Packing Capto™ S ImpAct using verified methods

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Packing Capto™ S ImpAct using verified methods

Capto S ImpAct chromatography medium (resin) is a strong cation exchanger. The medium is designed for polishing of monoclonal antibodies (MAbs) and a wide range of other biomolecules. To achieve effective purification, an efficient packing is important for any chromatography medium. Poorly packed columns can lead to costly disruptions and loss of valuable product. Robust and verified packing and testing methods will eliminate such concerns and risks. This procedure describes packing of Capto S ImpAct medium in large-scale chromatography columns (Fig 1).

Chromatography medium characteristics

Ionic groups and base matrix

Capto S ImpAct is a strong cation exchange (CIEX) medium. The medium offers high binding capacity through its polymer-grafted ligand that combines the functional group with a surface extender. High resolution is enabled by a 50 µm (average bead diameter) high-flow agarose base matrix that combines good pressure-flow properties with an optimized porosity. These properties, in combination with the polymer ligand, make Capto S ImpAct a good choice for reliable and robust polishing steps in industrial purification processes.

Large-scale columns for packing of Capto S ImpAct medium

Flow characteristics for different columns and bed heights are given in Table 1.

Fig 1. A robust and verified packing method contributes to effective purification of the target molecule.

Table 1. Expected flow velocities for different columns and bed heights (measured with 150 mM NaCl at 20°C)

Column type	Column diameter (mm)	Bed height (cm)	Flow velocity (cm/h)
AxiChrom™	≤ 1000	10	440*
AxiChrom	≤ 1000	20	220*
AxiChrom	≤ 1000	30	165*
AxiChrom	≤ 1000	40	110*
BPG	≤ 300	40	110
Chromaflow™	600	20	150

* Verified for columns up to 600 mm.

AxiChrom columns

AxiChrom columns are low-pressure, mechanical axial compression chromatography columns designed for process development and biopharmaceutical manufacturing environments. AxiChrom columns are simple to operate, enabling correct packing and fast start-up. Mechanical axial compression enables accurate and reproducible control of the packing, even with large-diameter columns. With AxiChrom columns, on-site maintenance is facilitated without the need for special tools or specially trained personnel.

AxiChrom columns are available in many different configurations and materials (see data file 28-9290-41 for more details). The columns are developed to accommodate the broad window of operation of Capto S ImpAct and other modern chromatography media. AxiChrom columns are designed to be scalable and will give predictable results over the entire range of scales by enabling a uniform plug flow through the bed irrespective of column size.

The columns feature Intelligent Packing with preprogrammed methods that support all column sizes. The Intelligent Packing feature allows full use of the medium specifications at all scales. Intelligent Packing enables straightforward operation and high packing success rates.

The AxiChrom platform simplifies column handling at all scales, from process development to full-scale production. The packing methods described here apply to bed heights up to 40 cm in AxiChrom columns up to 1600 mm in diameter.

BPG columns

BPG columns are glass columns for process development and manufacturing. The single-screw adapter facilitates efficient packing and running. The columns diameters range from 100 to 450 mm. The packing methods described here apply to all BPG columns, except BPG 450, which is not pressure rated for use with Capto S ImpAct medium.

Chromaflow columns

Chromaflow columns are acrylic or steel, pack-in-place columns for GMP manufacturing. The columns have diameters ranging from 400 to 2000 mm. The packing method described here applies to Chromaflow 600 with a standard configuration.

Packing

Definitions

The bed height of a gravity-settled bed differs from the bed height of a bed settled under low flow (consolidated). Therefore, the compression factor (*CF*) needs to be separated from the packing factor (*PF*). For example, for a 20 cm bed in 150 mM NaCl when consolidated at 60 cm/h, the *PF* is 1.20 and the *CF* is 1.23 for Capto S ImpAct medium. This information is helpful when the amount of gravity settled medium needed for a specific column size is calculated (amount of gravity settled medium needed to obtain a bed with a certain *PF* [e.g., 1.20] is calculated as column volume (V_c) × *CF* [e.g., 1.23])

Equations to calculate *CF*, *PF*, and V_c are shown below:

$$\text{Compression factor, } CF = \frac{L_{settled}}{L_{packed}}$$

$$\text{Packing factor, } PF = \frac{L_{cons}}{L_{packed}}$$

where

$L_{settled}$ = bed height measured after settling by gravity (cm)

