

PROCESS-SCALE CHROMATOGRAPHY

Tech note 0345

Packing Bio-Rad Nuvia™ HR-S media in the Verdot InPlace™ column

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Summary

This tech note presents the packing and unpacking capabilities of the Verdot Ips² InPlace column, using Bio-Rad Nuvia™ HR-S media⁽¹⁾.

VERDOT InPlace columns offer a unique solution for packing media such as Bio-Rad Nuvia™. One of the key features of the InPlace column is the slurry valves. The slurry valves can be used to transfer media into the column in a syringe mode, or to transfer media out of the column under slight air pressure. The valves being situated on the column wall, do not interfere with flow dynamics. The other key feature of the InPlace column is the possibility to combine air-sparging and tilting of the column for minimizing the amount of buffer needed for bed reslurrying and unpacking.

During the packing study, a 16.8cm bed of Bio-Rad Nuvia™ HR-S media was packed in a 80 cm diameter Verdot Ips² InPlace column, using a compression factor (CF) of 1.2. The packing was evaluated using HETP and Asymmetry tests and provided great results. Prior to removal, air sparging and the InPlace columns tilting feature were used to re-suspended the media within the column in less than 20 minutes. Through the column slurry valves, all media was transferred into the slurry tank in less than 5 minutes.

Materials and Methods

Material and Equipment

The VERDOT Ips² InPlace column can be equipped with filters with 25µm absolute porosity and an instrumentation package for semi-automated operations. Instrumentation package includes a rotary encoder and pressure transmitter. Column isolation valves are installed above the pressure transmitter and on the bottom process connection. The packing study involved an InPlace column 80 cm diameter, 60cm tube height in Acrylic.

The Piping & Instrument diagram (P&ID) in figure 2 shows the configuration for packing and unpacking. The numbering seen in the diagram will be used later in the instructions' section. The process systems are usually more equipped than seen on this P&ID.

In addition to this, a Basic Control Console or an automated Advanced Control Console is required to drive the packing motor. The VERDOT Ips² packing station can also be used for fully automated packing and unpacking.

Slurry

Carboys of media stored in 20% ethanol are poured into a slurry tank and exchanged 1x with packing buffer. During the packing study, packing buffer was 0.4M NaCl.

A slurry of 50% (v/v) is prepared and kept under suspension with agitator.



Fig.1. VERDOT Ips² InPlace column

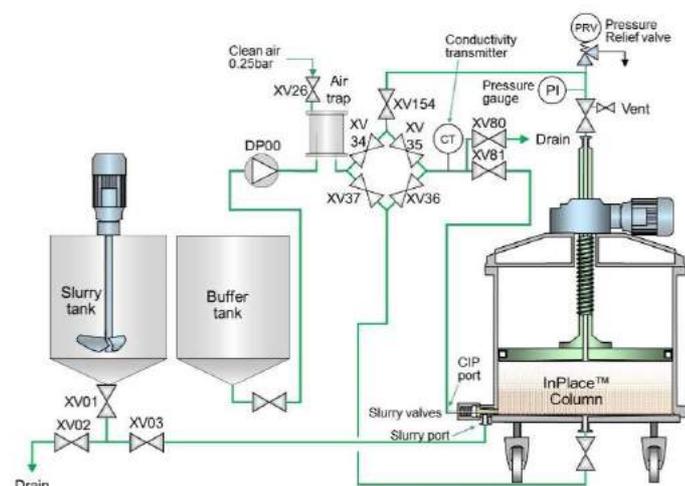
Media transfer

The topside of the columns top adapter is wiped clean to ensure that no debris or particulates are present. The column is leveled using leveling adjustments on column.

Column priming

The slurry valves being closed, their CIP ports and slurry ports are connected. Using DP00, the slurry valve manifold and transfer hoses are primed by injecting packing buffer through XV81, flowing through XV03 and XV02.

