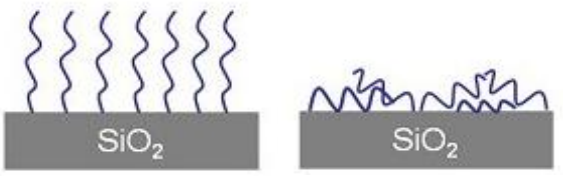
Sepax Unix™-C 1.8 µm UHPLC SEC Column

Ultra-High Efficiency and Resolution Size Exclusion Chromatography

General Description

Utilizing proprietary innovative surface technologies, the Unix™-C SEC-300 phase is made of uniform, hydrophilic, and neutral nanometer thick films chemically bonded to high purity and mechanically stabilized silica with a particle size of 1.8 µm. The combination of small particle size and large pore volume of the Unix™-C SEC-300 renders the highest separation efficiency and resolution of analytes. The well-controlled surface chemistry results in excellent lot-to-lot reproducibility. The unique bonding chemistry, coupled with maximized density, allows the Unix™-C SEC-300 to provide high stability and negligible non-specific interactions.

Unix™-C, uses lay-down monolayer on porous silica (as shown below), offers ideal phase chemistry for the separation of all biological sample types including challenging hydrophobic samples such as antibody drug conjugates (ADCs), hydrophobic mAb and its fragments, bispecific mAbs (BsMAbs), PEGylated proteins, glycoproteins, fusion proteins, membrane proteins and oligonucleotides.



Unix™ Phase
Stand-up Monolayer

Unix™-C Phase
Lay-down Monolayer

Featured Characteristics

- Particle size of 1.8 µm with a pore size of 300 Å
- Ultra-high separation efficiency, recovery, and resolution
- High stability and lot-to-lot reproducibility
- Innovative surface chemistry for minimized secondary interactions
- Reduced dependency on organic additives for hydrophobic samples
- Ideal for all sample types including challenging hydrophobic samples like ADCs, bsMAbs, PEGylated proteins, glycoproteins, fusion proteins, membrane proteins, and oligonucleotides.
- Great tool for universal platform high throughput method screening (HTS)
- Compatible with both UHPLC and HPLC

Technical Specifications

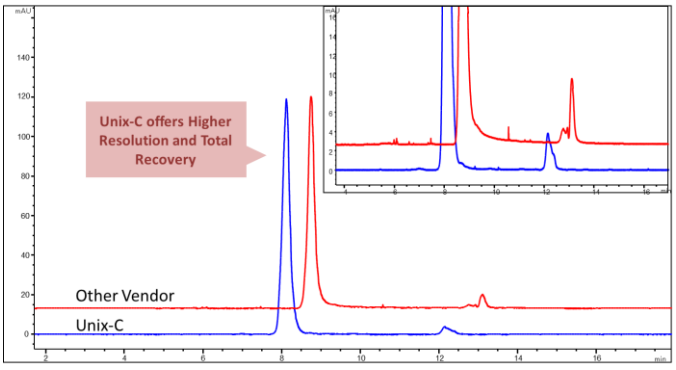
Phase	Unix™-C SEC-300
Material	Neutral, hydrophilic film bonded to silica
Average particle size	1.8 µm
Pore size	300 Å
Protein MW range (native)	5,000 – 1,250,000 Da
pH Stability [1]	2.0 – 8.5
Mobile phase compatibility	Aqueous and organic
Suggested salt concentration	< 0.5 M
Recommended flow rate range for maximum column lifetime	0.1 - 0.35 mL/min
Recommended operating column backpressure for maximum column lifetime	< 4,500 psi
Suggested operating temperature for optimal column lifetime	10 – 30 °C

[1] Store the column in neutral pH

Antibody Drug Conjugates Separation

Sepax Unix™-C UHPLC SEC-300 column offers better separation on antibody drug conjugates with higher resolution and recovery under native conditions without organic additives (Figure 1).

Figure 1. Cysteine ADC Separation – Unix™-C vs Competitor

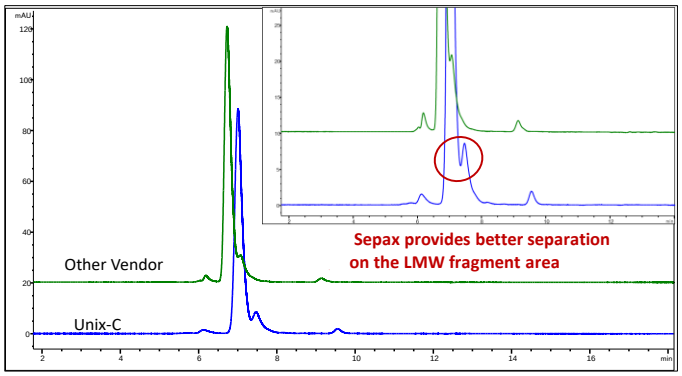


Column: Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm)
Sub 2 µm UPLC SEC column from other vendor (4.6 x 300 mm)
Mobile Phase: 150 mM sodium phosphate buffer, pH 7.0
Injection: 10 µL
Flow Rate: 0.3 mL/min
Instrument: UHPLC
Detection: UV 280 nm
Temperature: Ambient
Sample: 1 mg/mL ADC