L_{cons} = consolidated bed height, that is, bed height measured after settling the medium at a given flow velocity (cm)

L_{packed} = packed bed height (cm)

$$\text{Column volume, } V_C = L_{packed} \times A_C$$

where

A_C = cross-sectional area of the column (cm^2)

When packing BPG and AxiChrom columns, *PF* is used in the packing procedure to calculate the packed bed height after the consolidation step. *CF* is used in the medium preparation step to calculate the medium volume needed to pack a desired bed height. Because Chromaflow columns are pack-in-place columns, they have no registered consolidated bed heights and the *CF* is used throughout the packing process.

Properties of Capto S ImpAct in various packing solutions

Capto S ImpAct medium settle quickly in both water and in 20% ethanol. When using these solutions, it should be remembered that tubing and nozzles should be rinsed directly after packing to prevent clogging of the flow path. Adding salt to packing solutions slows the settling of the medium beads and also allows them to settle less tightly. To fully utilize the flow properties of Capto S ImpAct, salt (150 mM NaCl) should be added to the packing solution. The packing factor should be chosen in relation to the target bed height (Fig 2).

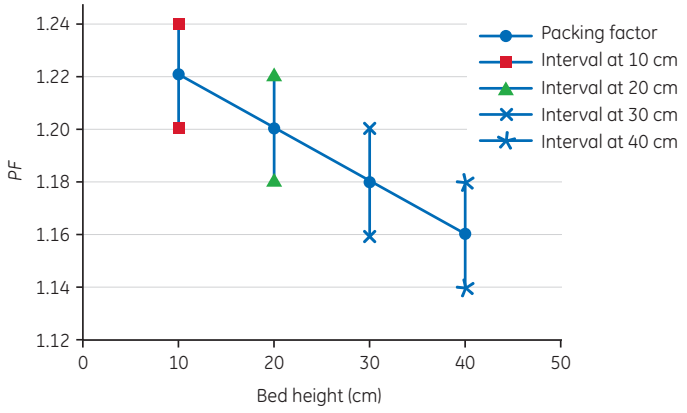

Fig 2. Bed height and packing factor curve for AxiChrom columns up to 600 mm, illustrating the packing factors' decreasing linearity for different bed heights. For each bed height, there is an acceptable interval for the packing factor. The packing factors are established for packing with 150 mM NaCl.

Slurry preparation

When preparing the slurry, start by calculating the chromatography slurry volume (*V*) needed to pack the desired bed height. The slurry concentration can be determined in a number of ways. However, to get an accurate slurry concentration determination of Capto S ImpAct medium in salt-containing solutions, use the method described below.

When calculating the slurry volume (*V*), use *CF* = 1.24 that is valid when the medium is in storage solution.

Note! The slurry concentration determined by the method below corresponds to the gravity-settled concentration in water.

$$\text{Chromatography slurry volume, } V = \frac{A_C \times L_{packed} \times CF}{C_{slurry}}$$

where

C_{slurry} = concentration of the slurry

Slurry preparation can be done manually, mechanically, or by using the Media Wand™ slurry mixing and transfer tool. Shaking gives good results, but is often not practical for larger volumes. When stirring, it is preferred to use soft stirrers without sharp edges. The Media Wand tool suspends the medium directly in the container and transfers the slurry to the slurry tank in one operation, which makes it suitable for large-scale packing.

Capto S ImpAct is supplied in 20% ethanol with 0.2 M sodium acetate. Before packing, transfer the medium to the packing solution as described in the packing instructions for the relevant column.

Measuring slurry concentration

It is important to measure the slurry concentration correctly to have the correct amount of chromatography medium in the slurry for packing to the target bed height at the correct level of compression. Measuring slurry concentration can be performed with a Tricorn™ 10/100 column.

GE Healthcare's Slurry Concentration Kit (see Ordering information) includes the materials required for determination of slurry concentration.

Packing of Capto S ImpAct in AxiChrom columns

When packing AxiChrom 50 to 200 columns for use with ÄKTA™ systems, Intelligent Packing control is managed by the UNICORN™ control software. For AxiChrom 300 to 1600 columns, Intelligent Packing is performed by AxiChrom Master, a separate unit that comprises a touchscreen operated user interface, or from the UNICORN software on the ÄKTAprocess™ system.