Fig. 2 P&ID of the installation for packing and unpacking



⁽¹⁾ Bio-Rad Nuvia™ HR-S is a Trade mark of Bio-Rad Laboratories

The slurry valves are opened and the column is filled with packing buffer to a height of 15cm. The slurry valves are cycled (opened and closed) to release any potential air that could be caught in the lines. The column is degassed by using the tilting actuator and by introducing packing buffer into the column until the inflatable seal is fully submerged. The inflatable seal is inflated and the column tilting actuator is disengaged, placing the column back into the level position. The top isolation valve is opened to the drain position (XV154-35-80). Using the control console, the top adapter is lowered at a speed of 200cm/h for 5cm in order to prime the top process line. Once primed, the top isolation valve is immediately closed. Conversely, the bottom process line is primed by opening the bottom process isolation valve to the drain position (XV36-80) and lowering the top adapter at a speed of 200cm/h until it is positioned approximately 5cm above the bottom frit. Once primed, the bottom isolation valve is immediately closed. The topside of the columns top adapter is wiped clean to ensure that no NaCl is left.

Media transfer via syringe method

Both the bottom slurry tank valve (XV01-03) and the column slurry valves are opened. Media transfer from slurry tank to column is performed using the syringe method. The top adapter, initially positioned at a height of 5 cm above the bottom frit (with the seal inflated) is raised at a speed of 200cm/hr initializing slurry transfer via suction. The travel of the piston is chosen to transfer enough slurry for the target bed height, considering the Compression Factor (CF):

$$\text{Length of travel (cm)} = \frac{\text{Packed height (cm)}}{\text{Slurry ratio}} \times CF + \frac{\text{slurry line volume (L)} \times 4000}{\pi \times \text{ID Column (cm)}^2}$$

Slurry line volume is the volume comprised between the tank outlet and the slurry valves, which starts with packing buffer and ends with slurry. The slurry valve is immediately closed after the slurry transfer.

Because the slurry valves and transfer lines contain residual slurry, approximately 20L of water is pumped through the slurry valve cleaning ports (XV81-03-01) to recover 100% of the unused slurry back to the slurry tank.

Figure 3 - Slurry transfer in syringe mode



Packing method -Dynamic Axial compression

Packing is accomplished using dynamic axial compression. DAC involves lowering the top adapter at a constant speed to remove the packing buffer and consolidate the bed.

The columns top process port is closed upstream the pressure gauge (close XV154) and the bottom process isolation valve is opened to the drain (XV36-80)

This configuration allows the bed to build from the bottom to the top while avoiding bed drying. The top adapter is lowered at 200 cm/hr for Bio-Rad Nuvia™ until the bed is consolidated. The top adapter speed is then reduced to 60 cm/hr to achieve the final bed height of 16.8cm with CF=1.2

Figure 4 - Axial compression

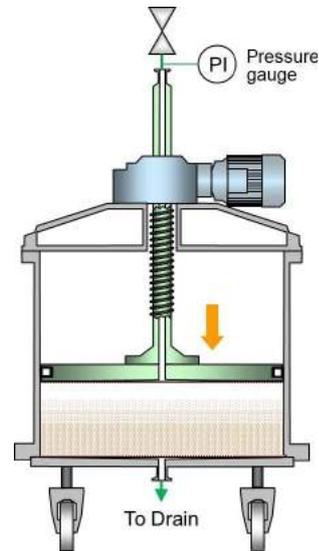
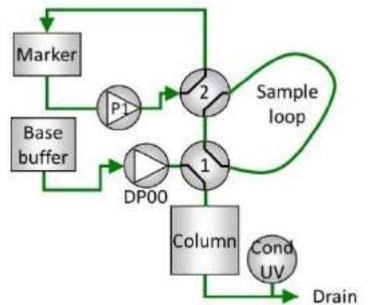


Figure 5 - Marker injection



Column equilibration and validation (HETP, As)

