glycoprotein fragments, and glycopeptides
- Analytical Standards and Reagents consumables and kits for MS and LC-MS applications of peptides, proteins, and other biomolecules

In addition, our ACQUITY UPLC Protein BEH SEC, 125Å, 200Å, and 450Å guards, columns, and quality controlled protein/peptide standards, as well as our ACQUITY UPLC Glycoprotein BEH Amide, 300Å offering, were developed for use on ACQUITY UPLC Systems to help obtain accurate, precise, and highly resolving quantitative analysis of therapeutic proteins such as mAbs.

Designed and QC tested with relevant biomolecules to help ensure column-to-column consistency.

Bioseparations Columns

www.waters.com/biosep

Analytical Standards and Reagents

asr.waters.com

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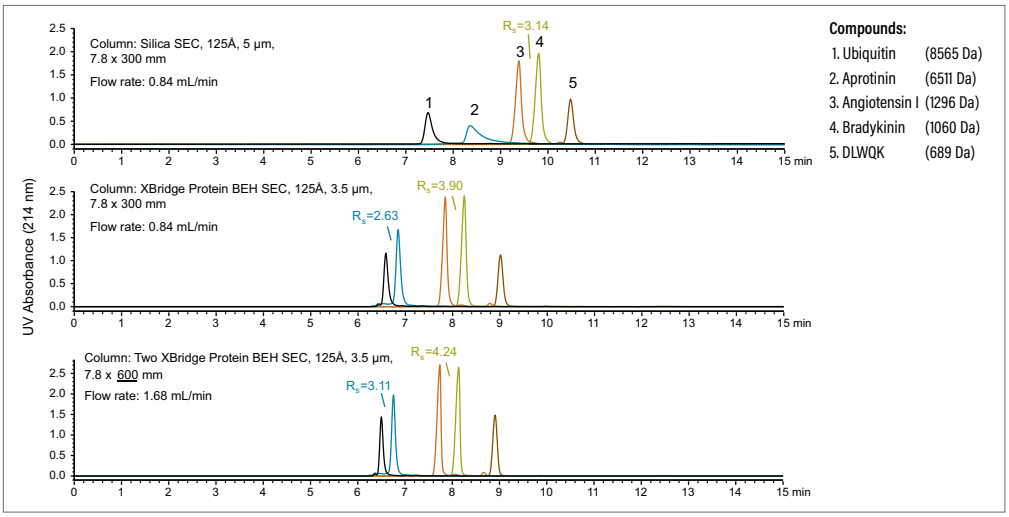
Factors to Consider when Investing and Using an HPLC, UHPLC, or UPLC Column for Bioseparations

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 an 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 "secondary interactions" can be beneficial or detrimental
- Particle composition can also influence column life (e.g., silica-based at pH >7)

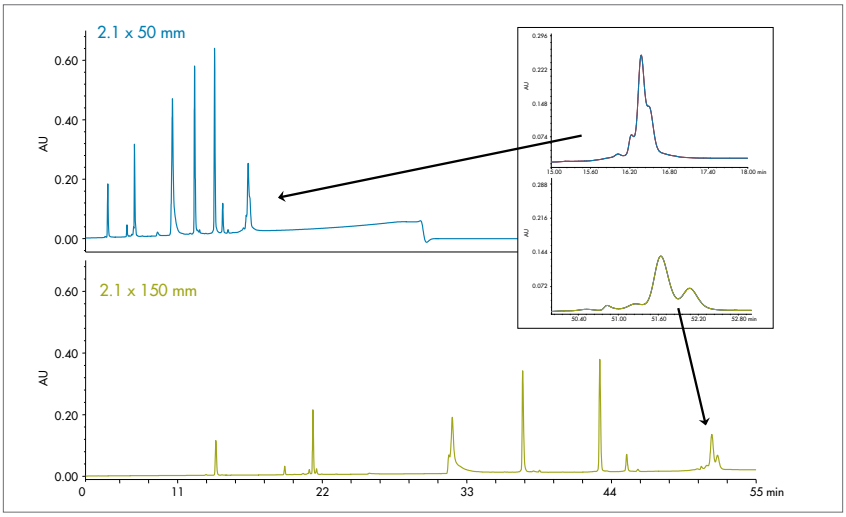


In the size-exclusion chromatographic (SEC) separation shown, a series of synthetic peptides are separated on a column containing 100% silica-based, diol-coated particles (top) vs. Waters diol-coated, bridged-ethylene hybrid (BEH Technology™) particles that have less-undesired-free silanols. Consequently, 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

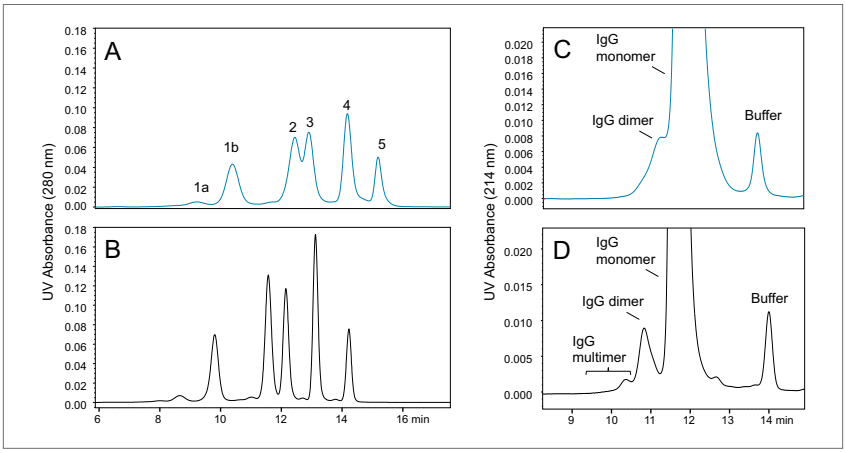


Increasing the length of the column will increase the resolving power for a separation. This is shown with the separation of a protein mixture. The additional small peaks surrounding the Phosphorylase b can be seen more readily on the longer column, as seen in the inset, but it comes at the cost of a 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 450Å, silica-based 8 µm (Frames A and C) and 450Å, BEH 3.5 µm (Frames B and D) SEC columns. Both columns were the same dimensions (7.8 x 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

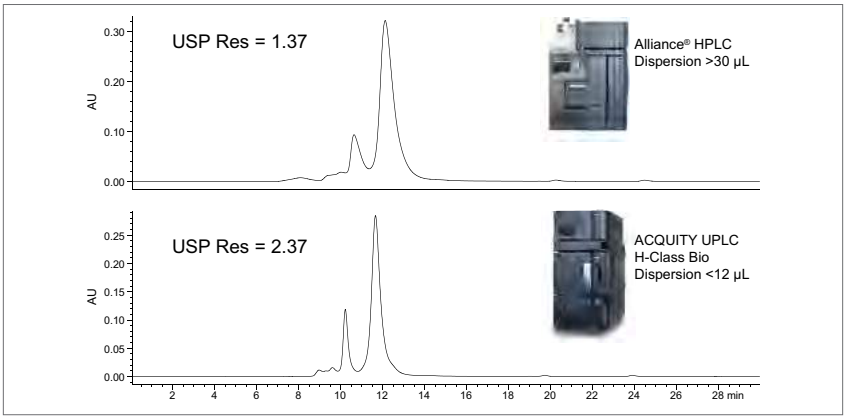
- Standard methods can be performed to measure system dispersion (band broadening)
- Different LC systems have different dispersion values (e.g., band broadening)
- Optimal chromatographic separations are obtained when the appropriate column (e.g, column I.D., particle size) and separation conditions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

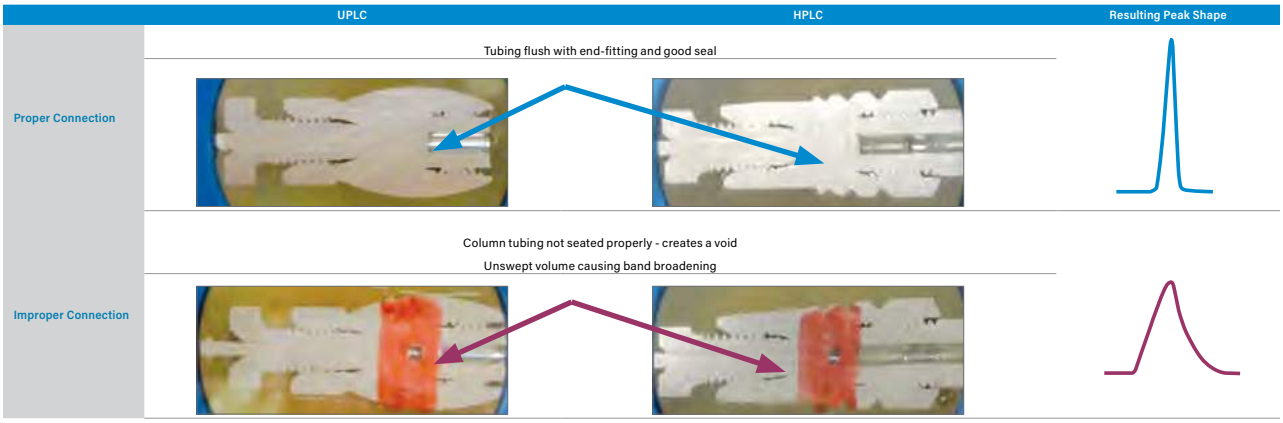
- LC Systems with lower system volumes better maintain column generated separations
- Isocratic-based SEC separations are most sensitive to deleterious band broadening effects



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, 200Å, 2.5 µm Column, SEC eluent, and sample.

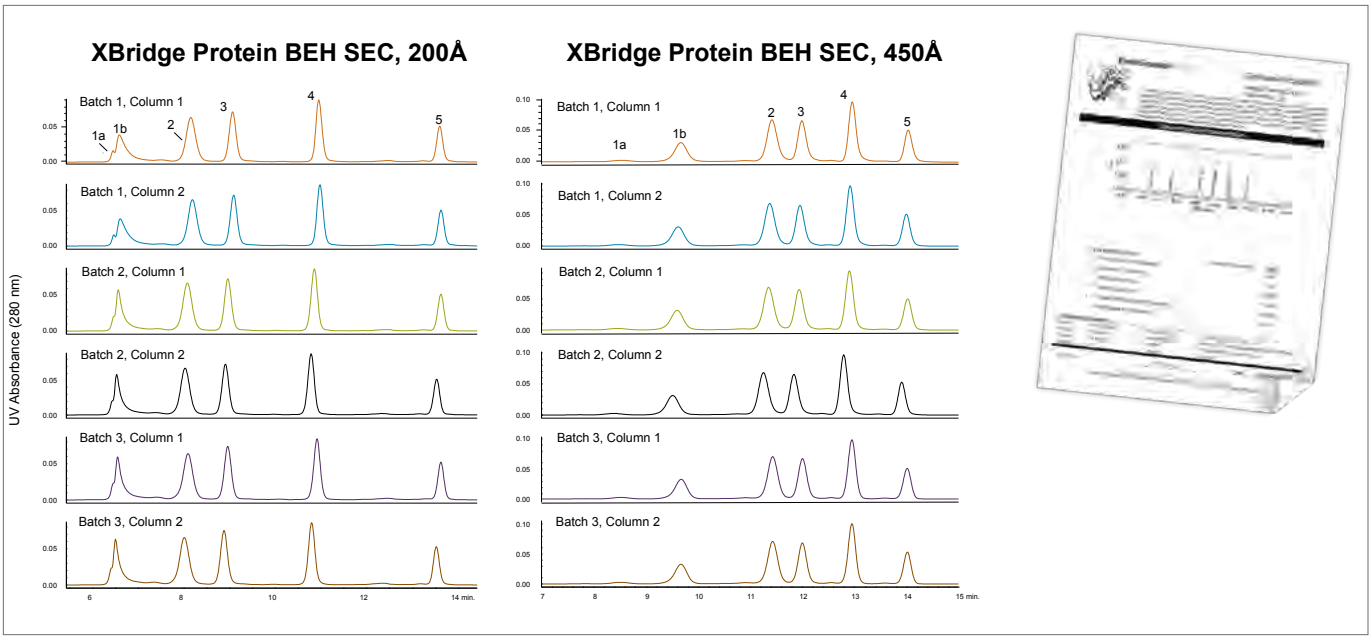
Importance of Making Good Connections from Column to LC System

- 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

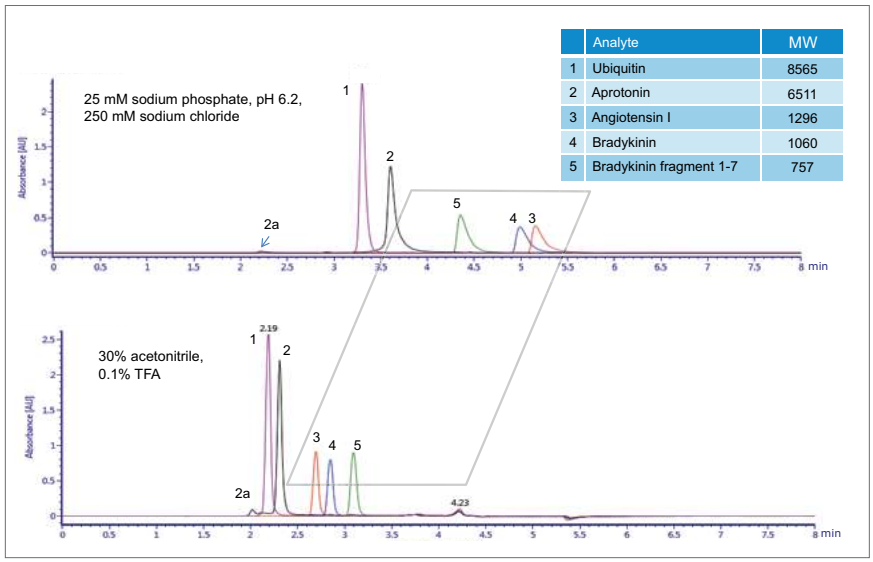


Shown are overlays of the separations of Waters BEH200 SEC Molecular Weight Standard (p/n: 186006518) on 200Å and 450Å BEH 3.5 µm SEC columns. Two columns (300 mm length x 7.8 mm I.D.) were packed from 3 individual manufactured batches of particles to evaluate both batch-to-batch and column-to-column 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



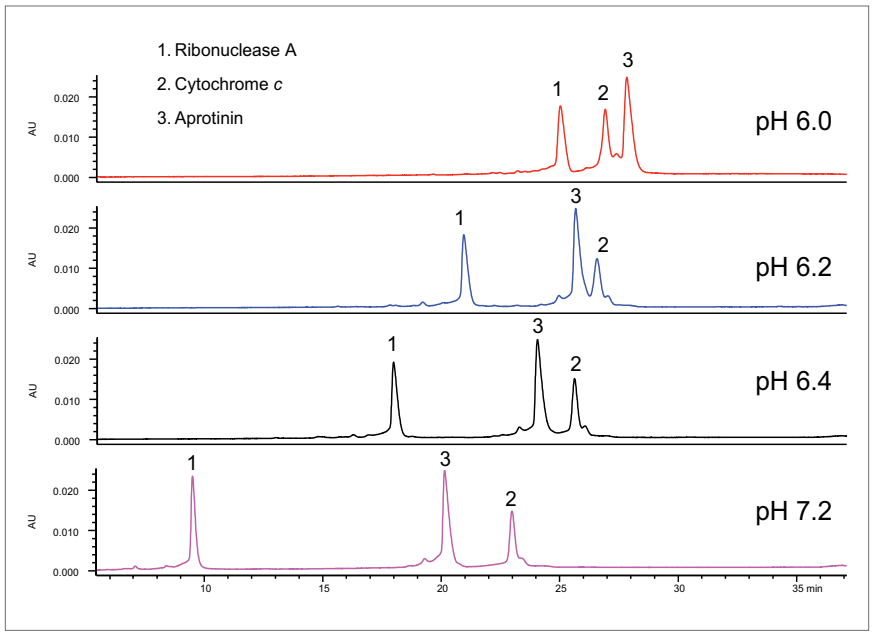
	Analyte	MW
1	Ubiquitin	8565
2	Aprotonin	6511
3	Angiotensin I	1296
4	Bradykinin	1060
5	Bradykinin fragment 1-7	757

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

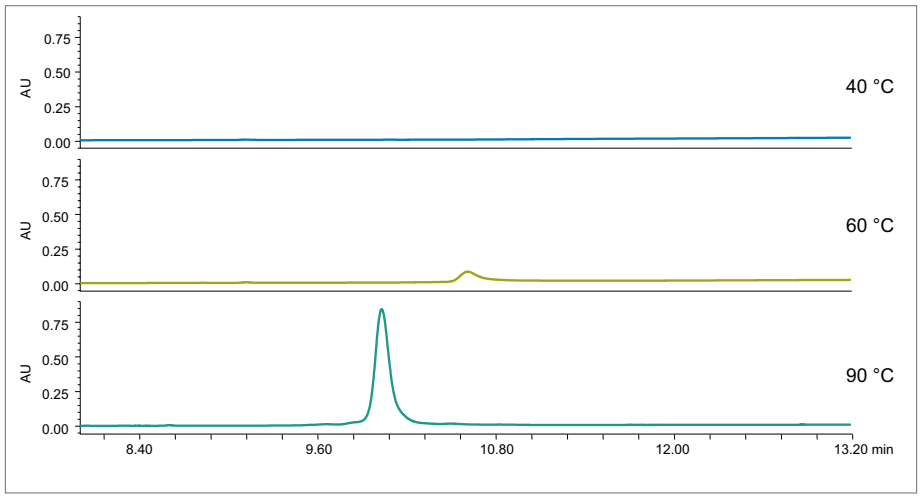
- pH has a significant influence on IEX separations
- Optimal pH for a separation is sample dependent



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 x 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

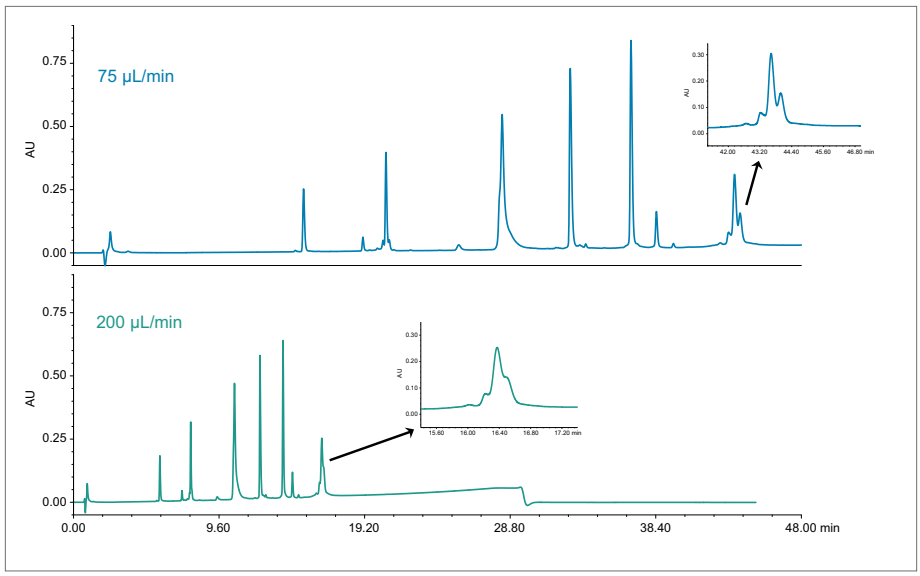


The intact IgG sample gave no observable peak at 40 °C, but recovery for the IgG sample improves with increasing temperature. There is not a measurable increase in recovery or improvement in peak shape above 80 °C. Column temperature has a large effect on reversed-phase separation of molecules. Changes 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

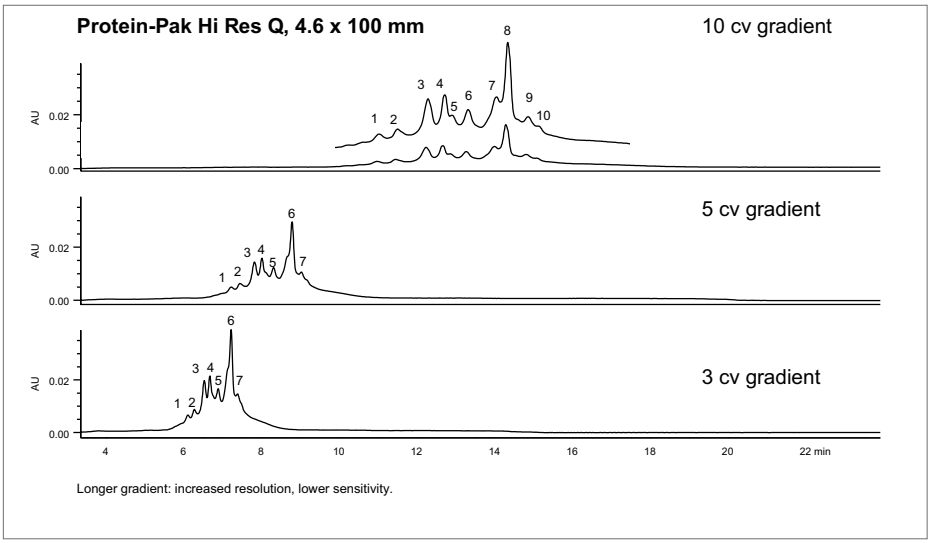


Decreasing the flow rate provides increased resolution without a compromise in the sensitivity, as seen in this separation of the protein mixture at 40 °C. The improved separation of the Phosphorylase b sub-units can be seen (inset) at the lower flow rate. The run time of the analysis is increased proportionally 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 of a Reversed-Phase Peptide Separation

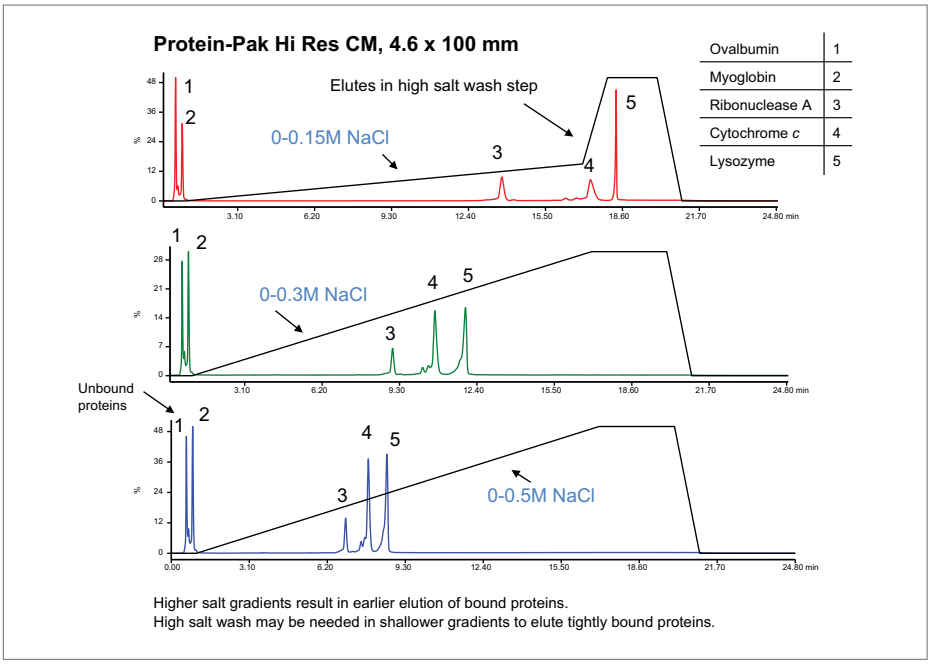
- Use of longer gradient time can translate into improved component resolution
- Analysis time will increase using longer gradients as will peak volume
- Sample complexity can influence selected gradient duration



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 0.5M 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 0-0.5M NaCl, while the top chromatogram has a gradient which ends at 0.15M NaCl, with the middle chromatogram ending at 0.3M NaCl — note effect gradient has on separation. Flow rates same for all separations.

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Amino Acid Analysis Solutions

Amino Acid Analysis Solutions

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Amino Acid Analysis Solutions

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.

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.

Pico•Tag Method	AccQ•Tag Method	AccQ•Tag Ultra 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 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

AccQ•Tag Ultra Chemistry and the UPLC 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 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 (binary), ACQUITY UPLC H-Class (quaternary), or ACQUITY UPLC H-Class Bio (quaternary) System with a tunable UV detector for enhanced chromatographic resolution and maximum-sensitivity detection
- AccQ•Tag Ultra derivatization chemistries including quality-controlled columns, reagents, and eluents
- Empower® 2 pre-configured projects, methods, and report templates
- Installation and application training and support
- Application-specific performance qualification
- Connections INSIGHT® ISDP instrument diagnostics to ensure continuous, consistent, and reliable operation
- Standards and kits to validate and troubleshoot

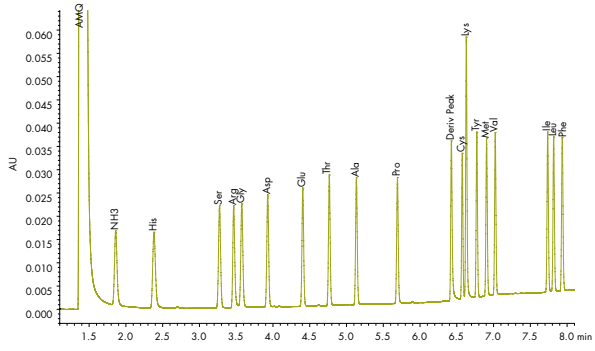
Accurate Amino Acid Analyses from Varied Sample Matrices

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:

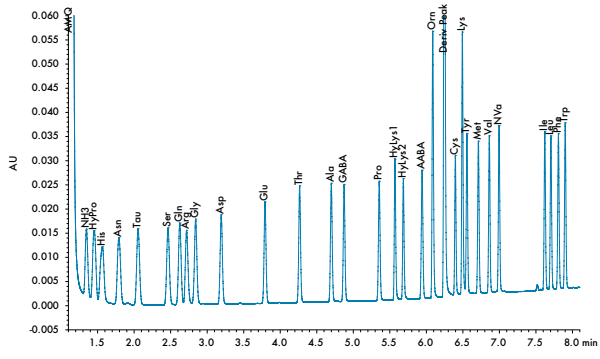
Sample:	10 pmole Hydrolysate Standard
Column:	AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 x 100 mm (p/n: 186003837)
Temp.:	55 °C
Eluent A:	AccQ•Tag Ultra Eluent A – 1:20 Dilution
Eluent B:	AccQ•Tag Ultra Eluent B
Flow rate:	0.7 mL/min
Gradient:	UPLC Amino Acid Analysis Solution Hydrolysate Gradient (provided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

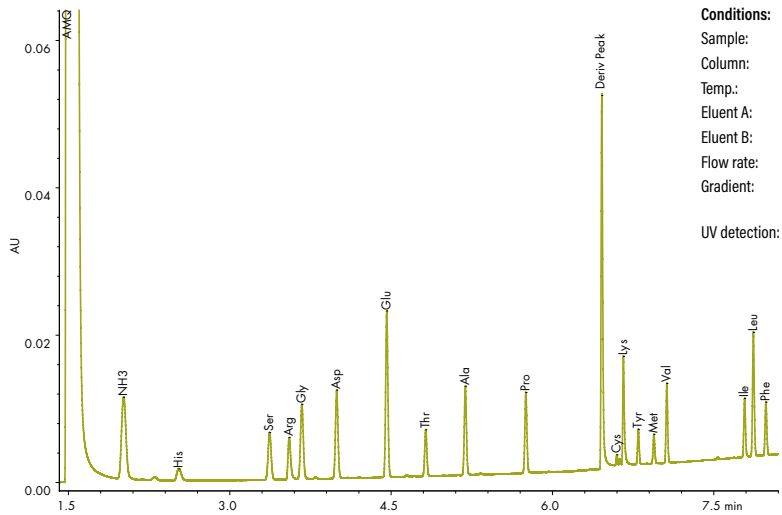
Cell Culture Standard 10 pmol/µL

Sample:	10 pmole Cell Culture Standard
Column:	AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 x 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

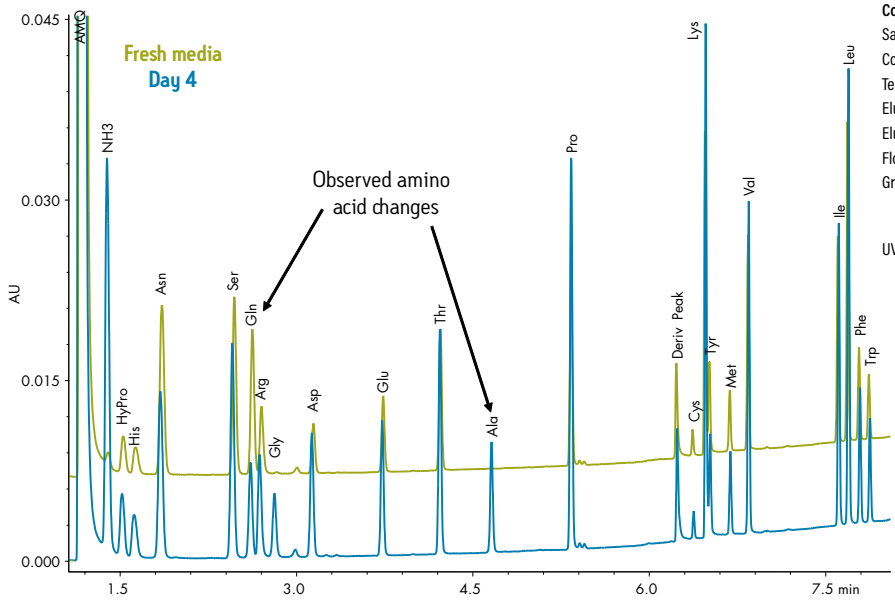


Conditions:

Sample:	Acid Hydrolysate of Poultry Diet, ~6 ng
Column:	AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 x 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



Conditions:

Sample:	Diluted Yeast Fermentation, ~5 nL analyzed
Column:	AccQ•Tag Ultra C_{18}, 1.7 µm, 2.1 x 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

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.

ACCQ•TAG ULTRA CHEMISTRY

The AccQ•Tag Ultra chemistry is an integral component of the 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 chemistry without the rest of the application solution is not supported as an Amino Acid Analysis method.

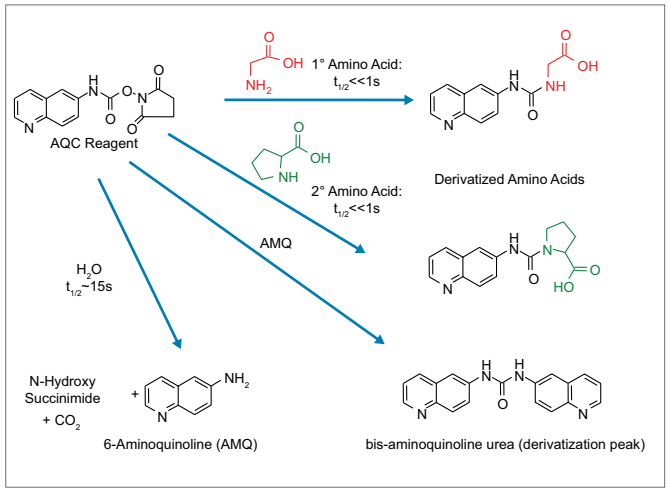
AccQ•Tag Ultra chemistry is different from the AccQ•Tag HPLC method described later in this chapter. Although the components of the two derivatization kits are the same, the QC tests are based on the specific separation and detection protocols. Both methods begin with the same derivatization chemistry but differ in all the other details such that components cannot be interchanged. Most importantly, the AccQ•Tag Ultra column has completely different chemistry from the AccQ•Tag Column. The AccQ•Tag Ultra Column leverages Waters 1.7 µm hybrid-silica BEH Technology particles that deliver the excellent column efficiency and resolution. The column also includes eCord™ Intelligent Chip Technology that is permanently attached to the column to easily track its history. The mobile phases in the AccQ•Tag Ultra chemistry are different from those used for the AccQ•Tag HPLC method, each being optimized for the specific column and detection technique.

Compared to traditional HPLC methods, the UPLC Amino Acid Analysis solution 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 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 bis urea
- Derivatized amino acids are separated chromatographically from the byproducts
- Requires no vacuum drying, sample prep, or extraction

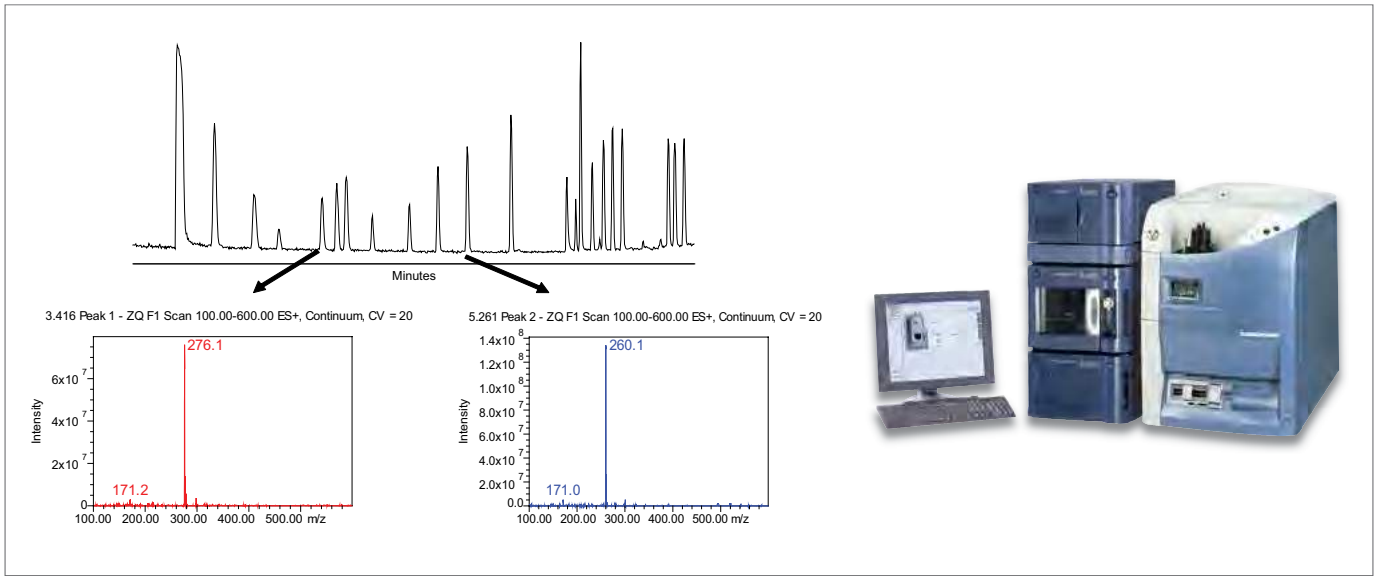
Chemistry of the AccQ•Tag Derivatization Reaction



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 ąL/min



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

AccQ•Tag Ultra Amino Acid Analysis Kits and Accessories

Description	Qty.	P/N
ACQUITY UPLC AAA Application Kit		176001279
This Kit is intended to enable existing ACQUITY UPLC Systems for AAA applications.		
Kit contains:		
Amino acid standard, hydrolysate	10 x 1 mL	
Sample tubes	4 x 72/pk	
Total recovery vials with caps	3 x 100/pk	
Column stabilizer kit, 150 mm		
AccQ•Tag Ultra Derivitization Kit		
AccQ•Tag Ultra C_{18} Column, 1.7 µm, 2.1 x 100 mm		
AccQ•Tag Ultra Eluent A, Concentrate	950 mL	
AccQ•Tag Ultra Eluent B	950 mL	
Tube inlet .0025 I.D. PEEK nut PDA assembly		
2 µL Sample loop		
Column in-line filter kit		
UPLC AAA solution information set		
UPLC AAA app. solution startup tests		
Cert., AAA Application and Familiarization		
UPLC AAA H-Class Applications Kit		176002983
This kit is intended to enable existing ACQUITY UPLC H-Class Systems for AAA applications.		
Kit includes:		
AccQ•Tag Ultra Derivatization Kit, 250 Analyses		
AccQ•Tag Ultra C_{18} Column, 1.7 µm, 2.1 x 100 mm		
AccQ•Tag Ultra Eluent A, Concentrate	950 mL	
AccQ•Tag Ultra Eluent B	950 mL	
Amino acid standard, hydrolysate	10 x 1 mL	
Total recovery vials	3 x 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		

Description	Qty.	P/N
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 for 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 includes:		
AccQ•Tag Ultra Derivitization Kit, 250 analyses		
AccQ•Tag Ultra C_{18} Column, 1.7 µm, 2.1 x 100 mm		
AccQ•Tag Ultra Eluent A, Concentrate	950 mL	
AccQ•Tag Ultra Eluent B	950 mL	
Amino acid standard, hydrolysate	10 x 1 mL	
Sample tubes	4 x 72/pk	
Total recovery vials with caps	3 x 100/pk	
AccQ•Tag Ultra Derivitization Kit, 250 Analyses		186003836
AccQ•Tag Ultra Borate Buffer	5 x 6 mL	
AccQ•Tag Ultra Derivitization Reagent Powder	5 x 3 mg	
AccQ•Tag Ultra Reagent Diluent	5 x 4 mL	
Amino Acid Standard, Hydrolysate	10 x 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 x 72/pk	WAT007571
Total Recovery Vials with Caps	3 x 100/pk	186000384C
AccQ•Tag Ultra C_{18} Column, 1.7 µm, 2.1 x 100 mm		186003837
AccQ•Tag Ultra Eluent A, Concentrate	950 mL	186003838
AccQ•Tag Ultra Eluent B	950 mL	186003839

AccQ•Tag Amino Acid Analysis Using HPLC

The HPLC AccQ•Tag method utilizes the same pre-column derivatization step as the AccQ•Tag Ultra method. 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

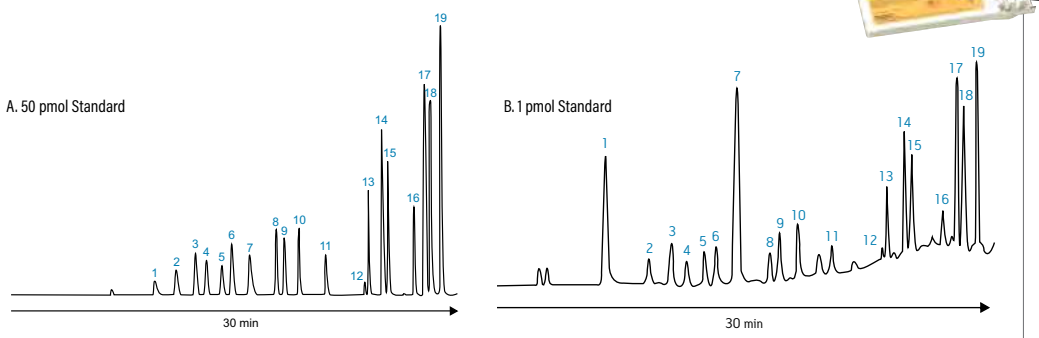
The AccQ•Tag Column is a high-efficiency HPLC column specifically certified for use with the AccQ•Tag method. This column separates the amino acid derivatives produced by the AccQ•Fluor derivatization reaction.