Intelligent Packing in AxiChrom columns—general considerations

Packing methods are created by entering values for the packing variables, for example, medium, slurry concentration, and target bed height, in the Intelligent Packing wizard.

When packing AxiChrom 50 to 200 columns, the slurry is introduced into the column by hand and adapter movement is driven by internal hydraulics. After the wizard method has been created and the medium has been equilibrated to the packing solution, the column is primed and filled with slurry. The method controls the flow of hydraulic fluid to drive the adapter and pack the bed (Fig 3).

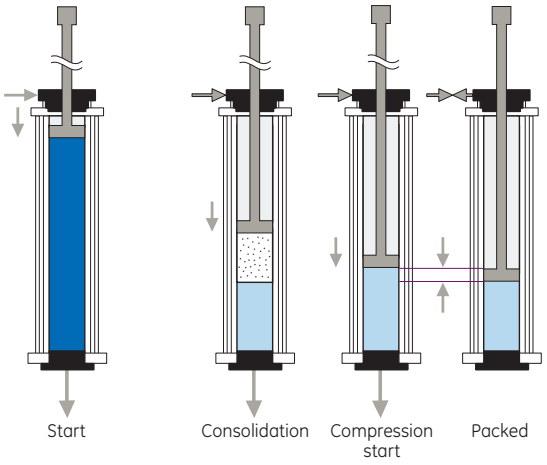

Fig 3. Intelligent Packing in AxiChrom 50 to 200 columns. The adapter is mounted to the column tube and the wizard is started (Start). The adapter moves down, forcing packing liquid out through the bottom bed support. The medium forms a consolidated bed (Consolidation). When the adapter comes in contact with the consolidated bed surface, the operator initiates bed compression in the UNICORN wizard (Compression start). Compression occurs according to a predetermined *PF*. Finally, the target bed height is attained (Packed).

In AxiChrom 300 to 1600 columns, slurry is introduced via a medium valve in the center of the bottom bed support and the adapter is driven by an electric servomotor. The two-position medium valve enables filling, packing, and unpacking without adjusting the assembled column.

After the column is primed, the adapter rises from its lowest position and the column fills with slurry via the medium valve. The slurry volume is calculated automatically from the target bed height, slurry concentration, and *PF*. Also the volume of the tubing connection between the column and slurry tank is taken into consideration. As an electric servomotor controls the movement of the adapter, its position is monitored with millimeter accuracy.

When the correct slurry volume has been drawn into the column, the adapter starts to lower and packing buffer is forced out through the bottom bed support and bed consolidation starts. The time to complete consolidation (i.e., when the adapter reaches the bed) is also automatically calculated (as for the AxiChrom 50 to 200 columns), allowing the operator to carry out other tasks in the meantime. As the adapter hits the consolidated bed, a very distinct dip is seen on the pressure curve, which is detected by the Intelligent Packing wizard. When this occurs, the operator confirms that the adapter has hit the bed.

The compression of the medium starts and a graphical interface is shown on the control screen of the UNICORN software or AxiChrom Master. This graphical interface assists the operator in finishing the packing, giving a well-packed bed. When the adapter symbol is within the range of approved packing factors and bed height limits, the operator can end the packing.

If selected in the UNICORN wizard, Intelligent Packing will automatically run a packed bed evaluation test after the packing. For large AxiChrom columns, automatic methods for priming and unpacking can also be created with the Intelligent Packing wizard.

Packing variables and recommended packing buffers are given in Table 2.