Fusion Protein Separation

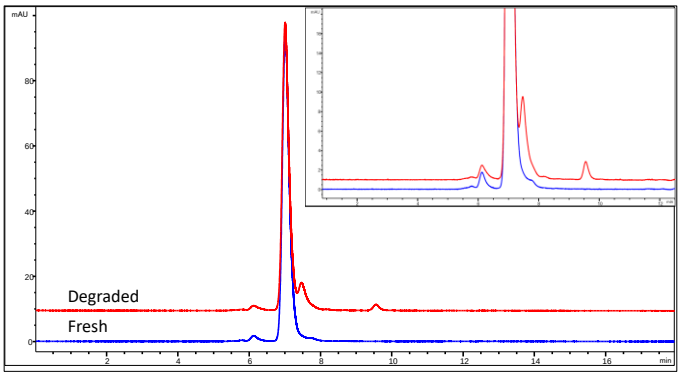
Sepax Unix™-C provides better separation on the LMW fragment area of degraded Enbrel fusion protein (Figure 2).

Figure 2. Degraded Enbrel Separation - Unix™-C vs. Competitor



Column:	Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm) Sub 2 µm UPLC SEC column from other vendor (4.6 x 300 mm)
Mobile Phase:	150 mM sodium phosphate buffer, pH 7.0
Injection:	0.5 µL
Flow Rate:	0.3 mL/min
Instrument:	UHPLC
Detection:	UV 280 nm
Temperature:	Ambient
Sample:	25 mg/mL Enbrel degraded

Figure 3. Enbrel Separation on Unix™-C: Fresh vs. Degraded

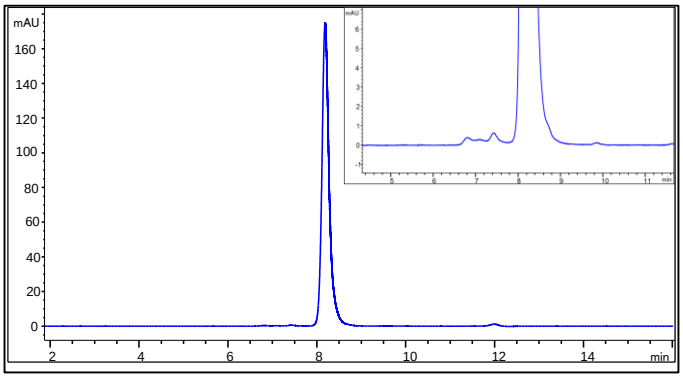


Column:	Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm)
Mobile Phase:	150 mM sodium phosphate buffer, pH 7.0
Injection:	0.5 µL
Flow Rate:	0.3 mL/min
Instrument:	UHPLC
Detection:	UV 280 nm
Temperature:	Ambient
Sample:	25 mg/mL Enbrel fusion protein

Monoclonal Antibody Separation

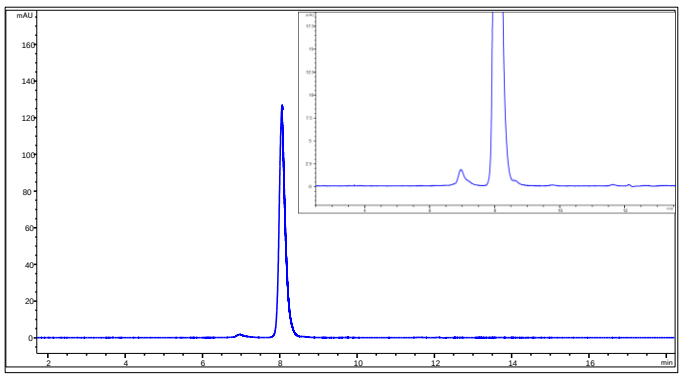
Sepax Unix™-C provides ultra-high resolution and efficiency mAb separation (Figure 4-5).