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 for up to 250 analyses includes:		
AccQ•Fluor Reagent 1	5 x 6 mL	
AccQ•Fluor Reagent 2A	5 x 3 mg	
AccQ•Fluor Reagent 2B	5 x 3 mL	
AccQ•Tag column, 3.9 x 150 mm		
AccQ•Tag Eluent A, Concentrate	2 x 1 L	
Sample tubes	4 x 72/pk	
Amino acid standard, hydrolysate	10 x 1 mL	
AccQ•Tag user guide		
Amino Acid Standard, Hydrolysate	10 x 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 includes:		
AccQ•Fluor Reagent 1	5 x 6 mL	
AccQ•Fluor Reagent 2A	5 x 3 mg	
AccQ•Fluor Reagent 2B	5 x 4 mL	
The components of this kit are not available separately		
AccQ•Tag Column, 3.9 x 150 mm		WAT052885
AccQ•Tag User Guide		WAT052874

AccQ•Tag Analysis of Hydrolysate Amino Acids



Sample:	AQC-Derivatized Amino Acid Standards
Column:	AccQ•Tag, 3.9 x 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:

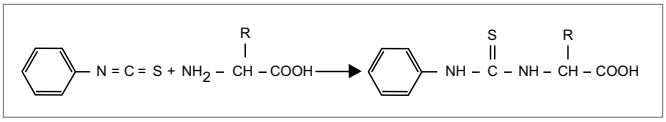
1. AMQ	6. His	11. Pro	16. Lys
2. Asp	7. NH_3	12. Cys	17. Ile
3. Ser	8. Arg	13. Tyr	18. Leu
4. Glu	9. Thr	14. Val	19. Phe
5. Gly	10. Ala	15. Met	

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).

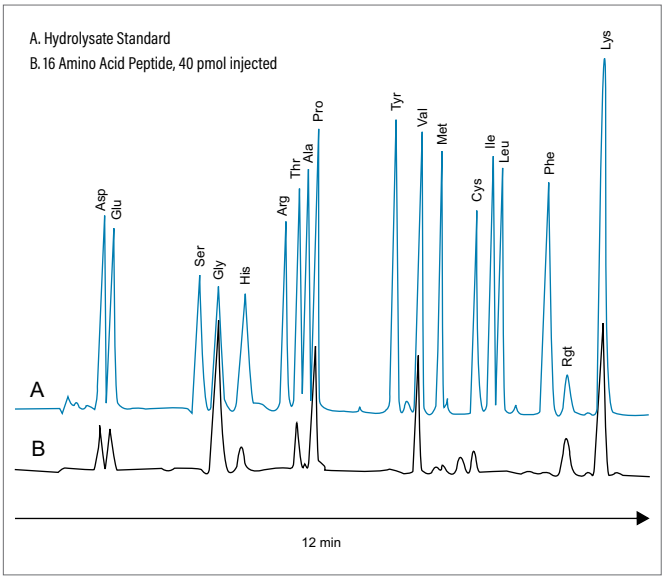
Pico•Tag HPLC Method

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

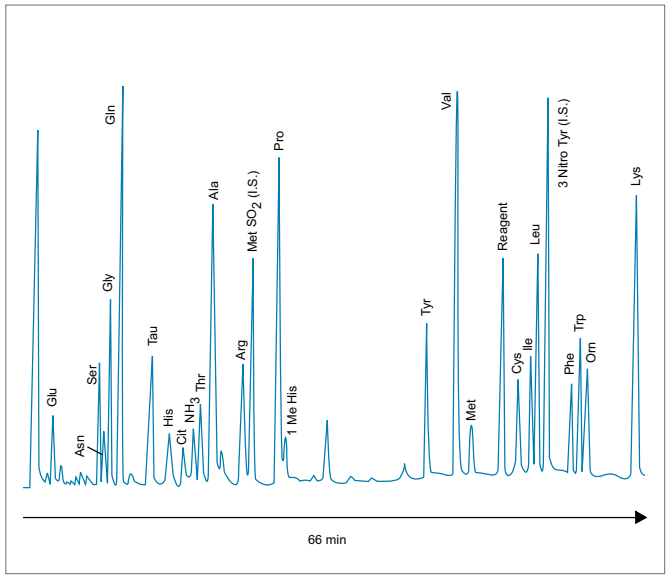


Peptide Hydrolysate Amino Acid Analysis Using the Pico•Tag Method



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
Designed for the analysis of physiologic amino acids.		
Kit includes: Free Amino Acid Analysis Column, 3.9 x 300 mm		
Pico•Tag Reagent Kit		
Pico•Tag Eluent 1	4 x 1 L	
Pico•Tag Eluent 2	4 x 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
Free Amino Acid Analysis Column, 3.9 x 300 mm		WAT010950
Pico•Tag Eluent 1	4 x 1 L	WAT010960
Pico•Tag Eluent 2	4 x 1 L	WAT010965
Pico•Tag Diluent	100 mL	WAT088119
Pico•Tag Eluent 2	1 L	WAT010985

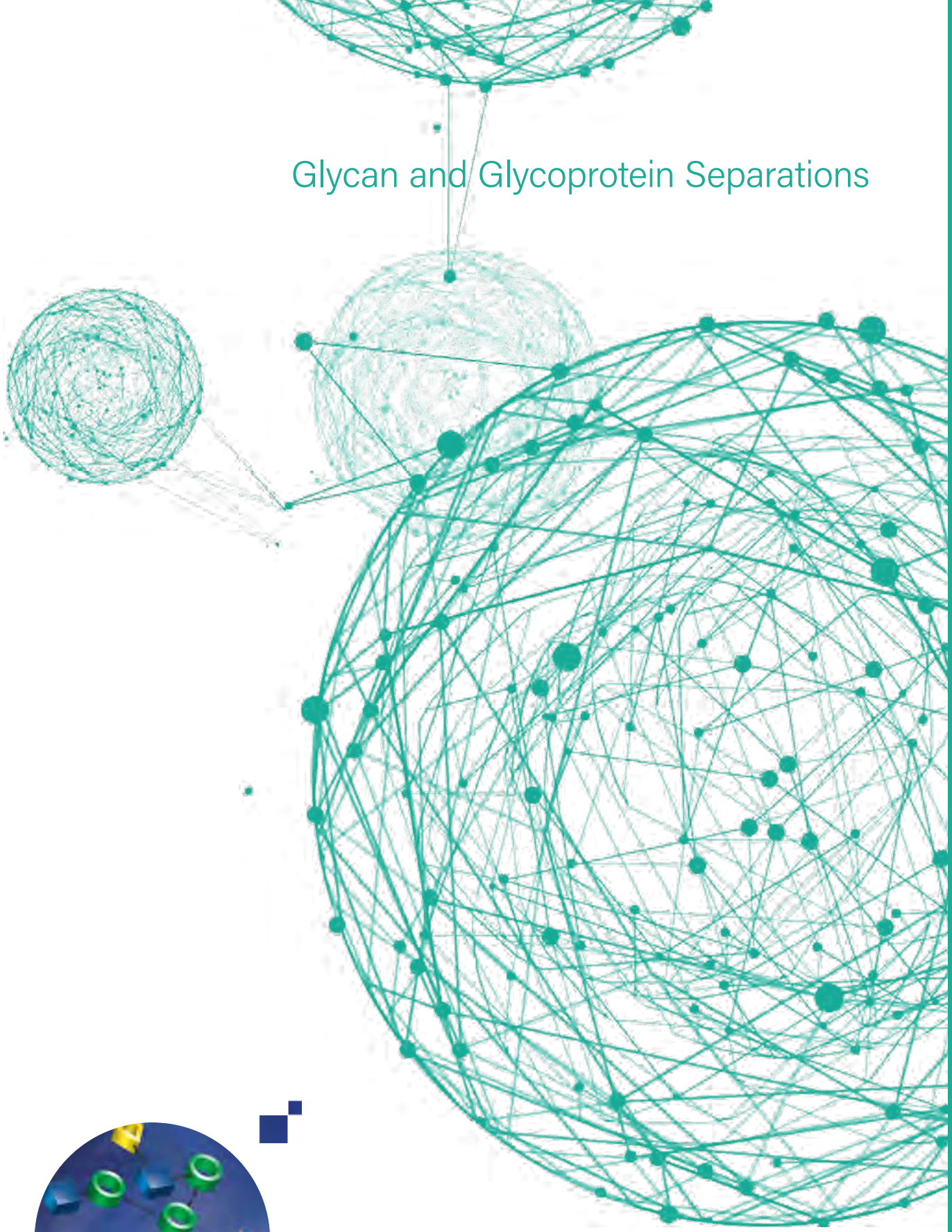
Pico•Tag Amino Acid Analysis for Protein Hydrolysates

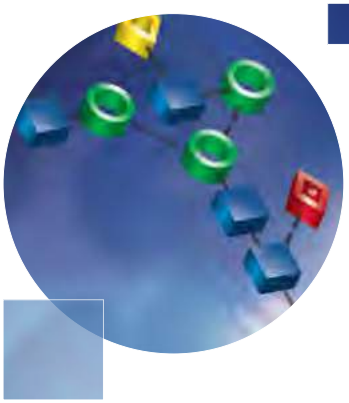
Description	Qty.	P/N
Chemistry Package for Amino Acid Analysis of Protein Hydrolysates		WAT007360
Designed for the analysis of protein hydrolysates.		
Kit includes: Pico•Tag Column, 3.9 x 150 mm		
Pico•Tag Reagent Kit (includes PITC, TEA, and standards)		
Pico•Tag Eluent A	4 x 1 L	
Pico•Tag Eluent B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

Reference Materials for Amino Acid Analysis Solutions

Title	Literature Code
Application Notebook	
Application Solutions for Biopharmaceuticals	720002487EN
Application Notes	
Amino Acid Analysis of Pure Protein Hydrolysate with Waters UPLC Amino Acid Analysis Solution	720002404EN
Application of the UPLC Amino Acid Analysis Solution to the Analysis of Animal Feed Hydrolysates	720002804EN
Enhancement of the UPLC Amino Acid Analysis Solution with Flexible Detector Options	720002913EN
Improving Food Process Control Using the UPLC Amino Acid Analysis Solution	720003357EN
Monitoring Cell Culture Media with the Waters Amino Acid Analysis Solution	720002381EN
Separation of Low Levels of Isoleucine from Leucine Using the ACQUITY UPLC H-Class Amino Acid System	720005263EN
UPLC Amino Acid Analysis Solution	720001683EN
For a complete list of application notes, visit waters.com/AppNotes	
Catalog	
Waters Analytical Standards & Reagents	asr.waters.com
Wall Chart	
Bioseparations Columns Wall Chart	720004232EN
Webinars	
[Meet the Experts] Webinar Series	www.waters.com/MeetTheExperts

Glycan and Glycoprotein Separations



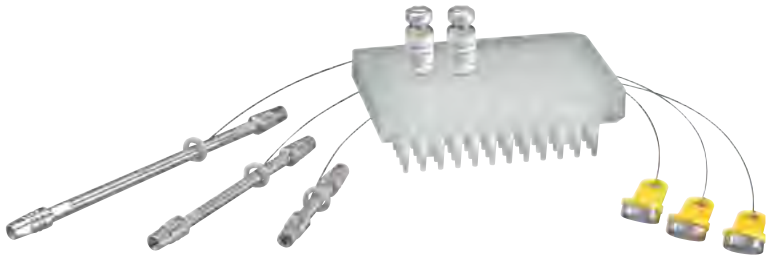


Glycan and Glycoprotein Separations

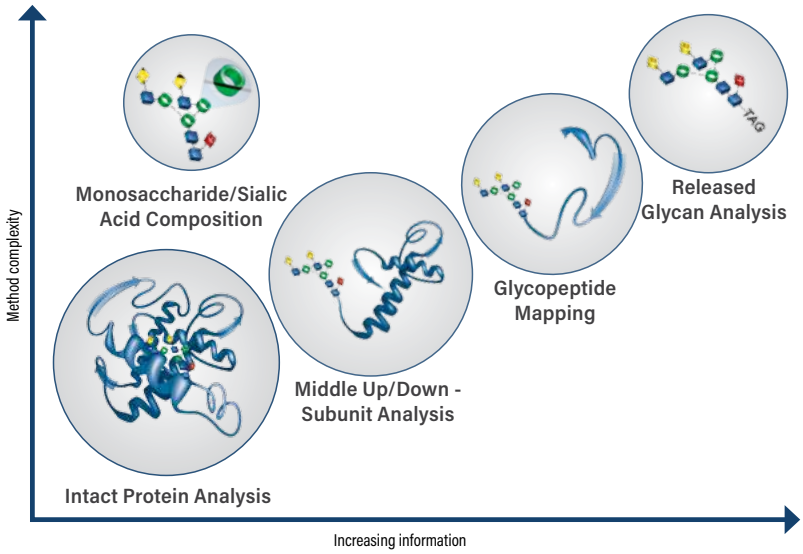
Contents

Glycan and Glycoprotein Separations

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:

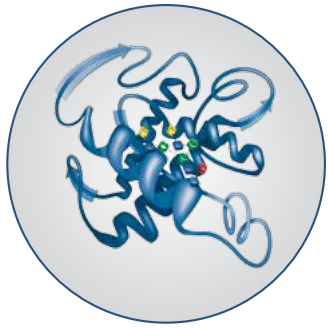
- Intact glycoprotein profiling (e.g., glycan occupancy determination)
- Middle up/down - Subunit analysis
- Glycopeptide mapping
- Released and labeled glycan analysis
- Monosaccharide/sialic acid composition

GLYCOPROTEIN AND GLYCOPEPTIDE ANALYSES

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, 300Å, 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)

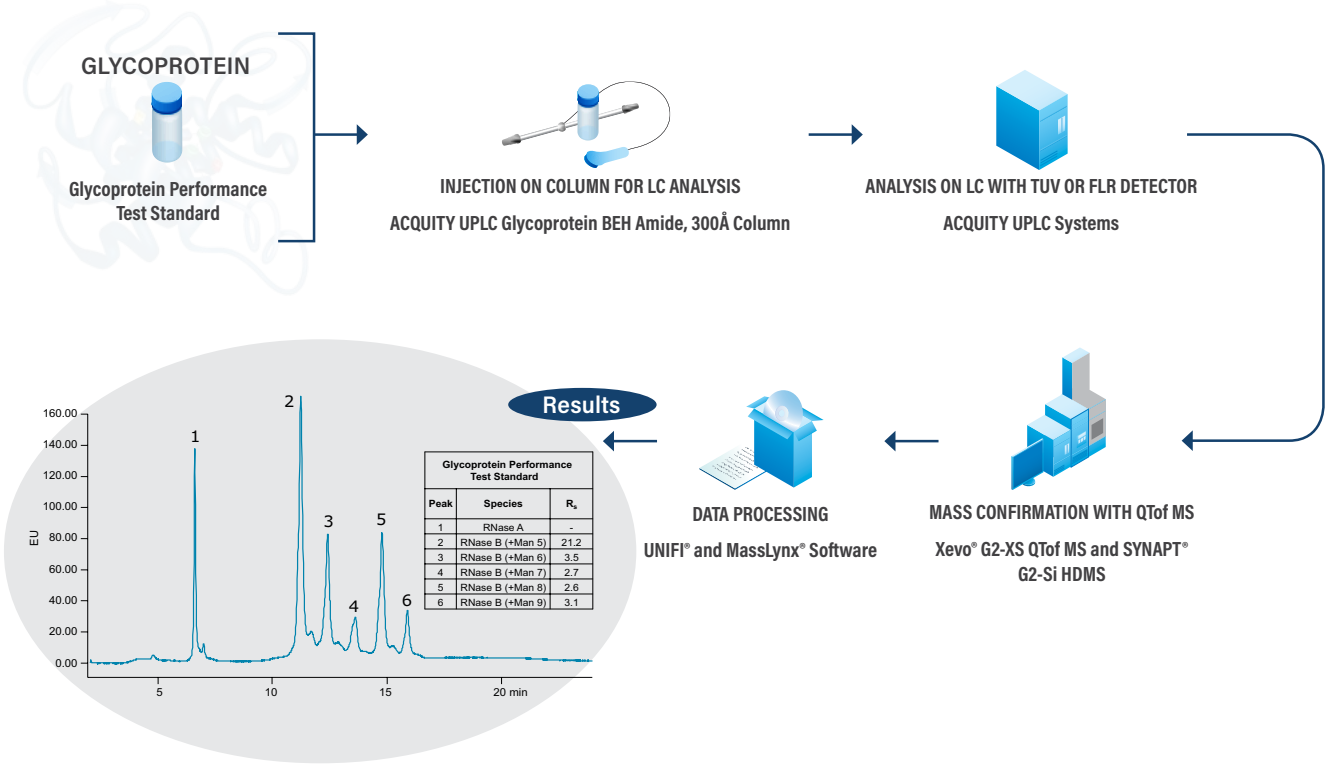
Intact Glycoprotein Analyses



Waters ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 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 page 21 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
- Widepore 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

LC system:	ACQUITY UPLC H-Class Bio System
Columns:	ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 100 mm (p/n: 176003701) that contains Glycoprotein Performance Test Standard (p/n: 186008010) ACQUITY UPLC Glycan BEH Amide, 130Å, 1.7 µm, 2.1 x 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

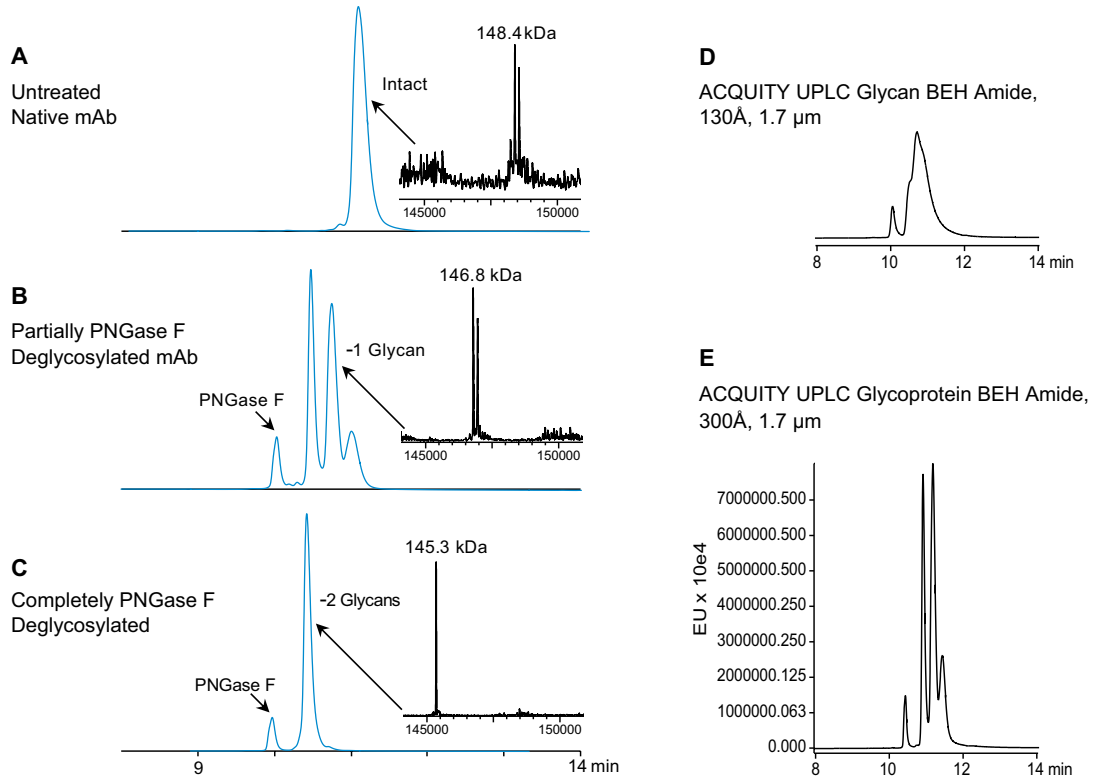
Flow rate:	0.2 mL/min
Injection vol.:	≤1 µL (aqueous diluents)

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

MS 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



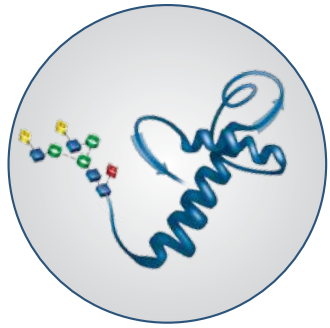
Glycoprotein BEH Amide, 300Å, 1.7 µm Column analyses of Waters mAb Mass Check Standard showing native (A), partially deglycosylated (B), and completely deglycosylated (C) samples. Also showing HILIC fluorescence profiles of partially deglycosylated Intact mAb Mass Check Standard using two ACQUITY UPLC Glycan BEH Amide, 130Å, 1.7 µm, 2.1 x 150 mm Columns in series (D) versus two ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 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 VanGuard Pre-column, 300Å, 1.7 µm, 2.1 mm x 5 mm, 3/pk with Standard	176003699
ACQUITY UPLC Glycoprotein BEH Amide Column, 300Å, 1.7 µm, 2.1 mm x 50 mm, 1/pk with Standard	176003700
ACQUITY UPLC Glycoprotein BEH Amide Column, 300Å, 1.7 µm, 2.1 mm x 100 mm, 1/pk with Standard	176003701
ACQUITY UPLC Glycoprotein BEH Amide Column, 300Å, 1.7 µm, 2.1 mm x 150 mm, 1/pk with Standard	176003702
ACQUITY UPLC Glycoprotein BEH Amide Method Validation Kit, 300Å, 1.7 µm, 2.1 mm x 100 mm, 3/pk with Standard	176003703
Glycoprotein Performance Test Standard	186008010
Intact mAb Mass Check Standard	186006552

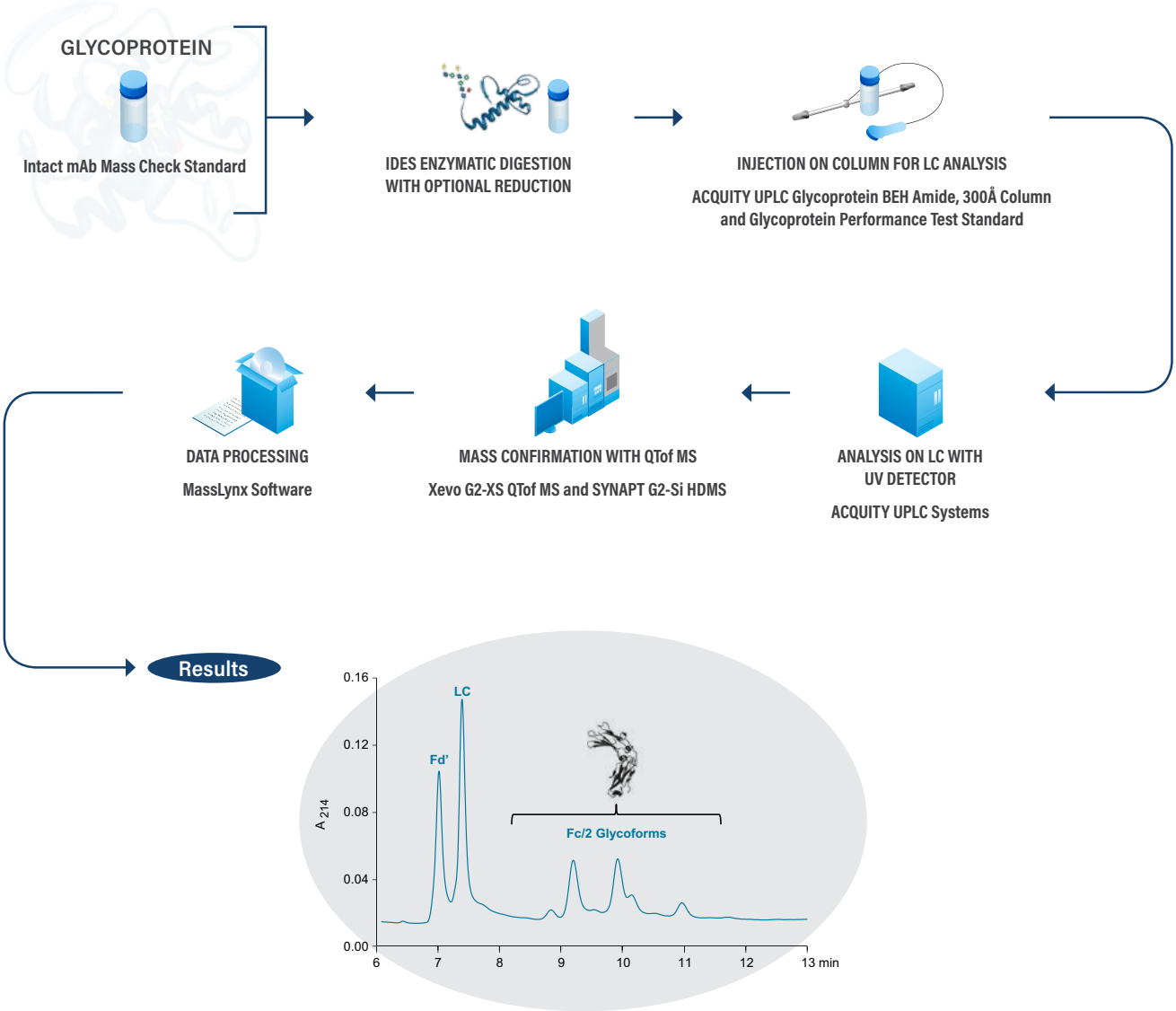
Glycoprotein Subunit Analysis



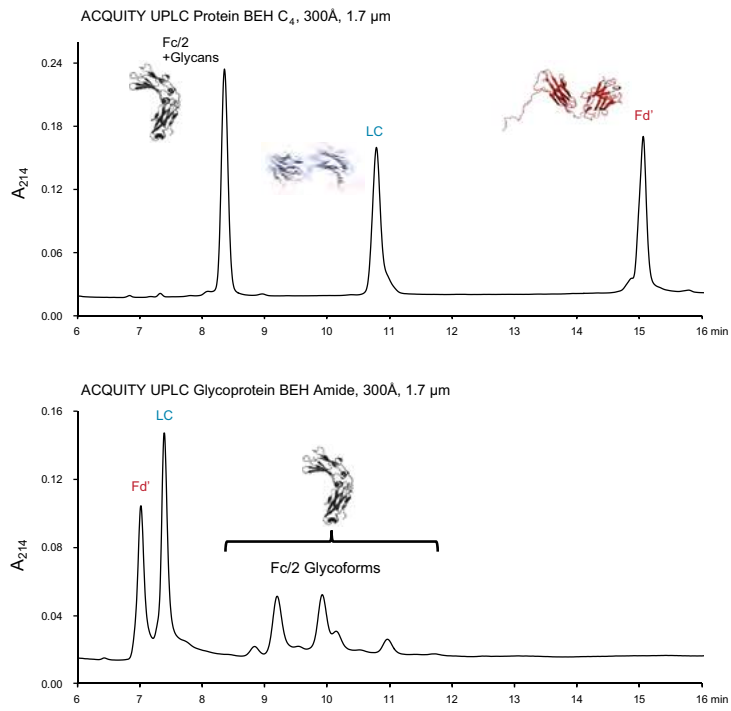
Reversed-phase chromatography is a well-established and commonly used technique to analyze intact protein of protein subunits generated from digestions with enzymes such as FabRICATOR (IdeS protease) that generates a site cleavage at the hinge region of a monoclonal antibody generating Fc and F(ab')2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

LC system:	ACQUITY UPLC H-Class Bio System, 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, 300Å, 1.7 µm, 2.1 x 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, 300Å, 1.7 µm, 2.1 x 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 digested separated using an ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm Column (0.7 µL aqueous injection). Bottom: 1 µg of reduced, IdeS digested separated using an ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm Column (0.7 µL aqueous injection).

Ordering Information

ACQUITY UPLC Glycoprotein BEH Amide Columns, Kits and Standards

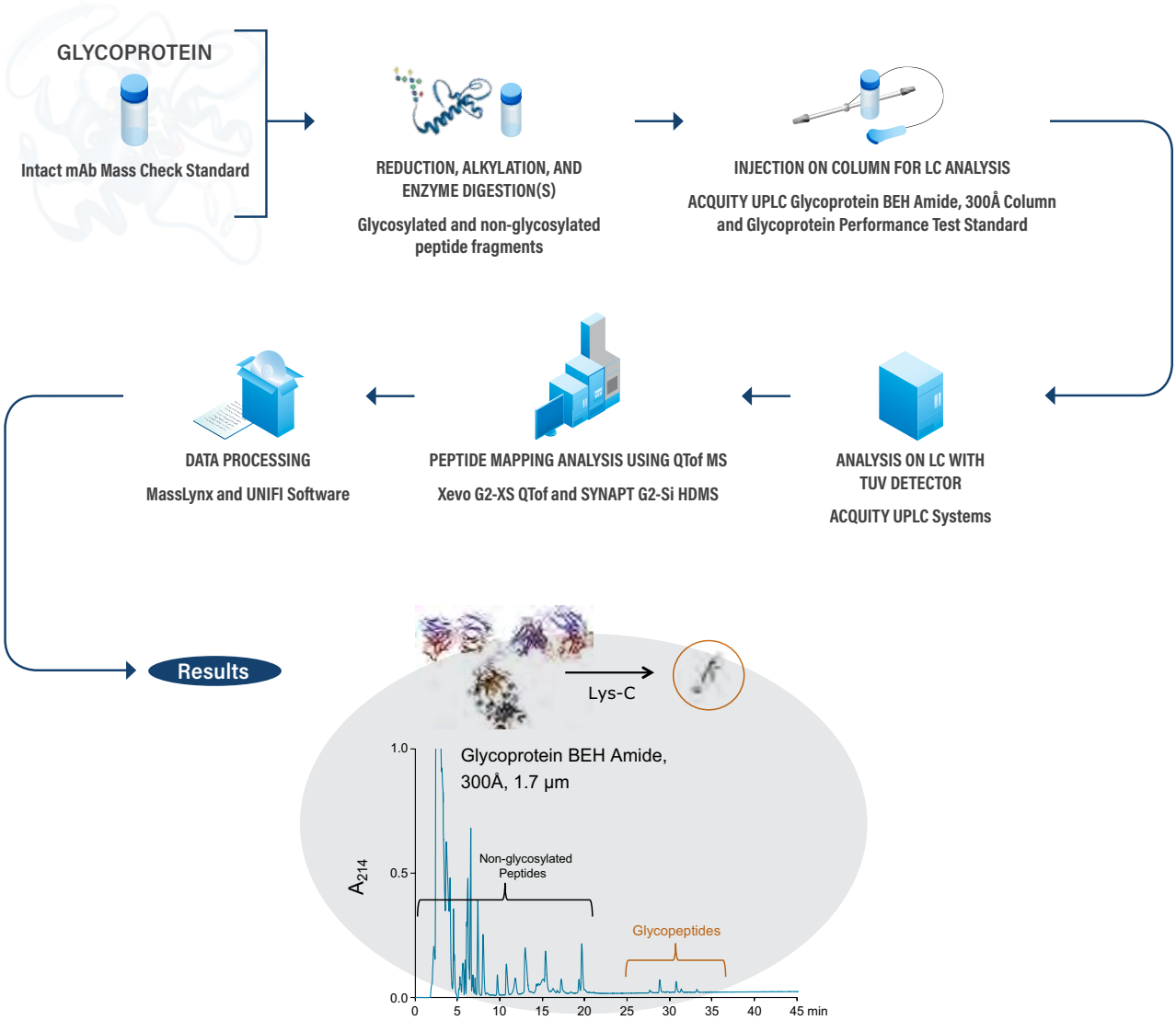
Description	P/N
ACQUITY UPLC Glycoprotein BEH Amide VanGuard Pre-column, 300Å, 1.7 µm, 2.1 mm x 5 mm, 3/pk with Standard	176003699
ACQUITY UPLC Glycoprotein BEH Amide Column, 300Å, 1.7 µm, 2.1 mm x 50 mm, 1/pk with Standard	176003700
ACQUITY UPLC Glycoprotein BEH Amide Column, 300Å, 1.7 µm, 2.1 mm x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

Glycopeptide Analyses

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



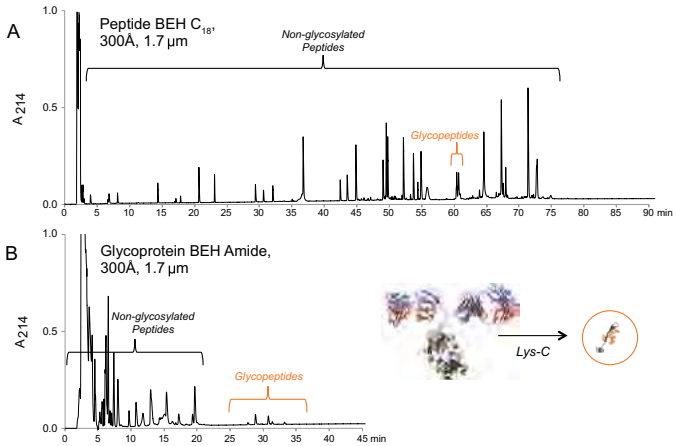
Reversed-Phase vs. HILIC-Based Analyses of a Lys-C Digest of Trastuzumab

LC Conditions

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

Reversed-Phase LC

Column:	ACQUITY UPLC Peptide BEH C_{18}, 300Å, 1.7 µm, 2.1 x 150 mm (p/n: 186003687)
Column temp.:	60 °C
Injection:	RP Injection Volume: 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, 300Å, 1.7 µm, 2.1 x 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

MS 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}, 300Å, 1.7 µm, 2.1 x 150 mm Column (top) vs. a HILIC separation of the Lys-C digest using an ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 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

Conditions (left)

LC system:	ACQUITY UPLC H-Class Bio System
Sample temp.:	10 °C
Vials:	Polypropylene 12 x 32 mm Screw Neck, 300 µL volume (p/n: 186002640)
Column:	ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 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 acetnonitrile/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

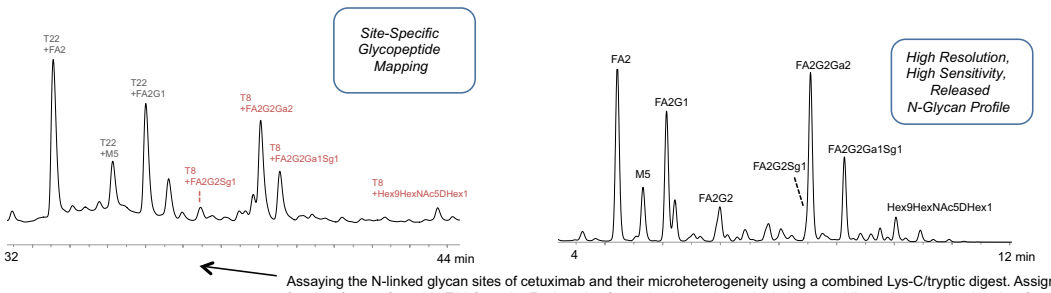
MS system:	SYNAPT G2-S HDMS
Ionization mode:	ESI+
Analyzer mode:	Resolution (~20 K)
Capillary voltage:	3.0 kV
Cone voltage:	25 V
Source temp.:	120 °C
Desolvation temp.:	350 °C
Desolvation gas flow:	800 L/hr
Acquisition:	50–2500 *m/z*, 0.1 sec scan rate
Data management:	MassLynx Software (v4.1)/ UNIFI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

Conditions (right)

LC system:	ACQUITY UPLC H-Class Bio System
Sample temp.:	10 °C
Vials:	Polypropylene 12 x 32 mm Screw Neck, 300 µL volume (p/n: 186002640)
Column:	ACQUITY UPLC Glycan BEH Amide 130Å, 1.7 µm, 2.1 x 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)



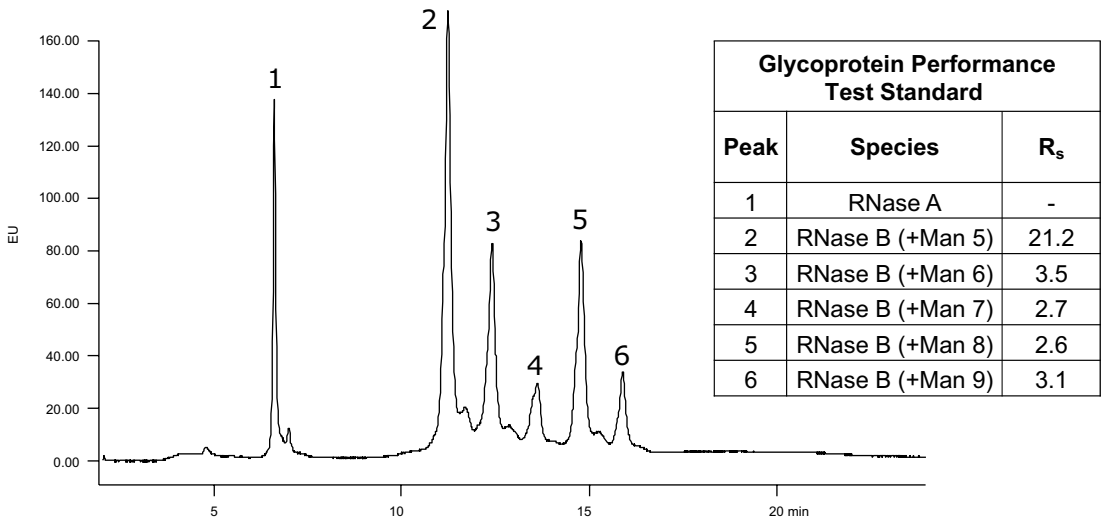
Assaying the N-linked glycan sites of cetuximab and their microheterogeneity using a combined Lys-C/tryptic digest. Assignments for glycoforms of peptide T22 from the Fc domain of cetuximab are shown in dark gray, while assignments to the glycoforms of peptide T8 from the Fab domain are shown in red. 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, 300Å, 1.7 µm, 2.1 x 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.