Table 2. Packing variables for packing of Capto S ImpAct medium in AxiChrom columns

Packing variable	AxiChrom 50 to 200	AxiChrom 300 to 1600
Packing solution	150 mM NaCl	150 mM NaCl
Packing speed/velocity	60 cm/h	60 cm/h
Packing factor	Bed height 10 cm: 1.22 ± 0.02 Bed height 20 cm: 1.20 ± 0.02 Bed height 30 cm: 1.18 ± 0.02 Bed height 40 cm: 1.16 ± 0.02	Bed height 10 cm: 1.22 ± 0.02 Bed height 20 cm: 1.20 ± 0.02 Bed height 30 cm: 1.18 ± 0.02 Bed height 40 cm: 1.16 ± 0.02
Flow conditioning	3 CV at 440 cm/h for 10 cm bed height 3 CV at 220 cm/h for 20 cm bed height 3 CV at 165 cm/h for 30 cm bed height 3 CV at 110 cm/h for 40 cm bed height	Optional for AxiChrom 300: 3 CV at 220 cm/h for 20 cm bed height 3 CV at 110 cm/h for 40 cm bed height

CV = column volumes

Packing of Capto S ImpAct in BPG columns

Capto S ImpAct medium can be packed in BPG 100 to 300 columns. The bed height interval recommended for BPG 100 to 300 columns is 10 to 40 cm. In BPG columns with small diameters (BPG 100), the increased influence of the wall support means that salt solution is required for packing. Capto S ImpAct medium is therefore packed with 150 mM NaCl in BPG 100 and BPG 300 columns. The packing factor for packing of Capto S ImpAct to various bed heights in BPG columns is 1.14.

Chromatography medium preparation

Equilibration of the medium to the packing solution can be performed by using the BPG column as a "filter". Pour the medium into the column (for calculation of amount, see Section Slurry preparation), mount the adapter, tighten the adapter O-ring, move the adapter down and compress the bed slightly, connect the pump, and wash the medium with at least three column volumes (CV) of the packing solution.

Unpack and resuspend the slurry and pack according to the method below.

Note! Equilibration is critical for Capto S ImpAct in BPG columns as the delivery solution contains 0.2 M sodium acetate in 20% ethanol, and the recommended packing solution is 150 mM NaCl.

Column and system preparation

A detailed description of column preparation is available in the BPG column instructions (18-1170-70). The packing pump should be as pulsation-free as possible. Screw and rotary lobe pumps are the most suitable for this task but multi-headed diaphragm pumps are also satisfactory.

Caution! Ensure that the column has no visible scratches in the glass tube and that the adapter moves smoothly in both upward and downward directions before packing. In addition, there should be no difficulty in tightening the adapter O-ring to the column inner wall.

1. Place a new 10 µm net on both the adapter and the bottom end pieces.
2. Level the column with the aid of a spirit level.
3. A pressure relief valve should be used for safety reasons, especially against pressure spikes. Position this valve on the pump outlet and add a pressure gauge to the adapter.
4. Mount one 4-port, 2-way valve on the bottom inlet and one on top of the pressure gauge, i.d. 10 mm for BPG 300 and i.d. 6 mm for BPG 100.

Packing

1. Set the pressure alarm or pressure relief valve according to the pressure rating of the column. Purge the system and tubing of air.
2. Purge the end piece net of trapped air by draining some packing solution through the column outlet. Leave about 2 cm of solution in the column and close the bottom valve. If air is still trapped under the end piece net, add more packing solution and connect tubing to the suction side of a pump. Start the pump and place the tubing on the bottom net and extract any remaining air.
3. Add the slurry to the column and, if needed, additional packing solution to about 40 cm. Mix the medium and the packing solution to a homogeneous slurry.

Note! The available height to allow the adapter to be inserted into a 50 cm column tube (for filling slurry) is 40 cm. Use a longer column tube when packing beds higher than 20 cm: 75 cm and 95 cm tubes are available.

4. Rinse the wall from particles and let the medium settle until there is about 1 cm clear liquid on top of the slurry to reduce the risk of particles sticking between the O-ring and the column wall, which can cause leakage.
5. Insert the top adapter and secure it to the column top flange. Lower the adapter to the surface of the slurry and allow some clear liquid to pass the O-ring. Tighten the adapter O-ring.
6. Make sure that the column top valve is open. Slowly move the adapter down until no air bubbles can be seen leaving the top valve.
7. Start the pump and adjust the settling velocity to 60 cm/h (4.7 L/h for BPG 100 and 42.4 L/h for BPG 300). Shift the top valve into the column and immediately open the bottom valve and lead the liquid to waste.
8. Run the settling flow until the bed is completely consolidated. Note the consolidated bed height and calculate the packed bed height. For BPG 100 and 300, *PF* = 1.14 in 150 mM NaCl for bed heights up to 40 cm. The packed bed height is the ratio between the consolidated bed height and the *PF*. Use a marker pen to indicate the packed bed height on the column.
9. Stop the flow and close the bottom valve. Loosen the O-ring and lower the adapter down to 1 cm above the settled bed and seal the adapter O-ring. Shift the top valve to waste and use the adapter to mechanically compress the bed to the mark on the column (step 8). Excessive packing solution is removed through the adapter tube.

