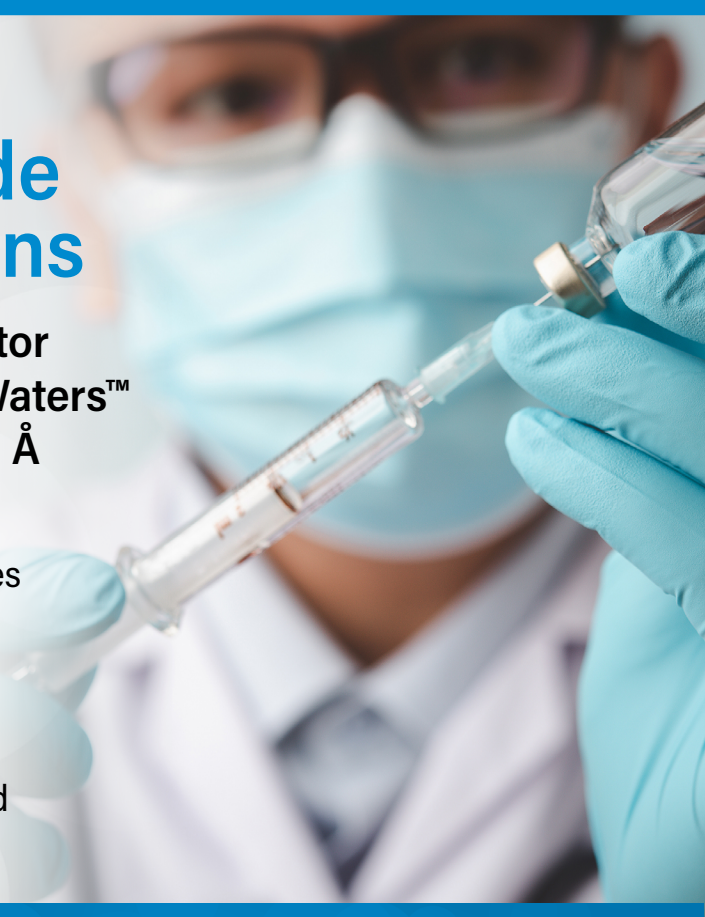
Faster, More Efficient Biotherapeutic Peptide Size-Based Separations



Enhance your Insulin and GLP-1 Receptor Agonist Size-Based Separations with Waters™ ACQUITY™ and XBridge™ 125 Å and 250 Å SEC Columns with MaxPeak™ Premier

MaxPeak Premier with High Performance Surfaces (HPS) Technology leverages decades of Waters SEC Column manufacturing and quality control testing expertise to provide better resolution with reduced secondary interactions for more reliable determination of HMWP species without the need for extensive column conditioning.

Obtain Improved Insulin Size-Base Separations

Up to 15%
Improved Resolution

6x
Faster Separations

7x
Solvent Savings

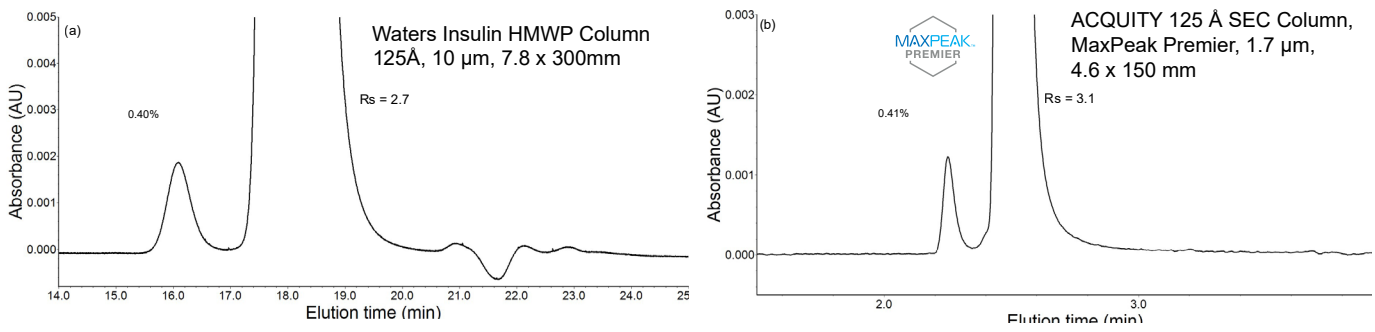


Figure 1. Separation of human insulin using USP <121.1> and EP prescribed SEC denaturing eluent (1 g/L L-arginine in water, glacial acetic acid, acetonitrile, 65/15/20, pH 2.4) on the recommended HPLC-based SEC column which required 100 mLs conditioning to obtain reliable results (left). Waters ACQUITY 125 Å SEC Column with MaxPeak Premier yielded "out of box" performance with comparable degree of component resolution and separated component quantitation. (right)

Generate Accurate HMWP and Insulin Monomer Quantitation

2.5x
increase in HMWPs areas

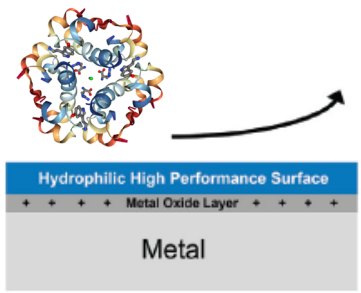


Figure 2: Use of MaxPeak Premier High Performance Surface Technology helps minimize secondary interactions between biomolecules and column hardware.

