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dSEC and dSEC-HRMS Characterization of GLP-1a and Related Peptide Therapeutics

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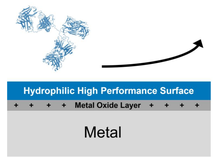
Abstract

Therapeutic peptides as a class of molecules are as diverse in their structural features as their targeted indications. Their resulting propensities to be highly surface-active and to form oligomeric dissociable higher-order structures in solution create challenges for size-based analyses critical to ensuring drug product efficacy. Here we explored the use of denaturing mobile phase conditions with appropriately selected highly efficient and inert SEC columns (dSEC) to achieve size-based separations of non-dissociable high molecular weight species (HMWS). Combining dSEC with high-resolution mass spectrometry (dSEC-HRMS) yielded additional valuable insights into low-abundant HMWS present in the GLP-1a drug products tirzepatide and semaglutide.

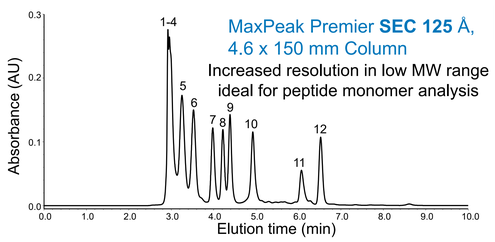
Column Considerations

SEC column selected: **ACQUITY™ 125 Å SEC Column, MaxPeak™ Premier Technology, 1.7 µm, 4.6 x 150 mm** (PN 186011350)

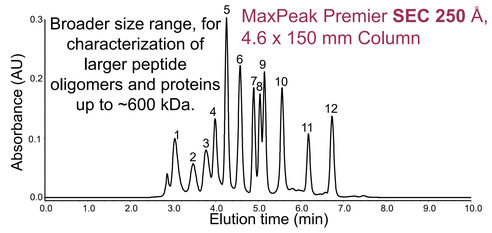
Reduced secondary interactions with hydrophilic MaxPeak HPS surface modified hardware

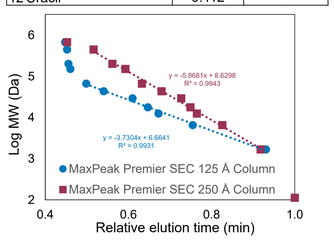


Optimal pore size for peptide monomer analysis



Analyte	MW (kDa)	Stokes radius (Å)
1 Thyroglobulin	669	86
2 Apo-Ferritin	443	61
3 beta-amylase	200	54
4 Alcohol dehydrogenase	150	46
5 BSA	66	36
6 Ovalbumin	43	28
7 Carbonic anhydrase	29	21
8 myoglobin	17.6	19
9 Cytochrome C	12.4	17
10 Aprotinin	6.5	13.5
11 Neurotensin	1.67	-
12 Uracil	0.112	-





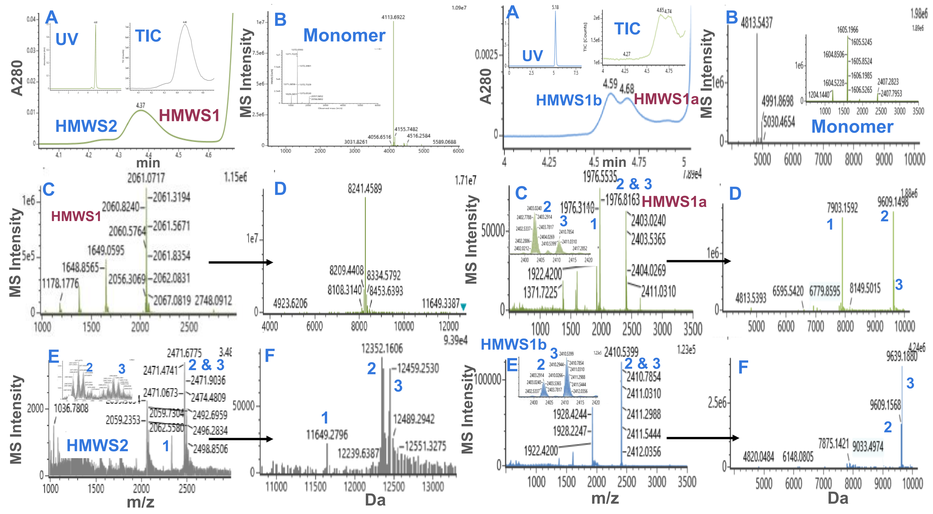
dSEC-HRMS of GLP-1a

Semaglutide

	Size Variant	Mass (Da)
Predicted Noncovalent Mass (average)	monomer	4113.58
	dimer	8227.16
	trimer	12340.74
Observed Predominant Mass (average)	monomer-1	4113.69 (Δ 0.11)
	HMWS1-1 (dimer)	8241.46 (Δ 14.3)
	HMWS2-1 (trimer)	11649.28 (Δ -691.46)
	HMWS2-2 (trimer)	12352.16 (Δ 11.42)
	HMWS2-3 (trimer)	12459.25 (Δ 118.51)