Note! Compressing Capto S ImpAct in BPG columns, especially the larger BPG 300, is physically demanding. Do not use extension rods on the adapter height adjuster to compress the medium.

To increase the performance and stability of the bed, flow condition the column downwards for 5 CV and upward for 5 CV at 110 cm/h with packing solution.

10. Connect the pump to the top of the column. Purge the system and tubing by running the mobile phase to waste by bypassing the column inlet with the 4-port valve. Start at a low flow velocity (approximately 60 cm/h).
11. Shift the top valve to direct the flow to the column and immediately open the bottom mobile phase to waste or connect it to the buffer tank for recirculation.
12. Increase the flow to 110 cm/h and run the column at this flow for 5 CV.
13. Slowly decrease the flow for 2 min to avoid disturbance of the conditioned bed.
14. Exchange the mobile phase connections. Connect the tubing from the pump to the bottom valve and open the top valve to waste or to the buffer tank for recirculation.
15. Repeat steps 10 through 13 with upward flow to complete the conditioning procedure.
16. Test the packing at the optimal test velocity (20 to 30 cm/h).

Packing variables and recommended packing buffers are given in Table 3.

Table 3. Packing variables for packing of Capto S ImpAct medium in BPG columns

Packing variable	BPG 100 to 300
Packing solution	150 mM NaCl
Packing speed/velocity	60 cm/h
Packing factor	Bed height 40 cm: 1.14
Flow conditioning	Optional for BPG: 3 CV at 110 cm/h

CV = column volumes

Packing of Capto S ImpAct in Chromaflow 600

The method described in this section has only been verified for packing of Capto S ImpAct in Chromaflow 600 to a bed height of 20 cm. As the pump is stopped 5 mm from the adapter, as seen in the packing method, lower beds will not be compressed sufficiently. Moreover, the extreme velocities needed to efficiently pack a shorter bed cannot easily be achieved using standard equipment.

To pack Capto S ImpAct medium in Chromaflow 600, the slurry is introduced from the top nozzle by the use of Chromaflow Packing Station Pack 100.

Note! The recommended operational flow velocity for Capto S ImpAct in Chromaflow 600 at 20 cm bed height is 150 cm/h.

Note! The flow capacity of Chromaflow Packing Station Pack 100 is required for packing Capto S ImpAct medium in Chromaflow 600.

Chromatography medium preparation

The recommendation is to use 150 mM NaCl as packing solution for Chromaflow columns and that this packing solution is used throughout the whole procedure.

Note! Water can potentially be used as packing solution, but compression and flow velocities have not been verified by GE Healthcare.

To avoid introducing air into the column when packing, additional slurry is required for the extra volumes in the tank and tubing. Add the slurry to the slurry tank and stir the medium. Dilute the suspension to a slurry concentration of about 50%.

Column and system preparation

For more detailed description about the column and packing station preparation, see Chromaflow column instructions (56-3193-25) and Chromaflow packing station instructions (56-3215-58). In this procedure, standard Chromaflow equipment is used for the connections on the column and the packing station.

Note! It is important that the supply air flow rate follows the specification of Chromaflow Packing Station Pack 100 (1000 L/min) and that the supply air pressure into the packing station is 6 to 7 bar.

