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NANOCHROM

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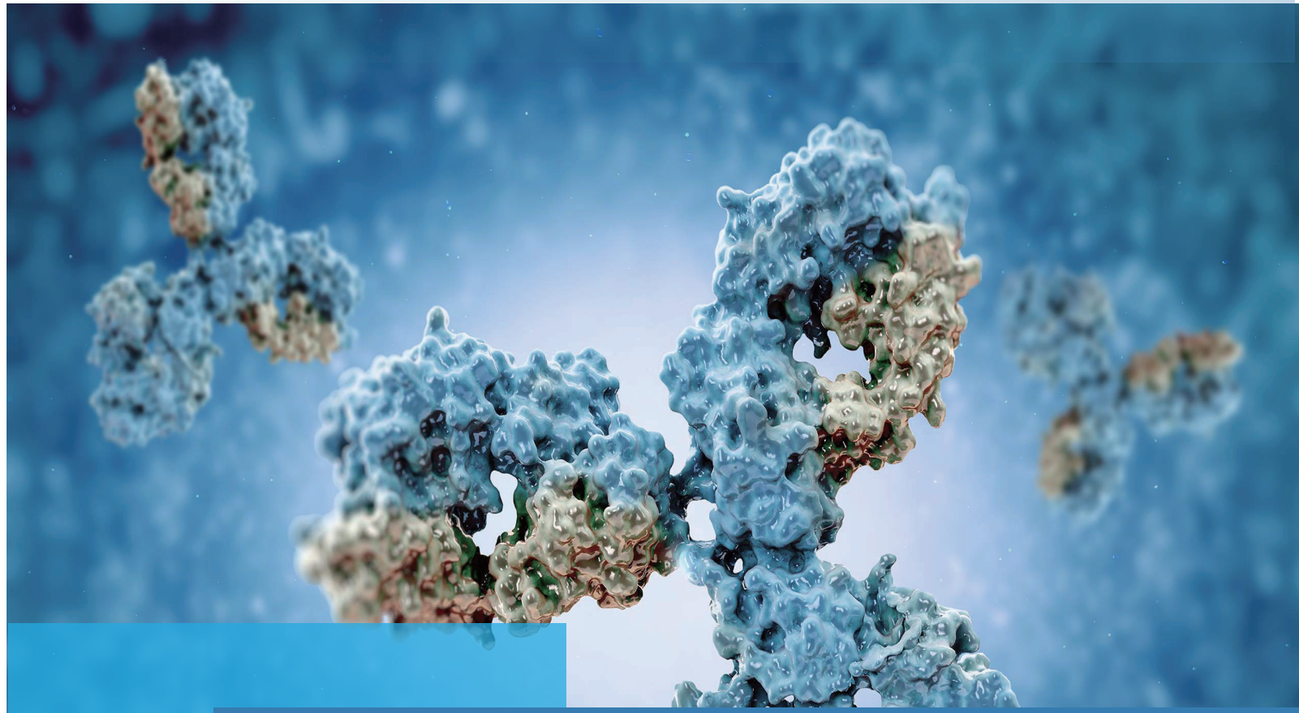
NanoChrom Technologies



BioCore SEC Columns

for biologics separation

Website

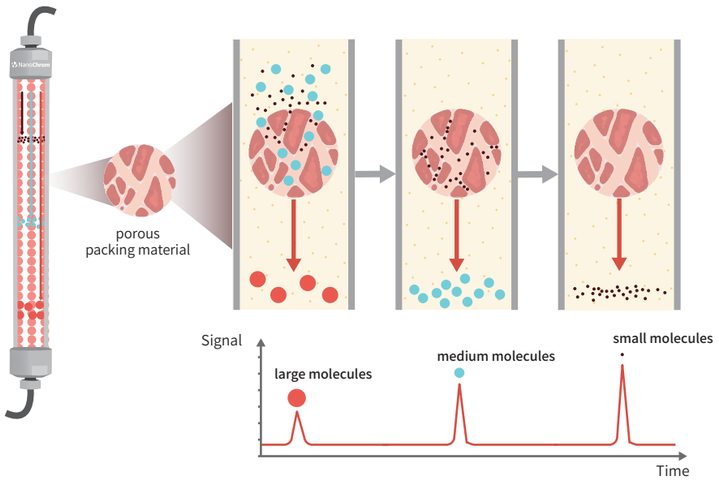
WeChat Official Account

Size Exclusion Chromatography

Size exclusion chromatography (SEC) is a chromatographic method in which molecules in solution are separated by their size, or hydrodynamic volumes. In SEC mode, a stationary phase containing a porous packing material is placed in a column and the mobile phase that contains the sample solution is injected into the stationary phase. The molecules that are larger than the pore size will be eluted first due to their inability to penetrate fewer pores, While the smaller molecules will take a longer time to be eluted because they can penetrate more pores.

