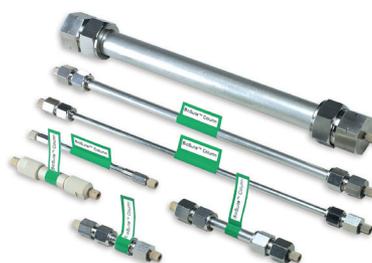


Column Technologies from the Leader in Liquid Chromatography and Bioseparation Sciences

BIOsuite HPLC COLUMNS FOR PROTEIN AND PEPTIDE SEPARATIONS

Waters BioSuite™ HPLC columns for protein and peptide separations contain high-performance chemistries dedicated to the isolation, analysis, and characterization of biomolecules. Separation offerings include ion-exchange, size-exclusion, hydrophobic interaction, and reversed-phase columns and support Waters array of LC and LC/MS systems for the characterization and lab-scale isolation of biotherapeutics and other related compounds.

Information on the BioSuite family of application-driven solutions that address separation needs in the areas of well-characterized biopharmaceuticals, proteomics and genomics are available online at:
www.waters.com/biosep



Waters Alliance® System

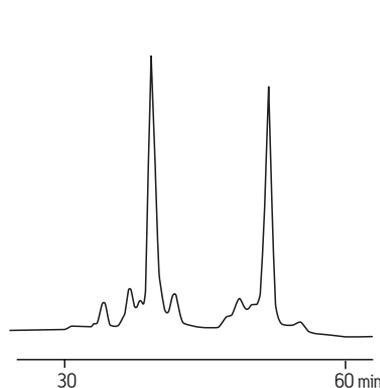
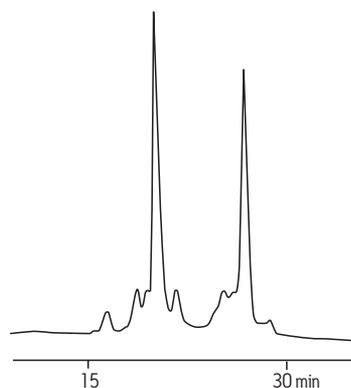
BioSuite™ Columns

- Ion-exchange, size exclusion, hydrophobic interaction and reversed-phase column offerings
- Excellent resolution and recovery of proteins and peptides
- Available in different particle and pore sizes
- Scalable from analytical to 'lab-scale' preparative applications

Predictable Ion-Exchange Chromatography on BioSuite Analytical and 'Lab-Scale' Preparative DEAE AXC Columns

A) BioSuite DEAE 10 µm, 7.5 mm x 75 mm

B) BioSuite DEAE 13 µm, 21.5 mm x 150 mm



Sample: Mixture of Ovalbumin and Trypsin inhibitor
 A: 0.2 mg each in 0.1 mL Eluent A
 B: 2 mg each in 1 mL Eluent A

Eluent:
 A: 20 mM tris-hydrochloride, pH 8.0
 B: 20 mM tris-hydrochloride, pH 8.0 containing 0.5 M sodium chloride

Flow Rate:
 A: 1.0 mL/min
 B: 6.0 mL/min

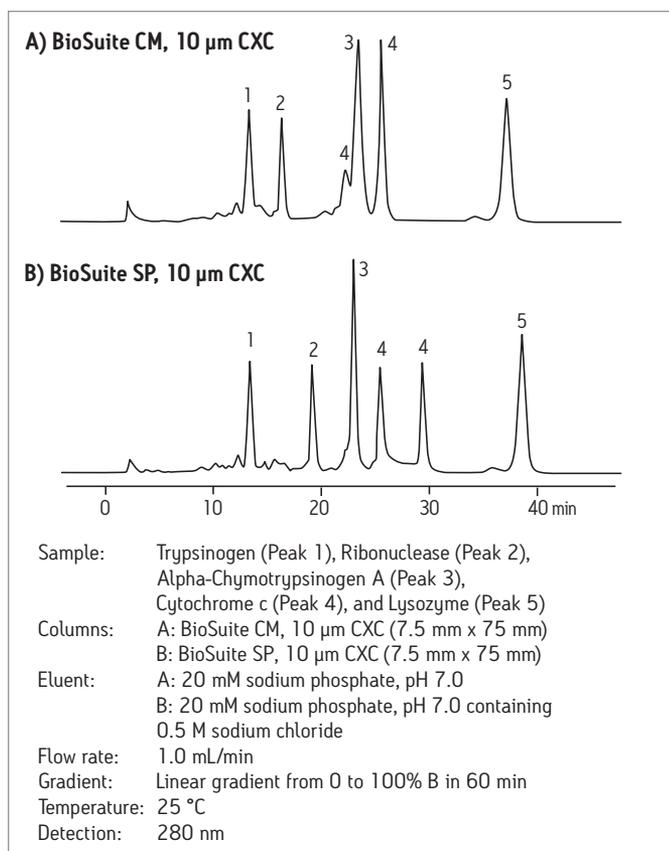
Gradient:
 A: Linear gradient from 0 to 100% B in 60 min
 B: Linear gradient from 0 to 100% B in 120 min