1. Set up the column according to the Chromaflow column instructions (56-3193-25).
2. A pressure relief valve should be used for safety reasons. Position the pressure relief valve on the slurry inlet top (SIT). Place a pressure gauge on the mobile phase top (MPT) to record the pressure during packing. Mount a 3-port, 2-way valve on top of the pressure gauge and the mobile phase bottom (MPB). The top valve should lead in two directions: one side into the system and one to the waste for purging the tubing. On the bottom valve, one side leads to the system and a 1.5" to 2" tubing leads to the waste (for packing). Part of the MPB waste tubing should be placed above the outlet valve to eliminate air entering through the MPB.
3. Connect appropriate tubing (i.d. 1" or 1.5") and tanks to the column and packing station. If a flow meter is used, place it between the SIT and the packing station.
4. Level the adapter to the desired bed height. Remember to loosen the nuts on the adapter rods to allow the adapter to be raised or lowered. Flush the adapter rods with 20% ethanol as lubrication.
5. Prime the column, packing station, and tubing with water according to the Chromaflow column instructions.

Packing

Note! Packing Chromaflow columns is a rapid procedure compared with other packing procedures and, therefore, it is important to thoroughly read the packing instructions and go through the packing steps in advance of starting the packing.

1. Set both nozzles in run position to prime the tubing with slurry. Lead the slurry outlet top (SOT) tubing back to the slurry tank and secure it. Stir the slurry to keep it homogeneous, select slurry and SIT on the packing station, open the slurry tank and start the packing pump.
2. The initial flow velocity should be at least 1400 cm/h, corresponding to an air pressure on the packing station of at least 2.5 bar for packed bed heights of 20 and 30 cm.
3. When the tubing is primed and the flow velocity set, set the SIT/slurry inlet bottom (SIB) to the position between SIT and SIB to block the flow during step 4 while maintaining the correct flow rate for the next step.
4. Move the top nozzle down into the packing position.
5. Open the bottom mobile phase valve to waste and turn the SIT/SIB valve to SIT on the packing station. This procedure can be facilitated if performed by two operators. The column starts to fill with slurry and the bed builds up slowly from the bottom as excess liquid exits via the MPB.

Note! Column pressure should not exceed the operating pressure limit of the column (i.e., 3 bar). If this pressure is reached, gently decrease the packing flow so that the pressure remains just below 3 bar. Typically, the final pressure in the column is 2 to 3 bar depending on the viscosity of the packing solution, column diameter, bed height, and so forth.

6. Stop the pump when the building bed is 5 mm from the top bed support by setting the SIT/SIB to the position between SIT and SIB, as described in 3. Once flow is stopped, the bed will expand to meet the adaptor.

Note! If a nontransparent column tube is used, stop the packing flow when the calculated volume of slurry has been introduced into the column. Check the volume in the slurry tank or use a volume totalizator.

7. Immediately move the top nozzle back to the run position.
8. Close the MPB valve when the pressure in the column is between 0.3 and 0.1 bar.
9. Use packing solution to rinse residual medium from the tubing and the top nozzle. Pump the packing solution through the top nozzle back into the slurry tank.
10. Close the slurry tank and empty the tubing between the tank and packing station.
11. Pump liquid upwards through the column until the air is expelled.

Figure 4 shows the increase in pressure and decrease in flow velocity when packing a 20 cm bed height in Chromaflow 600.

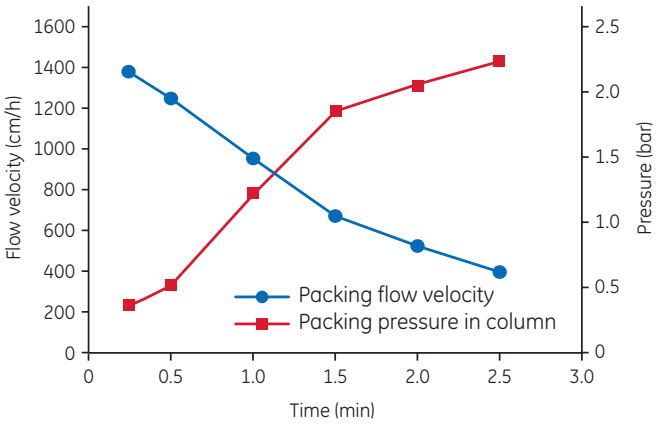

Fig 4. Column pressure and flow velocity during packing of a 20 cm bed height in Chromaflow 600 by the use of Chromaflow Packing Station Pack 100.

