Sepax SRT-10C SEC Media for High Throughput Preparative Size Exclusion Chromatography

Size Exclusion Chromatography (SEC) is a chromatographic technique that allows for separation of molecules based on their hydrodynamic size. In addition to being a common method for analytical characterization and quality control analysis of biological drug candidates, SEC is also a technique well employed in downstream biomolecule preparative purification (more commonly aggregate removal). Sepax Technologies, Inc. is a leading manufacturer of liquid chromatography (LC) related resins, consumables, and columns. The Sepax SRT-10C SEC column for Fast Purification, offers a robust, hydrophilic surface modified rigid silica matrix that allows for faster flow rates translating to shorter run times.

In the following paper, *Development of a robust and semi-automated two-step antibody purification process*, recently published by Eli Lilly, Yang et al. highlight the advantages of the Sepax SRT-10C SEC column chemistry and propose a quick but reliable cleaning method for Endotoxin removal.

Literature reference: Yang, Xiaomin, et al. "Development of a robust and semi-automated two-step antibody purification process." Mabs. Vol. 13. No. 1. Taylor & Francis, 2021.

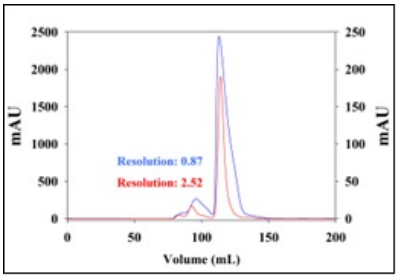
Table 1. Performance comparison of SRT-10 C SEC-300 compared to superdex 200 column.

Parameter / Results Column	Loading Vol. (mL)	Flow Rate (mL/ min)	Running Time (min)	Resolution*	Effluent Vol (mL)	Effluent Vol Increase	Yield %	aSEC Purity %
Superdex 200	6	1.5	80	1.05	16.0	2.7-fold	76.3	97.1
(16 x 600mm, 120 mL)	6	1.5	80	1.05	16.0	2.7-fold	75.9	97.1
SRT-10 C SEC-300 (30 x 300mm, 210 mL)	9	7.5	40	1.08	25.5	2.8-fold	79.1	97.1
	9	7.5	40	1.07	25.5	2.8-fold	79.5	97.0

*Resolution was calculated from [(Volume $_{peak2}$ – Volume $_{peak1}$)/((Width $_{1/2, peak2}$ + Width $_{½, peak1}$)/2)] by UNICORN software in AKTA pure.

Table 1, Sepax SRT-10C SEC-300 column showed the advantages of faster flow rate, shorter running time without sacrificing the resolution, yield and % purity of the antibody sample.

Figure 1 below illustrates that high resolution is achieved as per the red trace below. Based on the conditions outlined below, it is conceivable that 1-2% CV loading with a sample range of 2-7 mg/mL at 5 mL/min could be used to maximize loading while not drastically effecting resolution (or provide sufficient purification based on a acceptable range) .The blue trace below represents extreme conditions with a 25X load at nearly double the flow rate.



Overlaid chromatograms from the pSEC step representing the extremes of observed resolution. For the sample with good resolution (red), the loading volume was 2mL, at a concentration of 2mg/mL, and ran at a flow rate of 5mL/min. The sample with poor resolution (blue) used a loading volume of 8mL at a concentration of 12mg/mL, and was ran at a flow rate of 10mL/min. The resolution between monomer and aggregates as calculated by UNICORN software is shown.

From Table 2, endotoxin contamination was significantly reduced to less than 0.5 EU/mL in washes which IPA concentration was greater than 25%. Prep SEC column can be washed and stored in 25% IPA during routine operation and completely cleaned with 70% IPA in instances of endotoxin contamination.

Table 2. Endotoxin reduction following clean-in-place using various concentrations of isopropyl alcohol (IPA).

Recovery % IPA%	Pre-IPA Protein Recovery%	Pre-IPA Endotoxin Recovery%	Post-IPA Protein Recovery%	Post-IPA Total EU	Endotoxin Reduction %
0	95	107	98	220*	N/A
10	101	131	98	121	45
25	99	83	103	11	95
50	99	97	100	2	99
70	97	102	100	5	98

*baseline endotoxin level, which was used to calculate the endo removal%.

Endotoxin Wash: To establish an effective cleaning procedure for the SRT-10C column with IPA aqueous solution, a purified control antibody at 2 mg/mL alone and a mixture of antibody with bovine thyroglobulin at 3 mg/ml containing 3137 EU/mL endotoxin were used for testing. After the antibody/thyroglobulin mixture passes through the column, it was first washed with one CV of 70% IPA solution followed by one CV of PBS before injecting the pure antibody sample. The eluents from both injections were collected for concentration and endotoxin measurement; the protein and endotoxin recovery were calculated relative to the loading amount. This experiment

Full Literature Review

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Part Number: 239300-30030

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