Temperature: 25 °C
 Detection: 280 nm

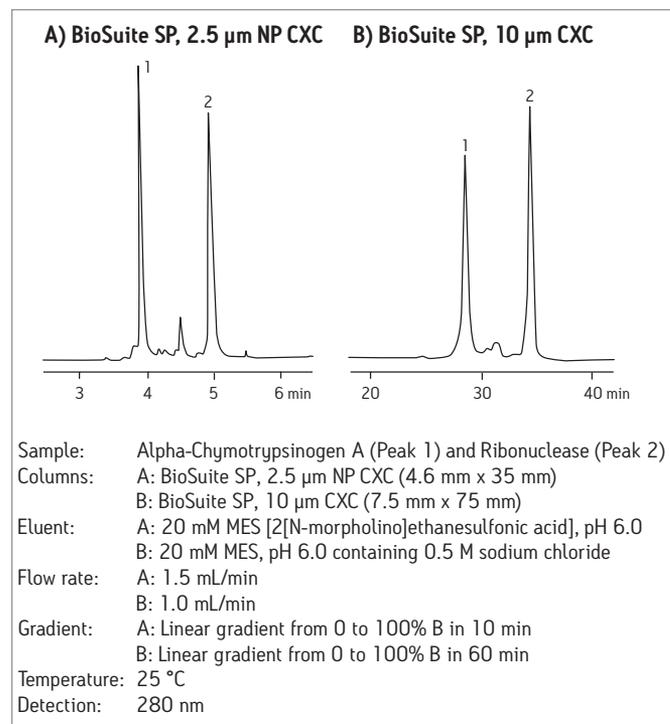
ION-EXCHANGE CHROMATOGRAPHY (IEX)

BioSuite ion-exchange column offerings include strong- and weak-cation (CXC) and strong-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 non-porous 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 preparative columns up to 21.5 mm in diameter.

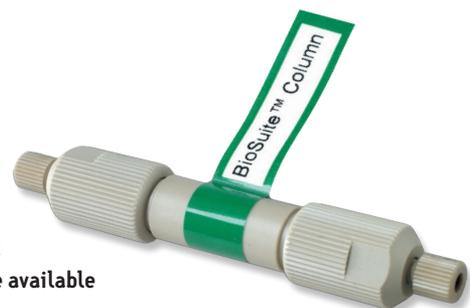
Protein Selectivity Differences Observed by Ion-Exchange Chromatography on BioSuite CM (Weak-Cation Exchange) vs. SP (Strong-Cation Exchange) Columns



Enhanced Compound Resolution by Ion-Exchange Chromatography on BioSuite SP Non-Porous (NP) vs. Porous CXC Columns



Description	Matrix	Diameter Width	Diameter Length	Part No.
BioSuite Q-PEEK 10 µm AXC	polymer	4.6 mm	50 mm	186002176
BioSuite SP-PEEK 7 µm CXC	polymer	4.6 mm	50 mm	186002182
BioSuite DEAE 2.5 µm NP AXC	polymer	4.6 mm	35 mm	186002179
BioSuite SP 2.5 µm NP CXC	polymer	4.6 mm	35 mm	186002183
BioSuite Q 10 µm AXC	polymer	7.5 mm	75 mm	186002177
BioSuite Q 13 µm AXC	polymer	21.5 mm	150 mm	186002178
BioSuite DEAE 10 µm AXC	polymer	7.5 mm	75 mm	186002180
BioSuite DEAE 13 µm AXC	polymer	21.5 mm	150 mm	186002181
BioSuite SP 10 µm CXC	polymer	7.5 mm	75 mm	186002184
BioSuite SP 13 µm CXC	polymer	21.5 mm	150 mm	186002185
BioSuite CM 10 µm CXC	polymer	7.5 mm	75 mm	186002186
BioSuite CM 13 µm CXC	polymer	21.5 mm	150 mm	186002187