Tirzepatide

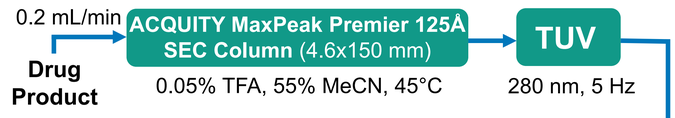
	Size Variant	Mass (Da)
Predicted Noncovalent Mass (average)	monomer	4813.53
	dimer	9627.05
	trimer	14440.58
Observed Predominant Mass (average)	monomer-1	4813.54 (Δ 0.01)
	HMWS1a-1 (dimer)	7903.16 (Δ -1723.86)
	HMWS1a-2 (dimer)	9609.15 (Δ -17.90)
	HMWS1b-3 (dimer)	9639.19 (Δ 12.14)



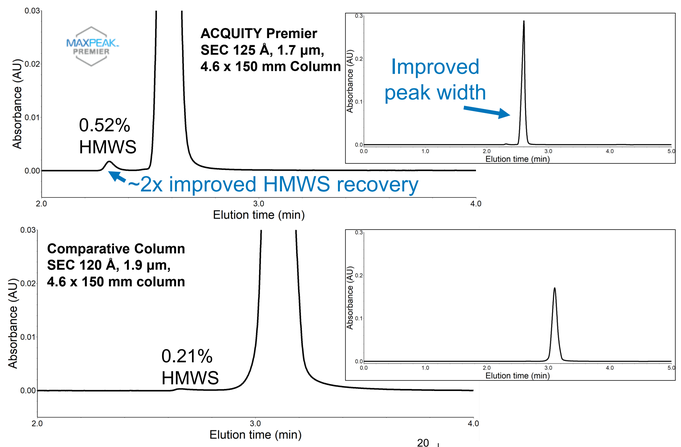
- Efficient separation of monomer from high molecular weight species (HMWS)
- Resolution of dimer-sized HMWS from higher-order HMWS in semaglutide
- Mass characterization revealed multiple distinct non-dissociable oligomeric species

Relevant application notes, simply search literature codes

- Denaturing SEC-MS Analysis of High Molecular Weight Impurities in the GLP-1a Lipopeptides Semaglutide and Tirzepatide: **Literature Code 720009256**
- Advancing Analysis of Covalent High Molecular Weight Insulin With ACQUITY® Premier SEC 125 Å Columns: **Literature Code 720008660**
- Development of Separation Methods for GLP-1 Synthetic Peptides Utilizing a Systematic Protocol and MaxPeak™ High Performance Surface Technology: **Literature Code 720008267**
- Zhang, X et al. Identification of GLP-1 analog oligomeric states using SEC-MALS. Wyatt Technology Application Note. **AN1901**, 2025.



Insulin dSEC: HMWS Recovery



LC System: ACQUITY Premier System with BioSample Manager (FTN) and BioQuaternary Solvent Manager (QSM)

Seal wash: 10% HPLC-grade methanol/ 90% 18.2 MΩ water (v/v)

SEC Eluent: 1 g/L arginine / acetonitrile / glacial acetic acid, 65/20/15 (v/v/v)

Sample: Human insulin reconstituted to 4 mg/mL with 0.01 N HCl

Injection Volume: 3.5 µL

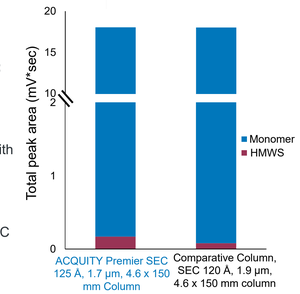
Sample manager washes: 18.2 MΩ water

Flow Rate: 0.4 mL/min

Detection: TUV detector with 5 mm Titanium Flow Cell

Wavelength: 276 nm

Column Temperature: 25°C



Consistent total peak areas across both columns (n=3) suggests that the HMWS peak partially co-eluted with the monomer for the comparative column due to secondary interactions. The ACQUITY Premier 125 Å SEC Column minimized secondary interactions to improve HMWS recovery.

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