Benchmarking, Method Development, and Troubleshooting: Glycoprotein Performance Test Standard

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, 300Å, 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, 300Å, 1.7 µm, 2.1 x 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

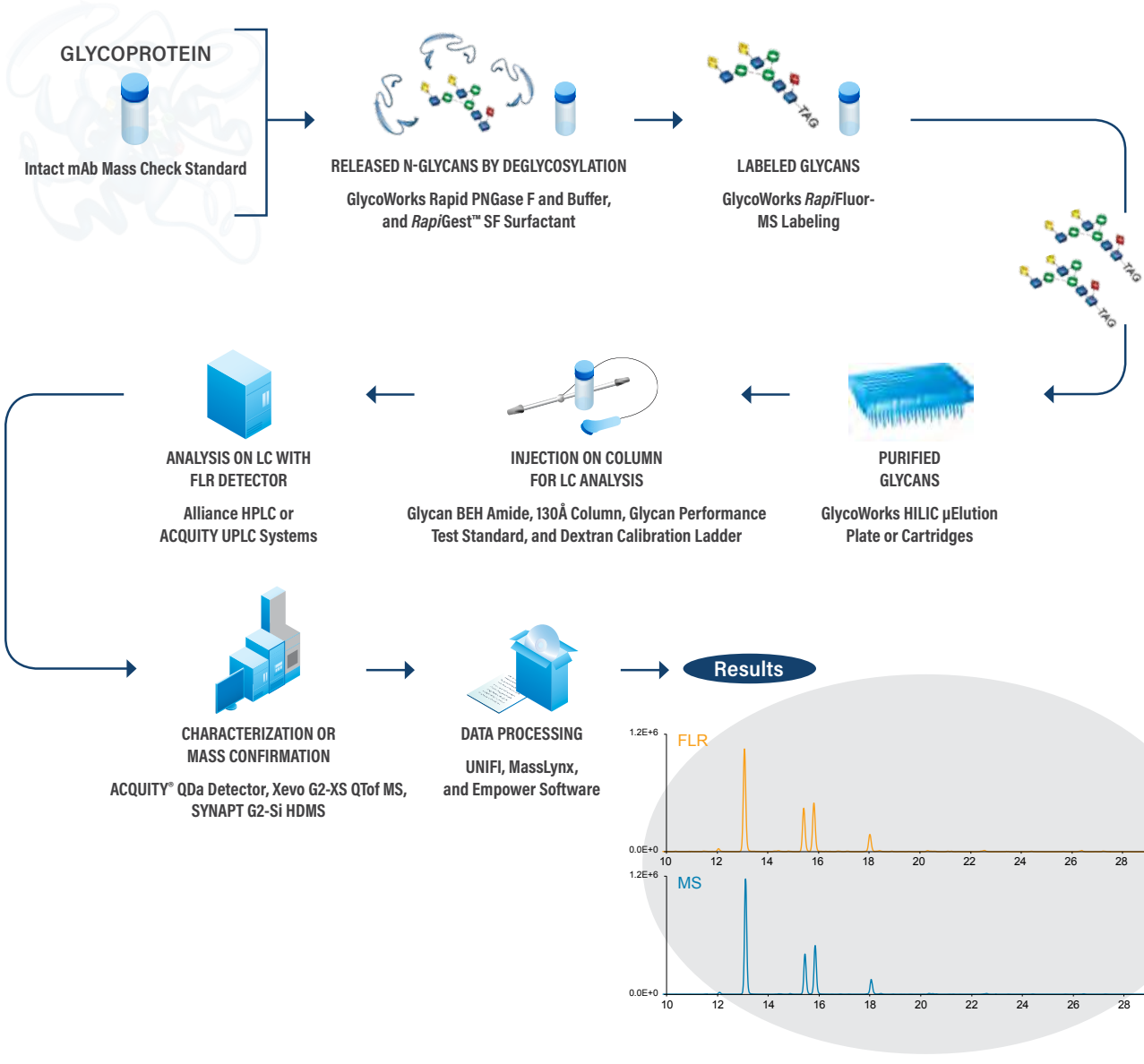
Description	P/N
ACQUITY UPLC Glycoprotein BEH Amide VanGuard Pre-column, 300Å, 1.7 µm, 2.1 mm x 5 mm, 3/pk with Standard	176003699
ACQUITY UPLC Glycoprotein BEH Amide Column, 300Å, 1.7 µm, 2.1 mm x 50 mm, 1/pk with Standard	176003700
ACQUITY UPLC Glycoprotein BEH Amide Column, 300Å, 1.7 µm, 2.1 mm x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

Released N-Glycan Analysis

Waters' GlycoWorks Sample Preparation Kits and Standards, along with the ACQUITY UPLC and HPLC Glycan BEH Amide 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
- Supreme sensitivity of both FLR and MS with the *Rapi*Fluor-MS derivitization label
- High resolving power due to the small particle size (1.7 µm) of the fully-porous material
- Column-to-column performance due to the chemical and mechanical stability of the Waters ethylene-bridged hybrid (BEH) particle and ligand binding technology
- Scale-up and transferability possible with UPLC and HPLC versions

Released N-Glycan Workflow

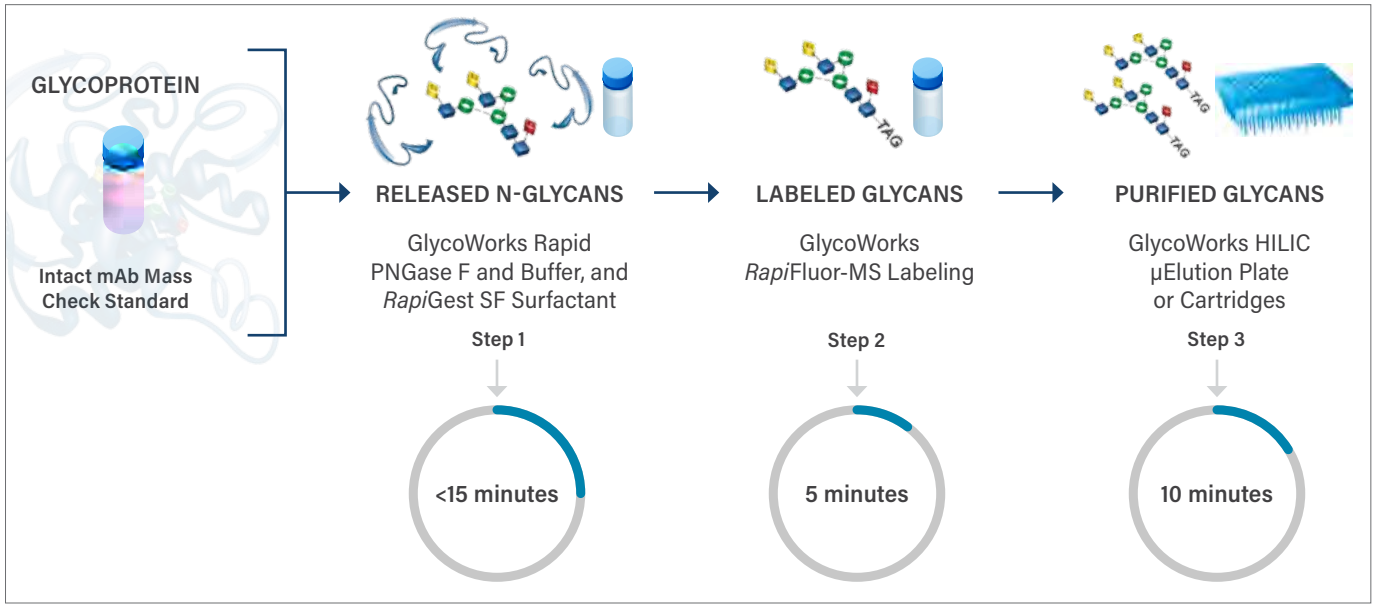


RELEASED N-GLYCAN SAMPLE PREPARATION WITH GLYCOWORKS

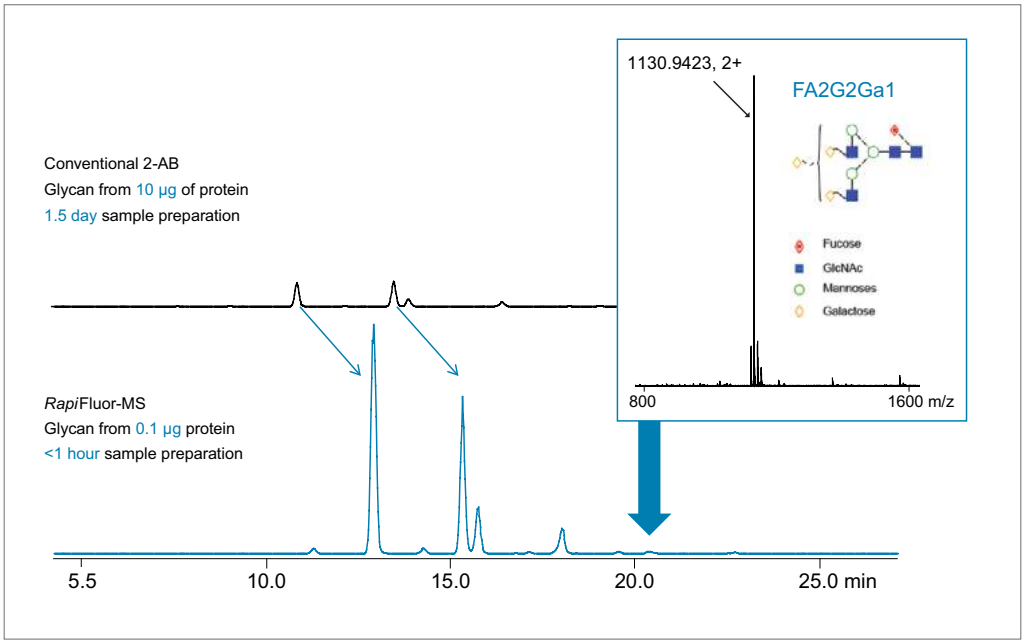
Waters GlycoWorks consumables offer a more convenient, comprehensive and effective sample-preparation solution for glycan analysis.

- The GlycoWorks *Rapi*Fluor-MS N-Glycan Kit ensures easy, quick preparation of released-labeled, N-glycan samples
- Streamlined protocols minimize errors and sample loss
- Greatly improved FLR and MS signal intensities help esely identiy low-abundance N-linked glycans
- Complete modules for processing 96 samples with flexibility of processing between 8 and 24 samples at a time depending on laboratory demands
- Support easy training of analysts and the transferring of methods throughout an organization.

3 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.

Ordering Information

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 Clean-up Module, GlycoWorks Sample Collection Module, ACQUITY UPLC Glycan BEH Amide, 1.7 µm, 2.1 x 150 Column, Ammonium Formate Solution – Glycan Analysis	176003635
GlycoWorks *Rapi*Fluor-MS N-Glycan Kit—96 Sample Kit contains: GlycoWorks Deglycosylation Module, GlycoWorks Labeling Module, GlycoWorks Clean-up Module, GlycoWorks Sample Collection Module	176003606
GlycoWorks *Rapi*Fluor-MS N-Glycan Starter Kit—24 sample Kit contains: GlycoWorks Deglycosylation Module (24 sample), GlycoWorks Labeling Module (24 sample), GlycoWorks Clean-up Module, GlycoWorks Sample Collection Module, ACQUITY UPLC Glycan BEH Amide, 1.7 µm, 2.1 x 150 mm Column, Ammonium Formate Solution – Glycan Analysis	176003712
GlycoWorks *Rapi*Fluor-MS N-Glycan Kit—24 sample Kit contains: GlycoWorks Deglycosylation Module (24 sample), GlycoWorks Labeling Module (24 sample), GlycoWorks Clean-up Module, GlycoWorks Sample Collection Module	176003713
GlycoWorks *Rapi*Fluor-MS N-Glycan Refill Kit—24 sample Refill Kit contains one of each: GlycoWorks Deglycosylation Module (25 sample) and the GlycoWorks Labeling Module (24 sample)	176003714
GlycoWorks Rapid Deglycosylation 1 x 24 Kit contains: one vial of GlycoWorks Rapid PNGaseF Enzyme and Buffer; and, one vial of 10-mg *Rapi*Gest SF Surfactant	176003867
GlycoWorks Rapid Deglycosylation 3 x 8	176003868

*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 Inatact 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
Ammonium Formate Solution – Glycan Analysis 5000 mM	186007081
GlycoWorks Rapid Buffer—5 mL	186008100

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
ACQUITY UPLC Glycan BEH Amide, 130Å, 1.7 µm, 2.1 x 150 mm Column	186004742

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

GlycoWorks RapiFluor-MS N-Glycan Kit—96 Sample.

Learn more about Waters latest Glycan Solutions.

Visit **waters.com/glycans**

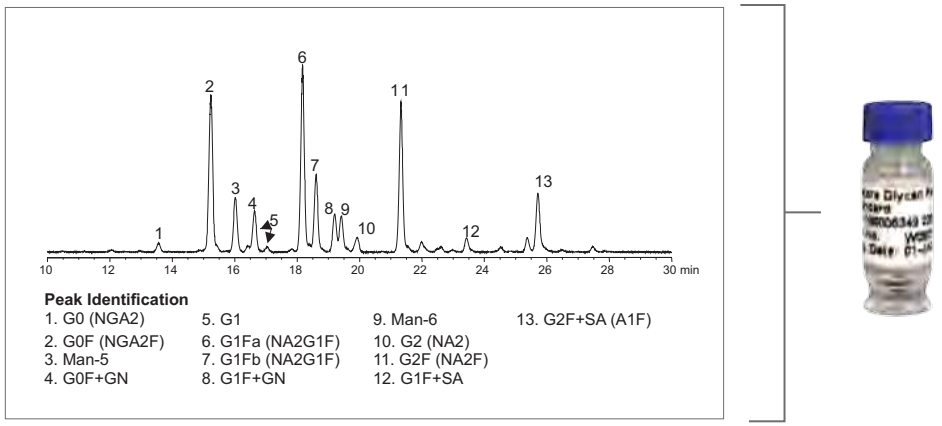
Benchmarking, Method Development, and Troubleshooting: Glycan Performance Test Standards and Dextran Calibration Ladders

GLYCAN PERFORMANCE TEST STANDARDS

The Glycan 2-AB Performance Test Standard is Human-like IgG spiked with Man-5 and Man-6 and is QC verified to contain the components needed to benchmark and evaluate ACQUITY UPLC Glycan BEH Amide Columns containing 1.7 µm particles and the XBridge Glycan BEH Amide Columns that contain either 2.5 µm or 3.5 µm particles. It is also valuable to use as an additional 2-AB labeled control to assess digestion and labeling reaction efficiencies.

Dextran Calibration Ladders

The Dextran Calibration Ladders allow the user to tie in the entire GlycoWorks Sample Preparation Solution seamlessly to the Waters ACQUITY UPLC System and GlycoBase Database Search. Using these labeled standards allows the user to calibrate their system based on GU units, and have confidence in results. Available in 2-AB, 2-AA, and the new *Rapi*Fluor-MS labels.



2-AB Glycan Performance Test Standard, FLR Trace.

Ordering Information

ACQUITY UPLC Glycan BEH Amide Columns and Method Validation Kits

Description	P/N
ACQUITY UPLC Glycan BEH Amide, 130Å, 1.7 µm, 2.1 x 5 mm VanGuard Column, 3/pk	186004739
ACQUITY UPLC Glycan BEH Amide, 130Å, 1.7 µm, 2.1 x 50 mm Column	186004740
ACQUITY UPLC Glycan BEH Amide, 130Å, 1.7 µm, 2.1 x 100 mm Column	186004741
ACQUITY UPLC Glycan BEH Amide, 130Å, 1.7 µm, 2.1 x 100 mm Column Method Validation Kit[1]	186004907
ACQUITY UPLC Glycan BEH Amide, 130Å, 1.7 µm, 2.1 x150 mm Column	186004742

[1]Three columns from three different batches of BEH Amide, 130Å material.

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.

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

Description	P/N
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 2.1 x 5 mm VanGuard Column, 3/pk	186007262
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 2.1 x 50 mm ***XP*** Column	186007263
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 2.1 x 100 mm ***XP*** Column	186007264
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 2.1 x 150 mm ***XP*** Column	186007265
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 2.1 x 150 mm ***XP*** Column Method Validation Kit[1]	186007266
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 3.0 x 30 mm ***XP*** Column	186008038
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 3.0 x 75 mm ***XP*** Column	186008039
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 3.0 x 150 mm ***XP*** Column	186008040
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 4.6 x 20 mm Guard Column, 2/pk[3]	186007267
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 4.6 x 50 mm ***XP*** Column	186007268
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 4.6 x 100 mm ***XP*** Column	186007269
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 4.6 x 150 mm ***XP*** Column	186007270
XBridge Glycan BEH Amide, 130Å, 2.5 µm, 4.6 x 150 mm ***XP*** Column Method Validation Kit[1]	186007271
XBridge Glycan BEH Amide, 130Å, 3.5 µm, 2.1 x 10 mm Guard Column, 2/pk[2]	186007505
XBridge Glycan BEH Amide, 130Å, 3.5 µm, 2.1 x 50 mm Column	186007502
XBridge Glycan BEH Amide, 130Å, 3.5 µm, 2.1 x 100 mm Column	186007503
XBridge Glycan BEH Amide, 130Å, 3.5 µm, 2.1 x 150 mm Column	186007504
XBridge Glycan BEH Amide, 130Å, 3.5 µm, 4.6 x 20 mm Guard Column, 2/pk[3]	186007272
XBridge Glycan BEH Amide, 130Å, 3.5 µm, 4.6 x 50 mm Column	186007273
XBridge Glycan BEH Amide, 130Å, 3.5 µm, 4.6 x 100 mm Column	186007274
XBridge Glycan BEH Amide, 130Å, 3.5 µm, 4.6 x 150 mm Column	186007275
XBridge Glycan BEH Amide, 130Å, 3.5 µm, 4.6 x 150 mm Column Method Validation Kit[1]	186007277
XBridge Glycan BEH Amide, 130Å, 3.5 µm, 4.6 x 250 mm Column	186007276

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

Reductive Amination Glycan Sample Preparation Kit and Standards

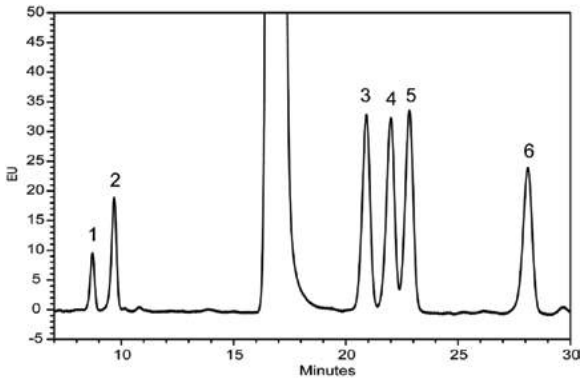
Description	P/N
GlycoWorks Reductive Amination High-throughput Prep Kit	176003090
GlycoWorks HILIC µElution Plate 96-well	186002780
*Rapi*Gest SF 1 mg vial	186001860
GlycoWorks Control Standard, 100 µg vial	186007033
GlycoWorks Reagent Kit	186007034
Manifold Waste Tray	600001282
GlycoWorks Reductive Amination Single Use Prep Kit	176003119
GlycoWorks HILIC 1 cc Cartridge (10/pk)	186007080
*Rapi*Gest SF 1 mg vial	186001860
GlycoWorks Control Standard, 100 µg vial	186007033
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
2-AA Dextran Calibration Ladder The 2-AA 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.	186007279
GlycoWorks HILIC 1 cc Cartridge, 20/pkg	186007080
GlycoWorks HILIC 1 cc Flangeless Cartridge	186007239
GlycoWorks HILIC µElution Plate	186002780
GlycoWorks Reagent Kit	186007034
GlycoWorks SPE Reagents	186007990
Ammonium Formate Solution – Glycan Analysis	186007081

Columns for Monosaccharide and Sialic Acid Analyses from Glycoproteins

MONOSACCHARIDE ANALYSES

Apart from charged sialic acid species, the primary monosaccharides found in N-linked and O-links 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 2M 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 (p/n: 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



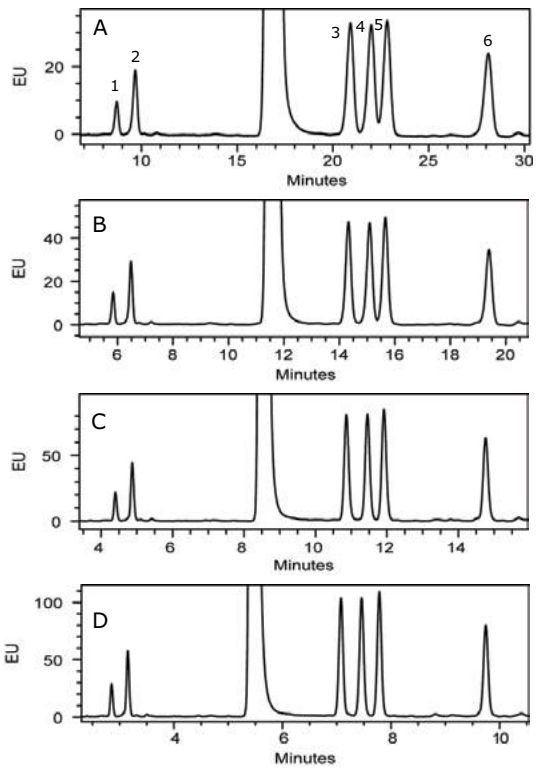
Conditions:

LC system:	ACQUITY UPLC H-Class
Sample:	Acid Hydrolyzed Bovine Fetuin followed by 2-AA derivatization
Injection:	4.8 µL
Column:	XBridge BEH C_{18}, 130Å, 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_g	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}, 130Å, 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



Conditions:

LC 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}, 130Å, 5 µm, 4.6 x 100 mm (p/n: 186003115) XBridge BEH C_{18}, 130Å, 3.5 µm, 2.1 x 100 mm (p/n: 186003033) XBridge BEH C_{18}, 130Å, 2.5 µm ***XP***, 2.1 x 100 mm (p/n: 186006031) ACQUITY UPLC BEH C_{18}, 130Å, 1.7 µm, 2.1 x 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)

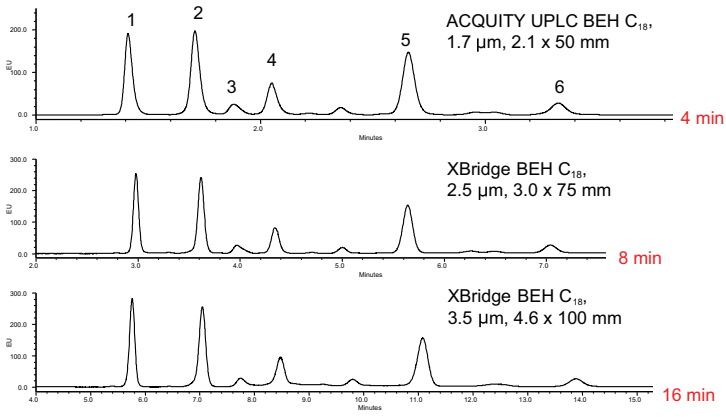
		5 µm		3.5 µm		2.5 µm		1.7 µm	
Step	%B[1]	Flow (mL min-1)	Time (min)	Flow (mL min-1)	Time (min)	Flow (mL min-1)	Time (min)	Flow (mL min-1)	Time (min)
1	7	0.480	0.00	0.685	0.00	0.200	0.00	0.294	0.00
2	7	0.480	7.78	0.685	5.45	0.200	3.89	0.294	2.64
3	17	0.480	27.78	0.685	19.47	0.200	13.88	0.294	9.44
4	100	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}, 130Å, (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 compenent 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 2 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. Neu,7Ac2, 4. Neu,Gc9Ac, 5. Neu5,9Ac2, 6. Neu5,7,(8)9Ac3

Conditions:

LC 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}, 130Å, 3.5 µm particle (bottom), 2.5 µm particle (middle), and ACQUITY UPLC BEH C_{18}, 130Å, 1.7 µm particle (top).

Ordering Information

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

Description	P/N
ACQUITY UPLC BEH C_{18}, 130Å,1.7 µm, 2.1 x 50 mm	186002350
ACQUITY UPLC BEH C_{18}, 130Å, 1.7 µm, 2.1 x 100 mm	186002352
ACQUITY UPLC BEH 130Å, 1.7 µm, 2.1 x 150 mm	186004742
XBridge BEH C_{18}, 130Å, 2.5 µm, 2.1 x 100 mm, ***XP***	186006031
XBridge BEH C_{18}, 130Å, 2.5 µm, 3 x 100 mm, ***XP***	186006035
XBridge BEH C_{18}, 130Å, 2.5 µm, 3 x 150 mm, ***XP***	186006710
XBridge BEH C_{18}, 130å, 3.5 µm, 2.1 x 100 mm	186003033
XBridge BEH C_{18}, 130å, 5 µm, 4.6 x 100 mm	186003115

Reference Materials for Glycan and Glycoprotein Separations

Title	Literature Code
Application Notebook	
Glycans Application Notebook	720005532EN
Application Notes	
Applying a Novel Glycan Tagging Reagent, *Rapi*Fluor-MS, and an Integrated UPLC-FLR/QTof MS System for Low Abundant N-Glycan Analysis	720005383EN
Comprehensive Characterization of the N and O-Linked Glycosylation of a Recombinant Human EPO	720005462EN
Developing High Resolution HILIC Separations of Intact Glycosylated Proteins Using a Wide-Pore Amide-Bonded Stationary Phase	720005380EN
DMB-Labeled Sialic Acid Analyses Using HPLC, UHPLC, and UPLC-Based BEH C_{18} Columns	720005550EN
Exploiting *Rapi*Fluor-MS Labeling to Monitor Diverse N-Glycan Structures via Fluorescence and Mass Detection	720005353EN
HILIC Glycopeptide Mapping with a Wide-Pore Amide Stationary Phase	720005409EN
Mapping IgG Subunit Glycoforms Using HILIC and a Wide-Pore Amide Stationary Phase	720005385EN
Measuring the Glycan Occupancy of Intact mAbs Using HILIC and Detection by Intrinsic Fluorescence	720005435EN
Profiling Released High Mannose and Complex N-Glycan Structures from Monoclonal Antibodies Using *Rapi*Fluor-MS Labeling and Optimized Hydrophilic Interaction Chromatography	720005516EN
Rapid Preparation of Released N-Glycans for HILIC Analysis Using a Novel Fluorescence and MS-Active Labeling Reagent	720005275EN
Robustness of *Rapi*Fluor-MS N-Glycan Sample Preparations and Glycan BEH Amide HILIC Chromatographic Separations	720005370EN
For a complete list of application notes, visit waters.com/AppNotes	
Catalog	
Waters Analytical Standards & Reagents	asr.waters.com
Wall Chart	
Bioseparations Columns Wall Chart	720004232EN
Webinars	
[Meet the Experts] Webinar Series	www.waters.com/MeetTheExperts

Oligonucleotide Separations

Oligonucleotide Separations

Contents

Oligonucleotide Separations

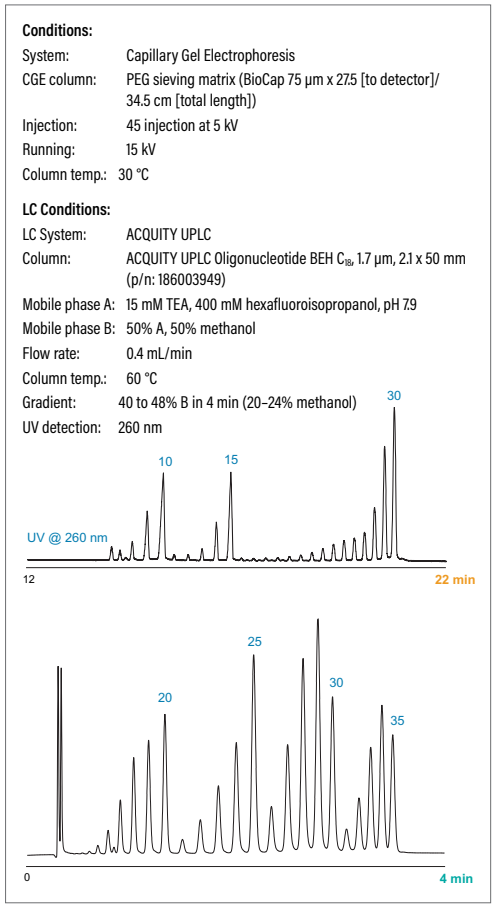
Waters Oligonucleotide columns contain second-generation hybrid silica BEH Technology particles functionalized with C_{18}. The separation of detritylated synthetic oligonucleotide samples is based on the well-established method of ion-pair, reversed-phase chromatography. The availability of 1.7 µm UPLC 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.

- Separation 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

EXCEPTIONAL RESOLUTION OF OLIGONUCLEOTIDE MIXTURES

ACQUITY UPLC Oligonucleotide C_{18}, 1.7 µm (designed for use with an ACQUITY UPLC System) and XBridge Oligonucleotide 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 analyses 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



Ordering Information

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

Description	Particle Size	Pore Size	Dimension	P/N
ACQUITY UPLC Oligonucleotide BEH C_{18}*	1.7 µm	130Å	2.1 x 50 mm	186003949
ACQUITY UPLC Oligonucleotide BEH C_{18}*	1.7 µm	130Å	2.1 x 100 mm	186003950
ACQUITY UPLC Oligonucleotide BEH C_{18}*	1.7 µm	130Å	2.1 x 150 mm	186005516
ACQUITY UPLC Oligonucleotide BEH C_{18} Method Validation Kit**	1.7 µm	130Å	2.1x 100 mm	186004898

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

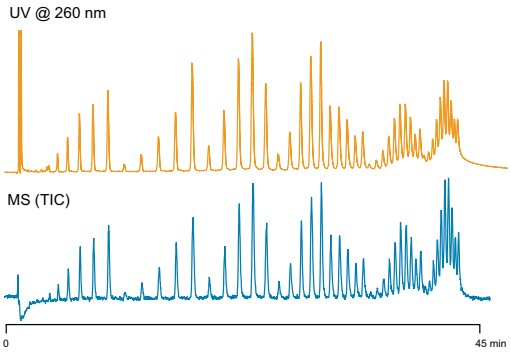
Description	Particle Size	Pore Size	Dimension	P/N
XBridge Oligonucleotide BEH C_{18}*	2.5 µm	130Å	2.1 x 50 mm	186003952
XBridge Oligonucleotide BEH C_{18}*	2.5 µm	130Å	4.6 x 50 mm	186003953
XBridge Oligonucleotide BEH C_{18}***	2.5 µm	130Å	10 x 50 mm	186008212
XBridge Oligonucleotide BEH C_{18} Method Validation Kit**	2.5 µm	130Å	4.6 x 50 mm	186004906

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

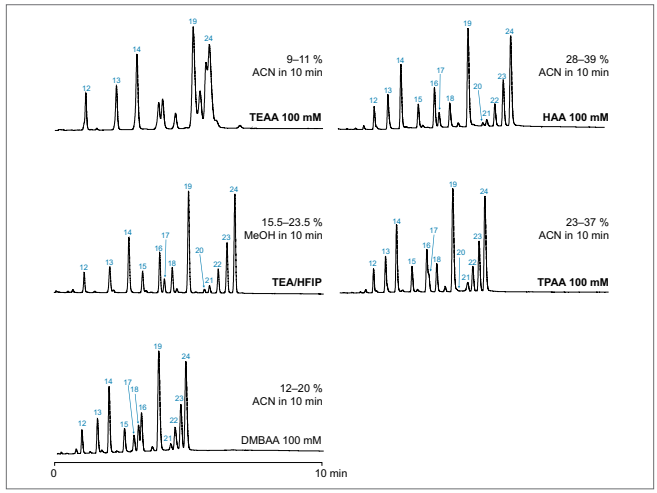
Separation of a 15–60 mer Detritylated Oligodeoxythymidine Ladder

Conditions:

LC System:	ACQUITY UPLC with PDA and Q-Tof micro™ MS	Flow rate:	0.1 mL/min
Column:	ACQUITY UPLC Oligonucleotide BEH C_{18}, 1.7 µm, 2.1 x 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 Oligoneucleotides separations can be achieved using alternative IP agents compared to use of traditional TEAA.

UPLC-MS ANALYSIS OF INTERFERING RNA OLIGONUCLEOTIDES

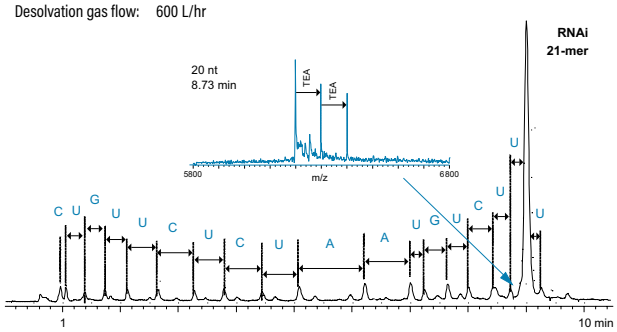
Discovery of the RNA interference (RNAi) mechanism now broadly used for silencing of target gene expression has prompted a need for the analysis of small interfering RNAs (siRNA) molecules. To satisfy the need for a robust, fast, and sensitive analysis of 20–25 nucleotides of small interfering RNA (siRNA), 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 Figure 2. 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)

Conditions:

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



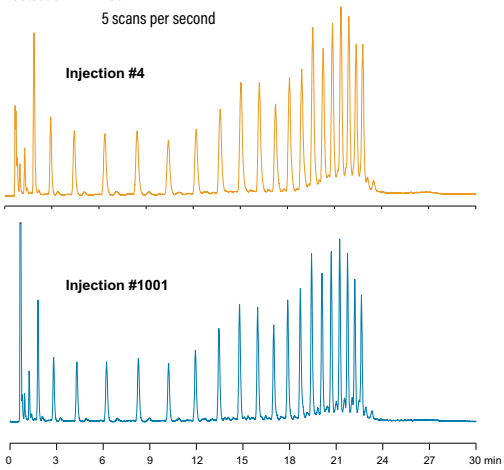
OUTSTANDING COLUMN LIFE

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.

Separation of 5–25 mer Detritylated Oligodeoxythymidine Ladder

Conditions:

HPLC System:	Alliance HPLC with PDA
Column:	XBridge Oligonucleotide BEH C_{18}, 2.5 µm, 2.1 x 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



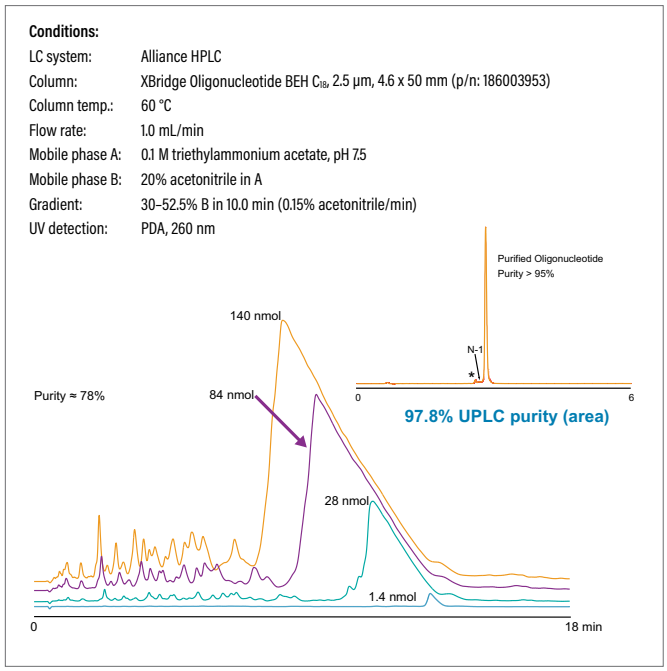
SCALABLE DNA AND RNAI SEPARATIONS WITH GOOD PRODUCT RECOVERY

XBridge Oligonucleotide BEH C_{18}, 130Å 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 x 50 mm column containing XBridge Oligonucleotide BEH C_{18}, 130Å, 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.

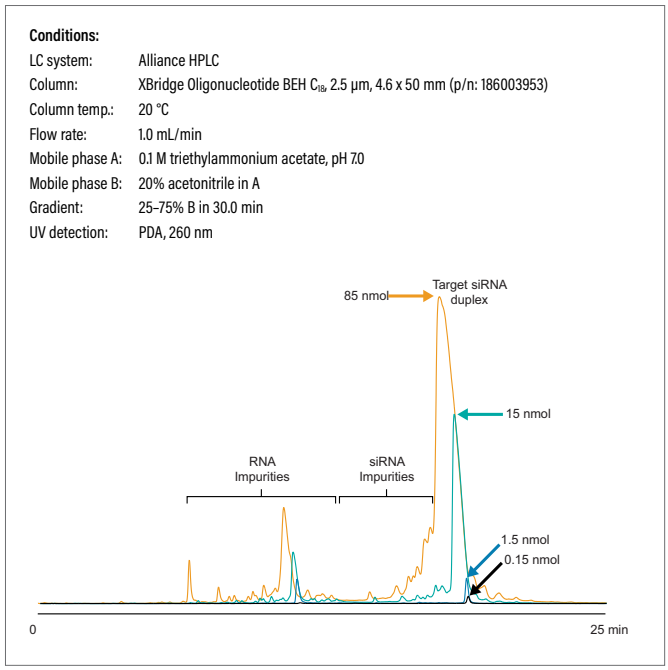
For researchers involved in gene silencing it is often necessary to work with RNA of high purity. Crude synthetic oligonucleotides used for gene knockout are typically purified. The figure below illustrates a lab-scale purification of 21 mer RNA at various column loads. Using an Oligonucleotide column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



Purification of siRNA Duplex from Impurities



Dimension	Approx Mass Load**	Yield***	Flow Rate
2.1 x 50 mm	0.04 µmoles	0.2 mg	0.2 mL/min
4.6 x 50 mm	0.20 µmoles	1.0 mg	1.0 mL/min
10 x 50 mm	1.00 µmoles	4.5 mg	4.5 mL/min
19 x 50 mm*	4.00 µmoles	16.0 mg	16.0 mL/min
30 x 50 mm*	9.00 µmoles	40.0 mg	40.0 mL/min
50 x 50 mm*	25.00 µmoles	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 BEH C_{18}, 300Å Reversed-Phase 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.

Ordering Information

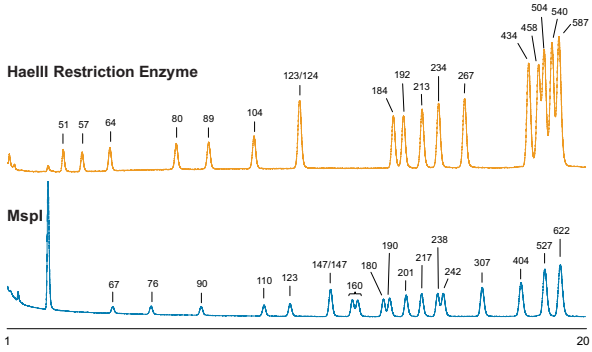
ACQUITY UPLC BEH C_{18}, 300Å Columns for DNA/RNA Fragments

Description	Particle Size	Pore Size	Dimension	P/N
ACQUITY UPLC BEH C_{18}	1.7 µm	300Å	2.1 x 50 mm	186003685

Separation of Duplex DNA Fragments: HaeIII and MspI Restriction Enzyme Digests of pBR322 Plasmid

Conditions:

LC system:	ACQUITY UPLC
Column:	ACQUITY UPLC Peptide BEH C_{18}, 1.7 µm, 2.1 x 50 mm (p/n: 186003685)
Column temp.:	50 °C
Flow rate:	0.2 mL/min
Mobile phase A:	0.1 M triethylammonium acetate, pH 7.0
Mobile phase B:	20% acetonitrile in A
Gradient:	57.5–84.5% B in 20.0 min
UV detection:	PDA, 260 nm



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.

