

Packing of Sephacryl™ S-500 in ReadyToProcess™ columns and evaluation of performance in a vaccine process

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Vaccine purification processes often involve processing through a soft chromatography resin operation based on size exclusion. Sephacryl™ S-500 HR is an example of a size exclusion resin that is used for vaccine processing. To prevent addition of bioburden, the process is kept closed without exposure to the outside. This also protects operators from being exposed to situations where the target is harmful. Closed, disposable products enable a better control of bioburden and remove the need to open and clean surfaces that have been exposed to harmful substances.

An example of this is the repacking of a chromatographic column in a process with an active virus. The requirement for cleaning validation is also removed. This poster presents the results from the development of a packing method that generates a stable bed in disposable ReadyToProcess™ column, and confirmation of column performance in a vaccine process. To generate a column bed height suitable for size exclusion chromatography, two, 20 cm disposable columns were connected in series.

Column packing method and evaluation at Cytiva

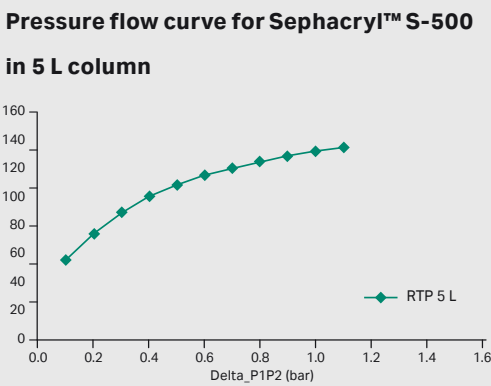
The Sephacryl™ S-500 resin is sensitive to mechanical compression that is required to set the ReadyToProcess™ column and make it stable for transportation. A packing method was developed and tested for flow stability and transportation.

The column was efficiency tested in 20% ethanol, where a sample of 0.02× CV containing 2.5% acetone was passed through the column at 30 cm/h.

Results

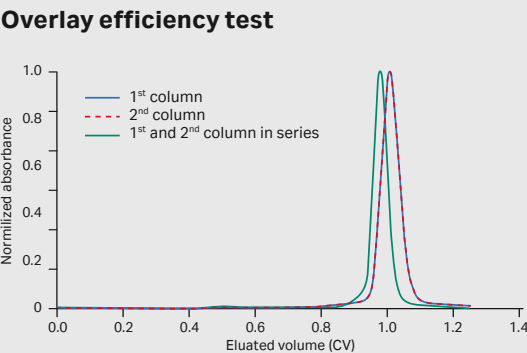
ReadyToProcess 5 L column

Column	1	2	3
Efficiency test (plates/m)	7473	7799	7655
Asymmetry (-)	1.12	1.17	1.12
Stability test pressure (bar)	0.4	0.5	0.6
Efficiency test post stab. (plates/m)	9494	7643	5912 (Fail)
Asymmetry post stab. (-)	1.05	1.11	0.88



ReadyToProcess™ 1 L column

Column	4	5	6	7	6 and 7 in series
Efficiency test (plates/m)	7535	8528	7493	7377	5824
Asymmetry (-)	1.07	1.31	1.27	1.15	1.11
	Stability test pressure: 0.05 Mpa (0.5 bar)	Transportation testing	Columns used in process evaluation		
Efficiency test post stab./transport test (plates/m)	7468	7709	NA	NA	NA
Asymmetry post stab. (-)	1.09	1.31	NA	NA	NA



Column packing evaluation at Amgen

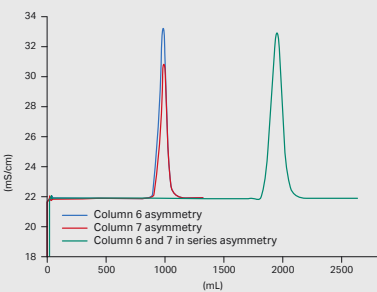
The ReadyToProcess™ columns were transported from Cytiva in Uppsala, Sweden to Amgen Woburn, USA.

The column efficiency was tested by Amgen in phosphate buffer containing 6% w/v sugars, where a sample of 0.02× CV buffer containing 0.6 M sodium chloride was passed through the column at 20 cm/h.

Results

ReadyToProcess™ 1 L column

Column	6	7	6 and 7 in series
Efficiency test (plates/m)	6611	6764	4621
Asymmetry (-)	0.81	0.95	0.96
HETP	0.015	0.015	0.022



Evaluation parameters met acceptance criteria

Column performance evaluation

The ReadyToProcess™ columns were loaded, in-series, with 14% CV of cation exchange chromatography purified virus feedstock at a 20 cm/h flow rate. The resulting eluate was collected and analyzed for virus and impurity content.

	Volume (mL)	Active virus yield (PFU)	Virus protein yield (mg)	Bovine serum albumin (ng/mL)	Host cellular proteins (ng/mL)	AUP (mL × mAU)
SEC load	291	8.53 × 10 ¹¹	744.96	< 25	267	145 509
SEC eluate	405	7.00 × 10 ¹¹	635.85	< 25	98	121 742
Acceptance criteria	NA	2.98 × 10 ¹⁰ to 2.19 × 10 ¹³	NA	< 50	< 315	NA
Step recovery	NA	82.0%	85.4%	NA	NA	83.7%

Summary

It is possible to pack Sephacryl™ S-500 in ReadyToProcess™ columns. Packed beds are flow stable up to 0.05 MPa (0.5 bar). A transportation test for the ReadyToProcess™ 1 L column passed acceptance criteria. Individual ReadyToProcess™ columns fulfill efficiency criteria well, but when run in series, the rather crude flow distribution system affects efficiency in a

negative way. The serial coupled columns, however, still fulfilled Amgen's requirements.

Use of the ReadyToProcess™ Sephacryl™ S-500 columns for virus vaccine purification produced comparable performance data to Amgen in-house packed columns.

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