SEC is mainly used for the separation of macromolecules including proteins, enzymes, antibodies, nucleic acids (DNA and RNA), as well as industrial polymers.

SEC Principle



BioCore SEC Columns

BioCore SEC is a family of high performance, size exclusion chromatography columns, designed for separating antibodies, proteins, peptides, oligonucleotides and related substances, etc. The column technology involves creation of an inert neutral hydrophilic layer onto high strength, high pore-volume, monodispersed porous silica particles, combined with well-established column packing processes. BioCore SEC columns have a broad application range in bio-technology, biopharmaceutical and academic research.

Main Features

- Innovative particle technology: monodispersed for high efficiency, high mechanical strength for better column lifetime, and high pore volume for high resolution
- Advanced column chemistry for minimal secondary interaction
- Multiple pore size options for broad application range
- Robust column packing for good column lifetime
- Good column-to-column consistency

Specification

Product Name	SEC-120	SEC-150	SEC-300	SEC-500
Functional Group	Diol			
Substrate	Monodispersed, high pore volume, porous, spherical, silica particles			
Particle Size	1.8, 3 & 5 µm			
Pore Size	120 Å	150 Å	300 Å	500 Å
Pressure Limit	5000 psi for 1.8 µm 2500 psi for 3 µm 1500 psi for 5 µm			
Temperature Limit	40 °C			
pH Range	2-8			
Calibration Curve (PEG)	300-10,000	500-15,000	1,000-50,000	5,000-200,000
Calibration Curve (Glucan)	NA	1,000-30,000	2,000-100,000	20,000-500,000
Calibration Curve (Globular Protein)	NA	5,000-150,000	10,000-750,000	20,000-1,500,000
Application	Small-molecule drugs, peptides, glycans, small oligos	Small-molecule drugs, peptides, glycans, small oligos and small proteins	MAbs and aggregates, proteins and DNA/RNA	MAbs and high order aggregates, large proteins and DNA/RNA

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Application

Aggregate and fragment analysis on BioCore SEC-300

Recombinant monoclonal antibodies (mAbs) have achieved great success in treating diseases such as immunology, oncology, and blood diseases. MAbs are large molecules composed of two heavy chains and two light chains, which are connected through interchain disulfide bonds. During manufacturing and storage, mAbs can form aggregates (or high molecular weight species [HMWs]) and fragments (or low molecular weight species [LMWs]). MAb aggregation can be formed through various mechanisms, such as molecular interactions and chemical cross-linking. The aggregates can be potentially immunogenic, and possibly promote the formation of particulates. MAb fragmentation can occur through several pathways, such as hydrolysis, free radical induced fragmentation, and enzymatic cleavage. The fragmented mAb may have reduced biological activity and pharmacokinetic compared to the intact mAb. Both aggregates and fragments are the critical quality attributes that need to be controlled to very low levels to ensure the efficacy, safety, and stability of mAb drugs.

Size exclusion chromatography (SEC) is an important and most commonly used technique to determine aggregate and fragment content of antibody biologics. These columns are usually packed with media of pore size and particle size in 200 to 300 Å range and 1.8 to 5 µm range, respectively. For routine analysis, 7.8 mm ID SEC columns can be used on most HPLC systems for its resistance to extra column volume effect. When sample amount is limited or mass spectrometry detection is required, the 4.6 mm ID SEC columns format is recommended.

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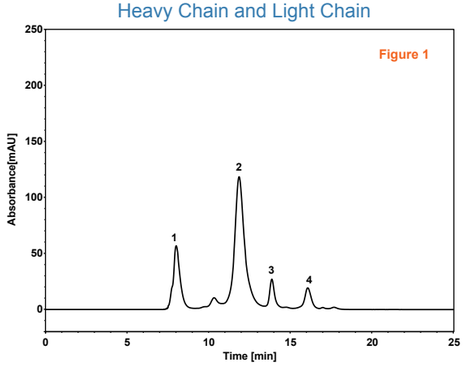
Figure 1 illustrates the separation of a mixture of a mAb (~150 kDa), its aggregates (tetramer, ~900 kDa) and fragments (HC, ~50 kDa, and LC, ~25 kDa) on a 5 µm, 7.8×300 mm BioCore SEC-300 column, demonstrating its good suitability for simultaneous separation of mAb, related aggregates and fragments.