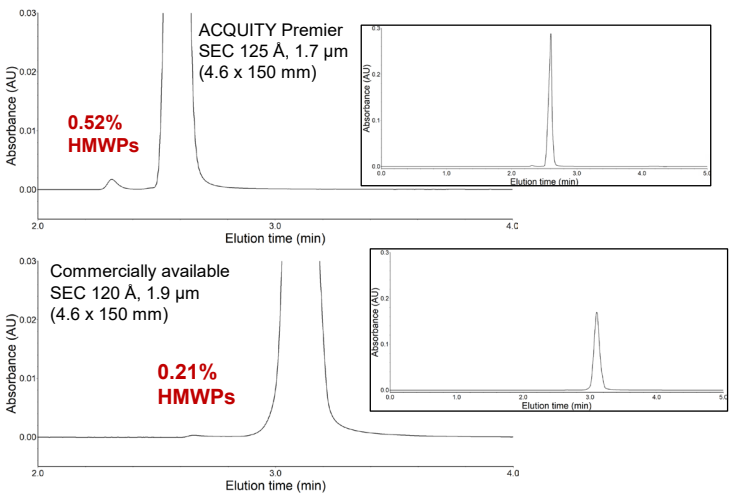


Figure 3. Analysis of human insulin sample (14 µg load) using denaturing SEC Eluent (See Figure 1) on Premier UPLC System. HMWP recovery on competitor column (bottom) was only 40% of HMWPs obtained on Waters ACQUITY 125 Å SEC Column with MaxPeak Premier HPS Technology.

Obtain High throughput resolution on any UPLC, UHPLC, or HPLC platform

Cross Platform

Obtain High Quality SEC Analysis on ANY LC System

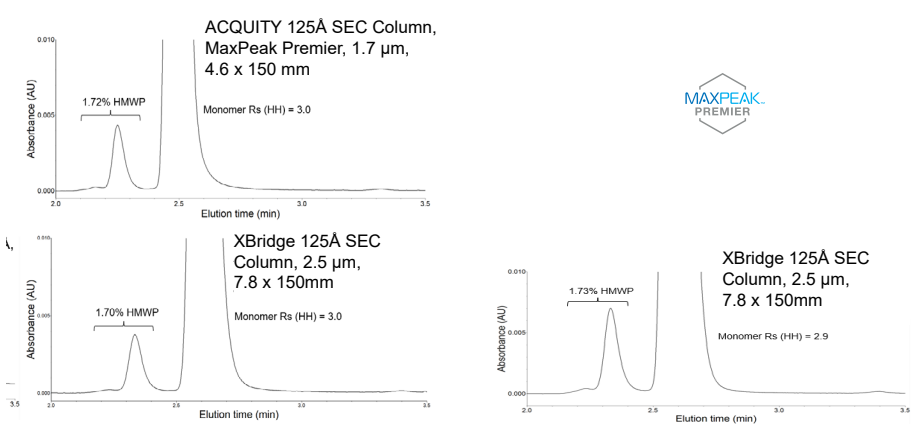


Figure 4. Insulin analyses on ACQUITY 125Å SEC Column, MaxPeak Premier, 1.7 µm and on a XBridge™ Premier SEC 125 Å 2.5 µm using denature SEC Eluent. (See Figure 1)

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Benefits of Denaturing vs Native SEC for GLP-1 Receptor Agonists

GLP-1 Receptor Agonists (*e.g.*, liraglutide and semaglutide) exist as noncovalent oligomers in non-denaturing formulation eluents which dissociate when SEC is performed using denaturing eluents. Addition of MALS detection to these two methods of separation provides critical information during the discovery, formulation development, and manufacture of these biotherapeutic peptides as required by regulatory agencies.

Obtain Reliable Quantitation of Covalent HMW GLP-1 Aggregates using SEC MALS

GLP-1 Receptor Agonist Covalent HMWPs vs Monomer Analyses Using Denaturing SEC Conditions

