## [product solution]

Waters THE SCIENCE OF WHAT'S POSSIBLE.

# ACQUITY UPLC SEC Columns

### Protein Separation Technology

Waters ACQUITY UPLC® technology continues to deliver enhanced component resolution and in less time compared to what's frequently attained using conventional HPLC-based chromatography. To date, more than 6,800 referenced ACQUITY UPLC literature publications support the effectiveness of this technology in a variety of applications including the analyses of biotherapeutics.

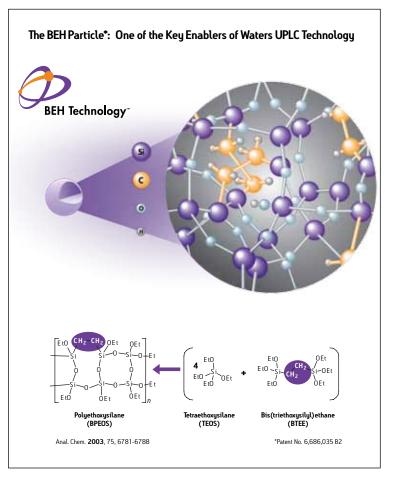
The ability to rapidly separate and accurately quantitate protein or peptide monomers from their respective aggregates is why several major biopharmaceutical accounts have invested in the ACQUITY UPLC SEC System Solution, enabled by the unique Ethylene-Bridged Hybrid (BEH) Diol coated particle technology.

- Accurately measures aggregation species up to 10 times faster and with less eluent compared to use of traditional SEC methods using either soft gels or >5 μm rigid particles.
- Significantly reduces the requirement for high salt concentration mobile phases to decrease non-desired, ionic secondary interactions between protein/peptide and SEC particle surface.
- QC tested with relevant proteins and peptides help ensure unmatched batch-to-batch consistency for increased confidence in validated methods.

#### **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.

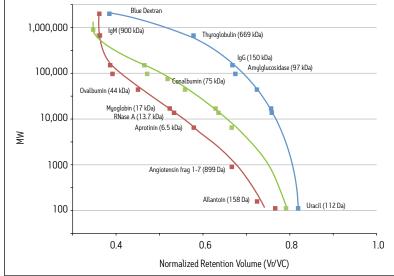
The first-generation methyl-hybrid particles of XTerra columns did not possess the mechanical strength or efficiency necessary to fully realize the potential speed, sensitivity and resolution capabilities of UPLC<sup>®</sup> Technology. Therefore, a new pressure-tolerant particle needed to be created. This new, second-generation hybrid material was developed that uses a 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 tolerance. In addition, our BEH-based, SEC particles are modified with a unique DIOL coating process to minimize undesired surface charges. In combination, our BEH SEC column chemistries provide outstanding component resolution, in less time, and with miminal non-desired secondary interactions that help users meet demanding regulatory requirements.



#### **ACQUITY UPLC SEC System Solution**

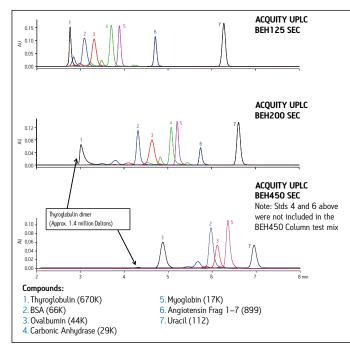
Waters ACQUITY UPLC offerings provide analysts with the enabling technology to see more and in less time. They have proven themselves to be valuable assets that improves the quality of collected data while increasing productivity. Biotherapeutic and biosimilar manufacturers can now choose the most appropriate UPLC-based, SEC column(s) (i.e., 125Å, 200Å, or 450Å pore size) to satisfy their application requirements based on this effective separation technology.

# Calibration Curves on ACQUITY UPLC BEH125, BEH200 and BEH450 SEC Columns





#### Separation of Same Protein and Peptide Standards on ACQUITY UPLC BEH450, BEH200, and BEH125 SEC Columns



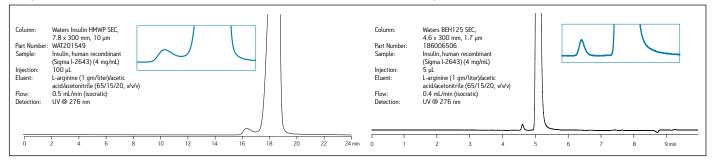
Columns	ACQUITY UPLC BEH125, 1.7 μm ACQUITY UPLC BEH200 1.7 μm	ACQUITY UPLC BEH450, 2.5 $\mu\text{m}$	
Column Configuration	4.6 x 150 mm		
Mobile Phase	100 mM Sodium Phosphate Buffer, pH 6.8		
Weak Needle Wash	100% Milli-Q® Water		
Strong Needle Wash	100% Milli-Q Water		
Seal wash	90/10 water/methanol		
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	



