PROCESS-SCALE CHROMATOGRAPHY

Tech note 0365

Packing Cytiva Source™ 15Q in VERDOT® InPlace™ column

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Summary

This tech note presents the results of a packing study of Source™ 15Q from Cytiva™ in VERDOT's InPlace columns. This packing study was performed with an InPlace column D596mm inner diameter. The packing method used was priorily developed with Source™ 30 D296mm inner diameter with 5cm bed, and hence makes this tech note applicable for both media.

This chromatography media is a synthetic chromatography strong anion exchanger for polishing steps based on a monosized 15 µm rigid polystyrene/divinyl benzene polymer matrix.

VERDOT InPlace® columns offer a unique solution for packing all media. One of the key features of VERDOT's InPlace column is the InPlace slurry valve, which enable transfer of media into the column in a syringe mode, and removal of under slight positive air pressure. The unique placement of the InPlace slurry valves around the perimeter of the column guarantees no interference with the distribution of liquid in the column or flow dynamics. The patented low-shear design of the InPlace valve minimizes damage to media.

InPlace columns from VERDOT utilize an axial compression method to consolidate chromatography media as opposed to packing by pressure often used for packing this media. With the InPlace column, the motor itself mechanically compresses the media to its target height, set with the compression factor.

Materials and slurry preparation

Material and Equipment

The VERDOT Ips² InPlace column was mounted with filters with ≤5µm absolute porosity, which is suitable for the 15µm particle size of the media. For automated operations, the InPlace column is fitted with an instrumentation package that includes a rotary encoder for precise positioning of the piston and a pressure sensor transmitter for monitoring packing conditions.

In addition to this, an InPlace Advanced Control Console, designed to control the speed and positioning of the top adapter was used for this packing study.

Slurry preparation

The slurry was prepared by performing a buffer exchange in the column from storage buffer to packing buffer, then was slurried in the column with sparged air. The slurry percentage was estimated via two methods: first, by centrifuge and second, using the rebound height after bed consolidation. Based on these two factors, the settled bed height was estimated to be 12.2 cm.

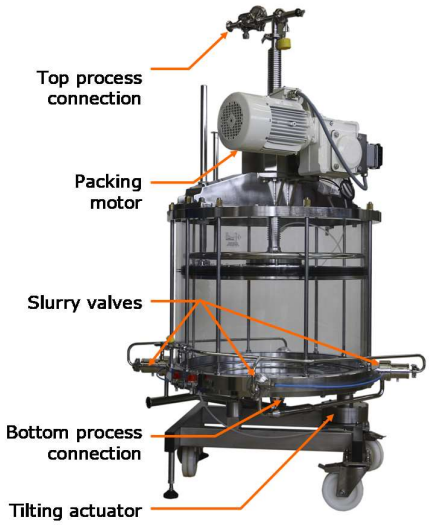

Figure 1 : VERDOT Ips² InPlace column

Packing procedure

The media was slurried in place with air sparging at approximately 40% for a minimum of 30 minutes. Once the air sparging was stopped, a brief upflow was performed to remove air from the bottom of the column. The media was then allowed to settle until a small supernatant layer was visible (approximately 2-3 mm). The top headplate was lowered into the supernatant and the column was tilted to remove the bubble.

The seal was then inflated to 4 Bar and the top piston was lowered at 50 cm/hr with the bottom process outlet closed and the top process inlet open in order to purge the top assembly of air. Once clear of air, the bottom process outlet was opened and the top process inlet was closed.

The piston was lowered at 50 cm/hr to consolidate the bed. The bed compression was calculated with Compression Factor (CF):

$$CF = \frac{\text{Settled bed height}}{\text{Packed bed height}}$$

This configuration allowed the bed to build from the bottom to the top while avoiding bed drying. This bed building is visible as the front line of the consolidated bed, slightly whiter, raised from bottom to top until it reached the slurry front line where the bed became fully consolidated.

The maximum pre-column pressure observed during the packing process was 2.4-2.5 Bar at 50 cm/hr, which is within the normal operating parameters of the standard InPlace column.