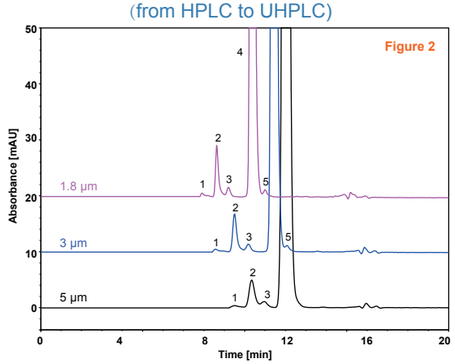
Separation of MAb, Aggregate, Heavy Chain and Light Chain



Columns:	**BioCore SEC-300**, 5 µm
Dimension:	7.8×300 mm
Mobile Phase:	300 mM NaCl in 50 mM phosphate buffer, pH 6.8
Flow Rate:	0.7 mL/min
Temperature:	25 ℃
Injection:	10 µL
Detection:	UV 214 nm
Sample:	MAb
Peaks:	1. Aggregate (M.W.=900 kDa) 2. MAb (M.W.=150 kDa) 3. Heavy chain (M.W.=50 kDa) 4. Light chain (M.W.=25 kDa)

For routine analysis, SEC columns packed with 5 µm media are often used. As shown in Figure 2, a 5 µm, 4.6×300 mm SEC-300 column provides suffice separation between the main peak of trastuzumab biosimilar and its aggregates but failing to resolve the fragment adjacent to the main mAb peak. When a 4.6×300 mm SEC-300 column packed with 3 µm media is used, both the resolutions between the mAb and its aggregates and among its aggregates can be improved significantly. Moreover, a fragment peak is partially resolved from the main mAb peak. The separation between the mAb and the fragment can be further improved on a 4.6×300 mm SEC-300 column packed with 1.8 µm media. It is clear that BioCore SEC columns family supports both routine HPLC and ultra-high resolution UHPLC applications for aggregate and fragment analysis.

Separation of MAb, Aggregates and Fragments (from HPLC to UHPLC)

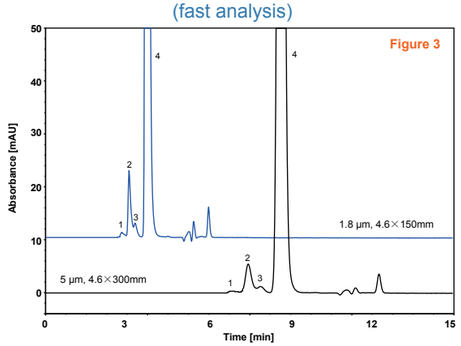


Column:	
Black:	**BioCore SEC-300**, 5 µm
Blue:	**BioCore SEC-300**, 3 µm
Red:	**BioCore SEC-300**, 1.8 µm
Dimension:	4.6×300 mm
Mobile Phase:	90/10 v/v 50 mM phosphate buffer, pH6.8/MeCN
Flow Rate:	0.25 mL/min
Temperature:	30 ℃
Injection:	5 µL
Detection:	UV 280 nm
Sample:	Trastuzumab Biosimilar (5 mg/mL)
Peaks:	1-3. Aggregates 4. MAb 5. Fragment

Particle Size	N(4)	Rs(1,2)	Rs(2,3)	Rs(3,4)	Rs(4,5)
1.8 µm	22668	2.21	1.95	3.83	2.33
3 µm	17006	2.84	1.88	3.58	1.06
5 µm	9616	2.29	0.94	1.68	/

Aggregate determination by SEC is one of the most frequently run assays in mAb drug development and drug manufacturing. Very often, fast analysis is desired for better productivity. For example, to analyze a mAb and its aggregates, the resolution and analysis time are compared on one BioCore SEC-300 (1.8 µm, 4.6×150 mm) and one BioCore SEC-300 (5 µm, 4.6×300 mm). As shown in Figure 3, while the resolutions between aggregates and mAb are comparable, the total analysis time on BioCore SEC-300 (1.8 µm, 4.6×150 mm) is two times faster than that on BioCore SEC-300 (5 µm, 4.6×300 mm) column, demonstrating that 1.8 µm UHPLC SEC columns can not only provide superior resolution power, but also support fast analysis.