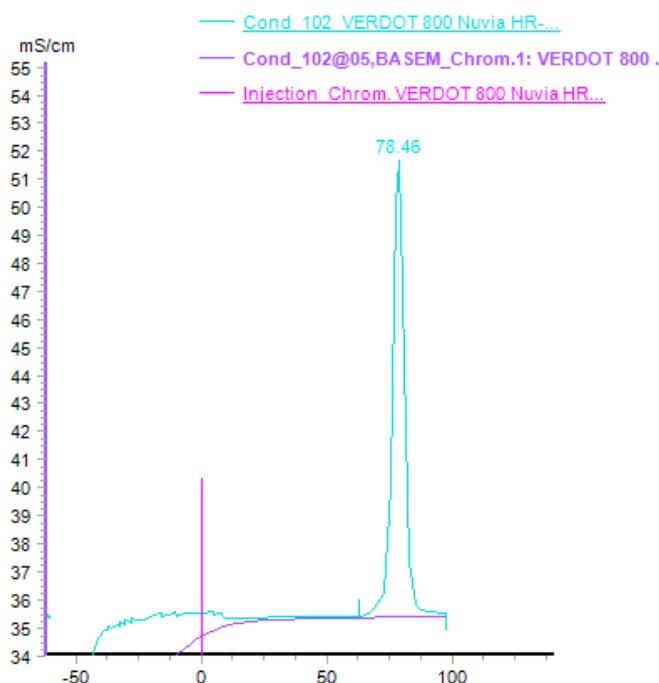
The installation described in figure 5 is ideal for HETP test as it allows injection of a sharp marker peak while keeping a constant flow. 1 and 2 are 4-ways valves. The sample loop is a flexible hose with a volume corresponding to 1-1.5% CV. Please refer to our tech note DP-MKT-338 HETP test for more details on column qualification.

Packing buffer is used as a baseline and 1.2M NaCl is used as marker. The column is equilibrated with 1CV of packing buffer in upflow followed with 3CV in downflow while pump P1 primes the loop with marker (valve 2 configured as valve 1 on picture). The loop is isolated by switching valve 2 as on picture. After column equilibration, valve 1 is switched as valve 2 on picture so as to push the sample in the column and the flow continues until the peak, measured with conductivity probe, elutes from the column.

The following results were observed during the packing study:

- Number of plates = 9101 plates per meter
- Asymmetry = 0.89
- HETP = 0.011cm/pl
- rHETP (= HETP / mean particle size) = 2.20

Fig. 5. HETP and symmetry test



Re-slurrying within the column

The following can be applied before repacking the column without transfer of the media or before unpacking. The unpacking buffer was 0.4M NaCl.

A 5cm headspace is created above the packed bed by injecting 0.4M NaCl at 220cm/h downflow while simultaneously raising the top adapter at 200cm/h to maintain a net positive down flow. Once the headspace is created, flow direction is changed to up flow and the top adapter is raised with the control console. Up flow continues until the top adapter reaches a height corresponding to 2 times the bed height (for obtaining a 50% slurry).

At this stage, if the media bed is still intact, the flow from the skid is reversed (down flow) and the direction is changed several times for 2 minutes between up flow and down flow to raise and lower the media bed until it collapses. Once the media bed is collapsed, the inflatable seal is deflated and the top adapter is moved 8 cm above the liquid level. The inflatable seal is re-inflated and the top process valve is connected to drain (XV36-80). Compressed air, injected through the cover of the bubble trap (XV26-37) sparges through the bottom frit at 0.25 Bar for at least 20 minutes with new media, and at least 1 hour in columns larger than 1m or with used media.

Unpacking method

Maintaining the air sparging, the top process isolation valve (XV154) is closed to pressurize the column at 0.25bar and the column tilting actuator is activated. The slurry valve located at the lowest point of the tilted column is solely opened to the slurry tank through a flexible hose (via XV03-01 or connected to the top of the tank). This allows transferring of the media from the column to the slurry tank in a few minutes.

Air sparging is maintained during the slurry transfer to keep the media suspended, maintain slight pressurization and help the media flow toward the slurry valve.

For removing the traces of media, close the slurry valve, disengage the tilting actuator and open the top process isolation valve. Equivalent to 1cm of water is injected in up flow (XV36-80), followed with air sparging for 30 seconds. The top process isolation valve is then closed to pressurize the column at 0.25bar and the column tilting actuator is engaged. The slurry valve located at the low point of the tilted column is opened to transfer any remaining media. This rinsing may need to be repeated one or two times more, eventually flowing to the drain (XV03-02) if media tracks is neglectable.

Usually less than 2.5CV of unpacking buffer is required to re-slurry the bed within the column, transfer the slurry back into the tank (maintaining a 50/50 slurry) and completely rinse the column, leaving no visible trace of media.

Fig. 6. Re-slurrying with air-sparging .



Conclusion

The VERDOT Ips² InPlace column is ideally suited for processes involving chromatography media, such as Bio-Rad Nuvia™ HR-S, or media of the same matrix. The InPlace columns dynamic axial compression provides quick and easy packing operations with a high level of reproducible performance results. The column can be easily unpacked in less than one hour using minimal equipment (via air sparging) and minimal buffer usage.