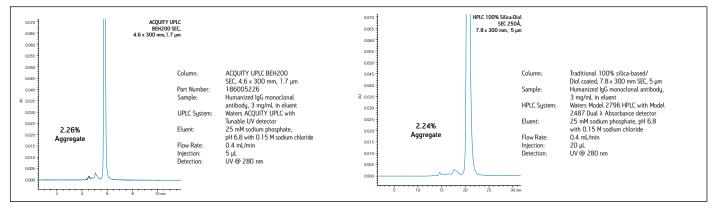
#### ACQUITY UPLC SEC Analysis of Therapeutic Insulin and Monoclonal Antibody

SEC is a well established and USP/EP approved method for the analysis of undesired, protein or peptide aggregates from active monomeric forms. As indicated in the figures below, Waters UPLC-based SEC separations deliver comparatively improved component resolution, while reducing analysis time and mobile-phase consumption. These attributes help get products to market faster with a consistency required by international drug regulatory agencies.

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



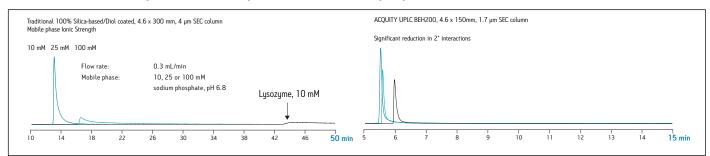
#### Comparative UPLC-Based SEC Benefits vs. Use of Traditional HPLC SEC for Biotherapeutic Monoclonal Antibody Characterization



#### **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 BEH SEC particles 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



#### Stringent Manufacturing Quality Assurance Delivers Confidence in Results

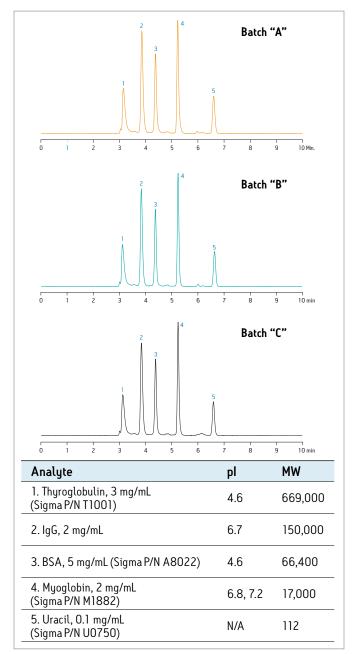
All Waters ACQUITY UPLC column chemistries are synthesized from high quality raw materials in state-of-the-art ISO certified manufacturing facilities and are extensively QC tested throughout the synthetic process. In addition, each batch of ACQUITY UPLC BEH SEC material is specifically QC tested with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

#### **ORDERING INFORMATION**

Description	Particle Size	Dimensions	Part No.
ACQUITY UPLC BEH125 SEC column	1.7 µm	4.6 x 150 mm	186006505
ACQUITY UPLC BEH125 SEC column	1.7 µm	4.6 x 300 mm	186006506
ACQUITY UPLC BEH125 SEC guard column kit	1.7 µm	4.6 x 30 mm	186006504
ACQUITY UPLC BEH200 SEC column	1.7 µm	4.6 x 150 mm	186005225
ACQUITY UPLC BEH200 SEC column	1.7 µm	4.6 x 300 mm	186005226
ACQUITY UPLC BEH200 SEC guard column kit	1.7 µm	4.6 x 30 mm	186005793
ACQUITY UPLC BEH450 SEC column	2.5 µm	4.6 x 150 mm	186006851
ACQUITY UPLC BEH450 SEC column	2.5 µm	4.6 x 300 mm	186006852
ACQUITY UPLC BEH450 SEC column w/ free standard	2.5 µm	4.6 x 150 mm	176002996
ACQUITY UPLC BEH450 SEC column w/ free standard	2.5 µm	4.6 x 300 mm	176002997
ACQUITY UPLC BEH450 SEC guard column kit	2.5 µm	4.6 x 30 mm	186006850
ELSD outlet tubing (0.004" id x	430001562		
0.005 x 1.75" SEC UPLC Conne	186006613		
BEH125 SEC Protein Standard N	186006519		
BEH200 SEC Protein Standard I	186006518		
BEH450 SEC Protein Standard	186006842		

Notes: Size-exclusion chromatography may require modifications to an existing ACQUITY UPLC system. Please refer to "Size Exclusion and Ion- Exchange Chromatography of Proteins using the ACQUITY UPLC System", (715002147 Rev A) or "Size Exclusion and Ion- Exchange Chromatography of Proteins using the ACQUITY UPLC H-Class System", (715002909 Rev A) for specific recommendations.





Waters ISO 2001 Manufacturing and Testing Processes Help Ensure Outstanding ACQUITY UPLC BEH200 SEC, 1.7  $\mu$ m Batch-to-Batch Reproducibility. This same approach is used to confirm consistent performance of the BEH125 and BEH450 SEC materials.

# Waters

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