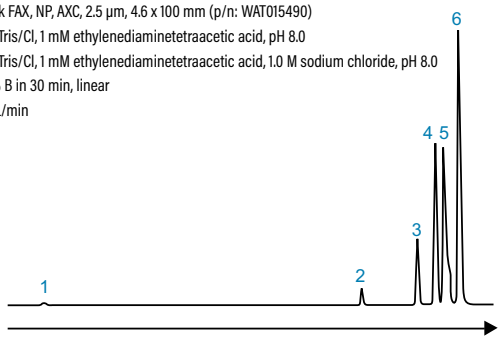
Separation of DNA Restriction Fragments

Conditions:

Sample:	3.0 µg of BstN1 digest of pBR322
Column:	Gen-Pak FAX, NP, AXC, 2.5 µm, 4.6 x 100 mm (p/n: WAT015490)
Eluent A:	25 mM Tris/Cl, 1 mM ethylenediaminetetraacetic acid, pH 8.0
Eluent B:	25 mM Tris/Cl, 1 mM ethylenediaminetetraacetic acid, 1.0 M sodium chloride, pH 8.0
Gradient:	25–75% B in 30 min, linear
Flow rate:	0.75 mL/min
Column temp.:	30 °C
UV detection:	260 nm

Compounds:

1. 13 bp
2. 121 bp
3. 383 bp
4. 929 bp
5. 1060 bp
6. 1857 bp



Ordering Information

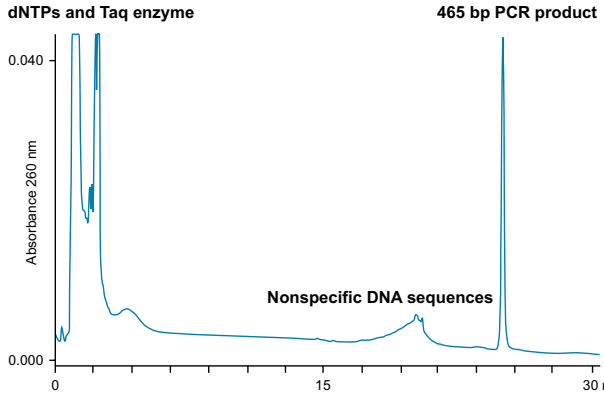
Gen-Pak FAX HPLC Column

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

Chromatography of a PCR Amplification Mixture Generated using 3 ng and 1 fg of HBV S-Gene Template

Conditions:

Sample:	PCR amplification mixture using 3 ng HBV-S gene template
Column:	Gen-Pak FAX, NP, AXC, 2.5 µm, 4.6 x 100 mm (p/n: WAT015490)
Eluent A:	25 mM Tris/Cl, 1 mM ethylenediaminetetraacetic acid, pH 8.0
Eluent B:	25 mM Tris/Cl, 1 mM ethylenediaminetetraacetic acid, 1.0 M sodium chloride, pH 8.0
Gradient:	40–75% B in 30 min, linear
Flow rate:	0.75 mL/min
Column temp.:	30 °C

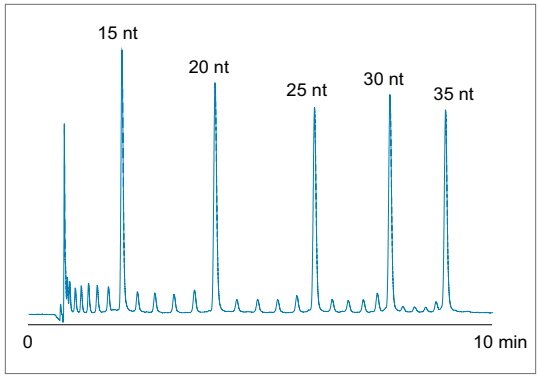


Benchmarking, Method Development, and Troubleshooting: MassPREP Oligonucleotide Standard

- 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 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}, 1.7 µm Column



Waters ACQUITY UPLC analysis of MassPREP Oligonucleotide Standard on an ACQUITY UPLC Oligonucleotide C_{18}, 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

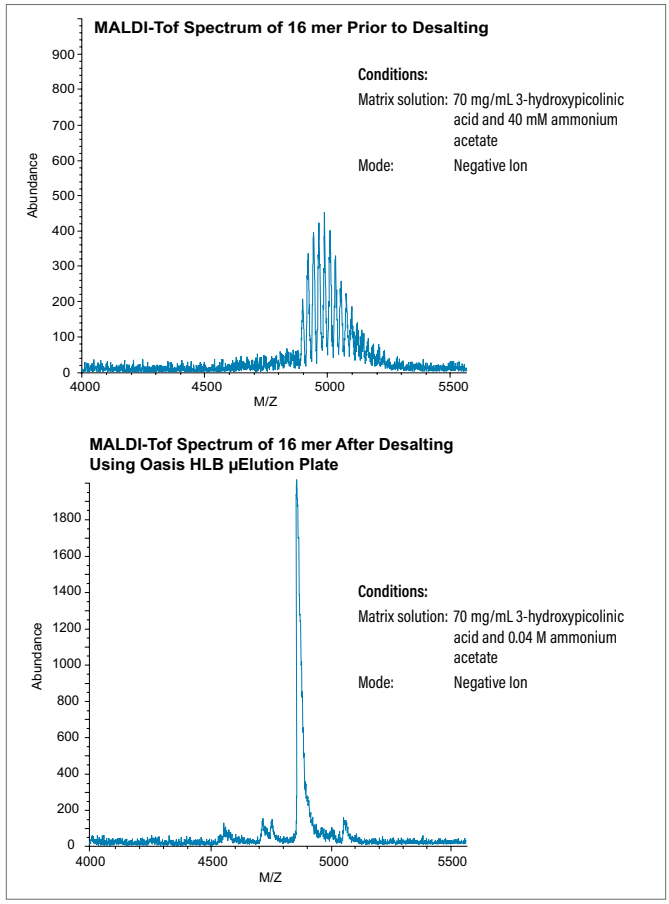
Oasis µElution Plates

Oligonucleotide Desalting by Solid-Phase Extraction

- 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. Now you can perform SPE clean-up 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

Reference Materials for Oligonucleotide Separations

Title	Literature Code
Application Notebook	
Application Solutions for Oligonucleotides	720002873EN
Application Notes	
Hexylammonium Acetate as an Ion-Pairing Agent for IP-RP LC Analysis of Oligonucleotides	720003361EN
HPLC and UPLC Column for the Analysis of Oligonucleotides	720002376EN
Real-Time Analysis of RNAi Duplexes	720002573EN
RNAi Duplex Analysis and Purification	720002800EN
Semi-Preparative Scale Single-Stranded RNA Purification	720002602EN
Successful Analysis of siRNA using the ACQUITY UPLC H-Class Bio System	720003645EN
UPLC Separation of DNA Duplexes	720002741EN
UPLC Separation of Oligonucleotides: Effect of Increased Flow Rate and Faster Run Time	720003386EN
UPLC Separation of Oligonucleotides: Method Development	720002383EN
UPLC/MS Analysis of Interfering RNA Oligonucleotides	720002412EN
For a complete list of application notes, visit waters.com/AppNotes	
Catalog	
Waters Analytical Standards & Reagents	asr.waters.com
Wall Chart	
Bioseparations Columns Wall Chart	720004232EN
Webinars	
[Meet the Experts] Webinar Series	www.waters.com/MeetTheExperts

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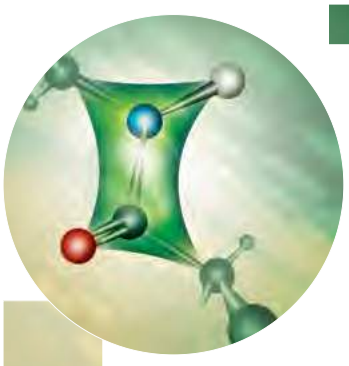
Peptide Separations



Peptide Separations

Contents

Peptide Separations

The desired separation, accurate quantitation, and identification of peptides ranging from proteomics investigations to biotherapeutics mAb characterization is challenging. To be successful, scientists acknowledge the importance of separation synergies that occur when a defined column, instrument, and method are assembled to address specific application needs.

Reversed-phase (RP) chromatography has become the separation mode of choice for many of these challenging applications. It offers relatively high resolving power and provides outstanding quantitative (UV) and qualitative (ESI-MS) information. In RP-based peptide separations, the size of the peptide as well as the hydrophobicity of the amino-acid side chains determine the elution order. Consequently, small, less hydrophobic peptide sequences elute first using a gradient of increasing organic solvent concentration.

Peptide BEH C_{18} (130Å, 300Å), Peptide CSH C_{18} (130Å), and Peptide HSS T3 (100Å) Columns

Waters Ethylene-Bridged Hybrid (BEH) and Charged Surface Hybrid (CSH) Column Technologies can be effectively used to generate high quality UPLC, UHPLC, or HPLC peptide separations via RP chromatography. Their effective use in either TFA- or FA-containing eluents makes them well suited for either LC or LC-MS applications. Our Peptide HSS T3 columns are designed for separations where silica-based selectivities are desired or when retention of hydrophillic peptides are required.

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 (130Å and 300Å) 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 130Å pore size is best suited for compounds less than 10,000 Daltons

HSS HIGH STRENGTH SILICA

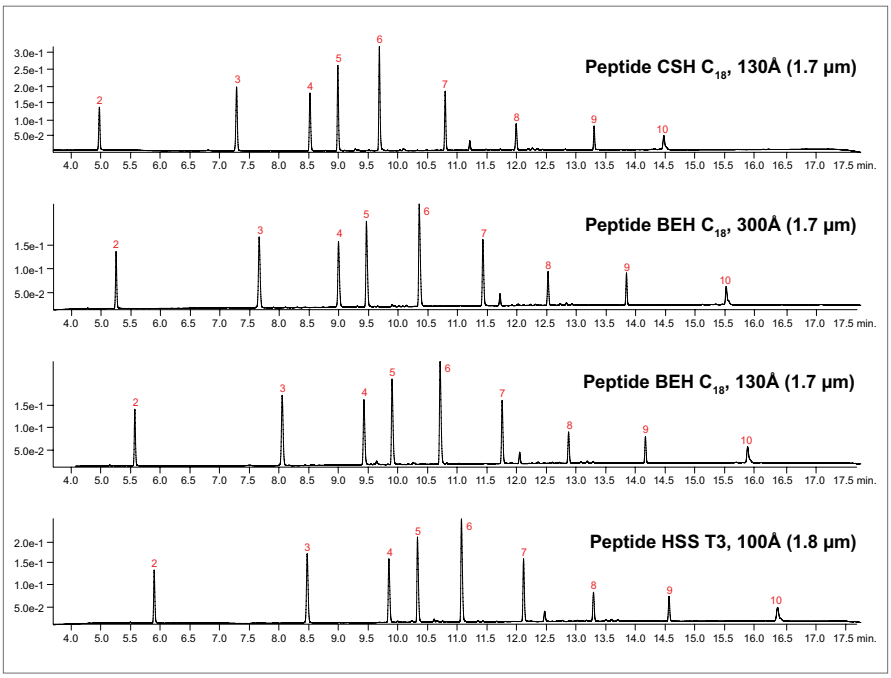
HSS (High Strength Silica)

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

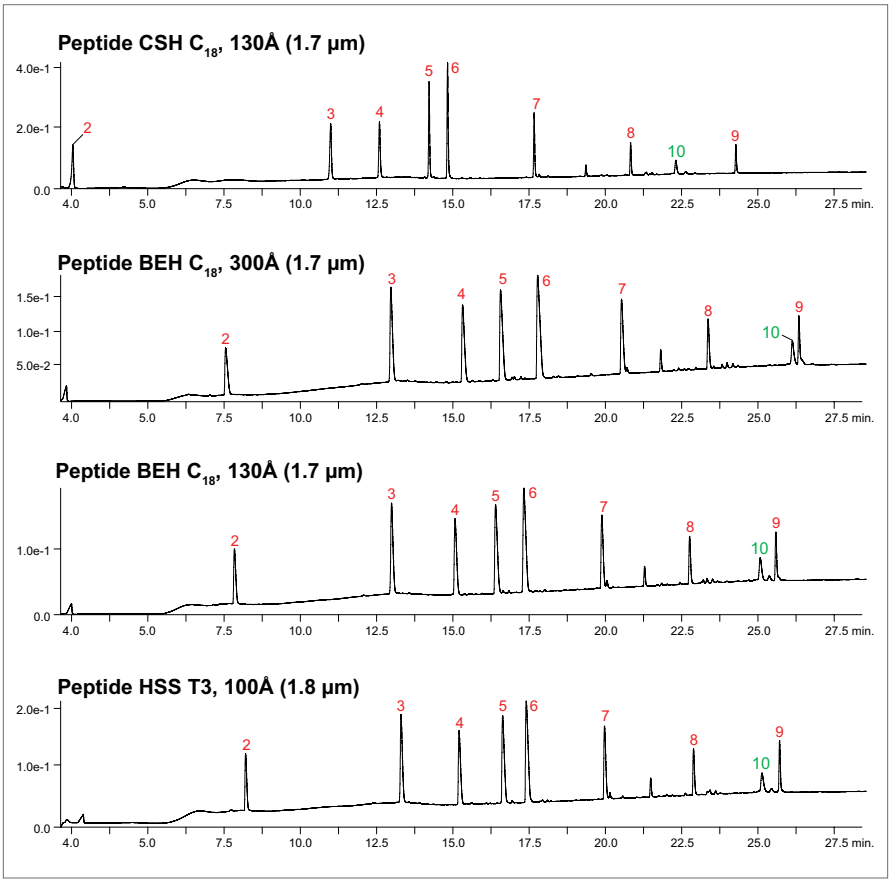
Separation of Peptide Standards Using 0.1% TFA Ion Pairing on Waters Peptide Separation Columns



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} 130Å (1.7 µm), Peptide BEH C_{18} 300Å (1.7 µm), Peptide BEH C_{18} 130Å (1.7 µm), or Peptide HSS T3 100Å (1.8 µm) UPLC-based particles on a Waters 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



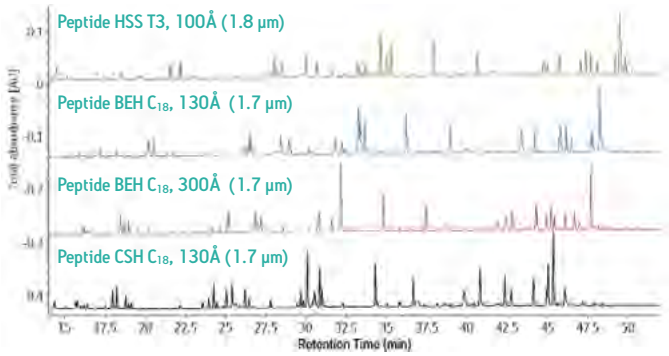
Peptides contained in Waters MassPREP Peptide Standard Mixture, p/n: 186002337, were separated on 2.1 × 150 mm columns containing Waters Peptide CSH C_{18} 130Å (1.7 µm), Peptide BEH C_{18} 300Å (1.7 µm), Peptide BEH C_{18} 130Å (1.7 µm), or Peptide HSS T3 100Å (1.8 µm) UPLC-based particles on a Waters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

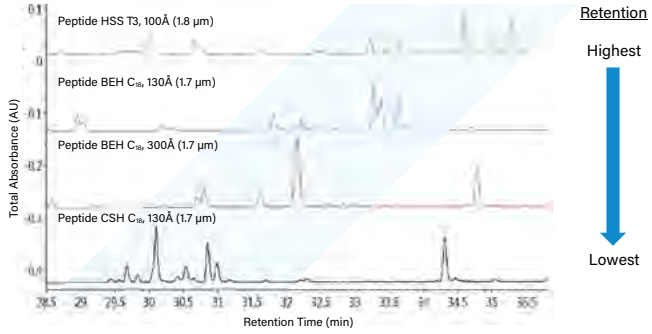
Sample as above.

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 National Institute of Standards and Technology's mAb on Waters Peptide Separation Columns





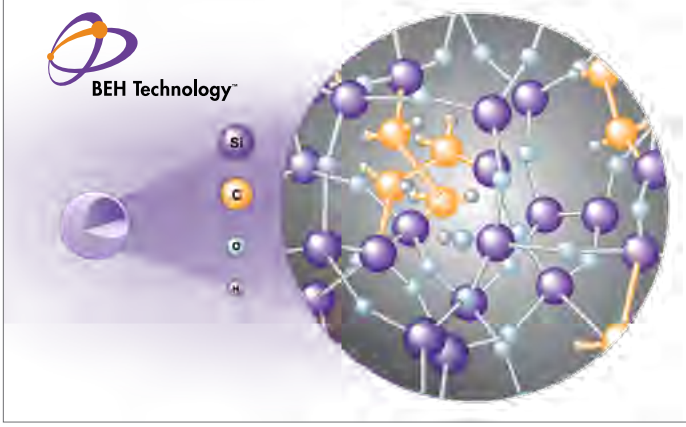
LC system:	ACQUITY UPLC® H-Class Bio	Eluent A:	Water with 0.1% TFA
Sample:	Tryptic Digest of NIST mAb reference material	Eluent B:	Acetonitrile with 0.1% TFA
		Gradient:	0.5% - 50% B in 60 min, linear
Columns:	2.1 x 150 mm	Flow rate:	0.20 mL/min
		Column temp.:	60 °C

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.

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}, 130Å, and BEH C_{18}, 300Å 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



Anal. Chem. **2003**, 75, 6781-6788

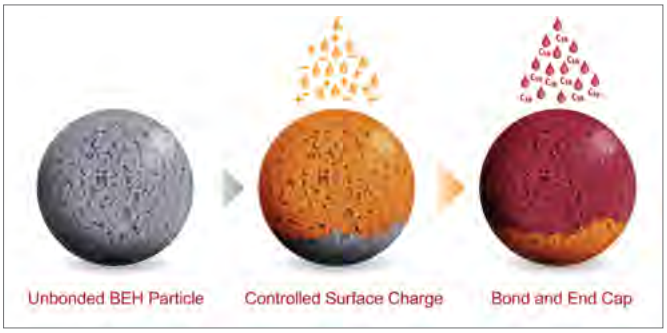
*Patent No. 6,686,035 B2

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}, 130Å offerings expands on the already successful and well recognized Peptide BEH C_{18}, 130Å and BEH C_{18}, 300Å columns. Based on comparative peptide separations, Peptide CSH C_{18}, 130Å Columns exhibit improved load ability, greater peak capacities, and unique selectivity compared to Peptide BEH C_{18}, 130Å. 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}, 130Å 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.

Benchmarking, Method Development, and Troubleshooting: Cytochrome *c* Digestion Standard

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

Peptide CSH C_{18}, 130Å Columns

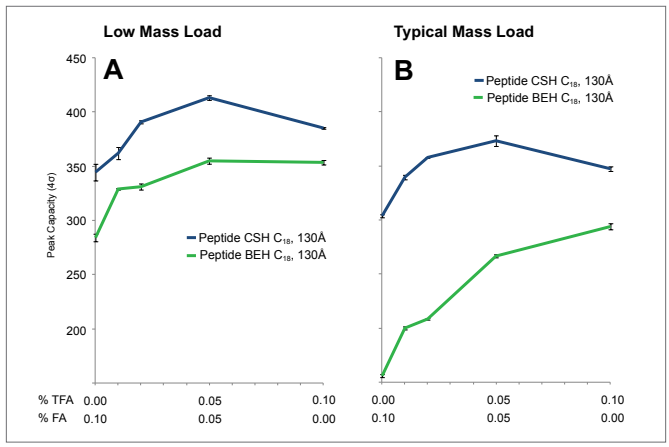
CHARGED SURFACE HYBRID PARTICLES DELIVER SUPERIOR PEPTIDE SEPARATIONS IN LC AND LC-MS APPLICATIONS

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}, 130Å 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 no longer 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}, 130Å 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}, 130Å 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}, 130Å vs. Peptide BEH C_{18}, 130Å Based Columns (2.1 × 150 mm) at Two Peptide Mass Loads and Differing Concentrations of Formic Acid and Trifluoroacetic Acid



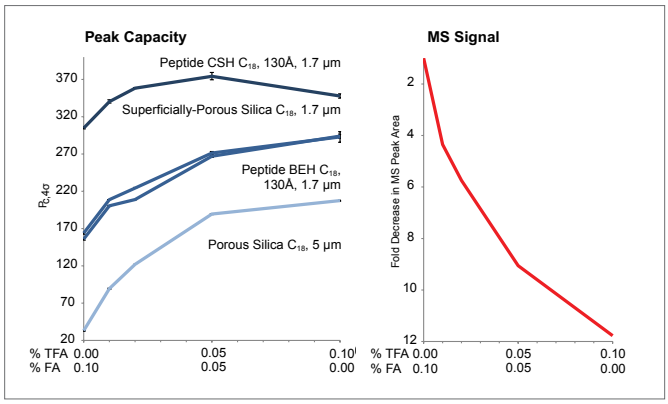
Effect of column mass load on separated peptide peak capacity in formic acid, trifluoroacetic acid, and eluent blends of formic acid and trifluoroacetic acid. (A) approximate sample load of 0.06 µg peptide mixture. (B) approx. 0.6 µg peptide mixture. Values were derived from two replicates. Waters MassPREP 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. The recent introduction of 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}, 130Å, 1.7 µm Column to a Peptide CSH C_{18}, 130Å, 2.5 µm ***XP*** Column simply by altering flow rate and gradient time. As shown below, 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}, 130Å 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}, 130Å 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



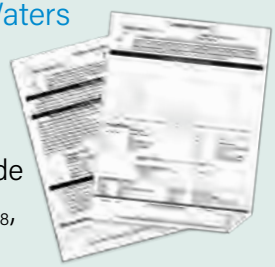
Effect of trifluoroacetic acid on peak capacity and MS signal. (A) Peak capacity as a function of acid modifier. Values were derived from two replicates. (B) Fold decrease in MS peak area as a function of acid modifier. Waters MassPREP Peptide Standard Mixture (p/n: 186002337) was used in study.

HIGH STRENGTH SILICA (HSS) TECHNOLOGY

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, 100Å, 1.8 µm as well as XSelect Peptide HSS T3, 100Å, ***XP*** 2.5 µm and 5 µm for UHPLC and HPLC-based separations for seamless transfer between UPLC and HPLC UHPLC instrument platforms.

Increased Assurance with Waters Peptide Columns



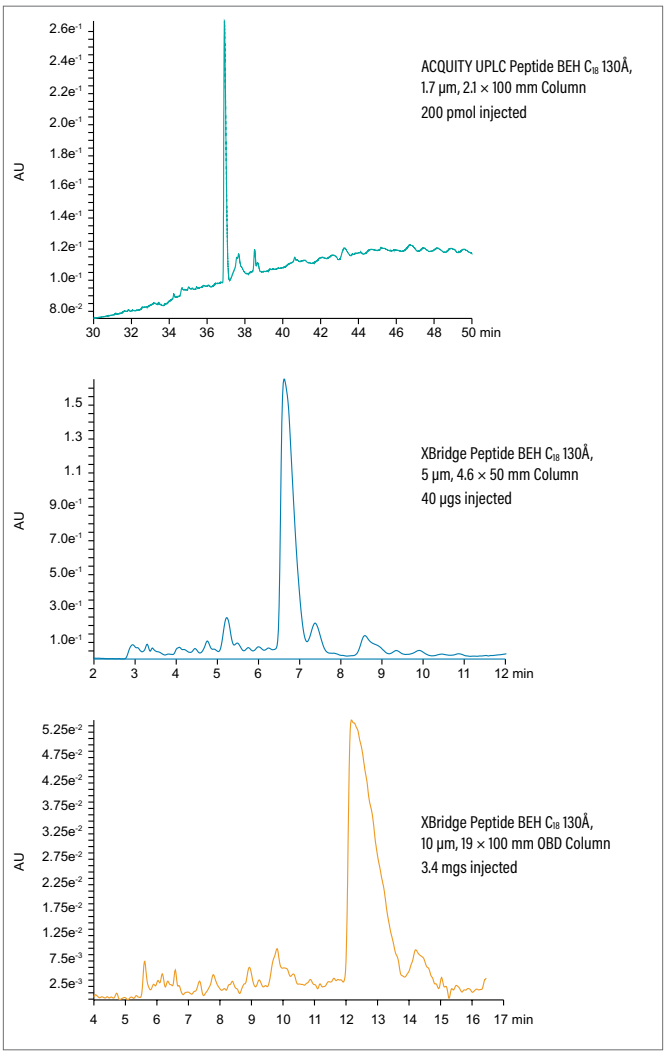
Waters rigorously tests each batch of our synthesized Peptide BEH C_{18}, 130Å; Peptide BEH C_{18}, 300Å; Peptide CSH C_{18}, 130Å; and Peptide HSS T3 100Å particles used in our manufactured columns. To pass, each batch of material must satisfactorily separate a complex protein digest using a gradient 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}, 130Å Column.

Simplifying Column Choice for Peptide Purifications

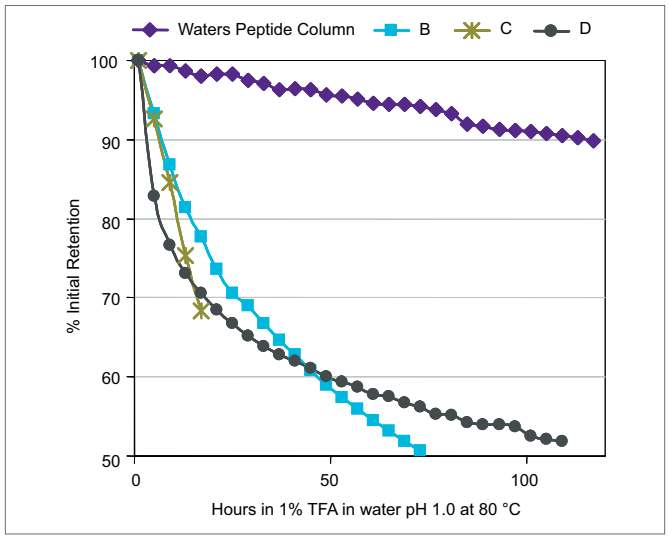
Our peptide columns are versatile. Often, a single C_{18}-based chemistry can separate a wide range of peptides, requiring little time and expense to obtain satisfactory results. We offer peptide packings in many particle sizes and column dimensions. (See the "Peptide Preparative Column Selection Guide,"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

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 µm and 10 µm				
130Å and 300Å				
I.D. (mm)	Length (mm)	µmoles 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				
130Å and 300Å				
I.D. (mm)	Length (mm)	µmoles 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

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 www.waters.com/OBD for details about OBD Technology.

Ordering Information

ACQUITY UPLC Peptide BEH C_{18} Guards and Columns

	Dimension	P/N
	Particle Size: 1.7 µm	
BEH C_{18}, 130Å	2.1 × 5 mm	186003975*
	2.1 × 50 mm	186003554
	2.1 × 100 mm	186003555
	2.1 × 150 mm	186003556
	Particle Size: 1.7 µm	
BEH C_{18}, 300Å	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*

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

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

XBridge Peptide BEH C_{18} Method Validation Kits*

	Dimension	P/N	Dimension	P/N
	Particle Size: 3.5 µm		**Particle Size: 5 µm**	
BEH C_{18}, 130Å	4.6 × 100 mm	186004904	4.6 × 100 mm	186005463
	Particle Size: 3.5 µm		**Particle Size: 5 µm**	
BEH C_{18}, 300Å	4.6 × 100 mm	186004905	4.6 × 100 mm	186005464

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

XBridge Peptide BEH C_{18} Guards and Columns

	Dimension	P/N	Dimension	P/N	Dimension	P/N
	Particle Size: 3.5 µm		Particle Size: 5 µm		Particle Size: 10 µm	
BEH C_{18}, 130Å	1.0 × 50 mm	186003560	1.0 × 50 mm	186003571	4.6 × 50 mm	186003648
	1.0 × 100 mm	186003561	1.0 × 100 mm	186003572	4.6 × 100 mm	186003649
	1.0 × 150 mm	186003562	1.0 × 150 mm	186003573	4.6 × 150 mm	186003650
	2.1 × 50 mm	186003563	2.1 × 50 mm	186003574	4.6 × 250 mm	186003651
	2.1 × 100 mm	186003564	2.1 × 100 mm	186003575	10 × 10 mm	186004465*,[1]
	2.1 × 150 mm	186003565	2.1 × 150 mm	186003576	10 × 50 mm	186008194
	2.1 × 250 mm	186003566	2.1 × 250 mm	186003577	10 × 100 mm	186008195
	4.6 × 50 mm	186003567	4.6 × 50 mm	186003578	10 × 150 mm	186008196
	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		

	Particle Size: 3.5 µm		Particle Size: 5 µm		Particle Size: 10 µm	
BEH C_{18}, 300Å	1.0 × 50 mm	186003604	1.0 × 50 mm	186003615	4.6 × 50 mm	186003663
	1.0 × 100 mm	186003605	1.0 × 100 mm	186003616	4.6 × 100 mm	186003664
	1.0 × 150 mm	186003606	1.0 × 150 mm	186003617	4.6 × 150 mm	186003665
	2.1 × 50 mm	186003607	2.1 × 50 mm	186003618	4.6 × 250 mm	186003666
	2.1 × 100 mm	186003608	2.1 × 100 mm	186003619	10 × 10 mm	186004467*,[1]
	2.1 × 150 mm	186003609	2.1 × 150 mm	186003620	10 × 50 mm	186008198
	2.1 × 250 mm	186003610	2.1 × 250 mm	186003621	10 × 100 mm	186008199
	4.6 × 50 mm	186003611	4.6 × 50 mm	186003622	10 × 150 mm	186008200
	4.6 × 100 mm	186003612	4.6 × 100 mm	186003623	10 × 250 mm	186008201
	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*,[1]	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

	Dimension	Column P/N	Kit P/N[1]
	Particle Size: 1.7 µm		
CSH C_{18}, 130Å	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 includes Peptide CSH C_{18}, 130Å Column plus one vial of Cytochrome *c* Digestion Standard, p/n: 186006371.

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

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

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

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

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

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

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

	Dimension	Column P/N	Kit P/N[1]	Dimension	Column P/N	Kit P/N[1]	Dimension	Column P/N (1/pk)
	Particle Size: 2.5 µm			Particle Size: 3.5 µm			Particle Size: 5 µm	
CSH, C_{18}, 130Å	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 includes Peptide CSH C_{18}, 130Å 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.

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

	Dimension	Column P/N	Kit P/N[1]	Dimension	Column P/N	Kit P/N[1]
	Particle Size: 2.5 µm			Particle Size: 3.5 µm		
CSH C_{18}, 130Å	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 3 columns, each from a different batch.
[1] Kit includes Peptide CSH C_{18}, 130Å Column plus one vial of Cytochrome *c* Digestion Standard, p/n: 186006371.

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

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

*Requires VanGuard Cartridge Universal Holder, p/n: 186007949.
[1] Kit includes Peptide CSH C_{18}, 130Å Column plus 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

	Dimension	Column P/N	Kit P/N[1]
	Particle Size: 1.8 µm		
HSS T3, 100Å	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

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

ACQUITY UPLC Peptide HSS T3 Method Validation Kits*

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

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

XSelect Peptide HSS T3 Columns

	Dimension	Column P/N	Kit P/N[1]	Dimension	Column P/N	Kit P/N[1]
	Particle Size: 2.5 µm			Particle Size: 5 µm		
HSS T3, 100Å	2.1 × 50 mm	186008758	176003998	2.1 × 50 mm	186008774	176004016
	2.1 × 100 mm	186008759	176003999	2.1 × 100 mm	186008775	176004017
	2.1 × 150 mm	186008760	176004006	2.1 × 150 mm	186008776	176004018
	4.6 × 50 mm	186008762	176004007	4.6 × 50 mm	186008778	176004019
	4.6 × 100 mm	186008763	176004008	4.6 × 100 mm	186008779	176004020
	4.6 × 150 mm	186008764	176004009	4.6 × 150 mm	186008780	176004021

[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*

	Dimension	P/N	Dimension	P/N
	Particle Size: 2.5 µm		Particle Size: 5 µm	
HSS T3, 100Å	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*

	Dimension	P/N	Dimension	P/N
	Particle Size: 2.5 µm		Particle Size: 5 µm	
HSS T3, 100Å	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.

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 1,000Å 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.

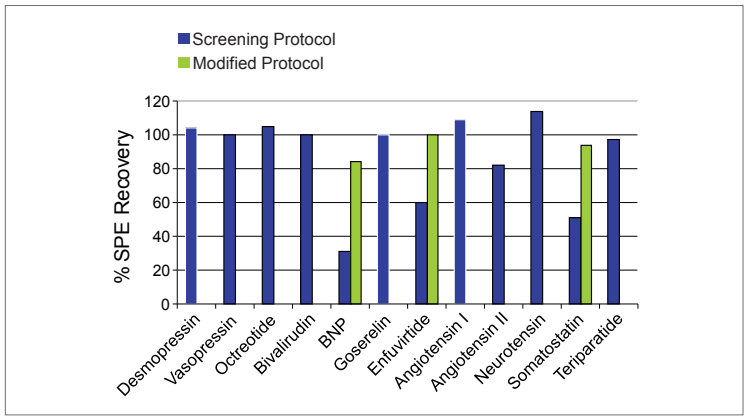
Therapeutic Peptide Method Development Kit

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 C_{18}, 300Å 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.

High Recovery of Peptides



The innovative Oasis µElution plate format 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.

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 www.waters.com/pepkit or contact your local Waters sales office.

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}, 300Å, 1.7 µm, 2.1 x 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}, 300Å, 3.5 µm, 2.1 x 50 mm Column	1	186003607
96-well 1 mL Collection Plate and Cap Mat	3	600001043

Available Waters Products Not Included in Kit:		
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 (includes 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	725000418

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 premier 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 100Å, 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

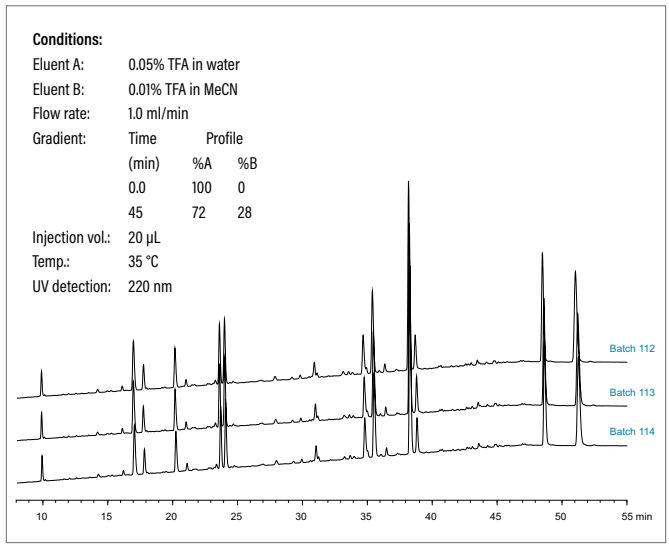
BioSuite C_{18}, 3.5 µm PA-B is a 300Å, high-ligand density, monofunctional, silica-based column.

- 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

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

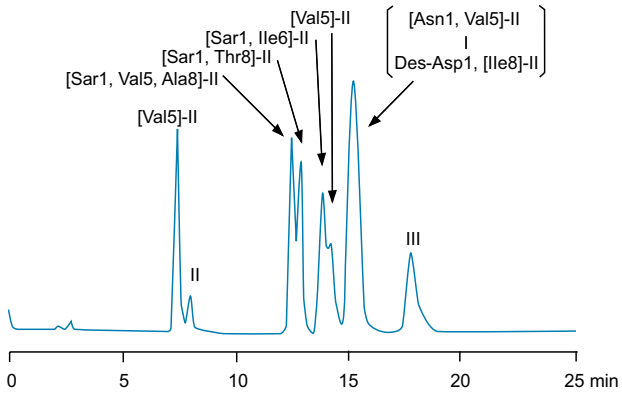
BioSuite Cation-Exchange HPLC Columns

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

Conditions:

Column: BioSuite SP-PEEK, 4.6 mm I.D. x 50 mm, PEEK (p/n: 186002183)
Eluent: A: 20 mmol/L sodium acetate buffer, pH 5.0
B: 20 mmol/L sodium acetate buffer containing 1.0 mol/L NaCl, pH 5.0
Linear gradient from eluent A to B for 20 minutes
Flow rate: 1.0 mL/min
Temp.: 25 °C
UV detection: 80 nm



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

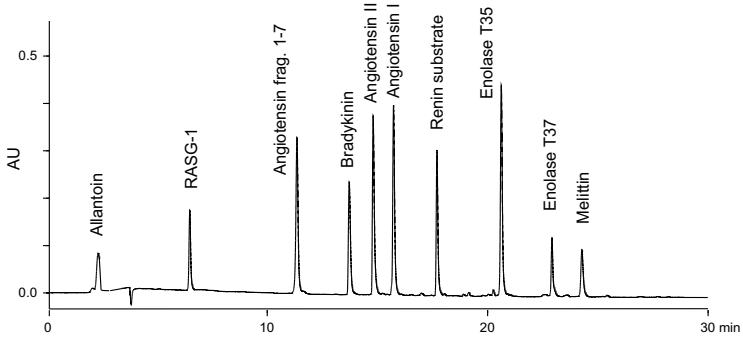
Description	Matrix	Pore Size	Exclusion Limit (Daltons) against Polyethylene Glycol	Inner Diameter	Length	Column Volume (mL)	# Approx Protein Binding Capacity Per Pre-Packed Column	P/N
BioSuite SP-PEEK, 7 µm CXC	Polymer	1300Å	>4,000,000	4.6 mm	50 mm	0.83	58 mg*	186002182
BioSuite SP, 2.5 µm NP CXC	Polymer	N/A	500	4.6 mm	35 mm	0.58	2.9 mg**	186002183
BioSuite SP, 10 µm CXC	Polymer	1000Å	1,000,000	7.5 mm	75 mm	3.31	132 mg**	186002184
BioSuite SP, 13 µm CXC	Polymer	1000Å	1,000,000	21.5 mm	150 mm	54.45	2,178 mg**	186002185

* Data generated with Gamma Globulin.
** Data generated with Hemoglobin.
NOTE: For best resolution of complex samples, do not exceed 20% of the column's protein binding capacity.

Benchmarking, Method Development, and Troubleshooting: MassPREP Peptide Standard

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 UPLC and HPLC 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 x 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

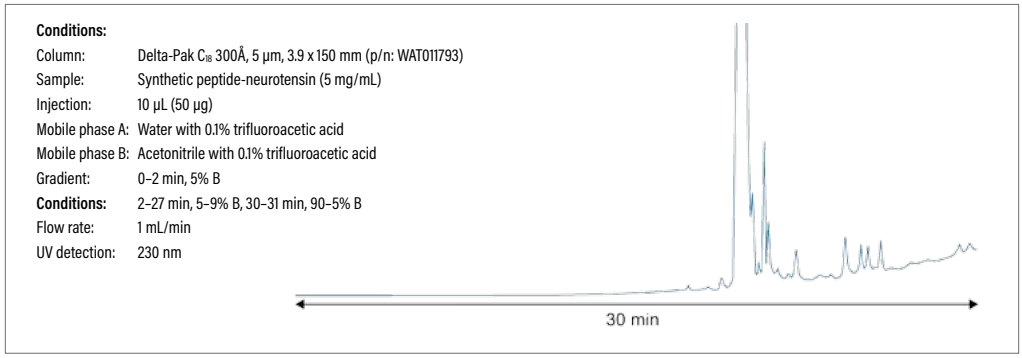
MassPREP Peptide Standards

Description	Volume	P/N
MassPREP Peptide Mixture One vial with approximately 1 nmole 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 This is a (5) pack of 186002337. Each vial contains approximately 1 nmole 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

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 µm or 15 µm spherical silica. Delta-Pak is available in two different pore size materials (100Å and 300Å) with a C_{18} or C_4 bonded phase.