ACQUITY 125 Å SEC Column, MaxPeak Premier, 1.7 µm, 4.6 x 150 mm

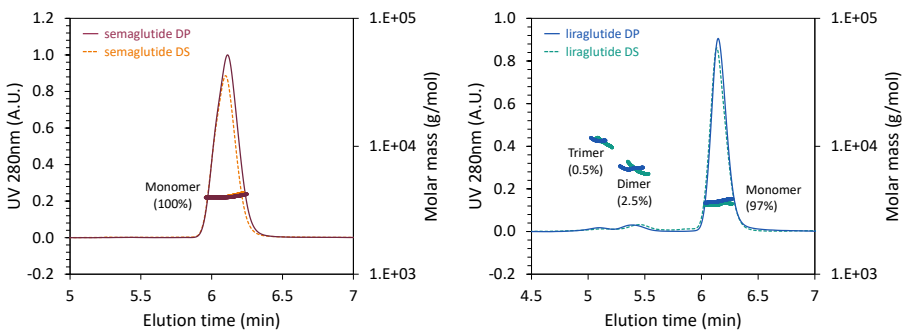
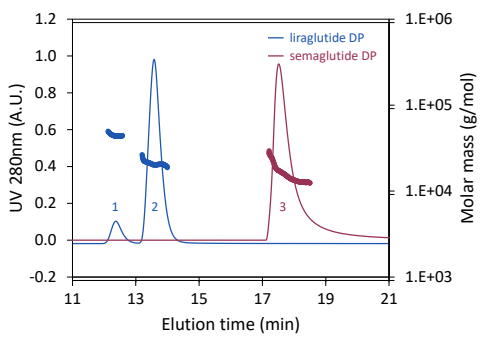


Figure 5: Separation of liraglutide and semaglutide on ACQUITY 125 Å SEC Column, MaxPeak Premier, 1.7 µm with MALS detection using denaturing SEC conditions. (See Figure 1)

Obtain Enhanced Characterization of GLP-1 Oligomers using SEC MALS

GLP-1 Receptor Agonist Oligomer Analyses Using Native SEC Conditions

ACQUITY 250 Å SEC Column, MaxPeak Premier, 1.7 µm, 4.6 x 150 mm



Attributes	Peak 1 (Liraglutide)	Peak 2 (Liraglutide)	Peak 3 (Semaglutide)
M_w (kDa)	48.6	22.6	13.8
R_h (nm)	3.5	2.6	2.1
Identity	13-mer	Hexamer	Trimer - Tetramer
Mass%	9	91	100

Note: Both analyzed under native conditions

Figure 6 Separation of liraglutide and semaglutide on ACQUITY 250 Å SEC Column, MaxPeak Premier, 1.7 µm with MALS detection using non-denaturing SEC conditions. SEC-MALS results suggests liraglutide has two states of oligomers, with hexamer being the dominant species and 13-mer being the minor species while semaglutide exists as a trimer and tetramer complexes.

Help Determine GLP-1 Oligomer Distributions using SEC /MALS Data

Monitor GLP-1 Receptor Agonist complexes under native conditions

ACQUITY 250 Å SEC Column, MaxPeak Premier 1.7 µm 4.6 x 150mm

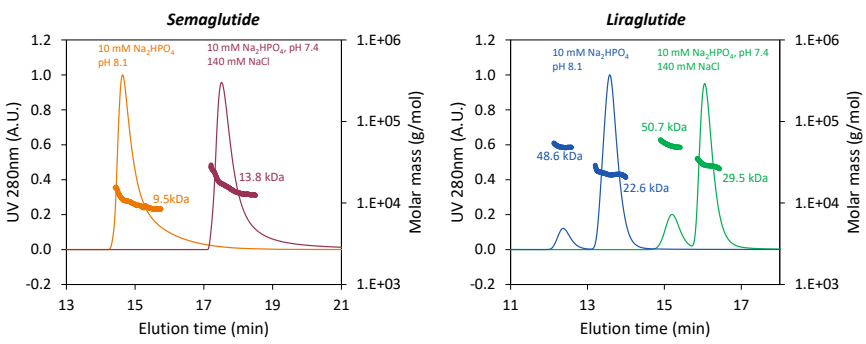
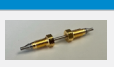


Figure 7. Oligomerization of GLP-1 is very sensitive to non-denaturing SEC separation conditions as shown by small pH and ionic strength changes in the SEC.

Ordering Information

Columns	Pore Size	Molecular Weight Range	Particle Size	4.6 x 30mm Guard	4.6 x 150 mm Column	4.6 x 300 mm Column	7.8 x 150 mm Column	7.8 x 300 mm Column
ACQUITY 125 Å SEC with MaxPeak Premier	125 Å	Approx. 1K - 80K Daltons	1.7 µm	–	186011350	186011351	–	–
XBridge 125 Å SEC with MaxPeak Premier			2.5 µm	186011356	186011352	186011353	186011354	186011355
ACQUITY 250 Å SEC with MaxPeak Premier	250 Å	Approx 10K - 650K Daltons	1.7 µm	–	186009963	186009964	–	–
XBridge 250 Å SEC with MaxPeak Premier			2.5 µm	186009969	186009959	186009960	186009961	186009962

Peptide, Protein and New Modality SEC Connections		
0.005 x 1.75 inch UPLC Connection Tubing with end ferrules and nuts (2 pack)		186006613
Short rigid column connector with end ferrules and nuts (1 pack)		WAT022681
U-shaped column connector with end ferrules and nuts (1 pack)		WAT084080

For a complete list of all SEC Columns visit: waters.com/SEC

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