Separation of MAb and Aggregates (fast analysis)



Column:	
Black:	**BioCore SEC-300**, 5 µm
Blue:	**BioCore SEC-300**, 1.8 µm
Dimension:	
Black:	4.6×300 mm
Blue:	4.6×150 mm
Mobile Phase:	300 mM NaCl in 50 mM phosphate buffer, pH6.8
Flow Rate:	0.35 mL/min
Temperature:	30 ℃
Injection:	5 µL
Detection:	UV 280 nm
Sample:	Trastuzumab Biosimilar (5 mg/mL)
Peaks:	1~3. Aggregates 4. MAb

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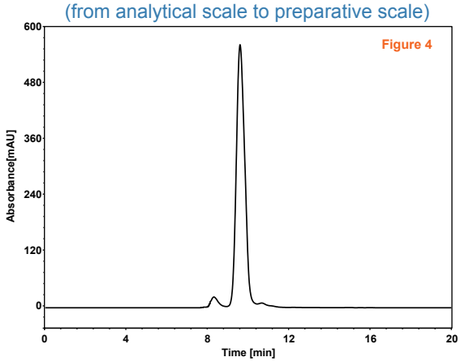
From analytical scale to preparative scale

In the development of new biologic drugs, it is desired to isolate related impurities for detailed study in order to assess their impact to drug safety and efficacy. The common strategy is to select a suitable separation media and develop a working chromatographic method on an analytical column as the first step, then pack a preparative column using the same media and scale and adjust the analytical method to a preparative method, and finally conduct purification using the developed method and collect fractions.

Figure 4 is an example of separation of a **fusion protein** (main peak), its aggregate (before the main peak) and fragment (next, adjacent to the main peak) on a 7.8×300 mm SEC-300 column packed with 5 µm media. Under the optimized condition, all three components can be well resolved from one another.

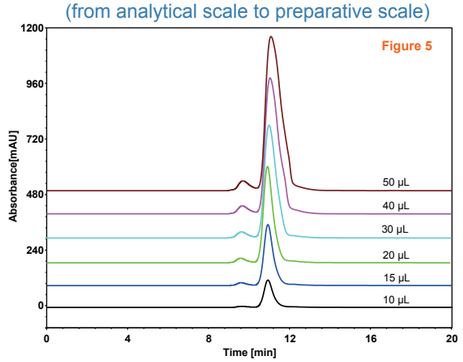
Fusion Protein
(from analytical scale to preparative scale)



Column:	**BioCore SEC-300**, 5 µm
Dimension:	7.8×300 mm
Mobile Phase:	100 mM Na_2SO_4 in 100 mM phosphate buffer, pH6.8
Flow Rate:	0.7 mL/min
Temperature:	30 ℃
Injection:	10 µL
Detection:	UV 280 nm
Sample:	Fusion Protein (M.W.=78 KD, 80 mg/mL)

Based on this result, one 20x300 mm preparative BioCore SEC-300 column can be used to isolate the aggregate. Figure 5 shows the separations with different sample loadings (from 0.8 to 4 mg), all with decent resolution, illustrating its suitability for mg-scale purification for minor components in biologics.

Fusion Protein
(from analytical scale to preparative scale)



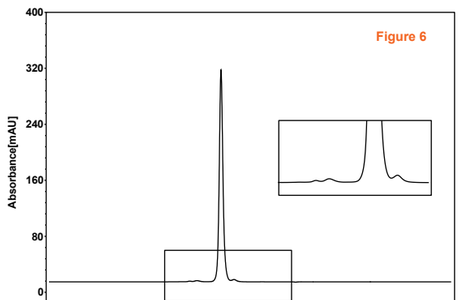
Column:	**BioCore SEC-300**, 5 µm
Dimension:	20×300 mm
Mobile Phase:	100 mM Na_2SO_4 in 100 mM phosphate buffer, pH6.8
Flow Rate:	4 mL/min
Temperature:	30 ℃
Injection:	10/15/20/30/40/50 µL
Detection:	UV 280 nm
Sample:	Fusion Protein (M.W.=78 KD, 80 mg/mL)