Testing of the performance of the packed column

Process-scale packed columns should perform with a high degree of efficiency over many processing cycles (i.e., display high stability). The efficiency of a packed column can be expressed in terms of height equivalent to a theoretical plate (HETP) and asymmetry factor (A_s). This test should be repeated regularly to monitor the state of the bed throughout the lifetime of the packed bed. If the test results are to be comparable over time, conditions such as flow velocity (cm/h), liquid pathway, sample composition, and elution buffer should be kept constant. The requirements for the test should be set in accordance with test conditions and the goal of the purification. Column efficiency testing is further described in application note 28-9372-07.

Test conditions used in this study

Sample:	2% v/v acetone in 150 mM NaCl or 0.8 M NaCl (AxiChrom 50 to 200)
Sample volume:	1% of the bed volume (V_C)
Flow velocity:	30 cm/h for AxiChrom and BPG 20 cm/h for Chromaflow
Eluent:	150 mM NaCl or 0.4 M NaCl (AxiChrom 50 to 200)

To compare the performance of columns packed with chromatography media of different particle diameters, the reduced plate height (h = *HETP/average bead diameter [dp]*) is typically used. As a guideline, a value of $h < 3$ is very good at the optimal test conditions.

Examples of results

The columns packed with the methods outlined above were tested for plate number, asymmetry, pressure-flow properties, and stability. Examples of efficiency and stability results for Capto S ImpAct packed in the different columns can be seen in Table 4.

AxiChrom columns

The results for Capto S ImpAct in column sizes ranging from 100 to 600 mm were very similar, showing that the verified packing methods available in Intelligent Packing give equal results, independent of column size and bed height. The stability test showed that the medium beds were stable when running in water at velocities given in Table 4.

Pressure-flow curves provide a simple, yet effective illustration of column performance in terms of the maximum operating flow velocity at which the purification process can be run. These curves also show the magnitude of the back pressure in the system at a certain flow velocity and for different bed heights. Pressure-flow curves for Capto S ImpAct packed in AxiChrom 300 to different bed heights are shown in Figure 5.

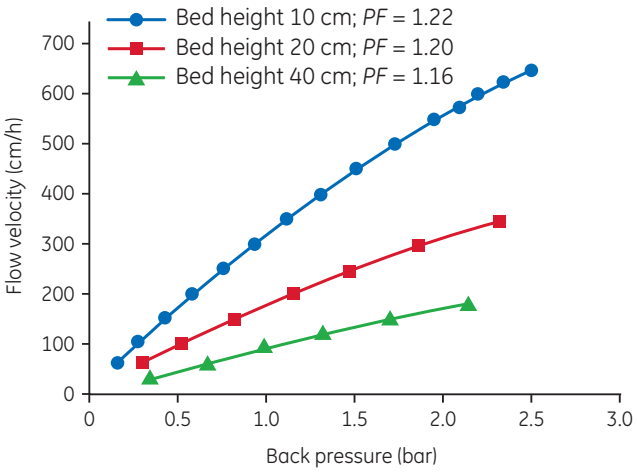

Fig 5. Pressure-flow curves for Capto S ImpAct in 150 mM NaCl packed in AxiChrom 300 to different bed heights using different packing factors. Capto S ImpAct can be run at 220 cm/h with a back pressure of less than 3 bar for bed heights up to 20 cm.

Table 4. Column efficiency data for Capto S ImpAct in different columns

Column	Bed height (cm)	Average plates/m	Reduced plates height (h) range	Asymmetry factor (A_s) range	Flow velocity for stability test (cm/h)	Change after stability test (%)	
						h	A_s
AxiChrom 100	20	12 000	1.6 to 1.7	1.1 to 1.2	250	-1	-3
AxiChrom 300	10	13 000	1.5 to 1.6	1.3 to 1.4	500	-6	4
AxiChrom 300	20	12 000	1.5 to 1.7	1.1 to 1.2	250	-1	2
AxiChrom 300	40	11 000	1.7 to 2.0	1.1 to 1.2	125	2	8
AxiChrom 300	30	11 000	1.7 to 1.8	1.0 to 1.2	167	1	3
AxiChrom 600	20	13 000	1.4 to 1.5	1.1 to 1.2	250	1	3
BPG 100	40	10 000	1.7 to 1.8	1.0 to 1.1	110	-4	1
BPG 300	20	11 000	1.5 to 1.7	1.0 to 1.3	250	-2	10
BPG 300	40	10 000	1.5 to 2.0	1.0 to 1.5	110	-4	-3
Chromaflow 600	20	9 500	1.9 to 2.0	1.1 to 1.2	200	2	-2

BPG columns

In general, the packing method works well for Capto S ImpAct in different sizes of BPG columns at different bed heights. The stability test showed that the bed was stable in water at velocities given in Table 4.