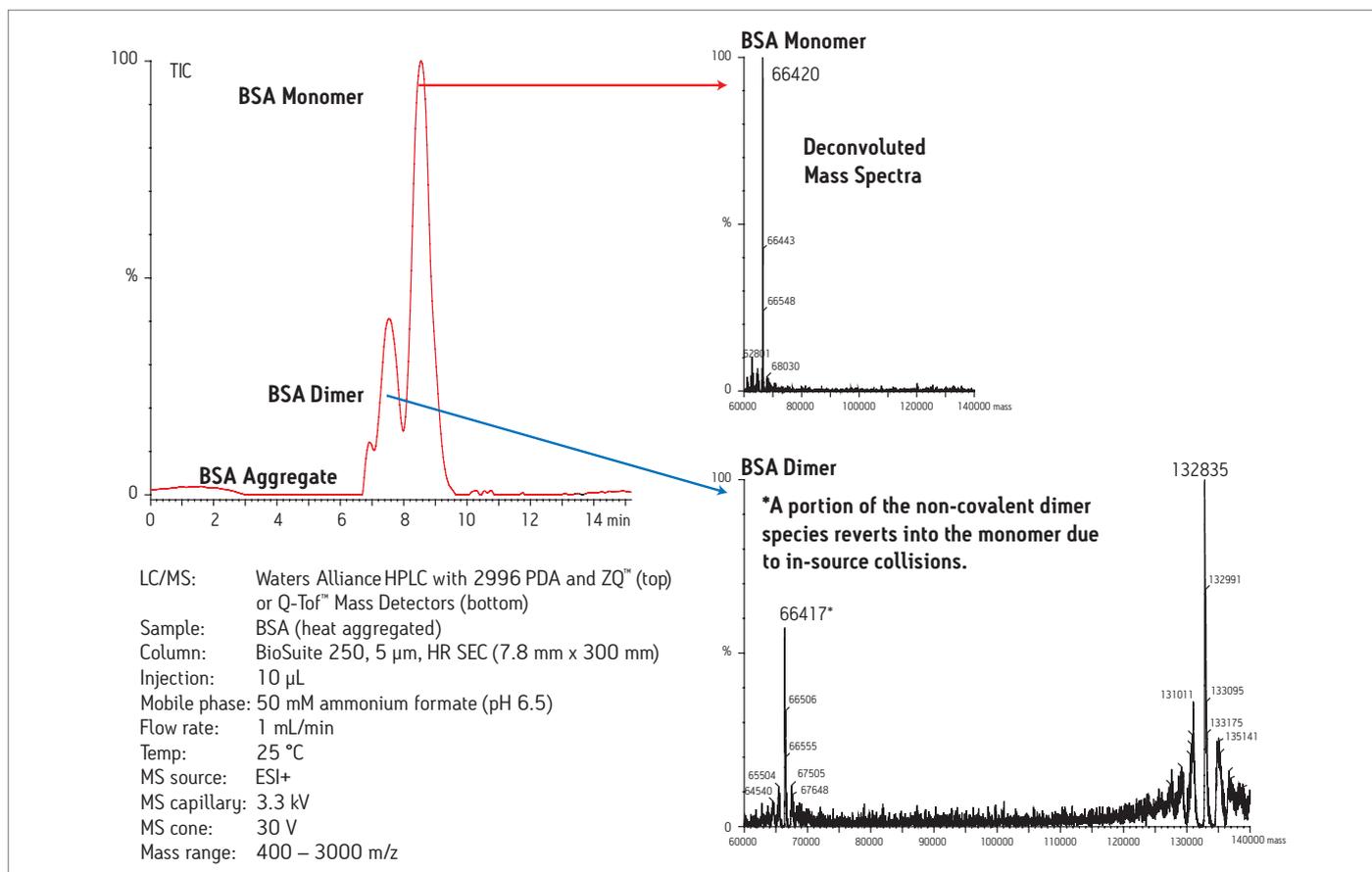


BioSuite Q-PEEK and SP-PEEK columns are available in 4.6 mm x 50 mm.

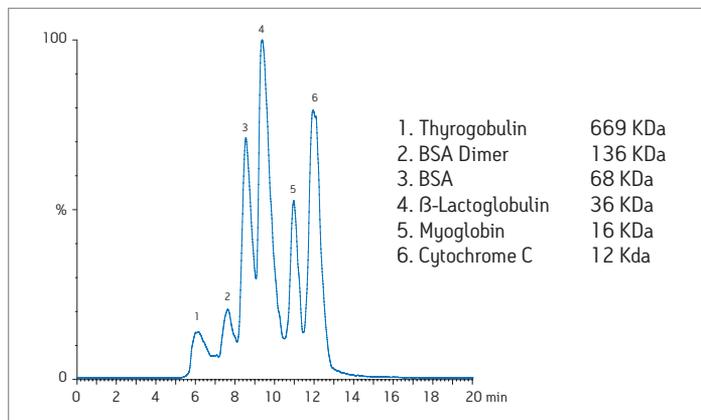
SIZE-EXCLUSION CHROMATOGRAPHY (SEC)

BioSuite Ultra-High Resolution (UHR), High Resolution (HR), and Standard Size Exclusion column packings use a rigid yet ‘wetable’ silica-based media that is stable from pH 2.5 to 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) or preparative (i.e., 21.5 mm) SEC columns, use of BioSuite guard columns is recommended.