Bispecific and trispecific antibodies, ADC and HSA

The ability of monoclonal antibodies to specifically bind a target antigen and neutralize or stimulate its activity is the basis for the rapid growth and development of the therapeutic antibody field. In recent years, traditional immunoglobulin antibodies have been further engineered for better efficacy and safety, and technological developments in the field enabled the design and production of engineered antibodies capable of mediating therapeutic functions unattainable by conventional antibody formats. Polyspecific antibodies and antibody–drug conjugates (ADCs) are representatives in this filed, each with several approved drugs and dozens more in the clinical development phase.

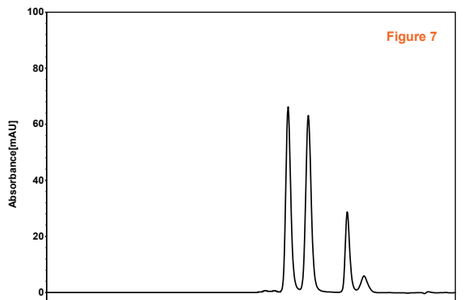
Figures 6, 7 and 8 show separations of a **bispecific antibody**, a **trispecific antibody** and **ADC** on 4.6×300 mm SEC-300 columns packed with 1.8 or 3 µm media. It is evident that BioCore SEC columns can provide good separation between the main component and related aggregates, and sometimes fragments can also be resolved from the main peak, except for the ADC. When developing a method, a small percentage of organic solvent can be added in the mobile phase to minimize secondary interactions between the analyte and the media, for better separation.

Bispecific Antibody



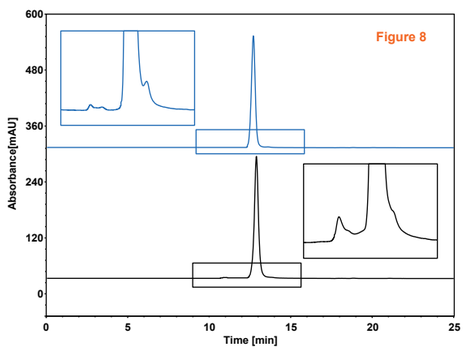
Column:	**BioCore SEC-300**, 1.8 µm
Dimension:	4.6×300 mm
Mobile Phase:	100 mM Na_2SO_4 in 50 mM phosphate buffer, pH6.8
Flow Rate:	0.25 mL/min
Temperature:	30 ℃
Injection:	10 µL
Detection:	UV 214 nm
Sample:	Bispecific Antibody

Trispecific Antibody



Column:	**BioCore SEC-300**, 3 µm
Dimension:	4.6×300 mm
Mobile Phase:	90/10 v/v 300 mM NaCl in 50 mM phosphate buffer, pH6.8/MeCN
Flow Rate:	0.21 mL/min
Temperature:	30 ℃
Injection:	2 µL
Detection:	UV 280 nm
Sample:	Trispecific Antibody (5 mg/mL)

Antibody-Drug Conjugate (ADC)



Column:	**BioCore SEC-300**, 3 µm
Dimension:	4.6×300 mm
Mobile Phase:	
Blue:	90/10 v/v 300 mM NaCl in 50 mM phosphate buffer, pH6.8/MeCN
Black:	300 mM NaCl in 50 mM phosphate buffer, pH6.8
Flow Rate:	0.21 mL/min
Temperature:	30 ℃
Injection:	2 µL
Detection:	UV 280 nm
Sample:	ADC (10.0 mg/mL)

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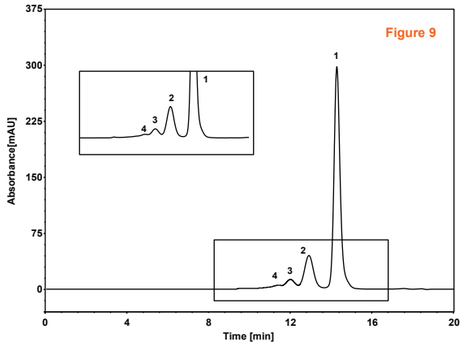
Human Serum Albumin (HSA) injection is mainly used to replace blood volume loss caused by trauma. Usually, it is required that the aggregates be below 5% for safety reason. The baseline separation of HSA from its aggregates can be obtained on a BioCore SEC-300 column for accurate aggregate content determination (shown in Figure 9).