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



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

For more information, visit waters.com/biosep

Ordering Information

Delta-Pak Analytical HPLC and UHPLC Columns and Guards

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

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

Delta-Pak Preparative HPLC and UHPLC Guard Columns

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

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

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

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

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
- 100Å and 300Å 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

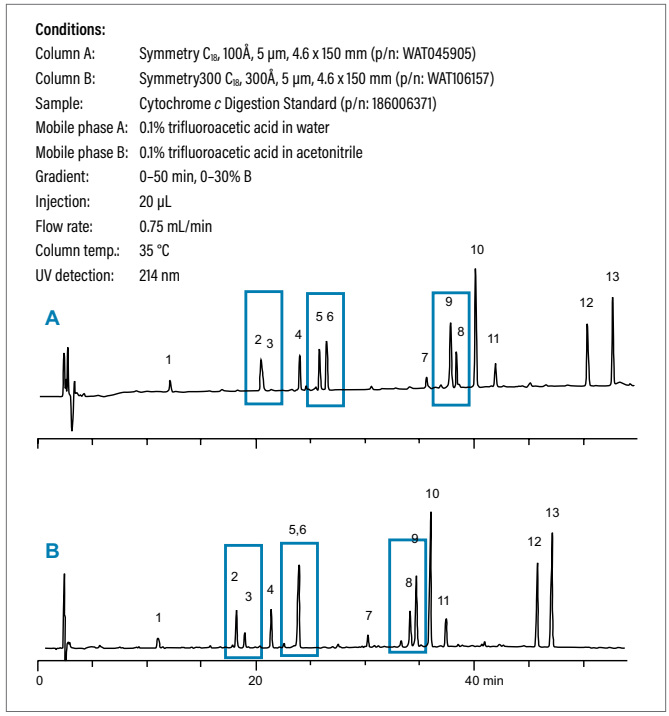
Symmetry300™ Columns are a 300Å reversed-phase addition to the existing Symmetry family of columns. They have been specifically designed to provide maximum batch-to-batch and column-to-column performance consistency and recovery of protein and peptide applications.

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.

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 100Å 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 100Å column vs. the 300Å 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.

Ordering Information

Symmetry300 HPLC and UHPLC Columns

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

Additional Peptide Consumables

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
MassPREP Digestion Standards		
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

Quantitative Peptide Standards

These sets of standards are 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 PhosB 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

Biopharmaceutical Training Kit

The Biopharmaceutical Training Kit was designed to provide trainers and customers a starting point for running LC and LC/MS systems dedicated to biopharmaceutical applications. It pulls together many of the individual components often used in demos and training classes. This kit provides a starting point for analysis and can be used for optimization of methods prior to running valuable samples to ensure system performance and confidence in results.

Ordering Information

Biopharmaceutical Training Standards (p/n: 186006921)

Description of Components Acid	Contents	Amount per Vial	Container	P/N
Horse Heart Myoglobin	1 x 15 mL	2.5 mg	15 mL Clear Nalgene Bottle	186006921-1
Intact mAb Mass Check Standard	1 vial	1 mg	TruView Max Recovery with non-slit PTFE/silicone Septa (p/n: 186005668CV)	186006921-2
Glu-fibrinopeptide B	1 vial	0.5 mg	4 mL Amber Screw Top Vial	186006921-3
MassPREP Enolase Digest	1 vial	1 nmol	TruView Max Recovery with non-slit PTFE/silicone Septa (p/n: 186005668CV)	186006921-4
Reverse Peptide Ser-Asp-Gly-Arg-Gly	1 vial	1.0 mg of each	4 mL glass amber bottle with screw top	186006921-5
Reverse Peptide Gly-Arg-Gly-Asp-Ser	1 vial	1.0 mg of each	4 mL glass amber bottle with screw top	186006921-6

Biopharmaceutical Prep Kit (p/n: 186007517)

Description of Components Acid	Contents	Amount per Vial	Container	P/N
Formic Acid	1 x 2 mL	2 mL	2 mL Ampoule	186007517-1
Sodium Cesium Iodide	1 x 30 mL	2 µg/µL	30 mL Nalgene	186007517-2
30 mL Nalgene Fludics Bottle (with cap)	3	Empty	30 mL Nalgene Fludics Bottle (with cap)	290002010

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Phosphorylated Peptide 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
4 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 4 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
A kit that allows one to mix and optimize a complex standard per specific applications. (2) Vials:		
MassPREP enolase digestion standard	Solid	186002325
MassPREP phosphopeptide standard enolase	Solid	186003285
MassPREP Enhancer (5 vials)	Solid	186003863
(5) 500 mg MassPREP Enhancer. A component in the MassPREP Phosphopeptide Enrichment Kit		186003864
MassPREP Phosphopeptide Enrichment Kit		186003864
Phosphopeptide enrichment µElution plate (2) MassPREP	Solid	186003820
Enhancer MassPREP enolase digest with phosphopeptides.	Solid	186003863
This kit is developed from selective enrichment of phosphopeptides from complex samples.		186003286

*RAPI*GEST SF PROTEIN DIGESTION SURFACTANT

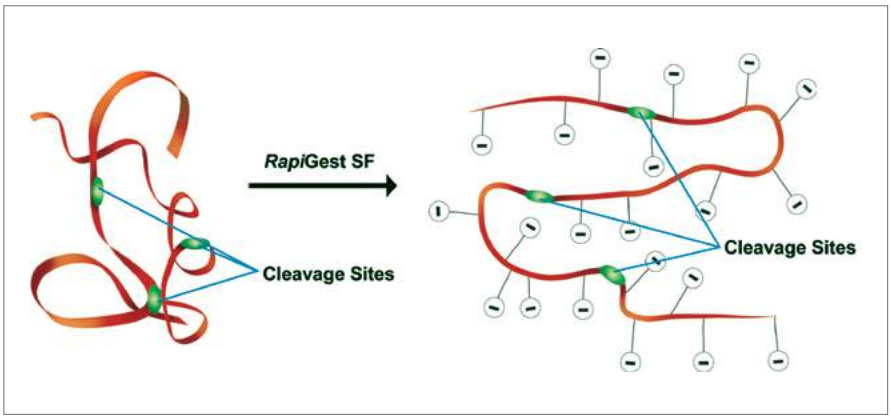
*Rapi*Gest™ SF (surfactant) radically enhances protein enzymatic digestions in terms of speed and percent recovery. *Rapi*Gest SF is a patented anionic surfactant that accelerates the production of peptides generated by proteases, such as trypsin, Asp-N, Glu-C, and Lys-C. Many hydrophobic proteins are 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.

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
*Rapi*Gest SF Custom	186002118

How *Rapi*Gest SF Works

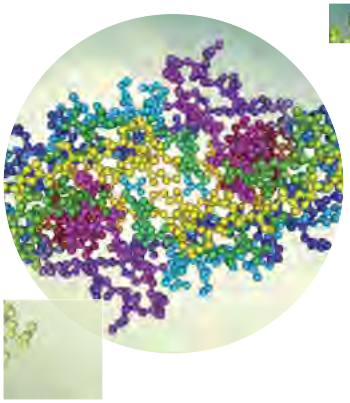


Waters RapiGest SF surfactant radically enhances protein enzymatic digestions in terms of speed and percent recovery. It's patented anionic surfactant accelerates the in-solution production of peptides generated by proteases such as trypsin, Asp-N, Glu-C, and Lys-C. Many hydrophobic proteins are resistant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

Reference Materials for Peptide Separations

Title	Literature Code
Application Notebook	
Peptide Separations	720005801EN
Application Notes	
A Flexible SPE/LC/MS/MS Platform for the Simultaneous Quantitation of Multiple Amyloid Peptides in Cerebrospinal Fluid	720003682EN
Effect of Pore Size in UPLC Peptide Mapping	720001792EN
Enabling Significant Improvements for Peptide Mapping with UPLC	720001339EN
High Mass Loading of Peptides with Hybrid Particle C_{18} Columns and Acetic Acid Mobile Phases	720004674EN
Increasing Peak Capacity in Reversed-Phase Peptide Separations with Charged Surface Hybrid (CSH) C_{18} Columns	720004568EN
Maximizing Chromatographic Resolution of Peptide Maps Using UPLC with Tandem Columns	720003362EN
Peptide Mapping and Small Protein Separations with Charged Surface Hybrid (CSH) C_{18} and TFA-Free Mobile Phases	720004571EN
Reliability of Peptide Mapping Using the ACQUITY UPLC H-Class System	720003288EN
Selecting a Reversed-Phase Column for the Peptide Mapping Analysis of a Biotherapeutic Protein	720005924EN
Simplifying Peptide Bioanalysis	720003253EN
For a complete list of application notes, visit waters.com/AppNotes	
Catalog	
Waters Analytical Standards & Reagents	asr.waters.com
Wall Chart	
Bioseparations Columns Wall Chart	720004232EN
Webinars	
[Meet the Experts] Webinar Series	www.waters.com/MeetTheExperts

Protein Separations

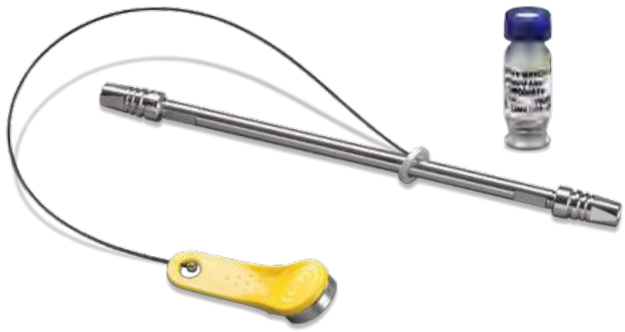


Protein Separations

Contents

ACQUITY UPLC SEC System Solution

ACQUITY UPLC Technology allows analytical chemists to reach far beyond conventional LC separations and has proven itself to be a major asset in increasing the productivity of laboratories around the world. The latest addition to this application-driven portfolio is the ACQUITY UPLC SEC System Solution, enabled by the unique ethylene-bridged-hybrid (BEH) Diol-coated particle technology.



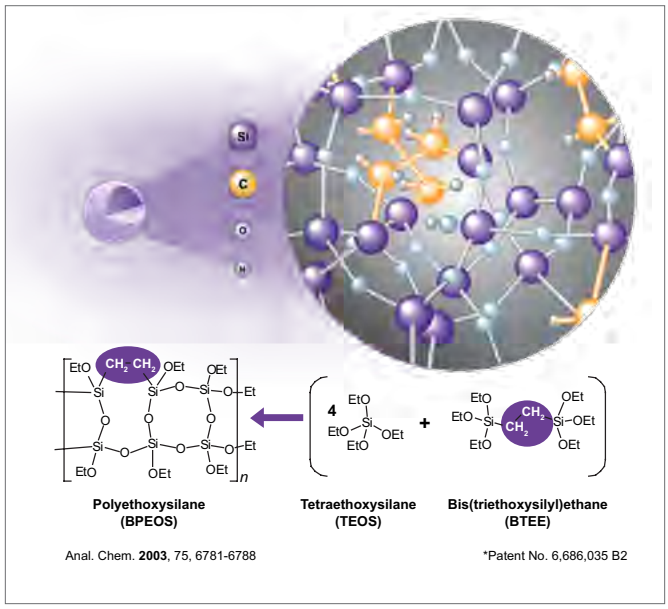
- Determines aggregation levels in therapeutic monoclonal antibodies up to 10x faster than traditional HPLC-based size-exclusion chromatography (SEC)
- Fully-optimized column chemistry significantly reduces the requirement for high salt concentration mobile phases
- QC tested with BEH protein standards, ensuring unmatched batch-to-batch consistency and increased confidence in validated methods
- Waters Protein Standard Mixes are available for the 125Å, 200Å, and 450Å SEC columns for additional validation (p/n: 186006519,186006518, and 186006842, respectively)

BEH TECHNOLOGY

In 1999, Waters launched the XTerra® Family of HPLC Columns featuring patented, first-generation hybrid-particle technology (HPT). HPT enabled XTerra Columns to become one of the most successful column products in 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 new, second-generation hybrid material was developed that 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.

The BEH Particle*: One of the Key Enablers of UPLC Technology

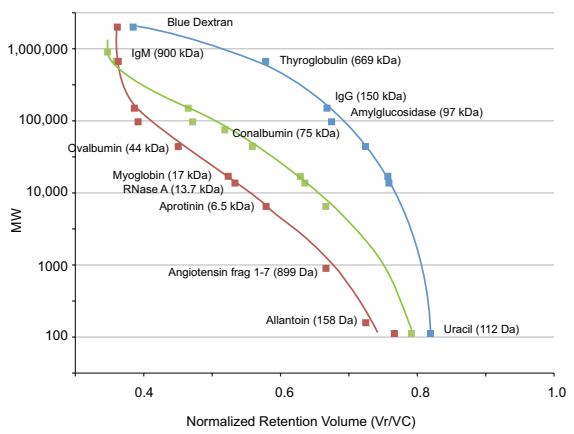


Ethylene-Bridged Hybrid (BEH) Technology synthesis creates particles that ensure extreme column performance and long column lifetime under harsh operating conditions.

ACQUITY UPLC TECHNOLOGY

Waters ACQUITY UPLC Technology allows analytical chemists to reach far beyond conventional LC separations and has proven itself to be a valuable asset that improves the quality of collected data while increasing sample throughput and productivity. Biotherapeutics and biosimilars manufacturers can now choose the most appropriate UPLC-based, Protein BEH SEC Columns (i.e., 125Å, 200Å, and 450Å pore size) to satisfy their application requirements based on this separation technology.

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



ACQUITY UPLC Protein BEH SEC, 125Å, 1.7 µm: 1K–80K Daltons

ACQUITY UPLC Protein BEH SEC, 200Å, 1.7 µm: 10K–450K Daltons

ACQUITY UPLC Protein BEH SEC, 450Å, 2.5 µm: 100K–1500K Daltons

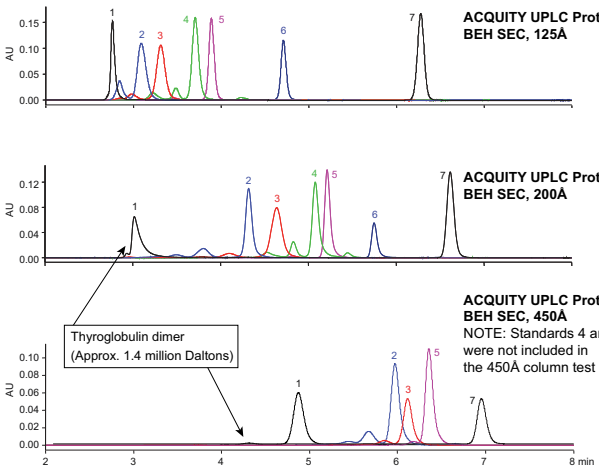
Protein standards
Temp.: 30 °C
Mobile phase: 100 mM sodium phosphate, pH=6.8

Size exclusion chromatography (SEC) separates compounds primarily based on their relative size in solution. Calibration curves on UPLC-based SEC columns of different pore size, using defined protein and peptides of known molecular weight, help chromatographers select the most appropriate SEC column for their specific application.

Separation of Protein and Peptide Standards on ACQUITY UPLC Protein BEH SEC, 125Å, 200Å, and 450Å Columns

Compounds:

1. Thyroglobulin	(670 K)	5. Myoglobin	(17 K)
2. BSA	(66 K)	6. Angiotensin Frag 1–7	(899)
3. Ovalbumin	(44 K)	7. Uracil	(112)
4. Carbonic Anhydrase	(29 K)		



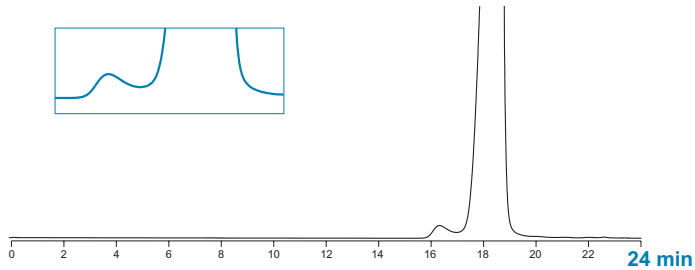
Columns	ACQUITY UPLC Protein BEH SEC, 125Å, 1.7 µm; ACQUITY UPLC Protein BEH SEC, 200Å, 1.7 µm	ACQUITY UPLC Protein BEH SEC, 450Å, 2.5 µm
Column configuration	4.6 x 150 mm	
Mobile phase	100 mM Sodium Phosphate Buffer, pH 6.8	
Weak needle wash	100% Milli-Q Water	
Strong needle wash	100% Milli-Q Water	
Seal wash	90/10 water/methanol	
Samples: Diluted in mobile phase	Thyroglobulin 0.3 mg/mL, BSA 0.3 mg/mL, Ovalbumin 0.3 mg/mL, Carbonic Anhydrase 0.3 mg/mL, Myoglobin 0.3 mg/mL, Angiotensin Frag. 1–7 0.1 mg/mL, Uracil 0.1 mg/mL	Thyroglobulin 3 mg/mL, BSA 5 mg/mL, Ovalbumin 3 mg/mL, Myoglobin 2 mg/mL, Uracil 0.1 mg/mL
Injection vol.	2 µL, Full Loop	
Flow rate	0.3 mL/min	
Column temp.	ambient	
Detection wavelength	UV @ 220 nm	UV @ 280 nm

Waters offers a family of BEH-based, diol-coated SEC columns of different pore size to satisfactorily address the molecular weight range of analytes to be separated.

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, 125Å, 1.7 µm Column with Waters UltraPerformance LC® (UPLC) instrumentation (shown below).

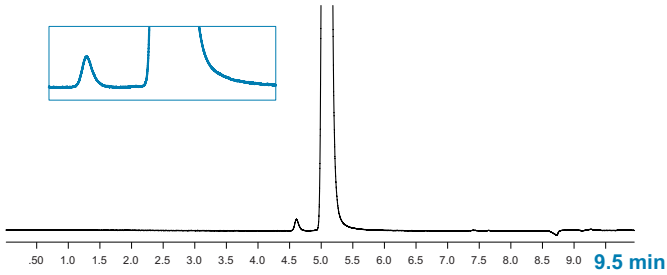
Insulin Analyses by Traditional HPLC-SEC vs. UPLC-SEC



Conditions:

Column:	Waters Insulin HMWP SEC, 125Å, 10 µm, 7.8 x 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



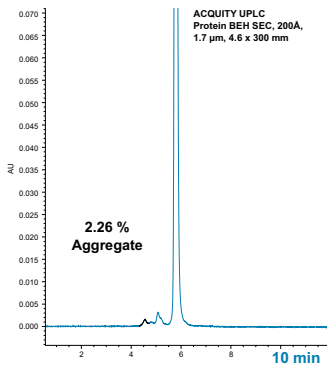
Conditions:

Column:	Waters ACQUITY UPLC Protein BEH SEC, 125Å, 1.7 µm, 4.6 x 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

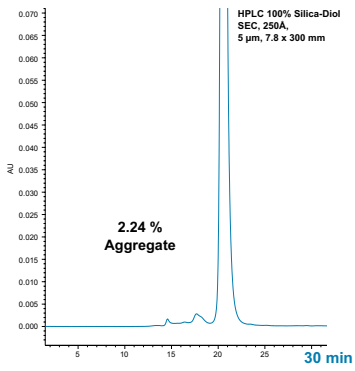
Compared to use of traditional HPLC-based SEC technology for the analysis of earlier eluting insulin aggregates from desired monomer species, Waters ACQUITY UPLC BEH SEC Technology delivers benefits of improved component resolution and in less time.

Comparative UPLC-Based SEC Benefits vs. Use of Traditional HPLC SEC for Biotherapeutic Characterization



Conditions:

Column:	ACQUITY UPLC Protein BEH SEC, 200Å, 1.7 µm, 4.6 x 300 mm (p/n: 186005226)
Sample:	Humanized IgG monoclonal antibody, 3 mg/mL in eluent
UPLC system:	Waters ACQUITY UPLC with Tunable UV Detector
Eluent:	25 mM sodium phosphate, pH 6.8 with 0.15 M sodium chloride
Flow rate:	0.4 mL/min
Injection:	5 µL
UV detection:	280 nm



Conditions:

Column:	Traditional 100% silica-based/ Diol coated SEC, 250Å, 5 µm, 7.8 x 300 mm
Sample:	Humanized IgG monoclonal antibody, 3 mg/mL in eluent
HPLC system:	Waters Model 2796 HPLC with Model 2487 Dual λ Absorbance Detector
Eluent:	25 mM sodium phosphate, pH 6.8 with 0.15 M sodium chloride
Flow rate:	0.4 mL/min
Injection:	20 µL
UV detection:	280 nm

Compared to use of traditional HPLC-based SEC technology, Waters ACQUITY UPLC BEH SEC Technology delivers benefits of the comparable determination on mAb aggregate vs. monomer (i.e., less time, higher sample throughput).

Waters Insulin HMWP HPLC and UHPLC Columns

The Waters Insulin HMWP Column is specifically designed for use in the manufacture and quality control of insulin products. This column is tested for performance in the analysis of impurities with molecular masses greater than those of insulin.

Ordering Information

Waters Insulin HMWP SEC HPLC and UHPLC Columns

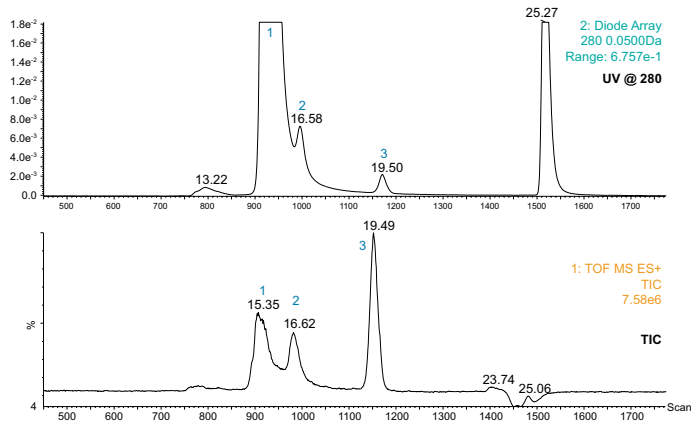
Description	Dimension	P/N
Waters Insulin HMWP Column	7.8 x 300 mm	WAT201549
Protein-Pak 125 Sentry Guard Column, 2/pk (requires holder)	3.9 x 20 mm	186000926
Sentry Universal Guard Column Holder	—	WAT046910

Tested to perform in the method published in PharmaEuropa Vol. 8, No 3, September 1996.

LC-MS Analyses Using SEC and Volatile Eluents

Size-exclusion chromatography (SEC), under non-denaturing conditions, is a standard method for testing biomolecules and their aggregates. MALS and AUC are established detectors but cannot provide exact mass for unknown species with a sufficient accuracy. The presence of an unexpected peak requires further investigation and/or confirmation of molecular weight, and SE-UPLC-MS under aqueous, non-denaturing conditions can provide valuable information that would more rapidly solve an organization's issues with characterization or quality.

LC-MS Analysis of Humanized Monoclonal Antibody on Protein BEH SEC, 200Å, 1.7 µm



LC Conditions:

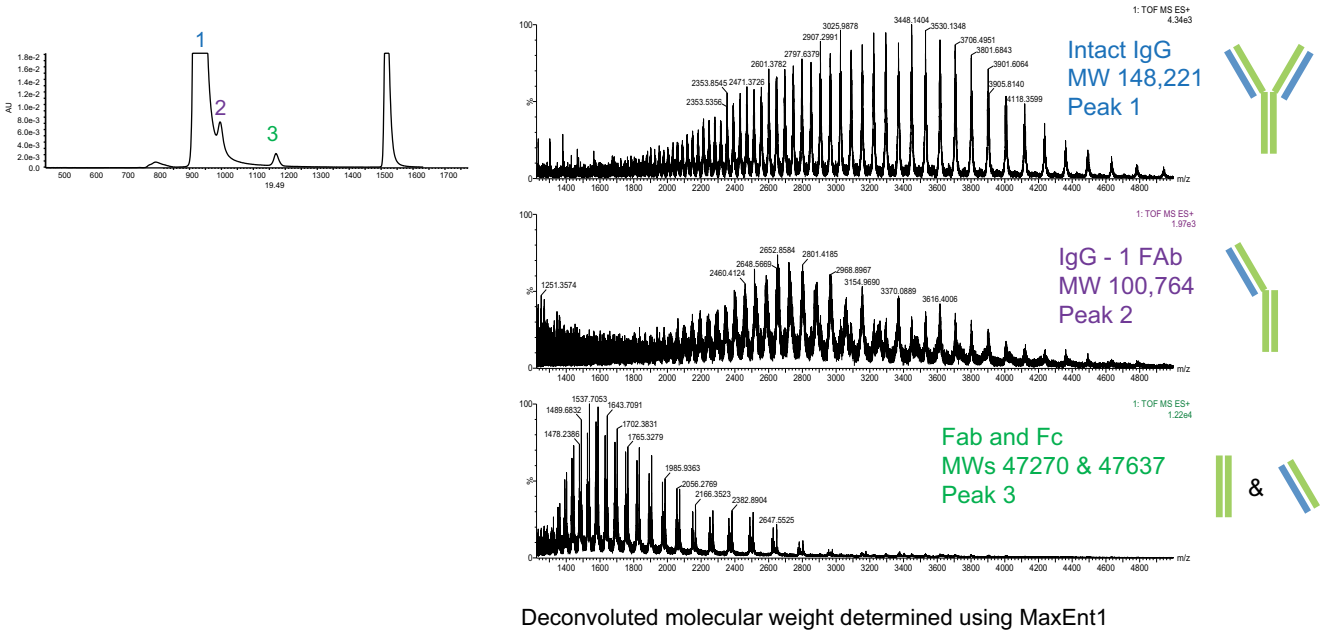
LC system:	ACQUITY UPLC H-Class Bio System with PDA Detector
Column:	ACQUITY UPLC Protein BEH SEC, 200Å, 1.7 µm, 4.6 x 300 mm (p/n: 186005226)
Column temp.:	30 °C
Sample temp.:	4 °C
Injection vol.:	2 µL
Flow rate:	0.15 mL/min
Mobile phase:	100 mM ammonium formate and 25 mM sodium phosphate, 150 mM sodium chloride, pH 6.8
Additive:	Acetonitrile, 0.8% formic acid, at 0.2 mL/min
External pump:	Waters 515 HPLC pump

MS Conditions:

MS system:	Xevo G2 QTof
Ionization mode:	ESI+
Analyser mode:	Sensitivity
Acquisition range:	500–5000
Capillary voltage:	3.00 kV
Cone voltage:	40.0 V
Source temp.:	150 °C
Desolvation temp.:	450 °C
Cone gas flow:	0.0 L/Hr
Desolvation gas flow:	800.0 L/Hr
Calibration:	NaI 2 µg/µL from 1000–4000 *m/z*

An intact biotherapeutic mAb, which was past expiry, was analyzed by using MS-friendly, non-denaturing conditions. In the UV chromatogram, not only are the mAb aggregate and monomer observed, but a low molecular weight (LMW) peak eluting after the intact mAb is partially resolved as well. In addition to these peaks, the UV chromatogram reveals two other LMW species.

Intact mAb Extracted and Deconvoluted Mass Spectra

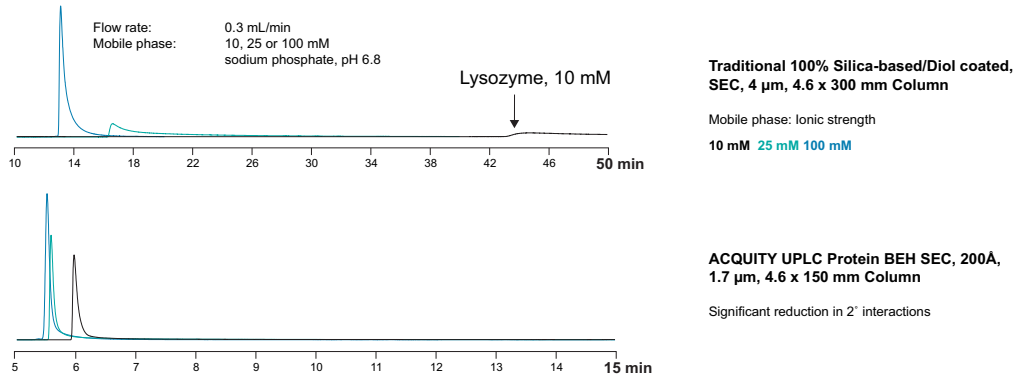


Shown are the raw MS spectra for the peaks shown in the chromatogram to the left. The calculated masses, using MaxEnt1 Software, were consistent with the structures shown.

Reduced Requirement for High Salt Concentration Mobile Phases

With conventional silica-based SEC Column chemistries, undesirable secondary ionic interactions between the silica surface and basic proteins can result in long retention times and excessive peak tailing. Traditionally, the solution to this issue is the inclusion of high concentrations of a salt to compete for the charged sites on the surface of the silica. The unique surface chemistry of the ACQUITY UPLC Protein BEH SEC, 200Å Column significantly reduces these secondary interactions, resulting in the ability to use less aggressive mobile-phase salt concentrations.

Effect of Eluent Ionic Strength on the SEC Analysis of the Basic Protein Lysozyme on 100% Silica vs. BEH SEC Particles



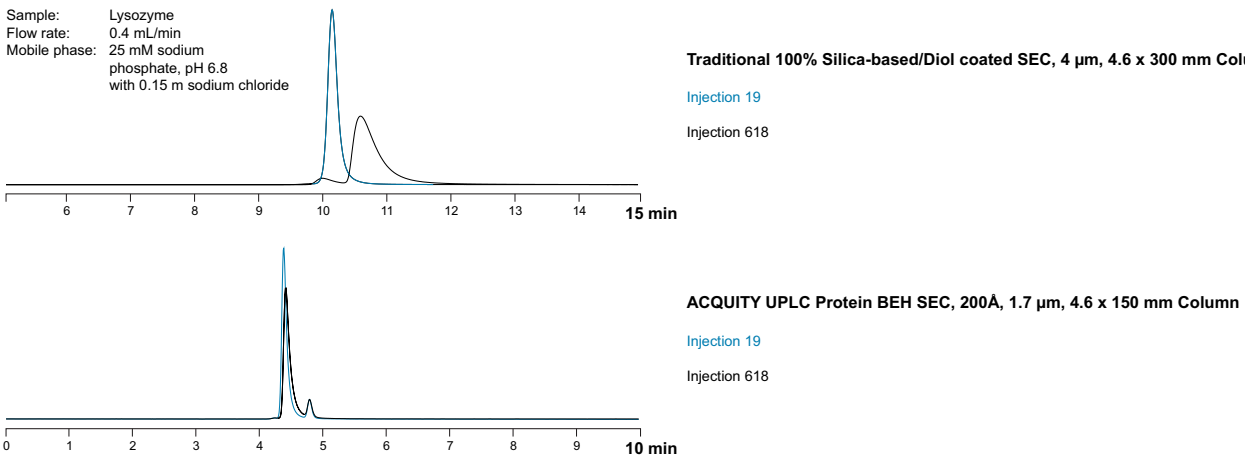
Conventional silica-based columns for SEC can exhibit significant secondary interactions, especially for basic proteins like lysozyme. In this example, a lower concentration of sodium phosphate (10 mM) causes lysozyme to be barely detectable. However, these non-desired secondary interactions are significantly reduced on the ACQUITY UPLC Protein BEH SEC, 200Å, 1.7 µm Column, as is shown with the same lysozyme analysis on the conventional silica-based SEC column. On the ACQUITY UPLC Protein BEH SEC Column, the peak shape is drastically improved with 10 mM salt, thereby eliminating the need to use high salt concentrations. This can lead to increased column and instrument lifetime.

A New Level of Column Stability for Size-Exclusion Chromatography

BEH particle technology is well established for chromatography of synthetic oligonucleotides, amino acids, peptides, proteins, and labeled glycans with stability and performance attributes not found with traditional, 100% silica-based particles.

The combination of the BEH base particle and the patent pending innovative diol bonding process, results in column stability, performance and lifetime not typical in traditional size-exclusion chromatographic columns.

ACQUITY UPLC Protein BEH SEC, 200Å Particle and Diol Bonding Technology Provides a Stable Chemistry with Outstanding Column Life

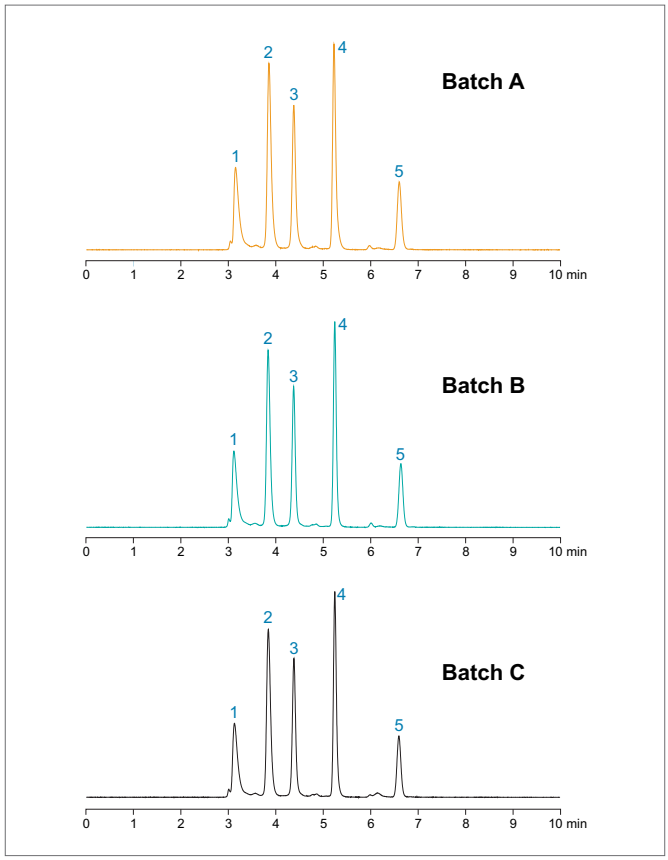


This example compares the lifetime of the conventional SEC column to the ACQUITY UPLC Protein BEH SEC, 200Å, 1.7 µm Column for lysozyme. The conventional SEC column not only shows a severe deterioration in peak shape, but also a difference in the retention that appears with increasing injections. This indicates that the conventional column is undergoing a chemical change that is not seen with the ACQUITY UPLC Protein BEH SEC Column. The ACQUITY UPLC Protein BEH SEC Column is stable, both mechanically and chemically, even for very basic proteins that are sensitive to small changes in the column over time.

Stringent Manufacturing Quality Assurance Delivers Confidence in Results

All Waters ACQUITY UPLC Columns chemistries are synthesised in state-of-the-art ISO-certified manufacturing facilities from high-quality raw materials, and are extensively QC tested throughout the synthetic process. In addition, each batch of Protein BEH SEC, 200Å, 1.7 µm material is specifically tested with relevant proteins to help ensure unmatched batch-to-batch consistency for supreme confidence in validated methods.

Waters ISO 2001 Manufacturing and Testing Processes Help Ensure Outstanding ACQUITY UPLC Protein BEH SEC, 200Å, 1.7 µm Batch-to-Batch Reproducibility



Waters BEH Protein Standards (125Å, 200Å, and 450Å formulated mixtures) are used to critically QC test our Protein BEH SEC columns to help ensure consistent batch-to-batch and column-to-column performance.

Ordering Information

ACQUITY UPLC Protein BEH SEC Columns and Guard Kits

Description	Configuration	Particle Size	Dimension	P/N
ACQUITY UPLC Protein BEH SEC, 450Å	Column and Standard	2.5 µm	4.6 x 150 mm	176002996
ACQUITY UPLC Protein BEH SEC, 450Å	Column	2.5 µm	4.6 x 150 mm	186006851
ACQUITY UPLC Protein BEH SEC, 450Å	Column and Standard	2.5 µm	4.6 x 300 mm	176002997
ACQUITY UPLC Protein BEH SEC, 450Å	Column	2.5 µm	4.6 x 300 mm	186006852
ACQUITY UPLC Protein BEH SEC, 450Å	Guard Kit	2.5 µm	4.6 x 30 mm	186006850
ACQUITY UPLC Protein BEH SEC, 200Å	Column	1.7 µm	2.1 x 150 mm	186008471
ACQUITY UPLC Protein BEH SEC, 200Å	Column and Standard	1.7 µm	4.6 x 150 mm	176003904
ACQUITY UPLC Protein BEH SEC, 200Å	Column	1.7 µm	4.6 x 150 mm	186005225
ACQUITY UPLC Protein BEH SEC, 200Å	Column and Standard	1.7 µm	4.6 x 300 mm	176003905
ACQUITY UPLC Protein BEH SEC, 200Å	Column	1.7 µm	4.6 x 300 mm	186005226
ACQUITY UPLC Protein BEH SEC, 200Å	Guard Kit	1.7 µm	4.6 x 30 mm	186005793
ACQUITY UPLC Protein BEH SEC, 125Å	Column and Standard	1.7 µm	4.6 x 150 mm	176003906
ACQUITY UPLC Protein BEH SEC, 125Å	Column	1.7 µm	4.6 x 150 mm	186006505
ACQUITY UPLC Protein BEH SEC, 125Å	Column and Standard	1.7 µm	4.6 x 300 mm	176003907
ACQUITY UPLC Protein BEH SEC, 125Å	Column	1.7 µm	4.6 x 300 mm	186006506
ACQUITY UPLC Protein BEH SEC, 125Å	Guard Kit	1.7 µm	4.6 x 30 mm	186006504
ELSD Outlet Tubing (0.004" I.D. x 6" length)				430001562
0.005 x 1.75" SEC UPLC Connection Tubing, 2/pk				186006613

NOTE: Size-exclusion c hromatography 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.

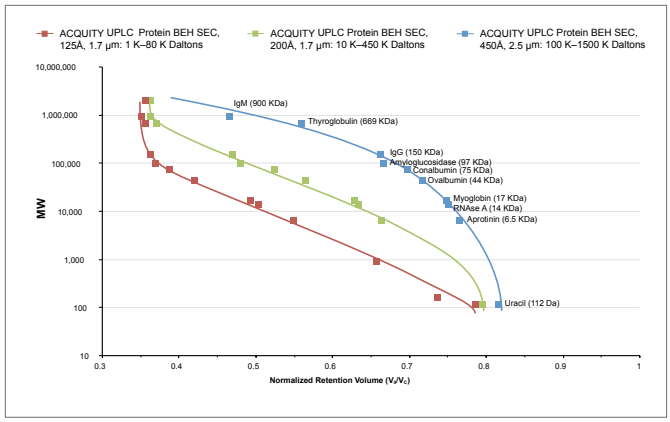
XBridge Protein BEH SEC, 125Å, 200Å, and 450Å Columns and Protein Standard Test Mixtures

Waters series of XBridge Protein BEH SEC, 125Å, 200Å, or 450Å, 3.5 µm Columns were developed to complement our 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- and 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 laboratory instrumentation and component resolution or sample throughput needs.

All of Waters BEH-based SEC columns are manufactured in a cGMP, ISO 9001 certified plant using stringent manufacturing protocols and ultra-pure reagents. Each batch of manufactured material undergoes a series of standard QC measurements (e.g., particle and pore size distribution) followed by an application-specific test using appropriate peptide and protein test mixtures. A packed column efficiency test is then performed on every batch approved, packed SEC column to further help ensure reproducible batch-to-batch and column-to-column performance for use in research or in a demanding validated method.