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

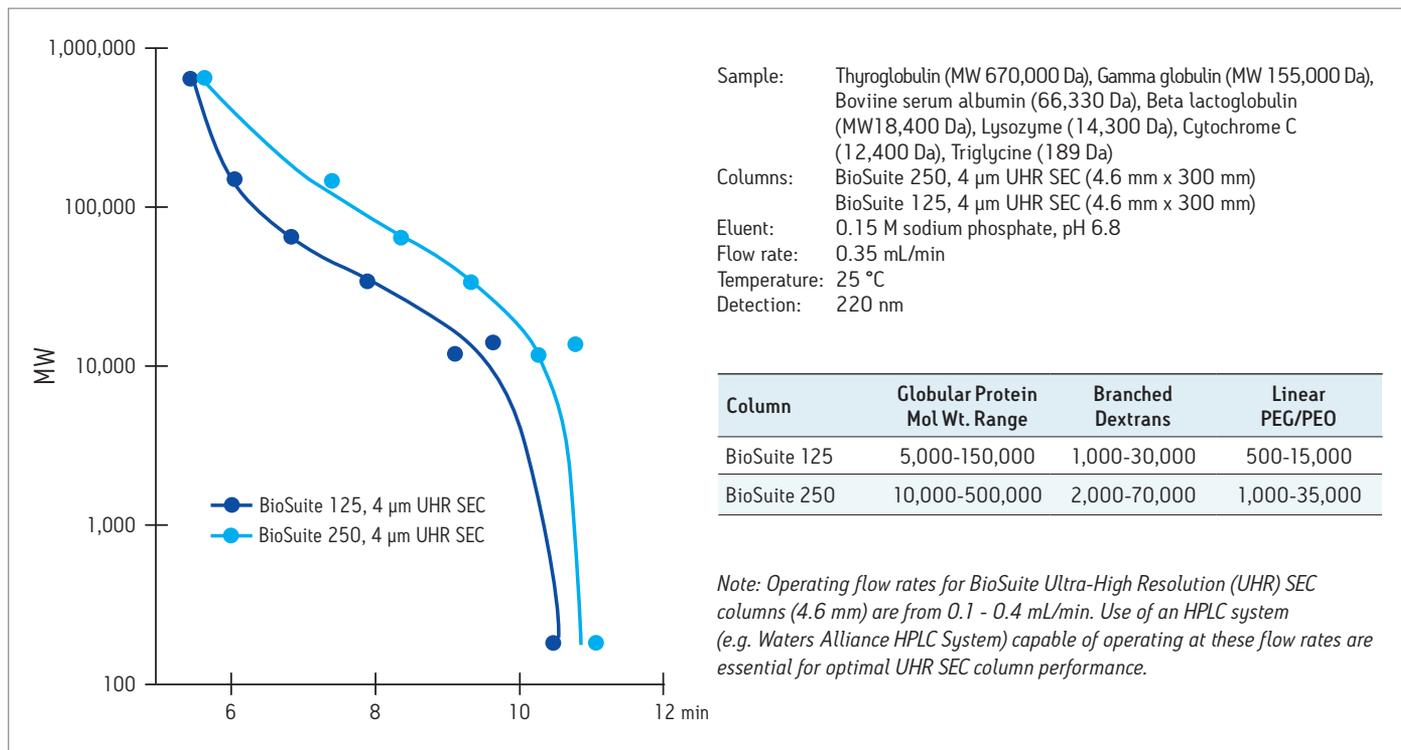


LC/MS Analysis of Protein Standards Using BioSuite 250, 5 μm High Resolution (HR) SEC Column (LC/MS conditions as above)