Human Serum Albumin



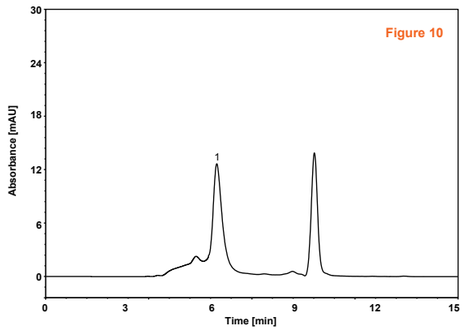
Column: **BioCore SEC-300**, 5 μm
Dimension: 7.8×300 mm
Mobile Phase: 100 mM phosphate buffer, pH7.0
Flow Rate: 0.7 mL/min
Temperature: 25 ℃
Injection: 10 μL
Detection: UV 280 nm
Peaks: 1. Human Serum Albumin
2~4. Aggregates

BioCore SEC-500 for larger proteins

Compared with the BioCore SEC-300 which is based on particles with 300 Å pore size, BioCore SEC-500 columns employ larger pore (~500 Å) silica particles, thus are suitable for separating larger proteins.

IgM is the largest immunoglobulin by size and exists mainly as pentamer in human serum. IgM polymer assembly depend on noncovalent interaction between subunit and disulfide bridging. Figure 10 shows the separation of IgM and its aggregates on a BioCore SEC-500 column.

IgM



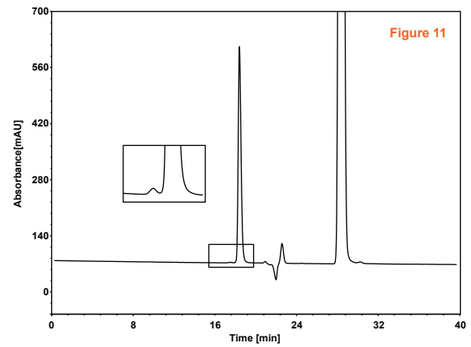
Column: **BioCore SEC-500**, 3 μm
Dimension: 4.6×150 mm
Mobile Phase: 10/90 v/v MeCN/300 mM NaCl in 50 mM phosphate buffer, pH6.8
Flow Rate: 0.21 mL/min
Temperature: 30 ℃
Injection: 1 μL
Detection: UV 280 nm
Sample: IgM in Human Serum
Peak: 1. IgM

BioCore SEC-150, for peptides and small proteins

BioCore SEC-150 columns are based on 150 Å pore silica particles, designed for for separating peptides and small proteins.

Exenatide, composed of 39 amino acids, is the active ingredient in an injectable medicine that helps to control blood sugar in the body. As shown in Figure 11, exenatide and its aggregate can be baseline separated on a BioCore SEC-150 column.

Exenatide Injection Solution

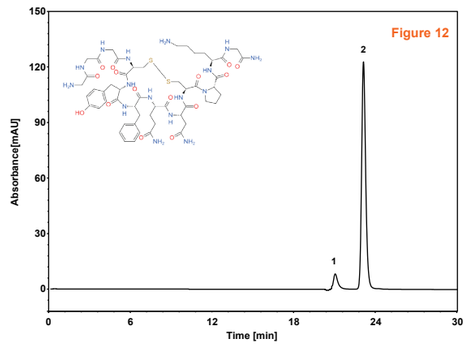


Column: **BioCore SEC-150**, 5 μm
Dimension: 7.8×300 mm
Mobile Phase: 25/75/0.1 v/v/v MeCN/340 mM Na_2SO_4 in H_2O/TFA
Flow Rate: 0.5 mL/min
Temperature: 30 ℃
Injection: 20 μL
Detection: UV 214 nm
Sample: Exenatide Injection

R.T. (min)	Theoretical Plate (USP)	Tailing Factor (USP)	Resolution (USP)
18.413	20924	1.25	1.65

Terlipressin is a peptide-like pro-drug to improve kidney function in adults. Terlipressin and impurity can be well separated on a BioCore SEC-150 column, as shown in Figure 12.