Figure 4. Rituximab Separation on Unix™-C SEC-300



Column:	Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm)
Mobile Phase:	150 mM sodium phosphate buffer, pH 7.0
Injection:	1 µL
Flow Rate:	0.3 mL/min
Instrument:	UHPLC
Detection:	UV 280 nm
Temperature:	Ambient
Sample:	10 mg/mL Rituximab

Figure 5. Erbitux Separation on Unix™-C SEC-300

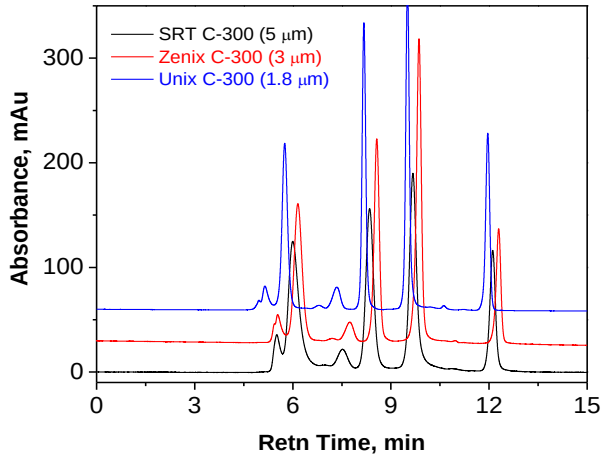


Column:	Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm)
Mobile Phase:	150 mM sodium phosphate buffer, pH 7.0
Injection:	5 µL
Flow Rate:	0.3 mL/min
Instrument:	UHPLC
Detection:	UV 280 nm
Temperature:	Ambient
Sample:	2mg/mL Erbitux

High Separation Efficiency

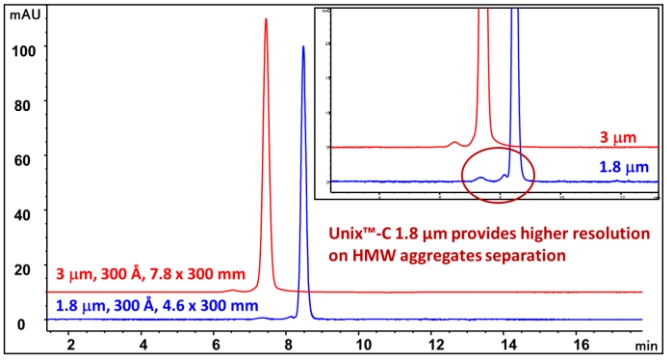
With smaller particle size, Unix™-C 1.8 µm SEC offers higher efficiency and higher resolution (Figure 6-7).

Figure 6. Comparison of Unix™-C (1.8 µm), Zenix®-C (3 µm) and SRT®-C (5 µm) SEC-300 on Protein Separation



Column:	Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm) Zenix®-C SEC-300 (3 µm, 300 Å, 4.6 x 300 mm) SRT®-C SEC-300 (5 µm, 300 Å, 4.6 x 300 mm)
Mobile Phase:	150 mM sodium phosphate buffer, pH 7.0
Injection:	3 µL
Flow Rate:	0.35 mL/min
Instrument:	UHPLC
Detection:	UV 214 nm
Temperature:	Ambient
Sample:	1. Thyroglobulin, 670 kD 2. BSA dimer, 132 kD 3. BSA, 66 kD 4. Ribonuclease A, 14 kD 5. Uracil, 120 Da

Figure 7. Comparison of Unix™-C (1.8 µm) and Zenix®-C (3 µm) on Vectibix IgG2 Separation

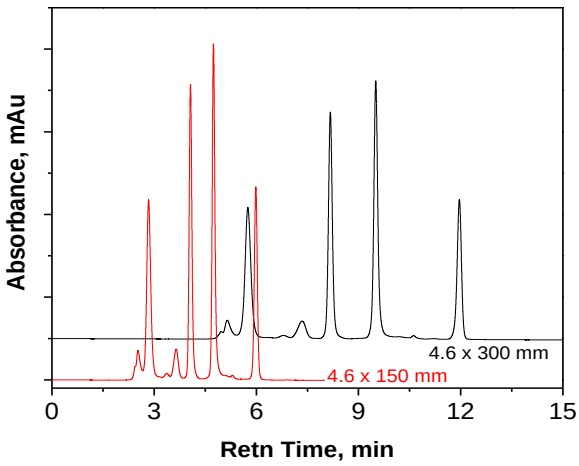


Column:	Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm) Zenix®-C SEC-300 (3 µm, 300 Å, 4.6 x 300 mm)
Mobile Phase:	150 mM sodium phosphate buffer, pH 7.0
Injection:	1 µL
Flow Rate:	0.3 mL/min
Instrument:	UHPLC
Detection:	UV 280 nm
Temperature:	Ambient
Sample:	20 mg/mL Vectibix IgG2

Shorter Dimension for a Fast Separation

Unix™-C SEC-300 column is available in the shorter dimension of 4.6 x 150 mm, allowing for faster separation (Figure 8).