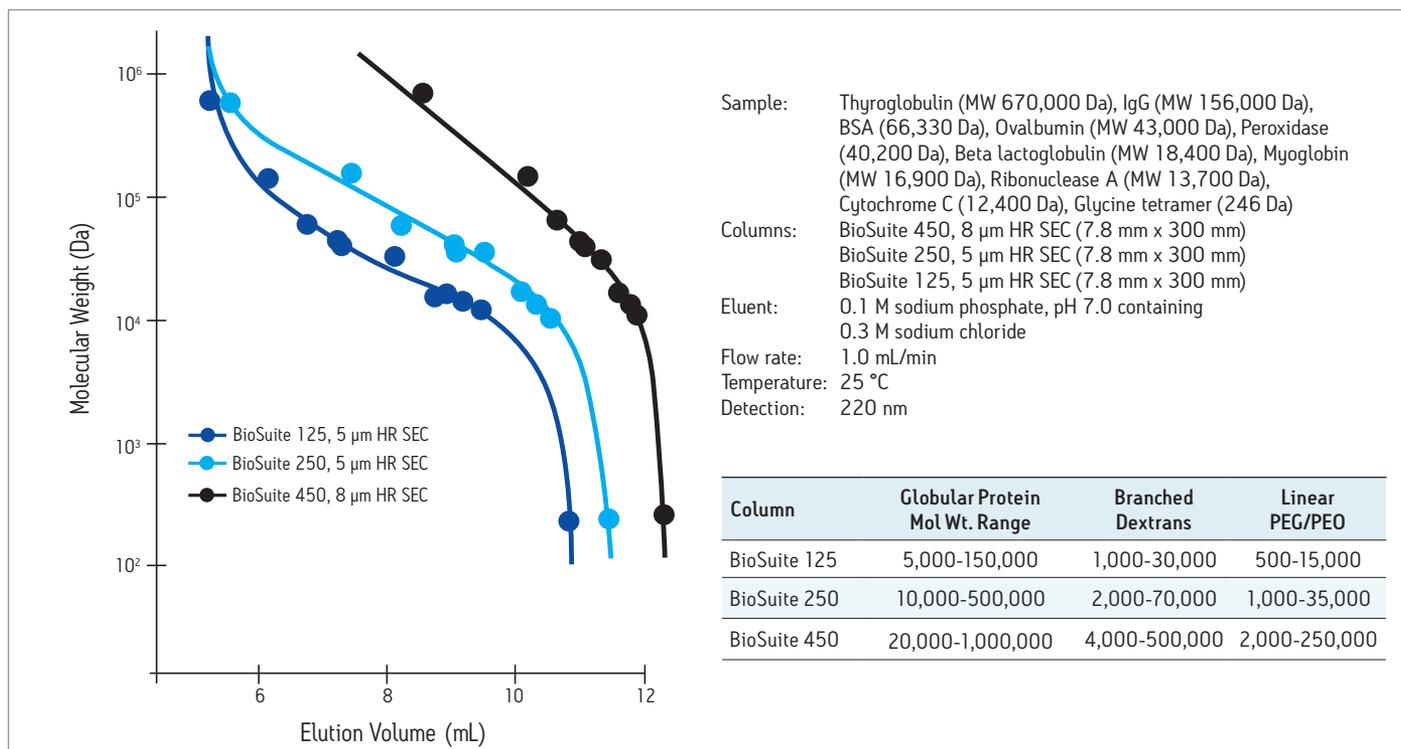


SIZE-EXCLUSION CHROMATOGRAPHY (SEC)

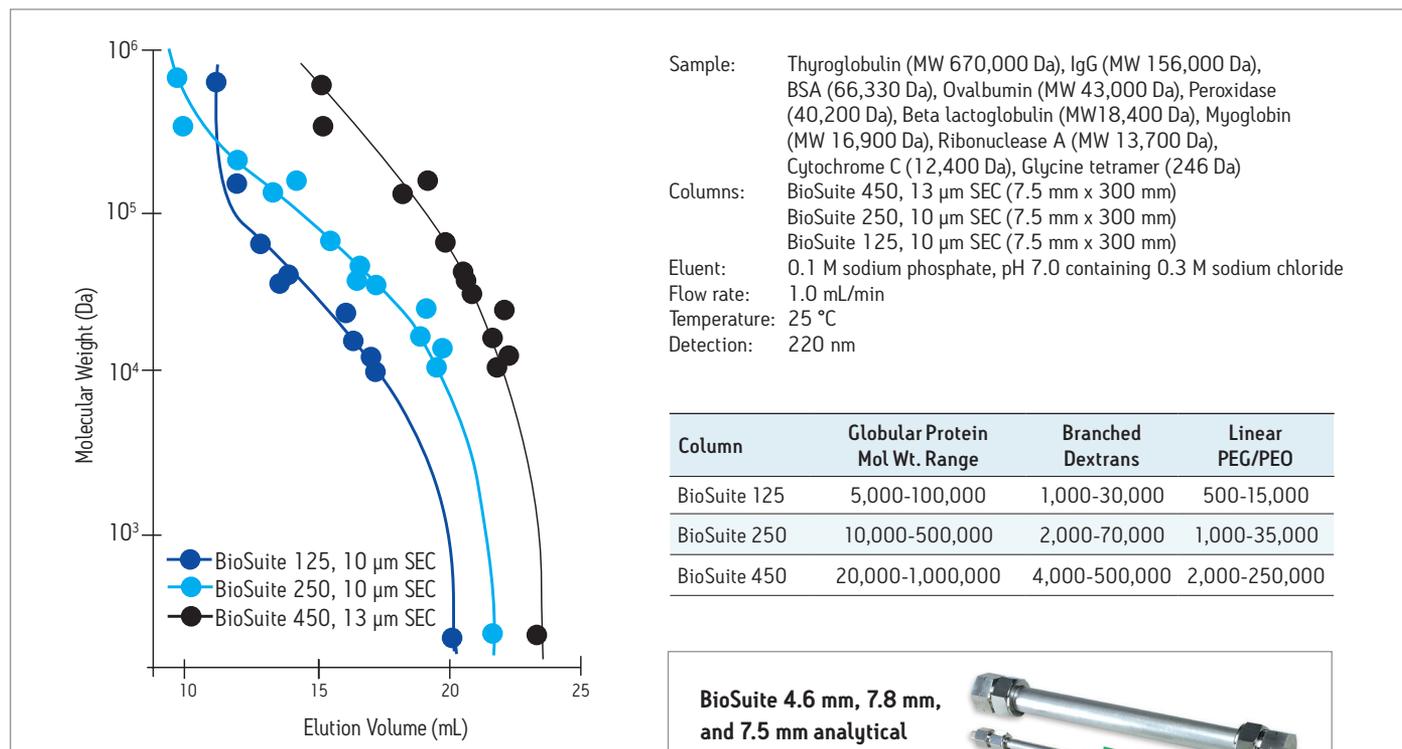
Protein Calibration Curves for BioSuite Ultra-High Resolution (UHR) SEC Columns