Terlipressin

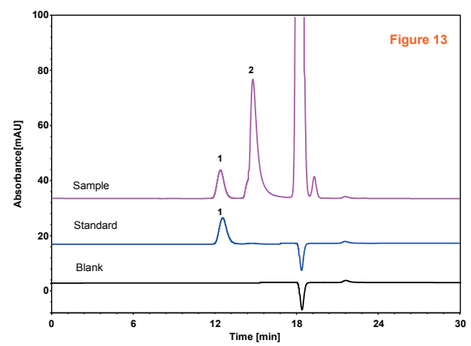


Column: **BioCore SEC-150**, 5 μm
Dimension: 7.8×300 mm
Mobile Phase: 100 mM Na_2SO_4 in 100mM phosphate buffer
Flow Rate: 0.5 mL/min
Temperature: 30 ℃
Injection: 10 μL
Detection: UV 274 nm
Peaks: 1. Impurity U
2. Terlipressin

R.T. (min)	Theoretical Plate (USP)	Tailing Factor (USP)	Resolution (USP)
23.080	29589	1.19	3.63

Poloxamer 188 (P188) is a nonionic triblock-copolymer surfactant widely used in producing biological formulations to protect proteins. The residual P188 in final drug product may cuase adverse reactions in human body. The BioCore SEC-150 can be used to determine the content of P188 in the formulation, free of interference from the API (protein) and other components in the sample (see Figure 13). and other components including protein are well separated. This method is well suited for quality control of protein-based formulations.

Poloxamer 188 (P188) in Protein Solution



Column: **BioCore SEC-150**, 5 μm
Dimension: 7.8×300 mm
Mobile Phase: 10 mM ammonium acetate solution, pH5.2
Flow Rate: 0.6 mL/min
Temperature: 25 ℃
Injection: 20 μL
Detection: RID
Peaks: 1. Poloxamer 188 (P188)
2. Protein

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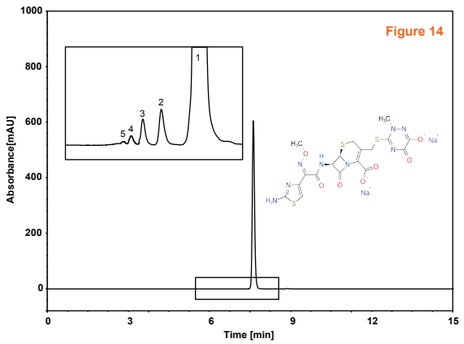
BioCore SEC-120, for small molecule drugs, peptides and heparins

BioCore SEC-120 columns use silica particles with 120 Å pore size, suited for lower molecular weight compounds, such as polymers in small molecule drugs, peptides, heparins, etc.

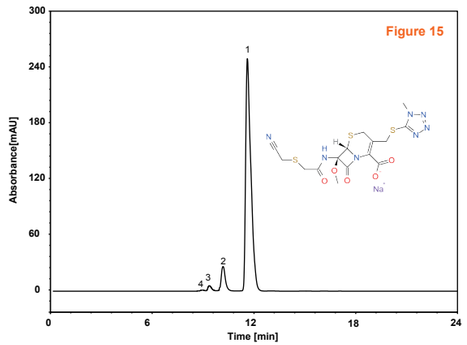
Ceftriaxone and cefmetazole are belong to cephalosporins which widely used to manage infections from gram-positive and gram-negative bacteria. In the process of synthesis, storage and transportation, it is inevitable to form polymers (aggregates) which may cause adverse reactions to patients. Figures 14 and 15 demonstrate that the BioCore SEC-120 can provide excellent separation for not only polymers and the drug molecule, but also among polymers themselves.

Ceftriaxone Sodium



Column: **BioCore SEC-120**, 5 µm
Dimension: 7.8×300 mm
Mobile Phase: 5 mM phosphate buffer, pH7.0
Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 2 µL
Detection: UV 231 nm
Peaks: 1. Ceftriaxone
2~5. Polymers of Ceftriaxone

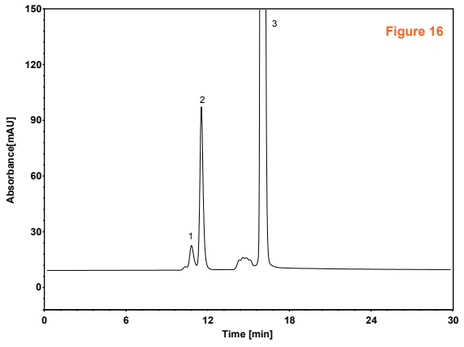
Cefmetazole Sodium



Column: **BioCore SEC-120**, 5 µm
Dimension: 7.8×300 mm
Mobile Phase: 90/10 v/v 5 mM phosphate buffer, pH7.0/MeCN
Flow Rate: 0.6 mL/min
Temperature: 25 ℃
Injection: 10 µL
Detection: UV 254 nm
Peaks: 1. Cefmetazole
2~4. Polymers of Cefmetazole

Figure 16 illustrates a separation of a small **peptide** (M.W.~5 kDa) and its aggregates on a BioCore SEC-120 column.