Figure 8. Impact of dimension on separation of Protein Standards: Unix™-C SEC-300 4.6 x 150 vs. 4.6 x 300 mm



Column:	Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 150 mm) Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm)
Mobile Phase:	150 mM sodium phosphate buffer, pH 7.0
Injection:	3 µL
Flow Rate:	0.35 mL/min
Instrument:	UHPLC
Detection:	UV 214 nm
Temperature:	Ambient
Sample:	1. Thyroglobulin 670 kD; 2. BSA dimer 132 kD; 3. BSA 66 kD; 4. Ribonuclease A 14 kD; 5. Uracil 120 Da

High Stability

Application of proprietary surface modification techniques to the Unix™-C SEC resin produces a densely bonded silica surface, which greatly hinders the diffusion of molecules that would attack the bond between the silica surface and tethered moieties, thus enabling high stability over a wide range of pH from 2.0 to 8.5.

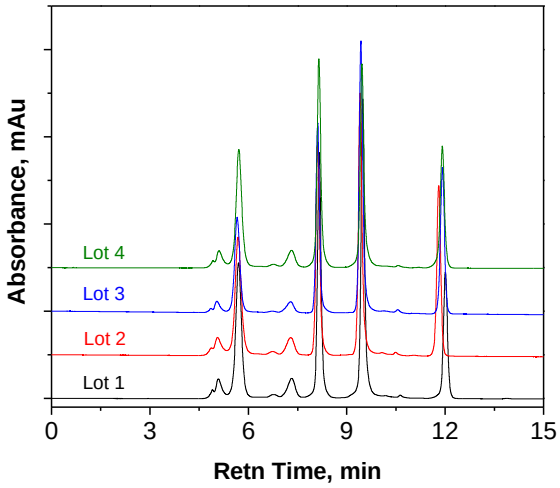
Mobile Phase Compatibility

The Unix™-C SEC-300 phase is compatible with most aqueous buffers, including ammonium acetate and phosphate, and can tolerate a high concentration of salts. Furthermore, Unix™-C SEC columns are stable in organic solvents (such as methanol, ethanol, THF, DMF, DMSO, etc.) as well as a mixture of water and organic solvents.

Lot-to-Lot Reproducibility

The controlled surface chemistry used to synthesize the Unix™-C SEC-300 phase makes the surface coating highly reproducible, leading to consistent column manufacturing. Separation variation from batch-to-batch is controlled to be within 5% for retention time (Figure 9).

Figure 9. Unix™-C Lot-to-Lot Reproducibility on Protein Standards



Column: Unix™-C SEC-300 (1.8 µm, 300 Å, 4.6 x 300 mm)
Mobile Phase: 150 mM sodium phosphate buffer, pH 7.0
Injection: 1 µL
Flow Rate: 0.35 mL/min
Instrument: UHPLC
Detection: UV 214 nm
Temperature: Ambient
Sample: 1. Thyroglobulin, 670 kD 2. BSA dimer 132 kD 3. BSA 66 kD 4. Ribonuclease A, 14 kD

Additional resources at www.sepax-tech.com

Creation of a customer account will provide you with:

- Access to application notes of your interested sample type
- Method development training webinars
- Up-to-date listing of where our columns have been cited in the scientific literature
- Easy way to view prices, request quotes and order products

Ordering Information

Unix™-C SEC-300 (1.8µm 300 Å)	
231300-4615	Unix™-C SEC-300, 1.8 µm, 300 Å. 4.6 x 150 mm
231300-4630	Unix™-C SEC-300, 1.8 µm, 300 Å. 4.6 x 300 mm
Precolumn Filters and Frits for Unix™-C SEC	
102000-P346	Precolumn Filter with 0.5 um stainless steel frit
102001-P346	0.5 um stainless steel refill frits (for Precolumn Filter)
Protein Standards for Unix™-C SEC	
201002-0000	Protein standard for Size Exclusion Chromatography and used for general correlation of elution time vs. protein MW (5 vials, 50ul/vial): -Thyroglobulin (1 mg/mL) -BSA (1 mg/mL) -Ribonuclease A (1 mg/mL) -Uracil (0.1 mg/mL)

Column Screening & Method Development Service

- Various column phases including SEC, IEX, HIC and RP from Sepax and other vendors available for screening
- Different buffer systems including mobile phases and gradients for development and optimization
- Cost effective and quick turnaround solutions
- Eliminate uncertainty, accomplish projects with higher success rates

Please contact techsupport@sepax-tech.com for further information or call toll free 1-887-SEPAX-US (option 3) to speak with our technical support team.

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