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



Protein Calibration Curves for BioSuite Standard SEC Columns



Description	Matrix	Diameter Width	Diameter Length	Column Volume	Suggested Volume Load for Maximum Multicomponent Resolution*	Multicomponent Resolution*	Part No.
BioSuite 125, 4 µm UHR SEC	silica	4.6 mm	300 mm	4.98 mL	Less than 8 mg/mL	Less than 40 µL	186002161
BioSuite 250, 4 µm UHR SEC	silica	4.6 mm	300 mm	4.98 mL	Less than 8 mg/mL	Less than 80 µL	186002162
BioSuite UHR Guard SEC	silica	4.6 mm	35 mm				186002163
BioSuite 125, 5 µm HR SEC	silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002164
BioSuite 250, 5 µm HR SEC	silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002165
BioSuite 450, 8 µm HR SEC	silica	7.8 mm	300 mm	14.33 mL	Less than 8 mg/mL	Less than 200 µL	186002166
BioSuite HR Guard SEC	silica	6 mm	40 mm				186002167
BioSuite 125, 10 µm SEC	silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002168
BioSuite 125, 13 µm SEC	silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mLs	186002169
BioSuite 250, 10 µm SEC	silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002170
BioSuite 250, 13 µm SEC	silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mLs	186002171
BioSuite 450, 13 µm SEC	silica	7.5 mm	300 mm	13.25 mL	Less than 8 mg/mL	Less than 200 µL	186002172
BioSuite 450, 17 µm SEC	silica	21.5 mm	300 mm	108.9 mL	Less than 8 mg/mL	Less than 1.6 mLs	186002173
BioSuite Guard SEC	silica	7.5 mm	75 mm				186002174
BioSuite Guard SEC	silica	21.5 mm	75 mm				186002175

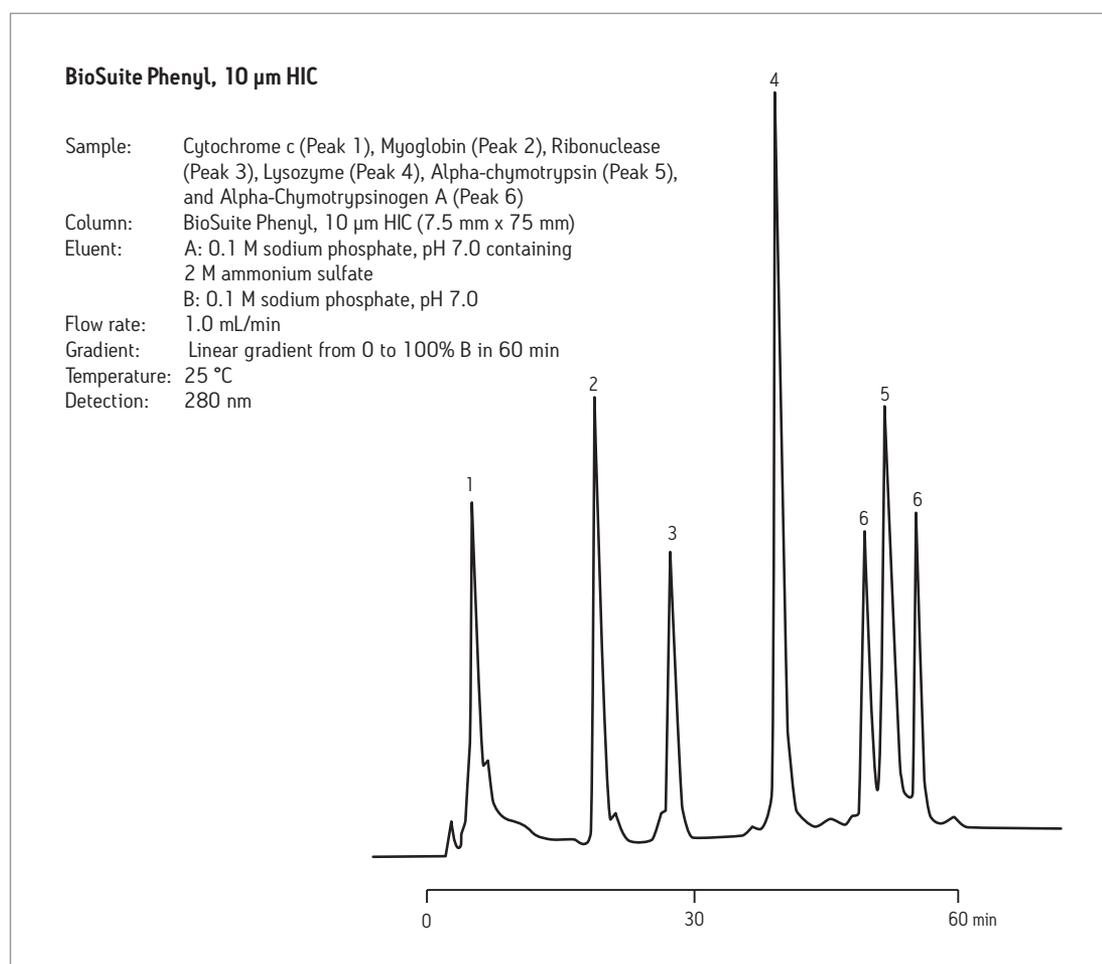
* Using a BSA protein standard in a 50 mM phosphate buffer containing salt (either 0.1 M NaCl or 0.1 M Na₂SO₄) eluent. Useful protein mass loads will vary depending upon separation eluent, complexity of sample, and on the type of proteins contained in mixture. In general, maximum component resolution is obtained by injecting the smallest possible volume of a dilute protein solution.