- Outstanding resolution of peptide and protein mixtures (from 1–1,000,000 K) obtained on high-efficient packed columns containing 3.5 µm particles or 125Å, 200Å, or 450Å pores
- Compared to SEC columns containing 100% silica particles, Waters BEH-based SEC columns are stable at pH values greater than 7 and exhibit less non-desired, secondary ionic interactions between the SEC particle and peptide/protein
- Each column is shipped with Waters SEC Protein Standard Mix to help users establish or confirm acceptable instrument and column performance
- HPLC- and UHPLC-based columns complement existing UPLC-based SEC Columns to assist in method transfer based on users' application and throughput needs

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



Size exclusion chromatography (SEC) separates compounds primarily based on their relative size in solution. Calibration curves on Waters HPLC-based, SEC Columns of different pore size, using defined protein and peptides of known molecular weight, help chromatographers select the most appropriate SEC column for their specific application.

Benchmarking, Method Development, and Troubleshooting: BEH SEC Protein Standards

The BEH SEC Protein Standards are specifically designed to help aid in the benchmarking of each set of columns. Each standard contains carefully chosen proteins unique to that chemistry, which has been worked out meticulously over time. These standards are used to QC the respective HPLC or UPLC columns which makes them an ideal choice for benchmarking a new column while also providing the capability to run the samples over time to monitor column performance.

Offers standards for:

- ACQUITY UPLC and XBridge Protein BEH SEC, 125Å
- ACQUITY UPLC and XBridge Protein BEH SEC, 200Å
- ACQUITY UPLC and XBridge Protein BEH SEC, 450Å

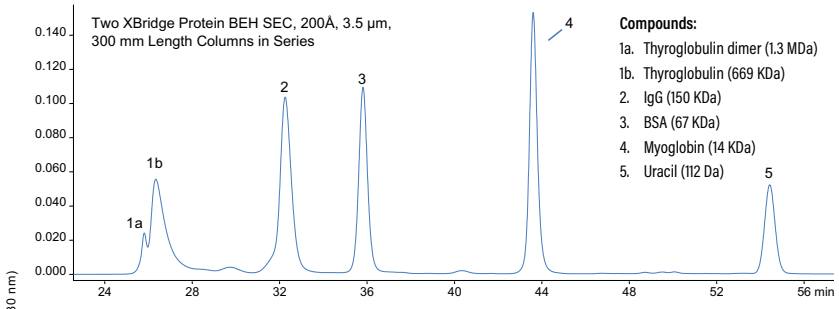
Ordering Information

ACQUITY UPLC BEH SEC Protein Standards

Description	P/N
BEH125 SEC Protein Standard Mix	186006519
BEH200 SEC Protein Standard Mix	186006518
BEH450 SEC Protein Standard Mix	186006842

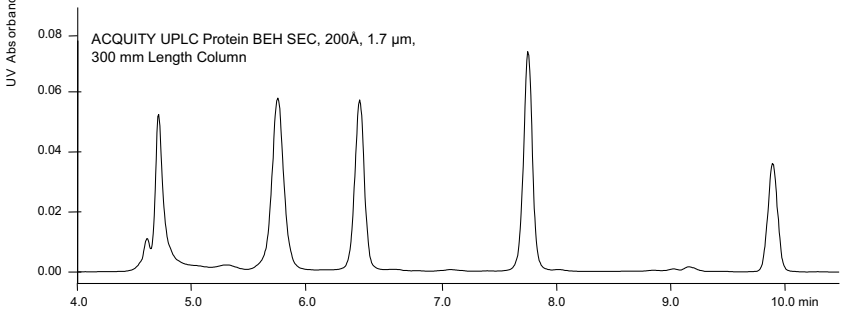
Scalable SEC Separations Using UPLC- vs. HPLC-Based SEC

Sample: BEH200 SEC Protein Standard Mix (p/n: 186006518)
Flow rate: 0.42 mL/min on 4.6 mm column
0.30 mL/min on 7.8 mm column
Mobile phase: 100 mM sodium phosphate, pH 6.8



Compounds:
1a. Thyroglobulin dimer (1.3 MDa)
1b. Thyroglobulin (669 KDa)
2. IgG (150 KDa)
3. BSA (67 KDa)
4. Myoglobin (14 KDa)
5. Uracil (112 Da)

Alliance HPLC System



ACQUITY UPLC H-Class Bio System

Comparison of separations of Waters BEH200 SEC Protein Standard Mix (p/n: 186006518) on two XBridge Protein BEH SEC, 200Å, 3.5 µm HPLC Columns (300 mm length X 7.8 mm I.D.) run in series using an Alliance HPLC (top panel) and on an ACQUITY UPLC Protein BEH SEC, 200Å, 1.7 µm Column (300 mm length X 4.6 mm I.D.) using an ACQUITY UPLC H-Class Bio System (bottom panel). The flow rates were scaled based on particle diameter and column I.D. to 0.42 mL/minute for the two HPLC columns run in series, and 0.3 mL/minute for the UPLC column. Samples loads were also adjusted for column volume.

Ordering Information

XBridge Protein BEH SEC HPLC and UHPLC Columns

Description	Configuration	Particle Size	Dimension	P/N
XBridge Protein BEH SEC, 125Å Column with BEH125 SEC Protein Standard Mix	Guard Column	3.5 µm	7.8 mm x 30 mm	176003591
XBridge Protein BEH SEC, 125Å Column with BEH125 SEC Protein Standard Mix	Column	3.5 µm	7.8 mm x 150 mm	176003592
XBridge Protein BEH SEC, 125Å Column with BEH125 SEC Protein Standard Mix	Column	3.5 µm	7.8 mm x 300 mm	176003593
XBridge Protein BEH SEC, 200Å Column with BEH200 SEC Protein Standard Mix	Guard Column	3.5 µm	7.8 mm x 30 mm	176003594
XBridge Protein BEH SEC, 200Å Column with BEH200 SEC Protein Standard Mix	Column	3.5 µm	7.8 mm x 150 mm	176003595
XBridge Protein BEH SEC, 200Å Column with BEH200 SEC Protein Standard Mix	Column	3.5 µm	7.8 mm x 300 mm	176003596
XBridge Protein BEH SEC, 450Å Column with BEH450 SEC Protein Standard Mix	Guard Column	3.5 µm	7.8 mm x 30 mm	176003597
XBridge Protein BEH SEC, 450Å Column with BEH450 SEC Protein Standard Mix	Column	3.5 µm	7.8 mm x 150 mm	176003598
XBridge Protein BEH SEC, 450Å Column with BEH450 SEC Protein Standard Mix	Column	3.5 µm	7.8 mm x 300 mm	176003599
Straight Connection Tubing and End-fittings for XBridge Protein BEH SEC Column	Column	—	—	WAT022681
U-Bend Connection Tubing and End-fittings for XBridge Protein BEH SEC Column	Column	—	—	WAT084080

Protein BEH C_4, 300Å 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.

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

- Separates proteins of various sizes, hydrophobicities, and isoelectric points
- Unique chemistry maximizes recovery and minimizes protein carryover
- 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)
- Couples directly to ESI-MS for protein identification

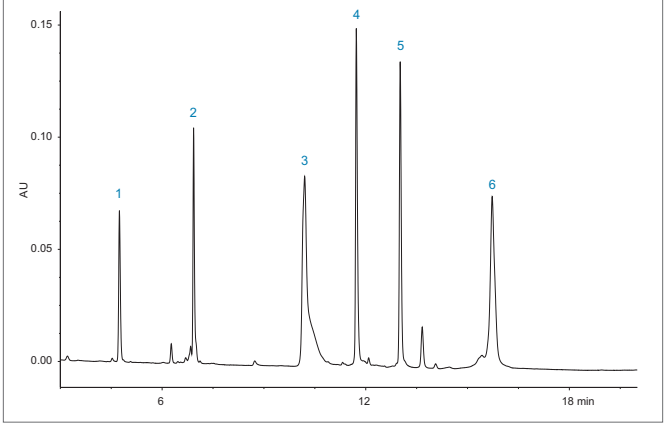
300Å C_4 Columns Developed for Protein Chromatography

Conditions:

Column:	XBridge Protein BEH C_4, 300Å, 3.5 µm, 2.1 x 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, 300Å 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.

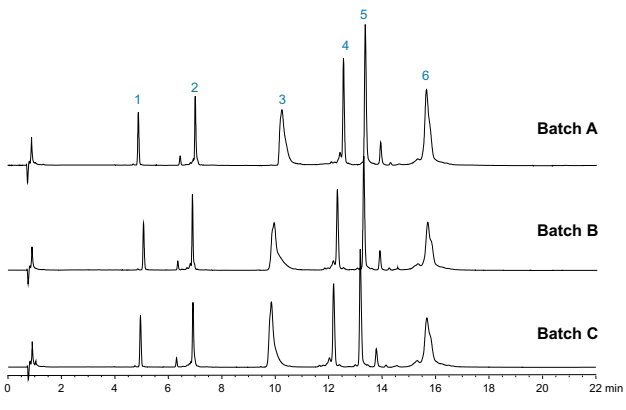
Batch-to-Batch Reproducibility

Conditions:

Column:	ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm, 2.1 x 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

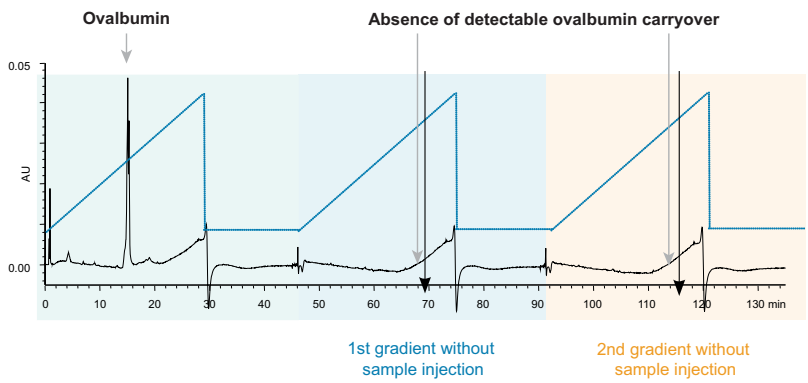
Compounds:

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



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

Minimal Protein Carryover

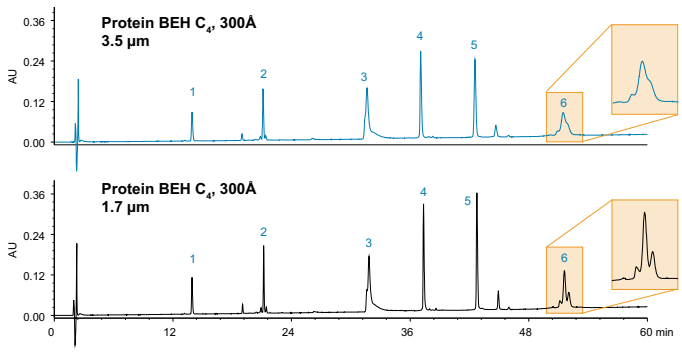


Conditions:

Columns:	ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm, 2.1 mm x 50 mm (p/n: 186004495)
Sample:	1.3 µg ovalbumin injected
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, hold at 100 %B for 2 min. then re-equilibrate column at 28% B for 18 min, gradient restarted at 45 min and at 90 min
Column temp.:	40 °C
UV detection:	220 nm

Column carryover was tested by running multiple gradients following a single injection. Protein peaks observed during the first gradient are not found in subsequent gradients.

Improved Protein Resolution with UPLC Technology



Conditions:

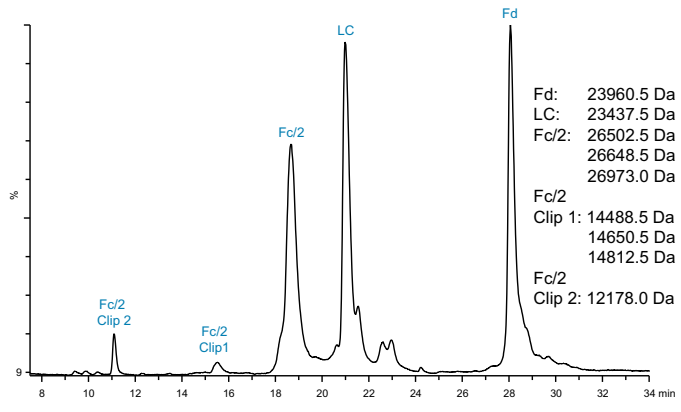
Columns:	ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm (top), 3.5 µm (bottom), 2.1 x 150 mm p/n's: 186004497 (1.7 µm); 186004500 (3.5 µm))
Sample:	MassPREP Protein Standard, 0.1% TFA in 5% acetonitrile (p/n: 186004900)
Mobile phase A:	0.1% trifluoroacetic acid in water
Mobile phase B:	0.1% trifluoroacetic acid in 100% acetonitrile
Flow rate:	0.2 mL/min
Gradient:	20–55% B in 60 min
Column temp.:	80 °C
Injection:	3.3 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

The protein test mixture was separated on two Protein BEH C_4, 300Å Columns, one with 3.5 µm particles and the other with 1.7 µm particles. The UPLC separation provides sharper peaks for all the proteins in the test mixture. This translates into better resolution as shown by the multiple peaks around phosphorylase, at approximately 50 minutes. This comparison, with both columns, was performed on a UPLC system to preserve the minimized band broadening. The benefits of the small particle UPLC BEH C_4, 300Å Column would be lost without the optimized ACQUITY UPLC System.

Protein BEH C_4, 300Å Columns for Protein Characterization with UPLC-MS



Conditions:

Column:	ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm, 2.1 x 50 mm (p/n: 186004495)
Sample:	Lys-C digested (limited) reduced monoclonal antibody
Eluent A:	0.1% formic acid in 100% HPLC grade water
Eluent B:	0.1% formic acid in 100% acetonitrile
Flow rate:	0.2 mL/minV
Gradient:	5% B for 5 min then 5–21% B in 0.1 min, then 21–29% B in 30 min
Column temp.:	80 °C

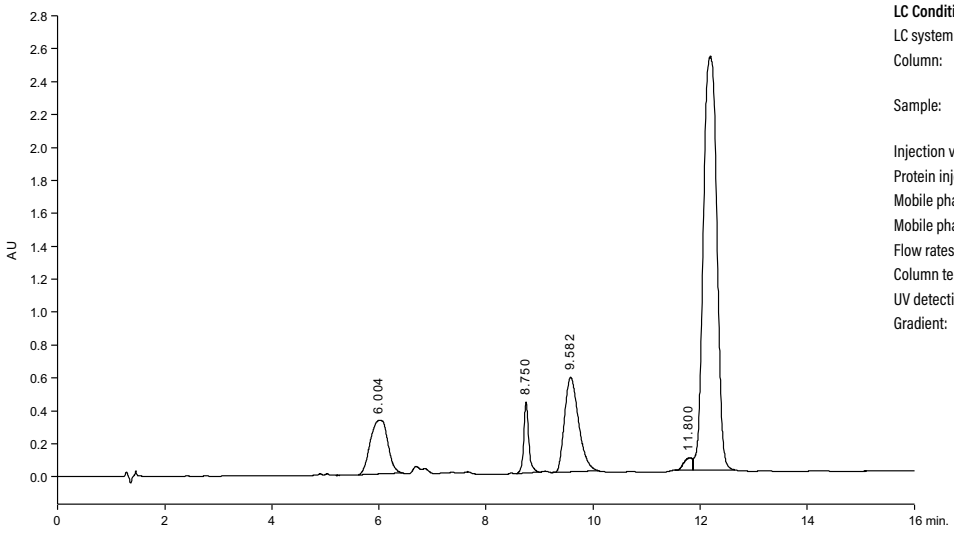
Waters LCT Premier™ XE MS Detection

Capillary voltage:	3200 V
Cone voltage:	45 V
Source temp.:	150 °C
Desolvation temp.:	350 °C
Desolvation gas:	000 L/h

The large fragments obtained through LysC digestion of a monoclonal antibody can be separated on the ACQUITY UPLC Protein BEH C_4, 300Å Column coupled directly to ESI/Tof MS for identification of the individual peptide products.

NOTE: ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm Columns are designed for use with the ACQUITY UPLC System. The benefits of the small particle packing in ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm Columns are only realized with the low system volume and low detector dispersion of an ACQUITY UPLC System.

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

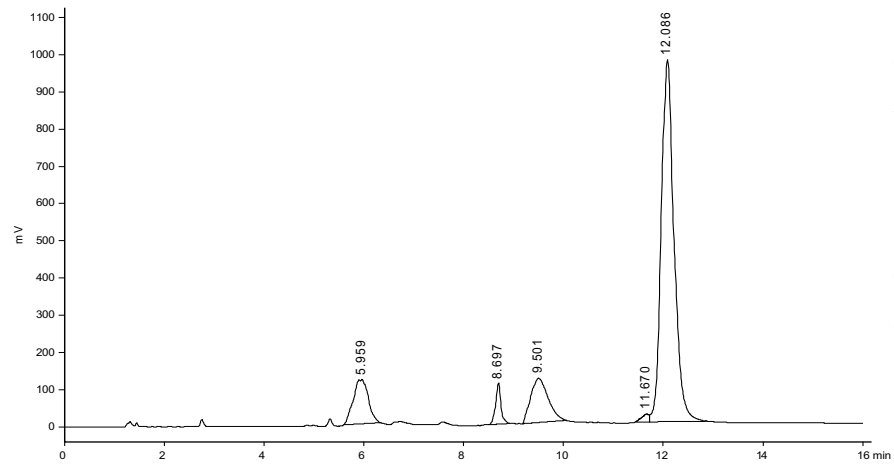


LC Conditions:	Analytical
LC system:	Alliance
Column:	XBridge Protein BEH C_4, 300Å, 3.5 µm, 4.6 mm x 150 mm (p/n: 186004504)
Sample:	Lyophilized chicken egg white, 10 mg/mL in 0.05% TFA in water
Injection vol.:	25 µL
Protein injected:	0.25 mgs
Mobile phase A:	0.05% TFA in water
Mobile phase B:	0.05% 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 ągs chicken egg white proteins on XBridge Protein BEH C_4, 300Å, 3.5 µm, 4.6 x 150 mm Column.

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



LC Conditions:	Preparative
LC system:	Prep 150 LC
Column:	XBridge Protein BEH C_4 OBD Prep, 300Å, 5 µm, 19 x 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 mgs
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, 300Å, 5 µm, 19 x 150 mm column results in chromatography showing an almost identical separation pattern.

Benchmarking, Method Development, and Troubleshooting: MassPREP Protein Standard Mix

The MassPREP Protein Standard Mixture 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, 300Å Columns.

MassPREP Protein Standard Mix		
Protein Sample	**Molecular Weight (MW)**	**Isoelectric Point (pI)**
Ribonuclease A, Bovine Pancrease	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. cereviasiae)	46.7 k	6.53
Phosphorylase b, Rabbit Muscle	97.0 k	7.18

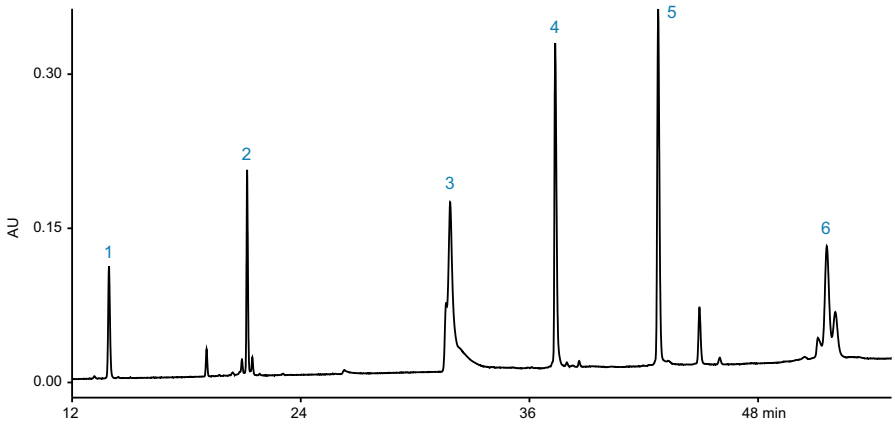
MassPREP Protein Standard Mix on an ACQUITY UPLC Protein BEH C_4, 1.7 µm, 2.1 x 150 mm Column

Conditions:

Column:	ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm, 2.1 x 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 Mixture 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

Each Waters Analytical Standards and Reagents standard comes 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.

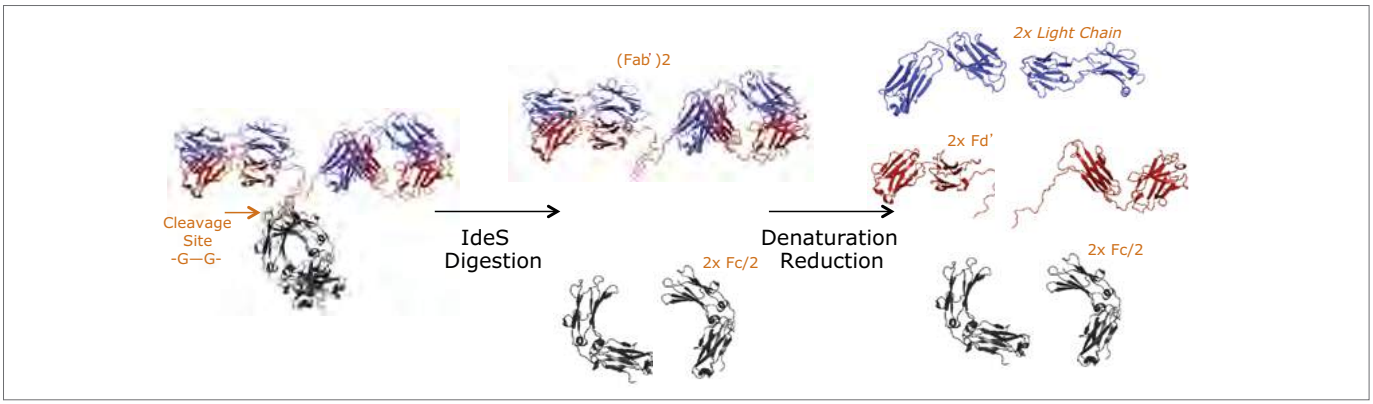
Ordering Information

Protein Standards

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

ACQUITY UPLC Glycoprotein BEH Amide, 300Å Column

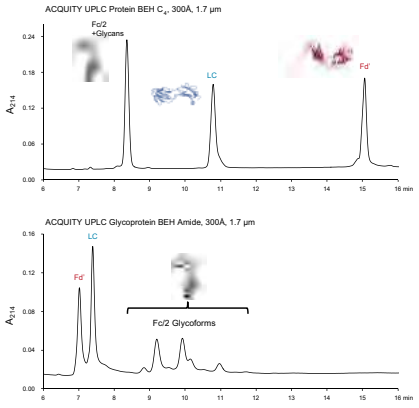
In what is commonly referred to as a middle-up or middle-down analysis, native mAbs can be proteolyzed into subunits to facilitate characterization. One increasingly popular way to produce subunit digests of mAbs is via the IdeS protease (Immunoglobulin Degrading Enzyme of S. pyogenes). IdeS cleaves 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 and reduction scheme for preparing IgG LC, Fd', and Fc/2 subunits.

IdeS digestion combined with reversed-phase (RP) chromatography on Waters ACQUITY UPLC Protein BEH C_4, 300å Column has, in fact, 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 based separation of glycoprotein subunits, HILIC-based chromatography on Waters ACQUITY UPLC Glycoprotein BEH Amide, 300å, 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

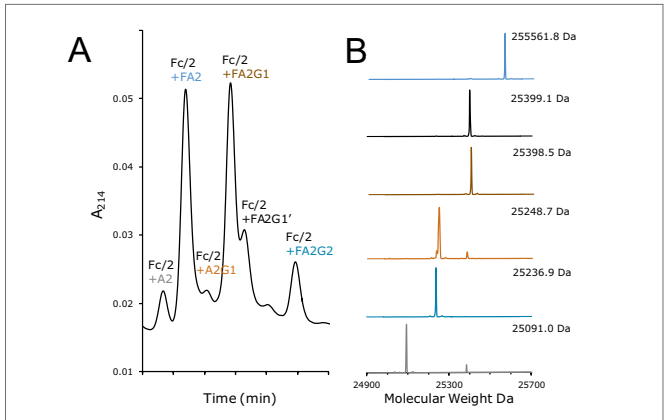
Gradient used for HILIC separations of IgG subunits:

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

Conditions:

LC system:	ACQUITY UPLC H-Class Bio System
Columns:	Top: ACQUITY UPLC Protein BEH C_4, 300Å, 1.7 µm, 2.1 x 150 mm (p/n: 186004497) Bottom: ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 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)

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



C

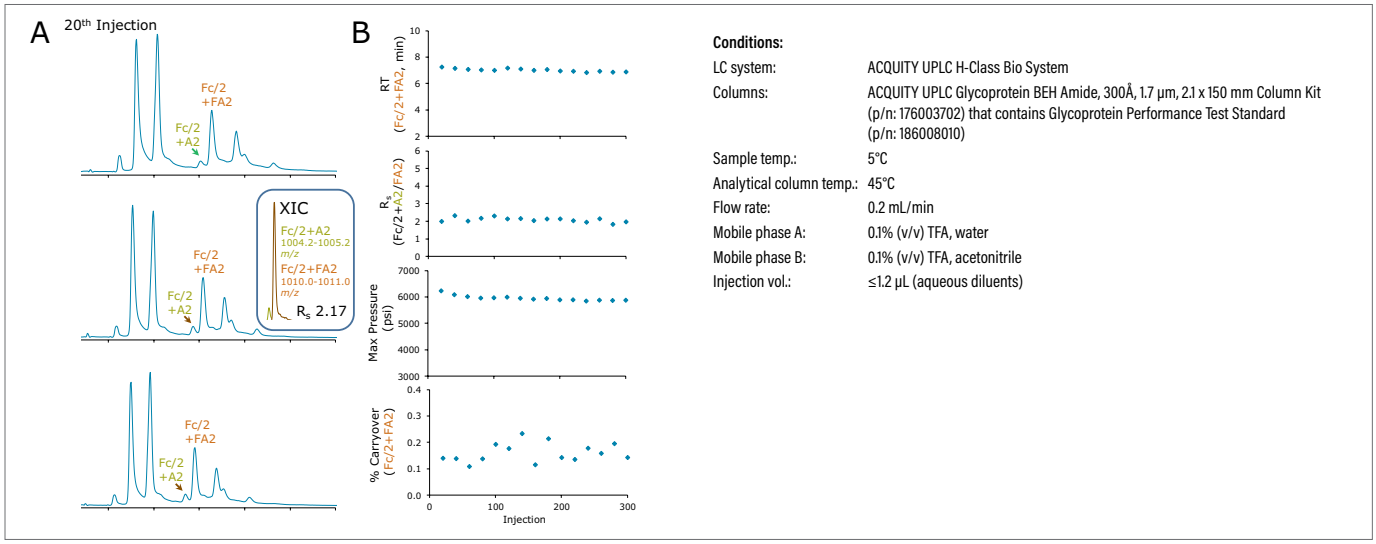
Species	MW_{Avg} Theoretical (Da)	MW_{Avg} Observed (Da)
Fd'	25383.6	25383.3
LC	23443.1	23443.1
Fc/2+A2	25090.2	25091.0
Fc/2+FA2	25236.3	25236.9
Fc/2+A2G1	25252.3	25248.7
Fc/2+FA2G1	25398.5	25398.5
Fc/2+FA2G1'	25398.5	25399.1
Fc/2+FA2G2	25560.6	25561.8

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, 300Å, 1.7 µm Columns for Profiling IgG Subunit Glycoforms

The ability of Waters BEH Amide 300Å, 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.

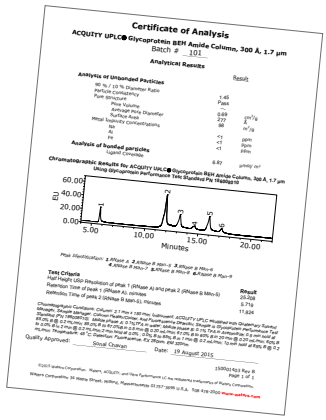
This was a potentially challenging use scenario given that the reduced, IdeS digested mAb sample contains both high concentrations of guanidine denaturant and TCEP reducing agent. Total ion chromatograms corresponding to the 20th, 180th, and 300th injections of this experiment are displayed. In these 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.



Lifetime testing of an ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 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, 300Å, 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, 300å, 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 shipped column to help benchmark, method development, and/or troubleshoot use of this column and instrumentation.



Ordering Information

ACQUITY UPLC Glycoprotein BEH Amide, 300Å Columns and Standards

Description	Qty.	P/N
ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 5 mm	3/pk with standard	176003699
ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 50 mm	1/pk with standard	176003700
ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 100 mm	1/pk with standard	176003701
ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 150 mm	1/pk with standard	176003702
ACQUITY UPLC Glycoprotein BEH Amide, 300Å, 1.7 µm, 2.1 x 100 MVK	3/pk with standard	176003703
Glycoprotein Performance Test Standard		186008010

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

Description	Particle Size	Dimension	P/N
ACQUITY UPLC Protein BEH C_4, 300Å	1.7 µm	1.0 x 50 mm	186005589
ACQUITY UPLC Protein BEH C_4, 300Å	1.7 µm	1.0 x 100 mm	186005590
ACQUITY UPLC Protein BEH C_4, 300Å	1.7 µm	1.0 x 150 mm	186005591
ACQUITY UPLC Protein BEH C_4, 300Å	1.7 µm	2.1 x 50 mm	186004495
ACQUITY UPLC Protein BEH C_4, 300Å	1.7 µm	2.1 x 100 mm	186004496
ACQUITY UPLC Protein BEH C_4, 300Å	1.7 µm	2.1 x 150 mm	186004497
ACQUITY UPLC Protein BEH C_4, 300Å	1.7 µm	VanGuard™ Pre-column, 3/pk	186004623
ACQUITY UPLC Protein BEH C_4, 300Å Method Validation Kit*	1.7 µm	2.1 x 100 mm	186004899
ACQUITY UPLC Protein BEH C_4, 300Å Method Validation Kit*	1.7 µm	2.1 x 150 mm	186006549

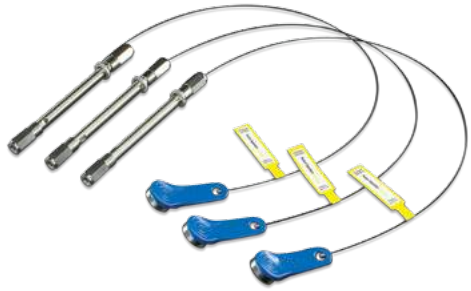
XBridge Protein BEH HPLC and UHPLC Columns and Method Validation Kits

	Dimension	P/N	Dimension	P/N	Dimension	P/N
	Particle Size: 3.5 µm		Particle Size: 5 µm		Particle Size: 10 µm	
BEH C_4, 300Å	2.1 x 10 mm Guard Cartridge	186007230[1]	10 x 10 mm Guard Cartridge	186007305[3]	10 x 10 mm Guard Cartridge	186007325[3]
	2.1 x 50 mm	186004498	10 × 50 mm	186007306	10 x 50 mm	186007326
	2.1 x 100 mm	186004499	10 × 100 mm	186007307	10 x 100 mm	186007327
	2.1 x 150 mm	186004500	10 × 150 mm	186007308	10 x 150 mm	186007328
	2.1 x 250 mm	186004501	10 x 250 mm	186007309	10 x 250 mm	186007329
	4.6 x 20 mm Guard Cartridge	186007235[2]	19 x 10 mm Guard Cartridge	186007310[4]	19 x 10 mm Guard Cartridge	186007330[4]
	4.6 x 50 mm	186004502	19 x 50 mm	186007311	19 x 50 mm	186007331
	4.6 x 100 mm (MVK)	186005465	19 x 100 mm	186007312	19 x 100 mm	186007332
	4.6 x 100 mm	186004503	19 x 150 mm	186007313	19 x 150 mm	186007333
	4.6 x 150 mm	186004504	19 x 250 mm	186007314	19 x 250 mm	186007334
	4.6 x 250 mm	186004505	30 x 10 mm Guard Cartridge	186007315[5]	30 x 10 mm Guard Cartridge	186007335[5]
			30 x 50 mm	186007316	30 x 50 mm	186007336
			30 x 75 mm	186007317	30 x 75 mm	186007337
			30 x 100 mm	186007318	30 x 100 mm	186007338
			30 x 150 mm	186007319	30 x 150 mm	186007339
			30 x 250 mm	186007320		

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

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.

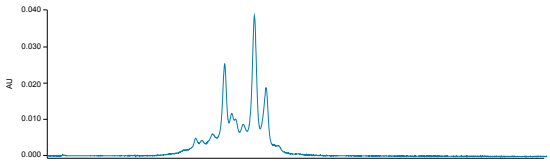


- Designed for the characterization of protein charge variants and other biocompounds
- Two cation-exchangers (carboxymethyl and sulfopropyl) and one anion exchanger (quaternary ammonium) that address selectivity needs
- Non-porous, high-capacity stationary phases deliver fast separations that address 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

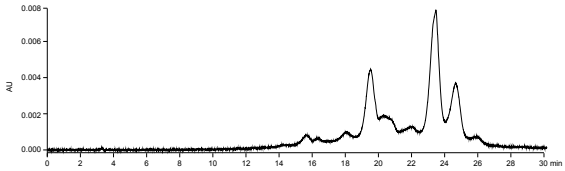
Conditions:

Column:	Waters Protein-Pak Hi Res CM, 7 µm, 4.6 x 100 mm (p/n: 186004929)
Flow rate:	0.67 mL/min
Eluent A:	20 mM disodium hydrogen phosphate pH 7
Eluent B:	0.7 M sodium chloride in B
Gradient:	0–10% B in 25 min
Column temp.:	30 ˚C
UV detection:	280 nm



Conditions:

Column:	Competitor's CM Cation Exchanger, 4 x 250 mm
Flow rate:	0.51 mL/min
Eluent A:	20 mM disodium hydrogen phosphate pH 7
Eluent B:	0.7 M sodium chloride in B
Gradient:	0–10% B in 62.5 min

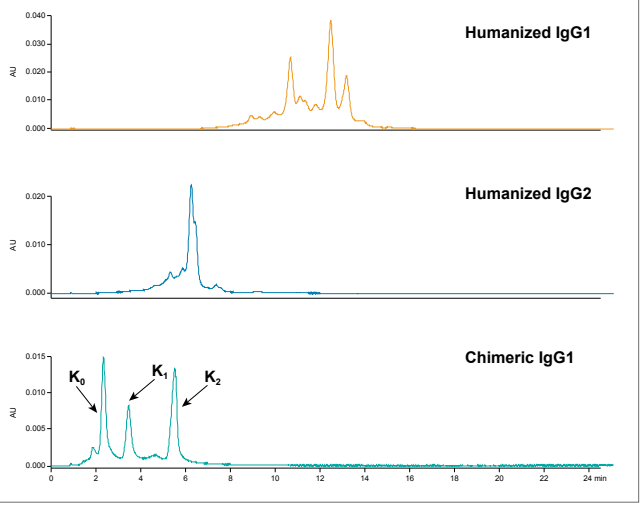


Cation-exchange chromatography is a useful tool for the characterization and quantitation of mAb or recombinant protein variants. Use of Waters Protein-Pak Hi Res CM Column on an ACQUITY UPLC System increases sample throughput while maintaining resolution between intended product and undesired variants.

Protein-Pak Hi Res CM Analysis of Three mAbs Containing Different Levels of Variants

Conditions:

Column:	Protein-Pak Hi Res CM, 7 µm, 4.6 x 100 mm (p/n: 186004929)
Flow rate:	0.67 mL/min
Mobile phase A:	20 mM sodium phosphate buffer, pH 7.0
Mobile phase B:	20 mM sodium phosphate buffer, 0.7 M sodium chloride, pH 7.0
Gradient:	0–10% B in 25 min
Column temp.:	30 ˚C
UV detection:	280 nm



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
Protein-Pak Hi Res CM	7 µm	4.6 x 100 mm	186004929
Protein-Pak Hi Res SP	7 µm	4.6 x 100 mm	186004930
Protein-Pak Hi Res Q	5 µm	4.6 x 100 mm	186004931

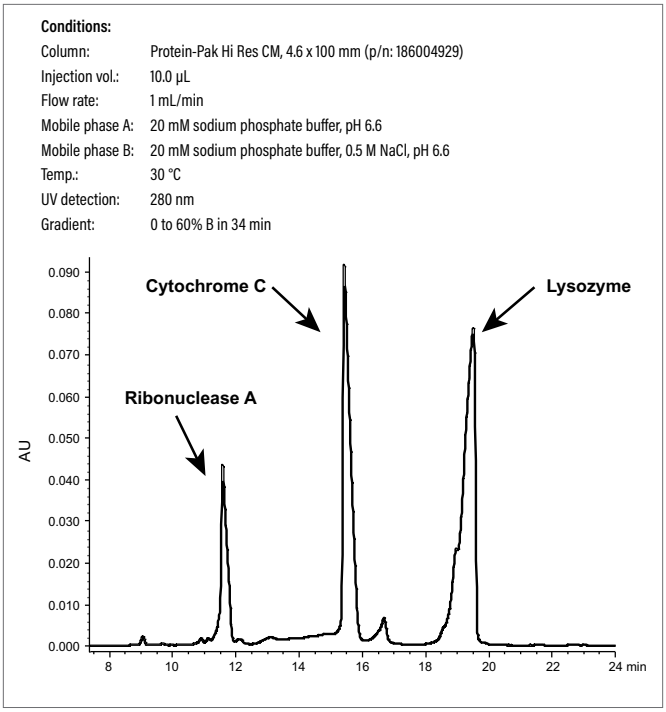
NOTE: Only when Protein-Pak Hi Res IEX Columns are combined with the ACQUITY UPLC System are the full performance benefits realized. See Waters service notes p/n: 715002147A for ACQUITY UPLC System configuration guidelines for ion-exchange chromatography.

Benchmarking, Method Development, and Troubleshooting: Ion Exchange Standards

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
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.

Novel IEX Particles Ideal for Biomolecule Characterizations

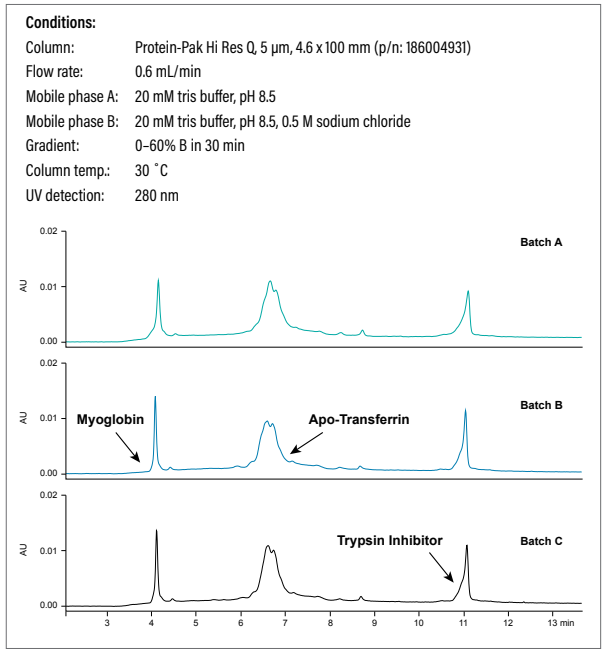
Protein-Pak Hi Res IEX Columns contain non-porous, pH tolerant, hydrophilic particles whose surface consists of a multi-layered network of either anion (5 µm) or cation (7 µm) exchange groups. This innovative particle and bonding chemistry produces particles with greater protein loading capacities than 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 (µm)	5	7	7
Pore size	Non porous	Non porous	Non porous
I.D. x L (mm)	4.6 x 100	4.6 x 100	4.6 x 100
Counter ion	Cl-	Na+	Na+
pH range	3–10	3–10	3–10
Temperature (°C)	10–60	10–60	10–60
pKa	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.