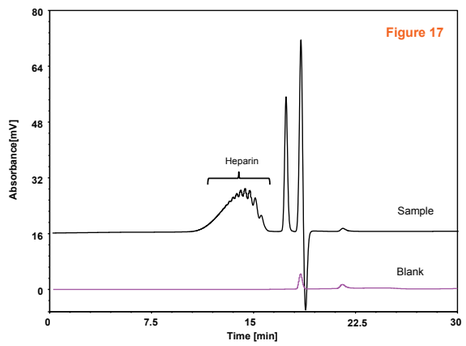
Peptide



Column: **BioCore SEC-120**, 5 µm
Dimension: 7.8×300 mm
Mobile Phase: 40/60 v/v MeCN/0.1% TFA in H_2O
Flow Rate: 0.7 mL/min
Temperature: 30 ℃
Injection: 20 µL
Detection: UV 276 nm
Peaks: 1. Aggregate of Peptide
2. Peptide
3. M-Cresol

Low molecular weight heparin is widely used in the prevention and treatment of venous thromboembolism. The assay on its molecular weight and distribution is important as it affects therapeutic effect. Figure 17 demonstrates the chromatographic profile of a low molecular weight heparin generated on a BioCore SEC-120 column, which can be used for calculating the molecular weight distribution of heparin in the sample.

Low Molecular Weight Heparin



Column: **BioCore SEC-120**, 5 µm
Dimension: 7.8×300 mm
Mobile Phase: 100 mM ammonium acetate solution
Flow Rate: 0.6 mL/min
Temperature: 35 ℃
Injection: 20 µL
Detection: RID, 40 ℃
Peaks: Heparin

Quality Assurance

Each batch of separation media is produced in accordance with a strict quality management system and tested with relevant biological molecules to ensure separation performance and batch-to-batch consistency.

Each BioCore column is produced by well-developed packing methods and tested individually using well-designed chromatography tests, to ensure quality and consistency. A certificate of assurance of the separation media and a column quality assurance report are shipped with every shipped column.

Ordering Information

Particle Size (µm)	Column Dimension L x ID (mm)	Product Name			
		BioCore SEC-120	BioCore SEC-150	BioCore SEC-300	BioCore SEC-500
5	300×4.6	B213-050012-04630S	B213-050015-04630S	B213-050030-04630S	B213-050050-04630S
	150×4.6	B213-050012-04615S	B213-050015-04615S	B213-050030-04615S	B213-050050-04615S
	50×4.6	B213-050012-04605S	B213-050015-04605S	B213-050030-04605S	B213-050050-04605S
	300×7.8	B213-050012-07830S	B213-050015-07830S	B213-050030-07830S	B213-050050-07830S
	150×7.8	B213-050012-07815S	B213-050015-07815S	B213-050030-07815S	B213-050050-07815S
3	300×4.6	B213-030012-04630S	B213-030015-04630S	B213-030030-04630S	B213-030050-04630S
	150×4.6	B213-030012-04615S	B213-030015-04615S	B213-030030-04615S	B213-030050-04615S
	50×4.6	B213-030012-04605S	B213-030015-04605S	B213-030030-04605S	B213-030050-04605S
	300×7.8	B213-030012-07830S	B213-030015-07830S	B213-030030-07830S	B213-030050-07830S
	150×7.8	B213-030012-07815S	B213-030015-07815S	B213-030030-07815S	B213-030050-07815S
1.8	300×4.6	B213-018012-04630S	B213-018015-04630S	B213-018030-04630S	B213-018050-04630S
	150×4.6	B213-018012-04615S	B213-018015-04615S	B213-018030-04615S	B213-018050-04615S
	50×4.6	B213-018012-04605S	B213-018015-04605S	B213-018030-04605S	B213-018050-04605S

NanoChrom