* Note: 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.

HYDROPHOBIC INTERACTION CHROMATOGRAPHY (HIC)

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 HIC columns provide 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 mm x 150 mm column is also available for 'lab scale' isolations.

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



Note: 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.

Description	Matrix	Diameter Width	Diameter Length	Part No.
BioSuite Phenyl 10 µm HIC	polymer	7.5 mm	75 mm	186002159
BioSuite Phenyl 13 µm HIC	polymer	21.5 mm	150 mm	186002160

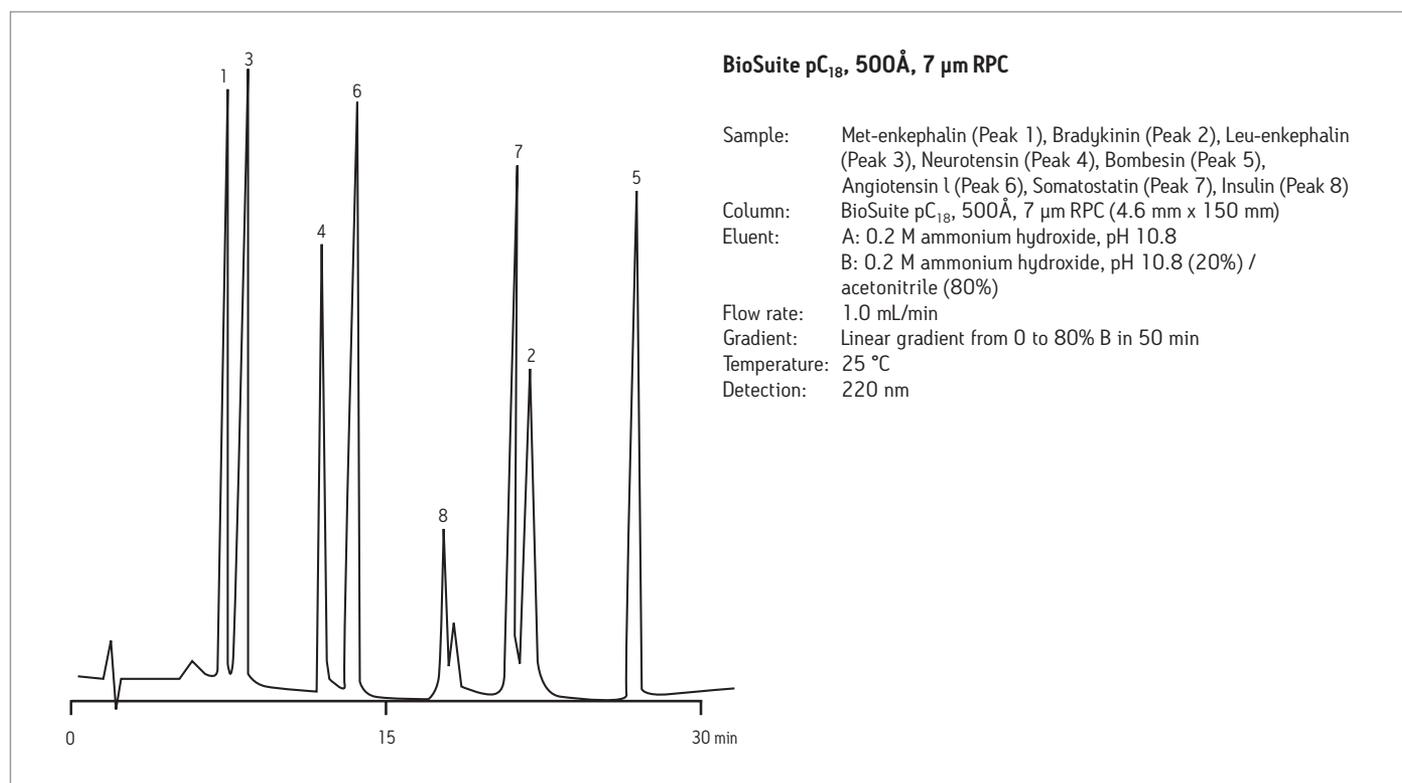
REVERSED-PHASE CHROMATOGRAPHY (RPC)