Flow conditioning

For flow conditioning of the column, two columns volume of packing buffer was injected in upflow at 50 cm/hr followed by two column volumes in downflow at 50 cm/hr. Then a short pressure/flow test was conducted with three setpoints of 25cm/hr, 50cm/hr, and 69cm/hr, to keep the column service pressure below 4bar.

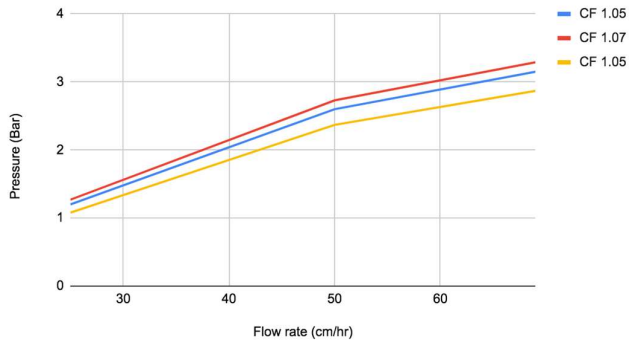

Figure 2 : Pressure vs flow curve

Results and discussion

For evaluating the column performance after packing, HETP and Asymmetry test, were performed. This test consists of evaluating the distortion of a marker pulse injected after its residence time through the column.

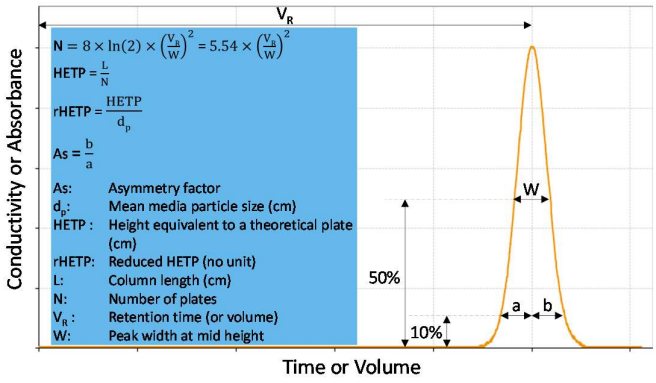

Figure 3: HETP and asymmetry test

The marker used was 20% EtOH + 300 mM NaCl, corresponding to 1% Column volume. The test was performed at 50cm/h.

A first test campaign was performed with a used resin, which explains the reduced performance. However it was beneficial for optimizing the compression factor.

Pack Number	Bed Height	Compression Factor	Asymmetry	N/m
1	11.6	1.05	0.75	7371.1
2	11.4	1.07	0.72	7284.5
3	11.6	1.05	0.95	7747.2
4	11.7	1.04	1.26	8558.9

Packing #2 indicated that the bed was overcompressed as evidenced by the fronting asymmetry value. The Compression Factor of 1.05 was found as optimal, as it provided good asymmetry results, and allowed a good filling of the outer edge below the top adaptor.

A second test campaign was performed with the same D596mm VERDOT InPlace column and with the same packing procedure but with new media. The tests were performed in triplicate. The same Compression Factor of 1.05 was used for the 3 tests.

	Test 1	**Test 2**	**Test 3**	**Average**
Asymmetry	1.00	1.64	1.50	1.38
HETP (µm)	108	79	73	87
Bed Height (cm)	13.1	13.4	13.3	13.3
N/m	9288	12657	13675	11873
HETP (um)	108	79	73	87
Reduced HETP	7.2	5.3	4.9	5.8

Conclusion

Packing speed at 50 cm/hr provided a rapid enough consolidation without exceeding the pressure rating of the column. The speed of consolidation is not expected to have a measurable impact on the quality of the packing.

The major influencing factor for asymmetry and HETP is the compression factor, where a small change can have a major impact.

As conclusion, these tests confirmed that the Source™ 15 and Source™ 30 media combined with VERDOT InPlace column can provide a performant chromatography bed. The axial compression method controlled by height is adapted providing that the media volume is accurately known or that the column tube is transparent. The packed bed height can hence be calculated based on the actual settled bed height.