ACQUITY UPLC Technology for biotherapeutic characterization.

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.

Protein-Pak Hi Res HIC Column 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 hyrdophobic 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 x 35 mm	176003575
Protein-Pak Hi Res HIC, 2.5 µm Column and HIC Protein Standard	4.6 x 100 mm	176003576
HIC Protein Test Standard	—	186007953

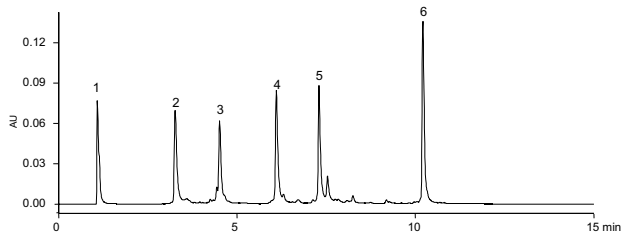
Protein-Pak Hi Res HIC Column and HIC Protein Standard

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 x 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

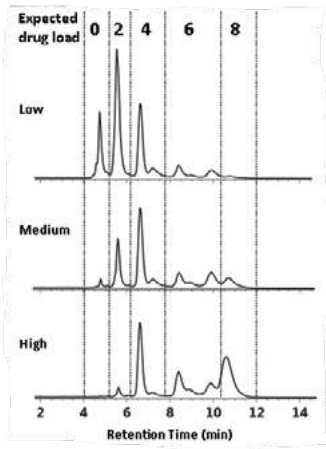


Using a gradient of decreasing salt concentration and on-denaturing eluents, Waters Protein-Pak Hi Res HIC Column is well suited for the separation of proteins of various molecular weights and hydrophobic interactions.

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 x 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

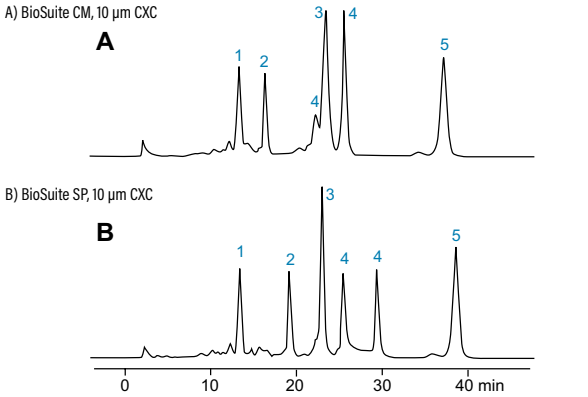


Monitoring drug load variability. Three batches of cysteine-linked ADCs were synthesized, each with a different level of drug conjugation (low, medium, high) and separated using hydrophobic interaction chromatography. The drug load distribution shifted from low to high corresponding to an increase in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

BioSuite 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

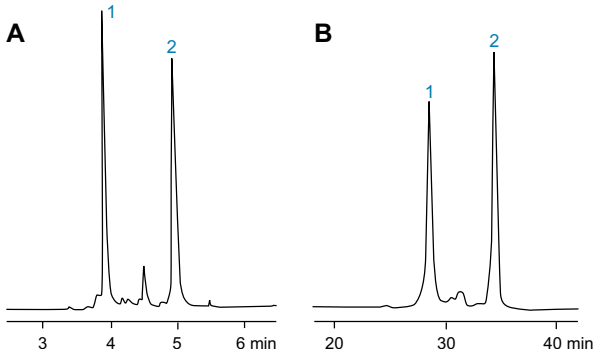


Conditions:

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 mm x 75 mm (p/n: 186002186) B: BioSuite SP, CXC, 10 µm, 7.5 mm x 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 mm x 35 mm (p/n:186002183) B: BioSuite SP, CXC, 10 µm, 7.5 mm x 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
BioSuite pC_{18}, 2.5 µm NP RPC	Polymer	4.6 x 35 mm	186002152
BioSuite pC_{18}, 500, 7 µm RPC	Polymer	2.0 x 150 mm	186002153
BioSuite pC_{18}, 500, 7 µm RPC	Polymer	4.6 x 150 mm	186002154
BioSuite pC_{18}, 500, 13 µm RPC	Polymer	21.5 x 150 mm	186002155
BioSuite pPhenyl, 1000, 10 µm RPC	Polymer	2.0 x 75 mm	186002156
BioSuite pPhenyl, 1000, 10 µm RPC	Polymer	4.6 x 75 mm	186002157
BioSuite pPhenyl, 1000, 13 µm RPC	Polymer	21.5 x 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	4000Å	>5,000,000	4.6 x 50 mm	0.83	58 mg[1]	186002176
BioSuite SP-PEEK, 7 µm CXC	Polymer	1300Å	>4,000,000	4.6 x 50 mm	0.83	58 mg[2]	186002182
BioSuite DEAE, 2.5 µm NP AXC	Polymer	n/a	500	4.6 x 35 mm	0.58	2.9 mg[1]	186002179
BioSuite SP, 2.5 µm NP CXC	Polymer	n/a	500	4.6 x 35 mm	0.58	2.9 mg[3]	186002183
BioSuite Q, 10 µm AXC	Polymer	1000Å	1,000,000	7.5 x 75 mm	3.31	331 mg[1]	186002177
BioSuite Q, 13 µm AXC	Polymer	1000Å	1,000,000	21.5 x 150 mm	54.45	5,445 mg[1]	186002178
BioSuite DEAE, 10 µm AXC	Polymer	1000Å	1,000,000	7.5 x 75 mm	3.31	99 mg[1]	186002180
BioSuite DEAE, 13 µm AXC	Polymer	1000Å	1,000,000	21.5 x 150 mm	54.45	1633 mg[1]	186002181
BioSuite SP, 10 µm CXC	Polymer	1000Å	1,000,000	7.5 x 75 mm	3.31	132 mg[3]	186002184
BioSuite SP, 13 µm CXC	Polymer	1000Å	1,000,000	21.5 x 150 mm	54.45	2,178 mg[3]	186002185
BioSuite CM, 10 µm CXC	Polymer	1000Å	1,000,000	7.5 x 75 mm	3.31	149 mg[3]	186002186
BioSuite CM, 13 µm CXC	Polymer	1000Å	1,000,000	21.5 x 150 mm	54.45	2,450 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			
Pre-packed Column	Q	DEAE	SP	CM
5 x 50 mm	60 mg	40 mg	40 mg	25 mg
5 x 100 mm	130 mg	150 mg	80 mg	45 mg
10 x 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 x 75 mm	WAT088044
Protein-Pak DEAE 5PW Glass Column	8 x 75 mm	WAT011783
Protein-Pak DEAE 5PW Steel Column	21.5 x 150 mm	WAT010640
Polymeric Strong Cation-Exchanger	7.5 x 75 mm	WAT088043
Protein-Pak SP 5PW Glass Column	8 x 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 1000Å 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.

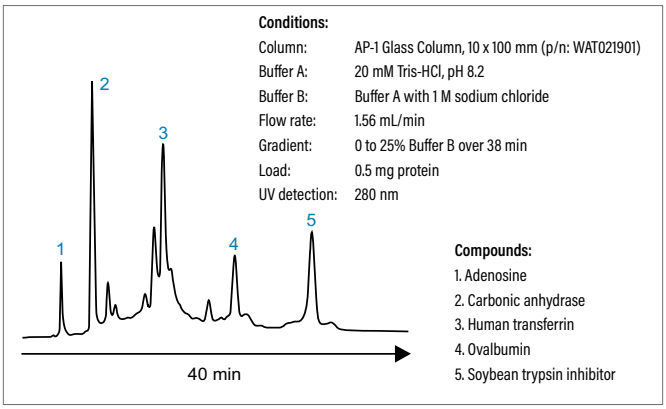
Protein-Pak HR ion exchangers are available with a Q functional group, a strong anion exchanger; DEAE, a weak anion exchanger; SP, a strong cation exchanger; and CM, a weak cation exchanger. The principal difference between a weak and strong ion exchanger does not lie in the protein binding capacity, 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	1000Å	5 x 50 mm	Polymeric strong anion exchanger	WAT039575
			5 x 100 mm		WAT039630
			10 x 100 mm		WAT035980
Protein-Pak Q 15HR	15 µm	1000Å	5 x 50 mm	Polymeric strong anion exchanger	WAT039782
			10 x 100 mm		WAT037663
Protein-Pak DEAE 8HR	8 µm	1000Å	5 x 50 mm	Polymeric weak anion exchanger	WAT039791
			5 x 100 mm		WAT039783
			10 x 100 mm		WAT035650
Protein-Pak DEAE 15HR	15 µm	1000Å	5 x 50 mm	Polymeric weak anion exchanger	WAT039780
			5 x 100 mm		WAT039786
			10 x 100 mm		WAT038564
Protein-Pak SP 8HR	8 µm	1000Å	5 x 50 mm	Polymeric strong cation exchanger	WAT039570
			5 x 100 mm		WAT039625
			10 x 100 mm		WAT035655
Protein-Pak SP 15HR	15 µm	1000Å	10 x 100 mm	Polymeric strong cation exchanger	WAT038567
Protein-Pak CM 8HR	8 µm	1000Å	5 x 50 mm	Polymeric weak cation exchanger	WAT039790
			5 x 100 mm		WAT039785
			10 x 100 mm		WAT035970
Protein-Pak CM 15HR	15 µm	1000Å	5 x 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

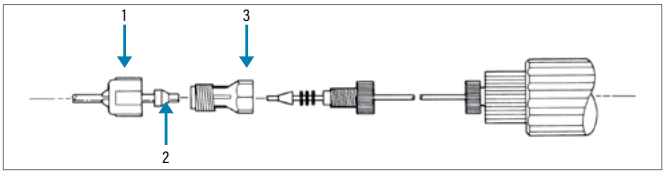
Advanced Purification (AP) Glass Columns

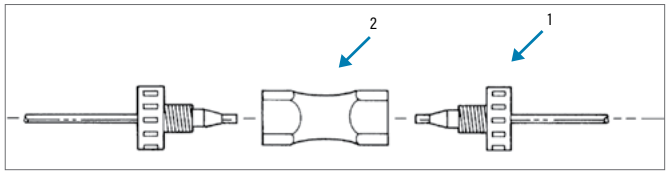
Dimension	Bed Volume (mL)	Flow Rate (mL/min)	Pressure Rating (psi/MPa)	P/N
5 x 50 mm	0.8–1.2	0–4	1500 psi/10 MPa	WAT064-01
5 x 100 mm	1.8–2.2	0–4	1500 psi/10 MPa	WAT064-02
10 x 100 mm	5–8	0–4	1500 psi/10 MPa	WAT021901
10 x 200 mm	13–16	0–4	1500 psi/10 MPa	WAT021902
10 x 300 mm	21–24	0–4	1500 psi/10 MPa	WAT021903
10 x 600 mm	45–48	0–4	1500 psi/10 MPa	WAT021906
20 x 100 mm	22–31	4–16	1000 psi/6.8 MPa	WAT027501
20 x 200 mm	53–62	4–16	1000 psi/6.8 MPa	WAT027502
20 x 300 mm	85–94	4–16	1000 psi/6.8 MPa	WAT027503
20 x 600 mm	179–188	4–16	1000 psi/6.8 MPa	WAT027506
50 x 100 mm	137–196	16–100	500 psi/3.4 MPa	WAT023321
50 x 200 mm	333–392	16–100	500 psi/3.4 MPa	WAT023332
50 x 300 mm	530–589	16–100	500 psi/3.4 MPa	WAT023323
50 x 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 x 'Z' Fitting	5/pk	WAT005137

AP MiniColumn Accessories and Spare Parts

Description	Dimension	P/N
Glass Tube	5 x 50 mm	WAT038802
	5 x 100 mm	WAT038803
Column Jacket	5 x 50 mm	WAT038804
	5 x 100 mm	WAT038805
Filters, 10/pk	–	WAT038806
O-rings, 13/pk (includes 10 inlet/outlet and 3 funnel)	–	WAT038807
Inlet Connector Assembly	–	WAT038800

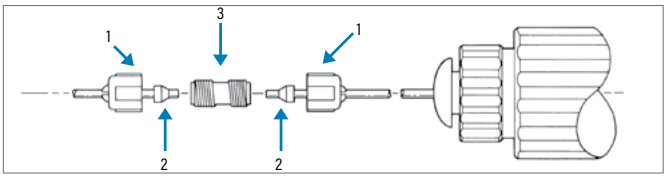
AP-1 Column

Description	Qty	P/N
1 Compression Screw and Ferrule 'Z' Fitting, Plastic	1/pk	WAT082708
2 Union 'Z' Fitting, Plastic	1/pk	WAT082745

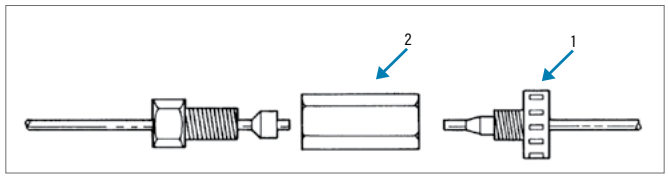
AP-1 Column Accessories and Spare Parts

Description	Dimension	P/N
Glass Tube	10 x 100 mm	WAT021992
	10 x 200 mm	WAT022033
	10 x 300 mm	WAT022034
	10 x 600 mm	WAT022035
Plastic Shield	10 x 100 mm	WAT021927
	10 x 200 mm	WAT021945
	10 x 300 mm	WAT021946
	10 x 600 mm	WAT021947
O-rings, 5/pk	–	WAT021907
Filters, 10/pk	–	WAT021910
Replacement Tubing (Tefzel) (1/16 in. O.D. x 0.009 in. I.D. x 10 feet) (1.6 mm O.D. x 0.23 mm I.D. x 3 m)	–	WAT021950
Inlet Connector Assembly	–	WAT021904

Connection of an AP-2 and an AP-5 Column to 1/8" OD Tubing



Connection of Pharmacia Fitting to 1/16" OD 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 x 3/8–24	1/pk	WAT082734

AP-2 Column Accessories and Spare Parts

Description	Dimension	P/N
Glass Tube	20 x 100 mm	WAT019891
	20 x 200 mm	WAT019892
	20 x 300 mm	WAT019893
Plastic Shield	20 x 100 mm	WAT027542
	20 x 200 mm	WAT027543
	20 x 300 mm	WAT027544
O-rings, 5/pk	–	WAT027528
Filters, 2/pk	–	WAT027530
Replacement Tubing (Tefzel) (1/8 in. O.D. x 0.040 in. I.D. x 10 feet) (3.2 mm O.D. x 1.02 mm I.D. x 3 m)	–	WAT023344
Inlet Connector Assembly	–	WAT027525
Distributors/Inserts, 5/pk	–	700004715

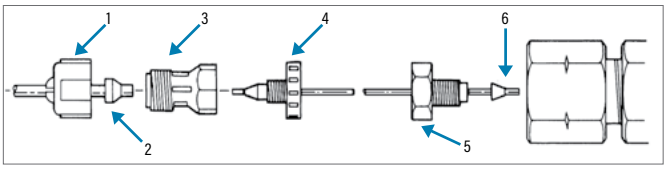
AP-5 Column

Description	Qty.	P/N
1. Compression Screw and Ferrule 'Z' Fitting, Plastic	1/pk	WAT082708
2. Union, Plastic	1/pk	WAT021951

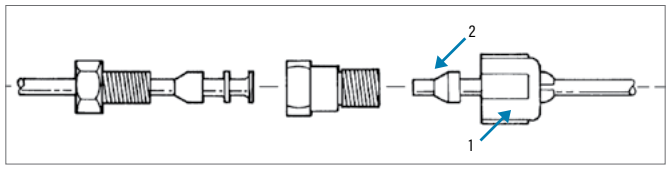
AP-5 Column Accessories and Spare Parts

Description	Dimension	P/N
Glass Tube	50 x 100 mm	WAT019876
	50 x 200 mm	WAT019877
	50 x 300 mm	WAT019878
Plastic Shield	50 x 100 mm	WAT023370
	50 x 200 mm	WAT023371
	50 x 300 mm	WAT023372
	50 x 600 mm	WAT023373
O-rings, 5/pk	–	WAT023345
Filter, 2/pk	–	WAT023343
Replacement Tubing (Tefzel) 1/8 in. O.D. x 0.040 in. I.D. x 10 feet) (3.2 mm O.D. x 1.02 mm I.D. x 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" OD Tubing



Connection of 1/8" or 1/16" Flanged Type Fitting to 1/8" OD 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 x '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

Accell Plus Ion-Exchange Packings

Solid-Phase Extraction for Protein Sample Preparation

Waters Accell Plus ion-exchange packings are 40 µm, 300Å polymer-coated, silica-based materials for both lab- and process-scale chromatography. Accell Plus, 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.

Accell Plus bulk material may be packed into our Advanced Purification (AP) Glass Columns.

1To estimate packed bed volume for a known amount of Accell Plus: **Accell Plus used (g) x 2 = packed bed volume (mL)**

Accell Plus Sep-Pak Cartridges

Sep-Pak™ Plus Cartridges packed with Accell Plus 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

Accell Plus Sep-Pak Cartridges

Description	Ion-Exchange Type	P/N
Accell Plus CM	Weak Cation Exchanger	WAT020550
Accell Plus QMA	Strong Anion Exchanger	WAT020545
Accell Plus QMA Plus	Strong Anion Exchanger	186004540

Accell Plus PrepPak Cartridges (47 x 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 **www.waters.com**

Protein Binding Capacity of Accell Plus	
Accell Plus QMA* 200 mg BSA/g packing	**Accell Plus 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 Accell Plus QMA.	
** Cytochrome *c* in 20 mM sodium phosphate pH 6.3 was used to measure protein binding capacity of Accell Plus CM.	

NOTE: For best resolution do not exceed 20% of the protein binding capacity.

Ordering Information

Accell Plus PrepPak Cartridges (47 x 300 mm)

Description	Particle Size	Pore Size	P/N
Accell Plus CM*	40 µm	300Å	WAT036545
PrepPak 1000 Module	—	—	WAT089592

* Requires PrepPak 1000 Module

Accell Plus Ion-Exchange Bulk Packings

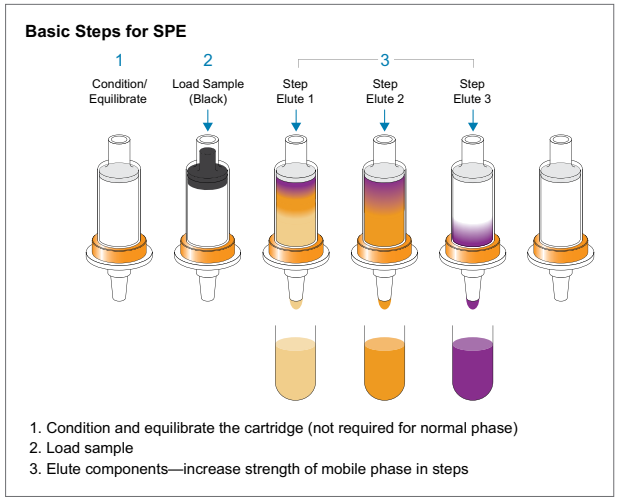
For all preparative isolations based on ionic interactions, particularly proteins, enzymes, and immunoglobulins.

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, Accell Plus 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, Accell Plus QMA, Accell Plus 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

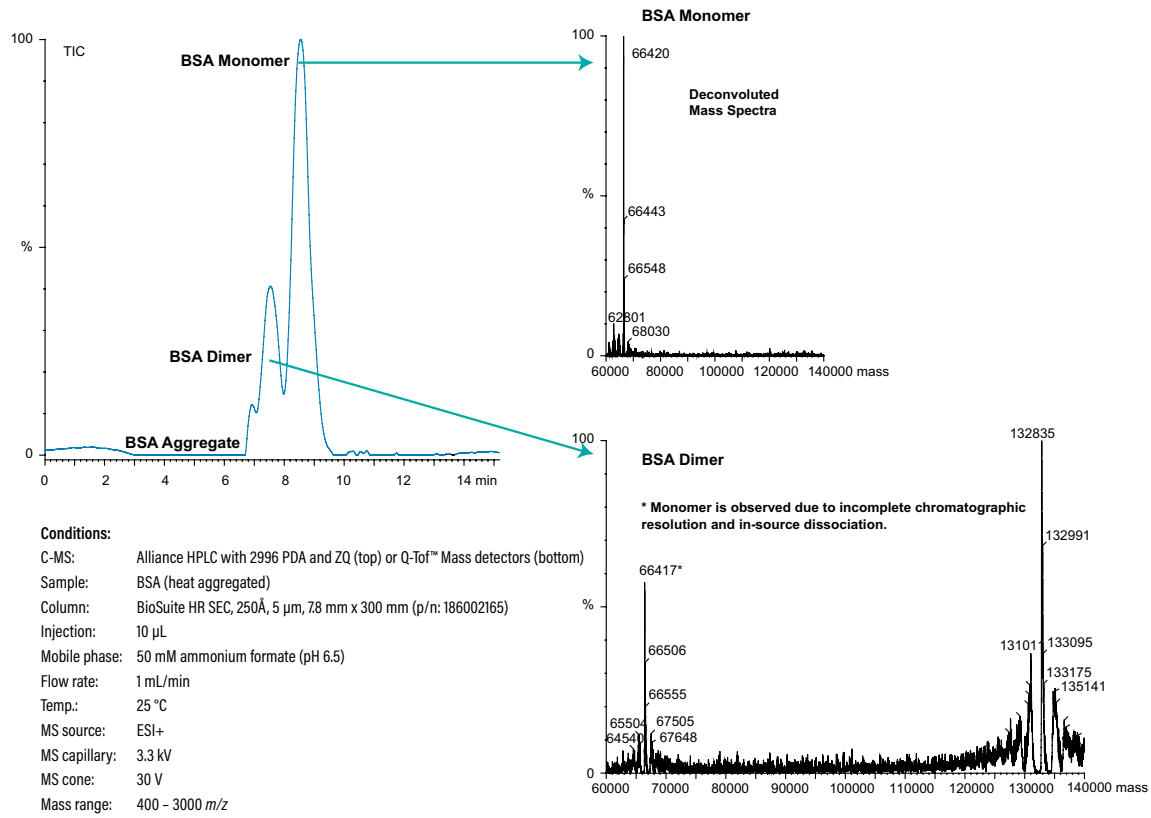
Accell Plus Ion-Exchange Bulk Packings

Description	Particle Size	Pore Size	Qty.	P/N
Accell Plus QMA	40 µm	300Å	100 g	WAT010742
Anion Exchanger	—	—	500 g	WAT010741
Accell Plus CM	40 µm	300Å	100 g	WAT010740
Cation Exchanger	—	—	500 g	WAT010739

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 250, HR 5 µm SEC Column

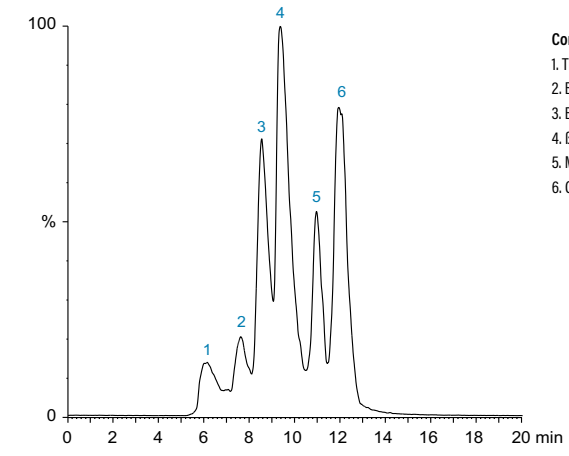


Conditions:

C-MS:	Alliance HPLC with 2996 PDA and ZQ (top) or Q-Tof™ Mass detectors (bottom)
Sample:	BSA (heat aggregated)
Column:	BioSuite HR SEC, 250Å, 5 µm, 7.8 mm x 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 250, 5 µm High Resolution (HR) SEC Column (LC-MS conditions as above)

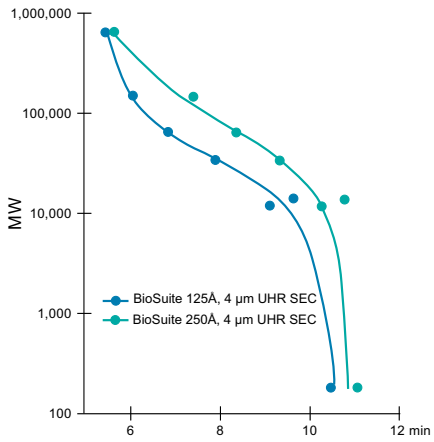


Compounds:

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

Eluent: 0.15 M sodium phosphate, pH 6.8

Flow rate: 0.35 mL/min

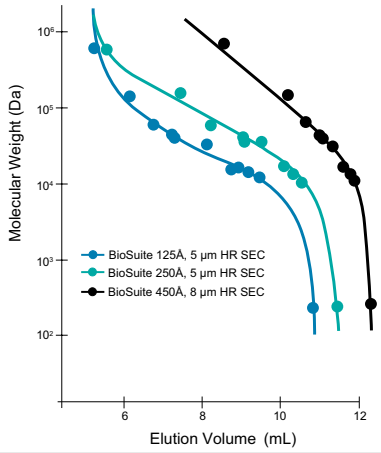
Column temp.: 25 °C

UV detection: 220 nm

Column	Globular Protein MW Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125Å	5000–150,000	1000–30,000	500–15,000
BioSuite 250Å	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, 450Å, 8 µm, 7.8 mm x 300 mm (p/n: 186002166)
BioSuite HR SEC, 250Å, 5 µm, 7.8 mm x 300 mm (p/n: 186002165)
BioSuite SEC, 125Å, 5 µm, 7.8 mm x 300 mm (p/n: 186002164)

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

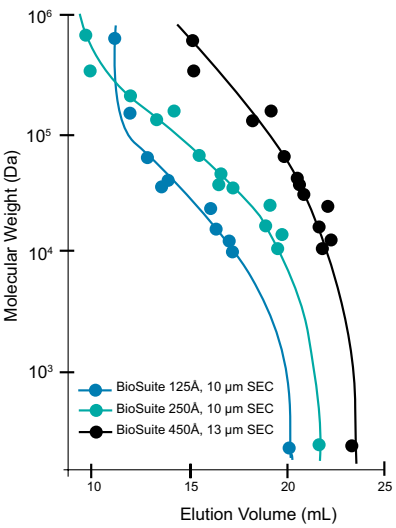
Flow rate: 1.0 mL/min

Column temp.: 25 °C

UV detection: 220 nm

Column	Globular Protein MW Range	Branched Dextrans	Linear PEG/PEO
BioSuite 125Å	5000–150,000	1000–30,000	500–15,000
BioSuite 250Å	10,000–500,000	2000–70,000	1000–35,000
BioSuite 450Å	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, 450Å, 13 µm, 7.5 mm x 300 mm (p/n: 186002172)
BioSuite SEC, 250Å, 13 µm, 7.5 mm x 300 mm (p/n: 186002170)
BioSuite SEC, 125Å, 10 µm, 7.5 mm x 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 125Å	5000–150,000	1000–30,000	500–15,000
BioSuite 250Å	10,000–500,000	2000–70,000	1000–35,000
BioSuite 450Å	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 125Å, 4 µm UHR SEC	Silica	4.6 mm	300 mm	4.98 mL	Less than 8 mg/mL	Less than 40 µL	186002161
BioSuite 250Å, 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 125Å, 5 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002164
BioSuite 250Å, 5 µm HR SEC	Silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002165
BioSuite 450Å, 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 125Å, 10 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002168
BioSuite 125Å, 13 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mL	186002169
BioSuite 250Å, 10 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002170
BioSuite 250Å, 13 µm SEC	Silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mL	186002171
BioSuite 450Å, 13 µm SEC	Silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002172
BioSuite 450Å, 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 Na2SO4) eluent. Useful protein mass loads will vary depending upon separation eluent, complexity of sample, and on the type of proteins contained in mixture. In general, maximum component resolution is obtained by injecting the smallest possible volume of a dilute protein solution.

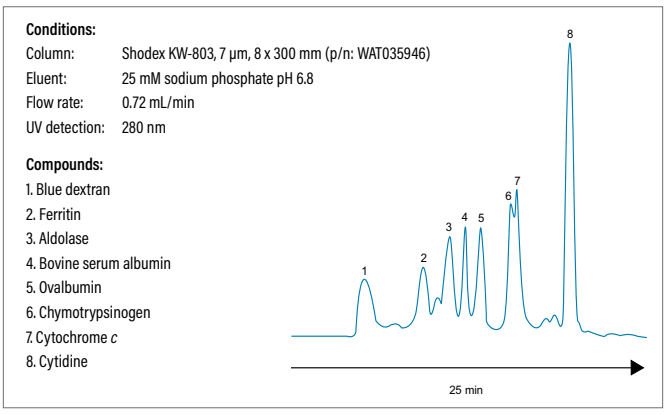
** Operating flow rates for BioSuite Ultra-High Resolution (UHR) SEC Columns (4.6 mm I.D.) are from 0.1–0.4 mL/min. Use of an HPLC system (e.g. Waters Alliance HPLC System) capable of operating at these flows is essential for optimal UHR SEC Column performance.

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

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

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

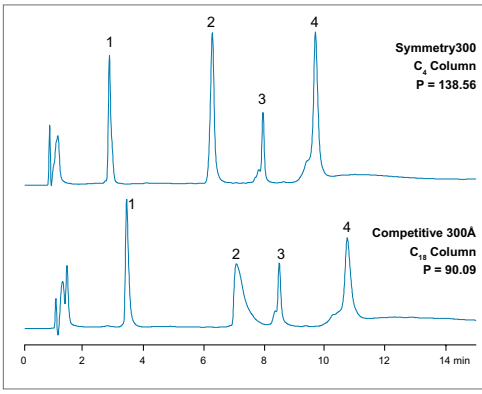
Description	Particle Size	Dimension	MW Range	P/N
Protein KW-802.5	7 µm	8 x 300 mm	100–50,000	WAT035943
Protein KW-803	7 µm	8 x 300 mm	100–150,000	WAT035946
Protein KW-804	7 µm	8 x 300 mm	500–600,000	WAT036613

Symmetry300 C_4 HPLC and UHPLC Columns

Compared to our Protein BEH C_4, 300Å 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.

- 300Å pore for peptide and protein applications
- Fully end-capped to minimize undesired secondary interactions
- Alternative separation selectivity compared to Waters BEH C_4, 300Å 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 C_4 HPLC and UHPLC Columns

Description	Particle Size	Dimension	P/N
Symmetry300 C_4	3.5 µm	1.0 x 150 mm	186000276
Symmetry300 C_4	3.5 µm	2.1 x 50 mm	186000277
Symmetry300 C_4	3.5 µm	2.1 x 100 mm	186000278
Symmetry300 C_4	3.5 µm	2.1 x 150 mm	186000279
Symmetry300 C_4	3.5 µm	4.6 x 50 mm	186000280
Symmetry300 C_4	3.5 µm	4.6 x 75 mm	186000281
Symmetry300 C_4	3.5 µm	4.6 x 100 mm	186000282
Symmetry300 C_4	3.5 µm	4.6 x 150 mm	186000283
Symmetry300 C_4	5 µm	2.1 x 150 mm	186000285
Symmetry300 C_4	5 µm	3.9 x 150 mm	186000286
Symmetry300 C_4	5 µm	4.6 x 50 mm	186000287
Symmetry300 C_4	5 µm	4.6 x 150 mm	186000288
Symmetry300 C_4	5 µm	4.6 x 250 mm	186000289

Symmetry300 HPLC and UHPLC Columns

			P/N	P/N
Description	Particle Size	Dimension	C_{18}	C_4
Symmetry300	3.5 µm	1.0 x 150 mm	186000185	186000276
Symmetry300	3.5 µm	2.1 x 50 mm	186000187	186000277
Symmetry300	3.5 µm	2.1 x 100 mm	186000188	186000278
Symmetry300	3.5 µm	2.1 x 150 mm	186000200	186000279
Symmetry300	3.5 µm	4.6 x 50 mm	186000201	186000280
Symmetry300	3.5 µm	4.6 x 75 mm	186000189	186000281
Symmetry300	3.5 µm	4.6 x 100 mm	186000190	186000282
Symmetry300	3.5 µm	4.6 x 150 mm	186000197	186000283
Symmetry300	5 µm	2.1 x 150 mm	WAT106172	186000285
Symmetry300	5 µm	3.9 x 150 mm	WAT106154	186000286
Symmetry300	5 µm	4.6 x 50 mm	WAT106209	186000287
Symmetry300	5 µm	4.6 x 150 mm	WAT106157	186000288
Symmetry300	5 µm	4.6 x 250 mm	WAT106151	186000289
Symmetry300	5 µm	19 x 10 mm	186001847	–
Symmetry300	5 µm	19 x 50 mm	186001848	–
Symmetry300	5 µm	19 x 100 mm	186001849	–

Protein-Pak Affinity Columns

The Protein-Pak Affinity Epoxy-Activated packing consists of 40 µm, 500Å pore size particles having a hydrophilic bonding layer with a glycidoxypropyl functionality resulting in a seven atom spacer arm. The epoxy-activated surface can immobilize a wide range of ligands via a covalent linkage with amino, hydroxyl or sulfhydryl groups using simple coupling procedures. For method screening or small scale separation, choose the convenience of pre-packed microcolumns. Larger-scale separations are easily achieved by packing bulk material in our Advanced Purification (AP) Glass Column.

To estimate packed bed volume for a known amount of Protein-Pak Affinity Epoxy-Activated packing:

Protein-Pak Affinity Epoxy-Activated used (g) x 2 = packed bed volume (mL)

Purification of Carbonic Anhydrase

Conditions:

Ligand: Sulfanilamide, 180 µmoles/g

Column: AP-1 Glass, 10 x 100 mm (p/n: WAT021992)

Buffer A: 100 mM tris sulfate with 200 mM sodium sulfate pH 8.7

Buffer B: 200 mM potassium thiocyanate in 50 mM tris sulfate pH 6.5

Gradient:

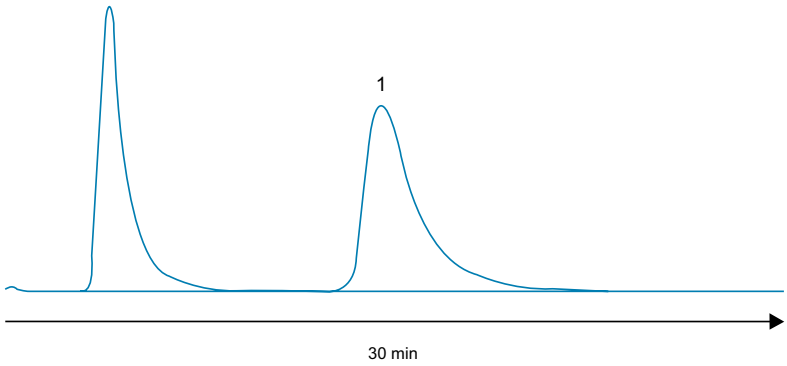
Time (min)	Profile %A	%B	Flow Rate (mL/min)	
Initial	1	100	0	
1	2	100	0	
2	7	0	100	
2	17	100	0	2

UV detection: 280 nm

Protein load: 24 mg of bovine hemolysate yielded 0.6 mg of carbonic anhydrase with a 100% recovery of esterase activity

Compound:

1. Carbonic anhydrase



Waters Protein-Pak Affinity material can be successfully used to create an affinity resin as shown in the example of the affinity purification for the protein carbonic anhydrase.

Ordering Information

Protein-Pak Affinity Columns

Particle Packing	Particle Size	Pore Size	Qty.	P/N
Protein-Pak Affinity	40 µm	500Å	25 g	WAT030653
Epoxy-Activated Packing	—	—	100 g	WAT030654
Protein-Pak Affinity Epoxy-Activated MicroColumn (500 mg of material in a 3 cc syringe barrel). Inquire for additional offerings.	40 µm	500Å	10/box	WAT035955

BioSuite pC_{18} and pPhenyl Reversed-Phase Chromatography (RPC) HPLC Columns

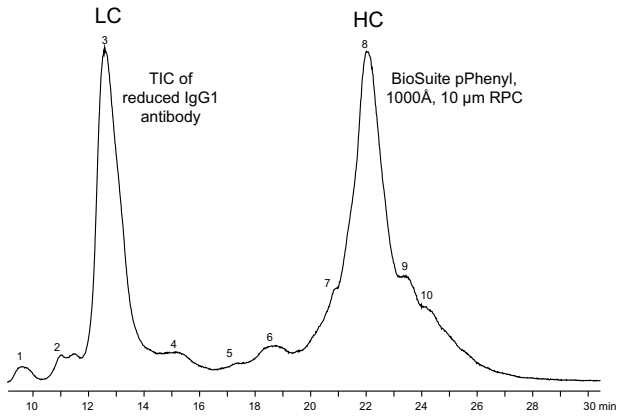
Reversed-phase chromatography (RPC) has become a widely accepted tool for the separation of proteins, peptides, synthetic oligonucleotides, and other biomolecules. For many applications, Symmetry and Symmetry300, Atlantis® T3, or BEH 130Å and BEH 300Å Chemistries can be successfully used for the isolation 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 500Å pore size of the pC_{18} base matrix accommodates proteins up to 2,500,000 Daltons while the 1000Å 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}, 500Å, 7 µm RPC selection. Waters porous, pC_{18}, 500Å, 7 µm RPC Column is available for applications requiring greater binding capacity. The pC_{18} and pPhenyl RPC chemistries are available in 21.5 x 150 mm columns for "lab-scale" isolations while a 2.0 x 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, 1000Å, 10 µm, 2.0 x 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-TofMS



The BioSuite pPhenyl, 1000Å RPC Columns have a higher ligand density compared to the BioSuite Phenyl, 1000Å 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 x 75 mm	186002159
BioSuite Phenyl 13 µm HIC	Polymer	21.5 x 150 mm	186002160

BioSuite Hydrophobic-Interaction Chromatography (HIC) HPLC Columns

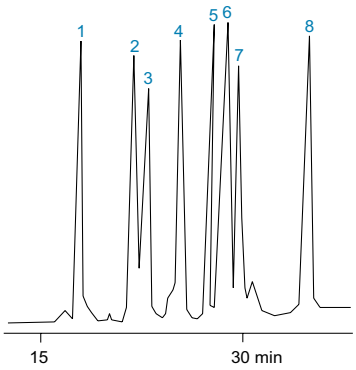
The separation of proteins and peptides based upon hydrophobic characteristics is a powerful chromatographic technique. However, some proteins denature at elevated organic solvent concentrations making reversed-phase chromatography (RPC) difficult. BioSuite Phenyl Hydrophobic-interaction Chromatography (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, 1000Å, 10 µm HIC column media consists of a phenyl group bonded to a methacrylic ester-based polymeric resin.