BioSuite pC₁₈ and pPhenyl RPC Columns

Reversed-phase chromatography (RPC) has become a widely accepted tool for the separation of proteins, peptides, and other biomolecules. For many applications, Waters XBridge™ BEH300, Symmetry® and Symmetry300™ 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 pC₁₈ and pPhenyl resin-based packings may be preferred. BioSuite RPC column offerings include a C₁₈ (pC₁₈) and a phenyl (pPhenyl) chemistry bonded to a pH stable, methacrylic ester-based polymeric resin. The 500Å pore size of the pC₁₈ base matrix accommodates proteins up to 2,500,000 Daltons while the 1,000Å pore size of the pPhenyl base matrix accommodates proteins up to 5,000,000 Daltons.

The BioSuite pC₁₈, 2.5 µm column contains a non-porous (NP) chemistry that yields superior chromatographic resolution in less time compared to chromatography performed on the porous, pC₁₈, 500Å, 7 µm RPC selection. Waters porous, pC₁₈, 500Å, 7 µm RPC column is available for applications requiring greater binding capacity. The pC₁₈ and pPhenyl RPC chemistries are available in 21.5 mm x 150 mm columns for 'lab scale' isolations while a 2.0 mm x 75 mm column is well suited for narrow-bore HPLC and LC/MS applications.

Reversed-Phase Chromatography at Elevated pH on BioSuite pC₁₈ RP Column Possible on Polymer Based Material



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

BioSuite pPhenyl, 1000Å, 10 µm RPC

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 pPhenyl, 1000Å, 10 µm RPC (4.6 mm x 75 mm)

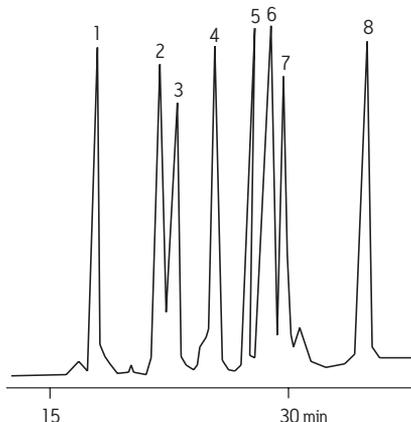
Eluent: A: HPLC grade water with 0.05% TFA
B: acetonitrile with 0.05% TFA

Flow rate: 1.0 mL/min

Gradient: Linear gradient from 5 to 80% B in 60 min

Temperature: 25 °C

Detection: 220 nm



Note: 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.

Description	Matrix	Diameter Width	Diameter Length	Part No.
BioSuite pC ₁₈ , 2.5 µm NPRPC	polymer	4.6 mm	35 mm	186002152
BioSuite pC ₁₈ , 500, 7 µm RPC	polymer	2.0 mm	150 mm	186002153
BioSuite pC ₁₈ , 500, 7 µm RPC	polymer	4.6 mm	150 mm	186002154
BioSuite pC ₁₈ , 500, 13 µm RPC	polymer	21.5 mm	150 mm	186002155
BioSuite pPhenyl, 1000, 10 µm RPC	polymer	2.0 mm	75 mm	186002156
BioSuite pPhenyl, 1000, 10 µm RPC	polymer	4.6 mm	75 mm	186002157
BioSuite pPhenyl, 1000, 13 µm RPC	polymer	21.5 mm	150 mm	186002158

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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.

Today, we continue to research, develop, and commercialize new sample preparation and innovative columns consumables that support the HPLC, UPLC® technology, and LC/MS analyses of peptides, oligonucleotides, proteins, amino acids, and glycans.

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