The large 1000Å pore size accommodates proteins up to 5,000,000 Daltons. A 21.5 x 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, 1000Å, 10 µm, 4.6 mm x 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

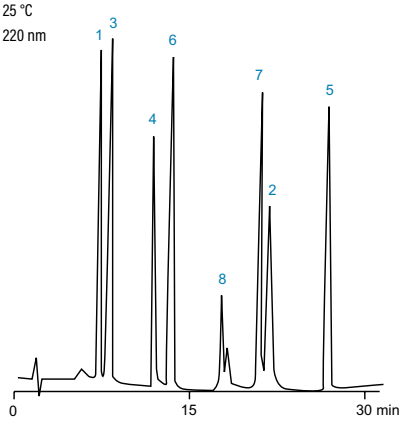


The BioSuite pPhenyl, 1000Å RPC Columns have a higher ligand density compared to the BioSuite Phenyl, 1000Å HIC Columns and are not recommended for hydrophobic-interaction separations.

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}, 500Å, 7 µm, 4.6 mm x 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

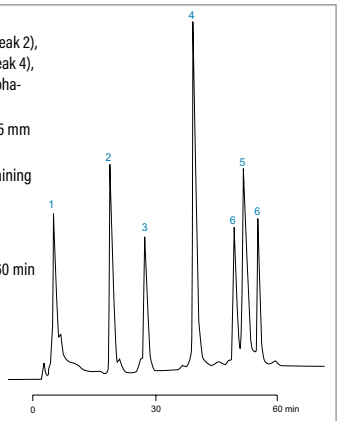


Use of "pH stable" methacrylate-based particles contained in Waters BioSuite pC_{18} Reversed-Phase Columns allow scientists to change separation selectivity by using a pH not possible with 100% silica-based C_{18} columns.

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, 1000Å, 10 µm, 7.5 x 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, 1000Å HIC Columns have a lower ligand density compared to the BioSuite pPhenyl, 1000Å RPC Columns and are not recommended for reversed-phase separations.

Ordering Information

Hydrophobic-Interaction HPLC and UHPLC Column

Description	Dimension	P/N
Protein HIC PH-814 Steel Column	8 x 75 mm	WAT035520

Reference Materials for Protein Separations

Title	Literature Code
Application Notebook	
Bioseparations: Tools, Techniques, and Insights Into Biopharmaceutical Analysis	720005536EN
Application Notes	
Advanced HPLC Size-Exclusion Chromatography for the Analysis of Macromolecular Proteins Using 3.5 µm Ethylene-Bridged Hybrid (BEH) Particles	720005202EN
Advances in Size-Exclusion Chromatography for the Analysis of Small Proteins and Peptides: Evaluation of Calibration Curves for Molecular Weight Estimation	720004412EN
Method Development Considerations for Reversed-Phase Protein Separations	720003875EN
Method Development for Hydrophobic Interaction Chromatography (HIC)–Based Protein Separations by Waters ProteinPak Hi Res HIC Columns	720005514EN
Method Development for Size-Exclusion Chromatography of Monoclonal Antibodies and Higher Order Aggregates	720004076EN
PEGylated Protein Analysis by Size-Exclusion and Reversed-Phase UPLC	720004782EN
Quantitation of Monoclonal Antibodies Using Reversed-Phase Liquid Chromatography using the BEH300 C_4 Column Chemistry	720003944EN
Size-Exclusion Ultra Performance Liquid Chromatography Method Development for the Analysis of the Degradation Products of the Trastuzumab Antibody	720004416EN
Successful Transfer of Size-Exclusion Separations between HPLC and UPLC	720005214EN
Ultimate Resolution HPLC-Based Size-Exclusion Chromatography for the Analysis of Small Proteins and Peptides Using 3.5 µm Ethylene-Bridged Hybrid (BEH) Particles	720005369EN
For a complete list of application notes, visit waters.com/AppNotes	
Catalog	
Waters Analytical Standards & Reagents	asr.waters.com
Wall Chart	
Bioseparations Columns Wall Chart	720004232EN
Webinars	
[Meet the Experts] Webinar Series	www.waters.com/MeetTheExperts

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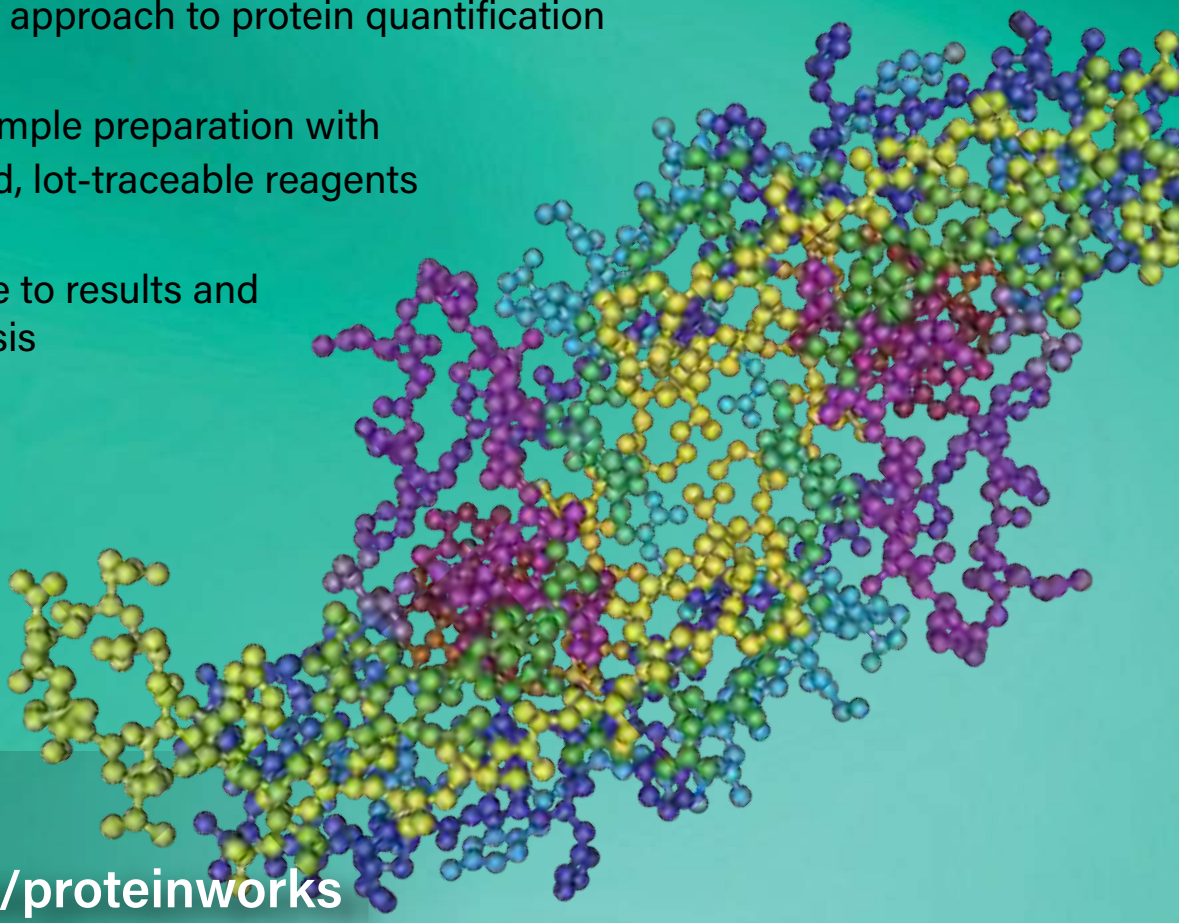


waters.com/proteinworks

Nano-Flow and Micro-Flow LC-MS

Nano-Flow and Micro-Flow LC-MS

Contents

Nano-Flow and Micro-Flow LC-MS

Our nano-flow and micro-flow LC Columns fully exploit the separation power of small, sub-2-µm particles to deliver superior chromatographic resolution.

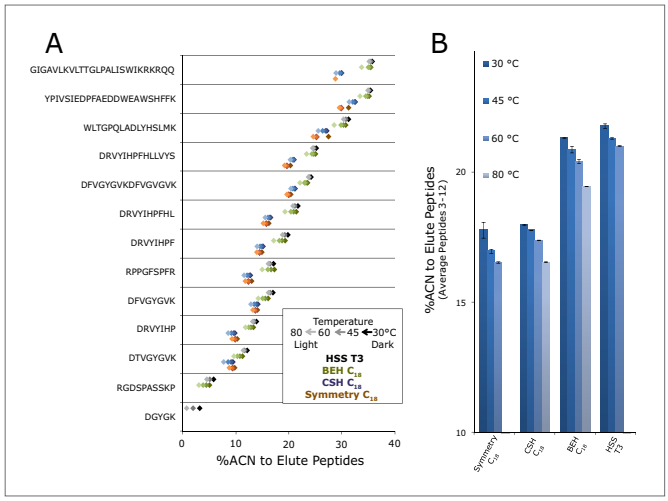
The selected stationary phases for nano-LC columns facilitate the efficiency and selectivity required for separations of complex peptide and protein separations as well as other sample-limited analyses.

Hybrid Particles			Silica-based Particles
			
BEH Technology™		CSH™ Technology	HSS HIGH STRENGTH SILICA
130Å	300Å	130Å	100Å
1.7 µm	1.7 µm	1.7 µm	1.8 µm
C_{18}	C_{18}, C_4	C_{18}	T3

Peptide Separation Technology stationary phases are specifically QC tested with tryptic digests of Cytochrome *c* to ensure consistent performance for peptide separations.

Protein Separation Technology stationary phases are specifically designed for the high resolution analysis of proteins of various sizes, hydrophobicities, and isoelectric points. Particles are QC tested using a protein standard mix.

Trap Elute Peptide Separation



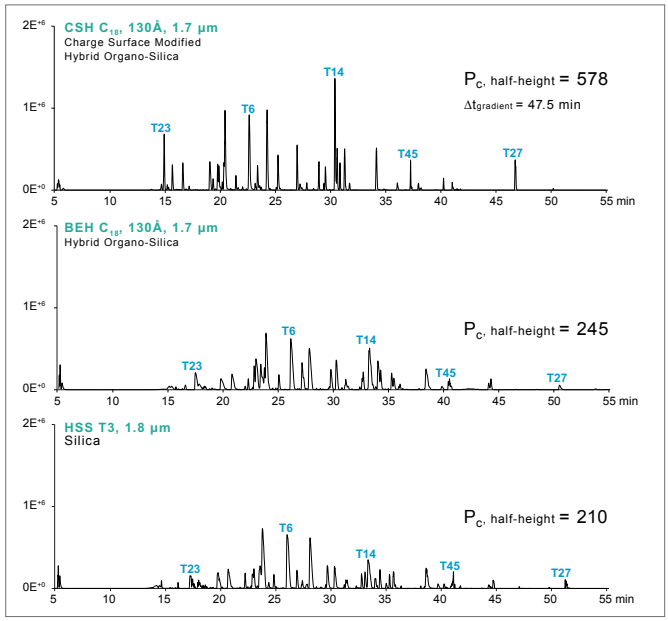
Peptide retentivity comparison of different stationary phases, including Symmetry Silica (the lower retention of Symmetry is used in trap-elute separations).

For more information on Waters Particle Technology, please refer to page 51.

In nano-flow and micro-flow LC-MS, analyzing large-volume samples using a single column can be impractical. In such cases, you can trap analytes at higher flow rates. It is preferable to perform online trapping of analytes at microscale flow rates and to subsequently elute and separate those analytes across an analytical column, wherein a significantly lower nanoscale flow rate is employed.

To be effective, the trapping column's retentivity must be lower than the analytical column's. This relationship between trapping and analytical columns ensures refocusing of analytes on the analytical column after elution from the trap at the start of the gradient, delivering high peak capacity separations.

Peak Capacity and Retentivity



Comparison of a base peak ion chromatogram of MassPREP Enolase Digestion Standard, 1 µg, direct injection on a 75 µm (I.D.) column.

Nano-flow and micro-flow LC-MS is becoming commonplace in areas of bio-separation such as peptide bioanalysis, intact antibody analysis, proteomics, lipidomics and metabolomics. This technique addresses limited sample availability and the need for high sensitivity and the requirement for low limits of detection or quantification.

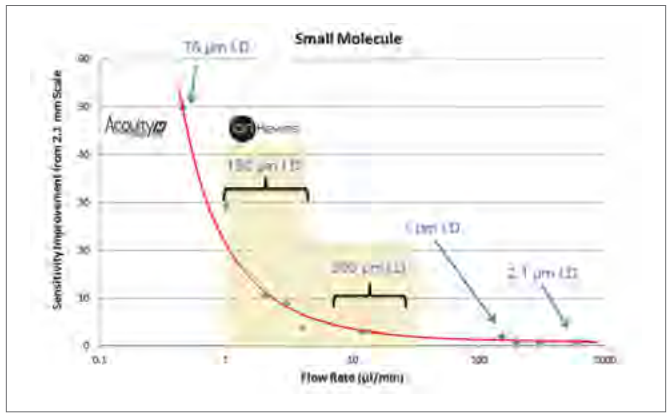
In micro flow LC-MS, the inner diameter of the separation column, and thus the flow rate of the mobile phase can dramatically alter the sensitivity of the mass spectrometry as follows:

- By increasing sampling efficiency
- By increasing ionization efficiency
- By reducing matrix effects

When performed using 75 µm I.D. columns, nano-flow LC-MS provides a higher sensitivity increase, compared with 2.1 mm UPLC Columns. Nevertheless, micro-flow separations, which use larger-diameter columns, increase sample throughput dramatically while continuing to deliver excellent sensitivity for many complex biomolecular analyses.

We offer solutions that satisfy the most demanding requirements for assays that rely on nano-flow and micro-flow LC-MS technology—solutions that ensure the assays' successful performance.

Gaining Sensitivity by Reducing Column Diameter and Flow Rate



Sensitivity enhancement for a series of small molecules relative to a 2.1 mm I.D. separation performed on an ACQUITY UPLC System. The volume and concentration of sample injected on each column format was identical.

Nano-flow and Micro-flow LC-MS Consumables

ionKey/MS™

- Includes a 150 µm I.D. separation channel, for highest sensitivity, and a 300 µm I.D. channel, for high throughput analysis
- Greatly simplified micro-flow LC-MS, with fitting-free connections
- The 150 µm I.D. iKey Separation Device demonstrates as much as 40 times the sensitivity of the 2.1 mm I.D. UPLC column
- The 300 µm I.D. iKey, during high-throughput UPLC-cycle times, delivers as much as 6 times the sensitivity of a 2.1 mm I.D. UPLC Column
- Easy post-column addition of MS-modifier solvents

nanoEase™ M/Z Columns

zenFiT® Connection Technology

- nanoEase™ M/Z Columns with easy-to-use ZenFit® Connection Technology
- Column inner diameters range from 75 to 300 µm
- Column lengths range from 50 to 250 mm
- Trapping columns range from 180 to 300 µm I.D.

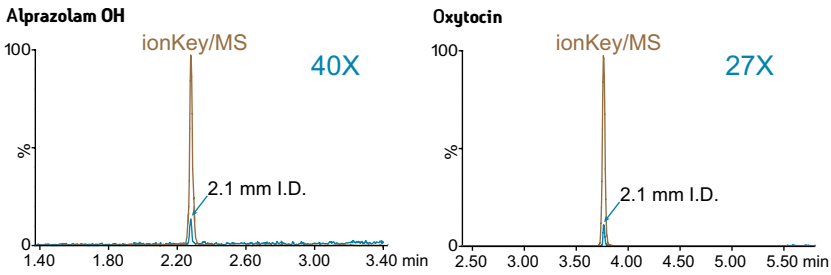
ionKey/MS

SIMPLIFIED MICRO-FLOW LC-MS WITH ENHANCED SENSITIVITY

The ionKey®/MS System integrates the micro-flow UPLC separation into the source of the mass spectrometer. This delivers LC-MS system performance and sensitivity that cannot be achieved any other way. ionKey/MS Systems are enabled by the iKey® Separation Device, which replaces the need for traditional fittings and columns and simplifies the user experience. The "plug and play" design of the iKey Separation Device eliminates operator variability common in traditional micro-flow LC-MS analyses.

The ionKey MS System with the ACQUITY UPLC M-Class System and Xevo TQ-S Mass Spectrometer.

150 µm I.D. iKey: Up to 40× Increase in Sensitivity Compared to 2.1 mm UPLC LC-MS Applications

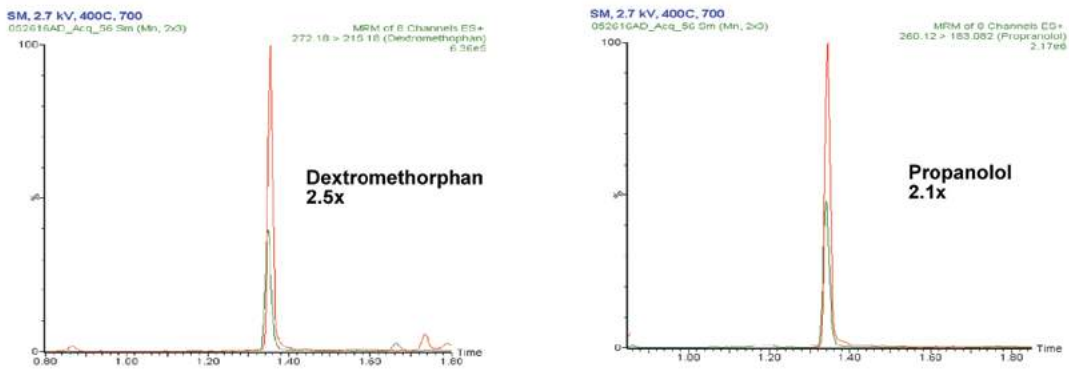


UPLC/MRM comparison of ionKey/MS relative to 2.1 mm I.D. chromatography

Compound name	Relative peak area	Relative S/N
Small Molecule		
Lidocaine	3	3
Fluconazole	15	39
Propranolol	2	2
Alprazolam OH	14	40
Alprazolam	7	12
Nefazodone	3	6
Tolbutamide	5	6
Warfarin	2	4
Clopidogrel	2	2
Mean value	6	13

Sensitivity comparison between ionKey/MS and 2.1 mm I.D. chromatography; 1 µL injection of equal sample load on each.

300 µm I.D. iKey HT: Increased LC-MS Sensitivity with UPLC Throughput



Sensitivity gains using (300 µm × 50 mm) iKey HT BEH C_{18} Separation Device (red) compared to (2.1 mm × 50 mm) UPLC BEH C_{18} Column (green) under identical injection volume and gradient conditions.

iKey Separation Device

In an ionKey/MS System, the iKey Separation Device contains the fluid connections, electronics, ESI interface, column heater, eCord, and chemistry needed to perform UPLC separations. As such, it replaces the need for traditional fittings and columns, simplifying the user experience. The "plug and play" design of the iKey eliminates user-dependent variation in results that often occurs in traditional micro-flow LC-MS analyses, regardless of users' skill level.

iKey Separation Device	iKey Separation Device with Post Column Addition (PCA)
	
The major component of the ionKey/MS System, the iKey Separation Device performs sub-2-µm UPLC separations, resulting in highly sensitive, efficient, micro-flow LC-MS analyses. The iKey Separation device is available with two inner diameters: 150 µm I.D. which provides the highest level of sensitivity, and the 300 µm I.D. iKey HT for higher throughput separations.	The PCA iKey incorporates a separation channel as well as a post-column addition (PCA) channel. The design allows for mixing the mobile phase, post-separation with a desired solvent. Both effluents are merged and collected at the inlet of the emitter. Post-column addition of solvents can enhance the electrospray process and increase sensitivity without adversely affecting the separation.

Robust, Reproducible, and Reliable

1 user X 1 system X 1 iKey	Peptide from P00924, yeast enolase		5 users X 5 systems X 18 iKeys
	6.84 min	Retention time	6.69 min
	0.01 min	SD of retention time	0.11 min
	3.01 sec	Average peak width FWHM	2.72 sec
0.15% retention time RSD			**1.64%** retention time RSD
QC reproducibility for one iKey with over 500 injections of protein precipitated plasma.			*QC reproducibility across multiple users, ionKey/MS Systems, and iKeys.*

The iKey Separation Device is LC-MS tested to ensure consistent performance not only for a particular iKey but from one iKey to another. The device also exhibits robust performance—performance that achieves high-quality results, even after hundreds of injections.

Ordering Information

iKey Separation Device

	Dimension	P/N
	Particle Size: 1.7 µm	
BEH C_{18}, 130Å	150 µm × 50 mm	186007256
	150 µm × 50 mm (PCA)	186007580
	150 µm × 100 mm	186007258
CSH C_{18}, 130Å	150 µm × 50 mm	186007244
	150 µm × 100 mm	186007245
HSS T3, 100Å	150 µm × 50 mm	186007260
	150 µm × 100 mm	186007261
	300 µm × 50 mm	186008727

iKey Peptide Separation Devices, 1/pk

	Dimension	P/N
	Particle Size: 1.7 µm	
BEH C_{18}, 130Å	150 µm × 50 mm	186006764
	150 µm × 50 mm (PCA)	186007557
	150 µm × 100 mm	186006766
CSH C_{18}, 130Å	150 µm × 50 mm	186007257
	150 µm × 100 mm	186007259
BEH C_{18}, 300Å	150 µm × 50 mm	186006969
	150 µm × 100 mm	186006970

iKey Protein Separation Devices, 1/pk

	Dimension	P/N
	Particle Size: 1.7 µm	
BEH C_4, 300Å	150 µm × 50 mm	186006765
	150 µm × 100 mm	186006968

iKey Utility Devices

	Dimension	P/N
iKey Infusion Device	85 µm × 50 mm	186007049
iKey Flow Injection Analysis Device	85 µm × 50 mm	186007051
iKey Diagnostic Device V3	n/a	186008450

For additional information, please go to www.waters.com/iKey

Nano- and Micro-flow Columns and Trapping Columns

Waters columns for nano-to-microscale LC-MS analyses are designed for low-dispersion nano-UPLC Systems. Our rigorous quality-control measures ensure that the columns achieve their full potential for sensitivity, resolution, and reproducibility for biomarker discovery and also for identifying and characterizing peptides and proteins.

SEPARATION COLUMNS

These columns enable nano- and microscale separations with MS detection under UPLC conditions at 15,000 psi. They take full advantage of the separation power of sub-2-µm particle technology. Columns of between 75 and 300 µm I.D. provide chromatographic separations, with flow rates between 200 nL/min and 100 µL/min, covering a 170-fold range of sample amounts. The varying characteristics of available particle technologies provide alternate selectivity, retentivity, and loadability, and thus the flexibility to achieve the most suitable separation for complex LC-MS analyses.

TRAPPING COLUMNS

Trapping columns are used to desalt and enrich the sample before eluting onto the analytical column for the final separation with MS detection. For fast loading of the trap column and to reduce the cycle time, trap columns are packed with larger 5 µm particles.

nanoEase M/Z Columns with ZenFit Connection Technology

Waters ZenFit Connection Technology introduces easy-to-use, re-usable, fingertight, liquid-line connectors to the family of nanoEase M/Z Columns. These columns are capable of withstanding pressures as high as 15,000 psi, eliminate dead volume, a frequent source of variability associated with regular fittings. ZenFit Connection Technology does not require user training or any further special attention.

*To use nanoEase M/Z Columns on the ACQUITY M-Class System, equip systems with the appropriate upgrade kit. The 300 µm I.D. ACQUITY M-Class Columns and traps are compatible with ZenFit Connections.

Ordering Information

nanoEase M/Z Peptide Columns

	Dimension	P/N
	Particle Size: 1.7 µm	
BEH C_{18}, 130Å	75 µm × 100 mm	186008792
	75 µm × 150 mm	186008793
	75 µm × 200 mm	186008794
	75 µm × 250 mm	186008795
	100 µm × 100 mm	186008796
	150 µm × 100 mm	186008797
BEH C_{18}, 300Å	75 µm × 100 mm	186008798
	75 µm × 150 mm	186008799
	75 µm × 200 mm	186008800
	75 µm × 250 mm	186008801
	100 µm × 100 mm	186008802
	150 µm × 100 mm	186008803
CSH C_{18}, 130Å	75 µm × 100 mm	186008807
	75 µm × 150 mm	186008808
	75 µm × 200 mm	186008809
	75 µm × 250 mm	186008810
	100 µm × 100 mm	186008811
	150 µm × 50 mm	186008812
	150 µm × 100 mm	186008813
	150 µm × 150 mm	186008814

nanoEase M/Z Protein Columns

	Dimension	P/N
	Particle Size: 1.7 µm	
BEH C_4, 300Å	75 µm × 100 mm	186008804
	100 µm × 100 mm	186008805
	150 µm × 100 mm	186008806

nanoEase M/Z HSS Columns

	Dimension	P/N
	Particle Size: 1.8 µm	
HSS T3, 100Å	75 µm × 100 mm	186008815
	75 µm × 150 mm	186008816
	75 µm × 200 mm	186008817
	75 µm × 250 mm	186008818
	100 µm × 100 mm	186008819
	150 µm × 100 mm	186008820

nanoEase M/Z Columns and ACQUITY UPLC M-Class Columns are preferred for use with the ACQUITY UPLC M-Class and nanoACQUITY UPLC Systems.

nanoEase M/Z Trap Columns*

	Dimension	P/N
	Particle Size: 5 µm	
Symmetry C_{18}, 100Å	180 µm × 20 mm	186008821

*For 300 µm I.D. traps please refer to M-Class Trap Columns.

ACQUITY UPLC M-Class Columns

	Dimension	P/N
	Particle Size: 1.8 µm	
HSS T3, 100Å	75 µm × 100 mm	186008006
	75 µm × 150 mm	186007473
	75 µm × 200 mm	186008007
	75 µm × 250 mm	186007474
	100 µm × 100 mm	186008008
	150 µm × 100 mm	186008009
	300 µm × 50 mm	186007559
	300 µm × 100 mm	186007560
	300 µm × 150 mm	186007472

ACQUITY UPLC M-Class Trap Columns

	Dimension	P/N
	Particle Size: 5 µm	
Symmetry C_{18}, 100Å	180 µm × 20 mm	186007496[4]
	180 µm × 20 mm	186007497[5]
	180 µm × 20 mm	186007500[6]
	180 µm × 20 mm	186007592[7]
Symmetry C_{18}, 100Å	300 µm × 25 mm	186007499[3]
	300 µm × 50 mm	186007498
Peptide BEH C_{18}, 130Å	300 µm × 50 mm	186007471
BEH C_4, 300Å	300 µm × 50 mm	186008470
HSS T3, 100Å	300 µm × 50 mm	186008029

[3]Configuration HCP (2D).
[4]Configuration: 2G, V/M.
[5]Configuration: 2D, V/M.
[6]Configuration: 2G, V/V.
[7]Configuration: 2D, V/V.

ACQUITY UPLC M-Class Peptide Columns

	Dimension	P/N
	Particle Size: 1.7 µm	
BEH C_{18}, 130Å	75 µm × 100 mm	186007481
	75 µm × 150 mm	186007482
	75 µm × 200 mm	186007483
	75 µm × 250 mm	186007484
	100 µm × 100 mm	186007485
	150 µm × 100 mm	186007486
	300 µm × 50 mm	186007564
	300 µm × 100 mm	186007565
	300 µm × 150 mm	186007566
BEH C_{18}, 300Å	75 µm × 100 mm	186007487
	75 µm × 150 mm	186007490
	75 µm × 200 mm	186007491
	75 µm × 250 mm	186007492
	100 µm × 100 mm	186007488
	150 µm × 100 mm	186007489
	300 µm × 50 mm	186007570
	300 µm × 100 mm	186007571
	300 µm × 150 mm	186007572
CSH C_{18}, 130Å	75 µm × 100 mm	186007475
	75 µm × 150 mm	186007476
	75 µm × 200 mm	186007477
	75 µm × 250 mm	186007478
	100 µm × 100 mm	186007479
	150 µm × 50 mm	186007513
	150 µm × 100 mm	186007480
	150 µm × 150 mm	186007514
	300 µm × 50 mm	186007561
	300 µm × 100 mm	186007562
	300 µm × 150 mm	186007563

ACQUITY UPLC M-Class Protein Columns

	Dimension	P/N
	Particle Size: 1.7 µm	
BEH C_4, 300Å	75 µm × 100 mm	186007493
	100 µm × 100 mm	186007494
	150 µm × 100 mm	186007495
	300 µm × 50 mm	186007567
	300 µm × 100 mm	186007568
	300 µm × 150 mm	186007569

ACQUITY UPLC M-Class with HDX Technology

ACQUITY UPLC M-Class System.

Hydrogen-deuterium exchange mass spectrometry (HDS-MS) is used to study a protein's structural dynamics and conformational changes, a component of understanding its higher-order structure. Information about protein conformation from an HDX MS study can serve to compare a control compound with an analyte by measuring the relative amount of deuteration uptake. HDX-MS can monitor domain interaction, localized-protein breathing, and folding or unfolding in the solution phase. The ACQUITY UPLC M-Class System can quantify small changes in protein conformation by extending its pressure range to effect a higher-efficiency separation. An integral part of the ACQUITY UPLC M-Class HDX System is the Waters Enzymate® BEH Pepsin Column, which performs online protein digestion.

The technology offers these benefits:

- True UPLC separations for peptide and protein HDX
- Reproducible, robust, and rapid separations (nano-to-micro-scale at 0 °C and pressure to 15,000 psi)

ENZYMATE PEPSIN ONLINE DIGESTION COLUMN

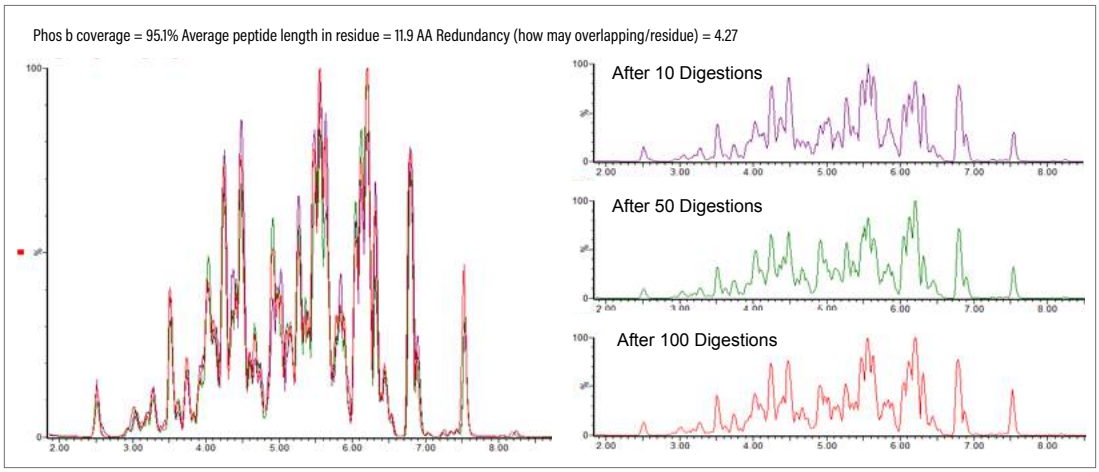
Waters Enzymate Pepsin Online Digestion Column digests intact proteins into peptides. The peptic peptides are then retained on a trapping column. Peptides eluting from the trapping column are refocused onto a sub-2-µm ACQUITY UPLC Column and then eluted into a high-resolution mass spectrometer.

Enzymate Pepsin Online Digestion Column.

Enzymate Pepsin Online Digestion Columns, an integral part of the ACQUITY UPLC M-Class HDX System, offer these benefits:

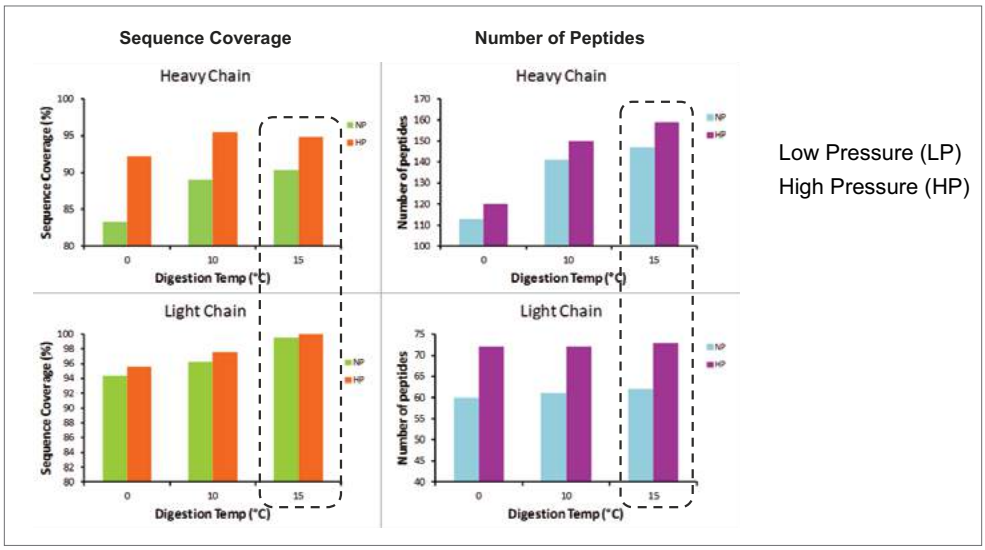
- Fast, reproducible, and efficient online protein digestion, typically within 30 seconds
- Shortened preparation time (overall) for protein samples
- Ability to optimize the efficiency of protein digestion by changing temperature, flow rate, or both

Overlay of Three Phos B Digestions within a 130-Injection HDX MS Study



Reproducible online pepsin digestions of phosphorylase b. A total of 130 digestions were performed using an Enzymate Pepsin Column. The 10th, 50th and 100th digestions are shown. The sequence coverage is shown on the right.

Comparisons of Low- and High-Pressure Digestion Efficiencies



The Waters Enzymate BEH Pepsin Column was used for digestion of IgG2, at 1000 psi (NP), and 13,000 psi (HP). Results show high-pressure digestion increases protein-sequence coverage and spatial resolution of IgG2, compared with low-pressure digestion.

Ordering Information

Enzymate Pepsin Online Digestion Column

Description	Dimension	P/N
	Particle Size: 5 µm	
Enzymate Pepsin Online Digestion Column	2.1 × 30 mm	186007233

LC-MS Accessories

TRUVIEW LCMS CERTIFIED VIALS

TruView LCMS Certified Vials include stringent dimensional tolerances plus UV and MS cleanliness testing. The additional product attribute of TruView vials is low polar analyte adsorption. The vials are manufactured by a process that limits the concentration of free ions on the surface of glass; ionic sites can cause analyte adsorption. Waters TruView LCMS Certified Vials are tested for high recovery of analyte at 1 ng/mL concentration using UPLC-MS/MS (MRM) and yield little adsorption. These vials exhibit the lowest adsorption of autosampler vials in the market.

Ordering Information

TruView LCMS Certified Vials





Description	Clear Glass	Amber Glass	Max Recovery	Total Recovery	Amber Max Recovery
TruView LCMS Certified Vials, 100/pk with cap and pre-slit silicone/PTFE septa	186005666CV	186005661CV	186005662CV	186005663CV	186005670CV
TruView LCMS Certified Vials, 100/pk with cap and silicone/PTFE septa	186005660CV	186005667CV	186005668CV	186005669CV	186005664CV

WATERS CERTIFIED CONTAINERS

Waters Certified Containers are uniquely processed, treated, and certified in the same unique manner as our highly regarded low TOC vials.

Ultra-clean containers can be used on any LC system, including UPLC, LC/UV, and LC-MS, among others. Manufactured to stringent standards, they prevent extraneous peaks and baseline noise stemming from high TOC. To help assist with contamination prevention and facilitate recommended care and use, each container carries the Waters certified mark for easy differentiation in operational use.

Ordering Information

Certified Containers

Description	P/N
Certified Container Kit Kit contains: 4 certified 1 L bottles, 3 certified 500 mL bottles, 1 clean container cap kit	186007088
Low Volume Certified Container Kit Kit contains: 5 certified 250 mL clear bottles, 1 certified 500 mL clear bottle, 1 clean container cap kit	186007278
Certified Container, 1 L	186007089
Certified Container, 500 mL	186007090
Clean Container Cap Kit	205000642

PH BUFFERS

These pH Buffers are directly traceable to NIST SRMs, mercury free, guaranteed stable for at least one year after your receipt, and are supplied with a full certificate of analysis.

Ordering Information

pH Buffers

Description	Volume	P/N
pH 4 Liter pH 4 Buffer	1 L	129
pH 7 Liter pH 7 Buffer	1 L	133
pH 10 Liter pH 10 Buffer	1 L	137
pH 4 Pint pH 4 Buffer	1 pint	127
pH 7 Pint pH 7 Buffer	1 pint	131
pH 10 Pint pH 10 Buffer	1 pint	135

Reference Materials for Nano-Flow and Micro-Flow LC-MS

Title	Literature Code
Application Notebook	
Application Solutions for Biopharmaceuticals	720002487EN
Application Notes	
An Introduction to the Capabilities of Microscale 2D-RP/RP Peptide Chromatography with an ACQUITY UPLC M-Class System	720004934EN
Charged Surface Hybrid C_{18} for High Peak Capacity nanoLC Peptide Separations	720004917EN
Desalting of Proteins Using MassPREP On-Line Desalting Cartridges Prior to Mass Spectrometry	720001077EN
High Sensitivity Analysis of Opioids in Oral Fluid Using ionKey/MS	720005296EN
High Sensitivity Intact Mass Analysis of Antibodies (IgG1) Using ionKey/MS	720005378EN
Illustration of the Selectivity of Collision Cross Section Ion Mobility Screening for the Analysis of Pesticide Residues in Food Using ionKey/MS	720005427EN
Performance of ACQUITY UPLC M-Class in Proteomics Nanoscale Applications	720005244EN
Reducing Sample Volume and Increasing Sensitivity for the Quantification of Human Insulin and Five Analogs in Human Plasma Using ionKey/MS	720005119EN
Targeted Lipidomics Using the ionKey/MS System	720004968EN
For a complete list of application notes, visit waters.com/AppNotes	
Catalog	
Waters Analytical Standards & Reagents	asr.waters.com
Wall Chart	
Bioseparations Columns Wall Chart	720004232EN
Webinars	
[Meet the Experts] Webinar Series	www.waters.com/MeetTheExperts

ionKey/ms™

NOW YOU CAN START THINKING OF

MICROSCALE SENSITIVITY

IN TERMS OF POSSIBILITIES,

NOT RESTRAINTS.

Introducing Waters ionKey/MS™ System. Open the door to the new world of robust, reliable and reproducible microscale with dramatically enhanced sensitivity and significantly lower solvent consumption compared to 2.1 mm I.D. chromatography. This plug & play MS system gives your whole lab access to UPLC chromatography performance and highly sensitive, reliable data. This is where the industry's going. You can get there today. To learn more about what's possible, visit waters.com/ionkey

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