The pressure-flow curves for Capto S ImpAct at 40 cm bed height in BPG 100 and BPG 300 are shown in Figure 6. Capto S ImpAct shows linear behavior in these columns.

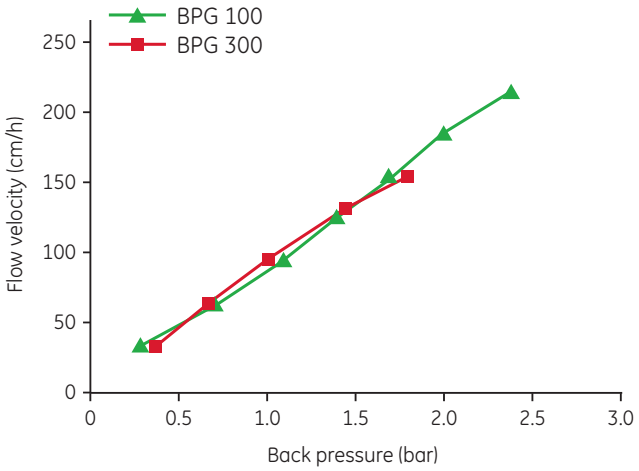

Fig 6. Pressure-flow curves for Capto S ImpAct in 150 mM NaCl packed to 40 cm bed height (*PF* = 1.14) in BPG 100 and BPG 300. System/tubing pressure is excluded.

Chromaflow 600

A bed height of 20 gives good plate number and asymmetry factor. In addition, the stability test shows that the bed is stable when running in water at the velocity given in Table 4.

The pressure-flow curves are shown in Figure 7. As the optimal compression factor is difficult to achieve in standard pack-in-place columns, the maximum flow velocity that can be run through the packed bed is limited. The highest operating flow velocity recommended for Capto S ImpAct in the Chromaflow 600 column is 150 cm/h. Note that bed efficiency and bed stability are very good, provided this 150 cm/h guideline is met.

When comparing data from AxiChrom (Fig 5) and Chromaflow (Fig 7) columns, a slightly higher back pressure at the same flow velocity can be seen for the AxiChrom columns. The increased back pressure results from the higher compression when packing AxiChrom columns (*CF* = 1.23) compared with when packing Chromaflow columns (*CF* = 1.10). This higher compression is needed to utilize the full flow velocity of Capto S ImpAct medium. Still, the back pressure over the column at full flow velocity is far from the maximum operating range of AxiChrom columns (4 bar).

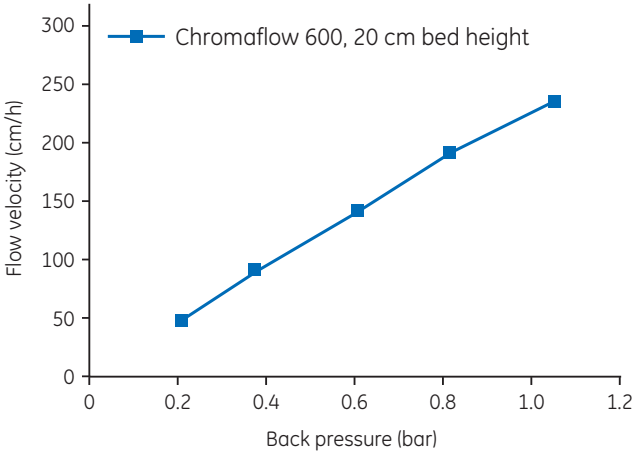

Fig 7. Pressure-flow curve for Capto S ImpAct in 150 mM NaCl packed to a bed height of 20 cm in Chromaflow 600 with a *CF* = 1.10.

Efficiency tests at different flow velocities

Efficiency tests were run at different flow velocities. Figure 8 shows a van Deemter plot of the results. The asymmetry factor is stable at the different flow velocities. The reduced plate height increases with the flow velocity and optimal result in this study is achieved at 10 to 40 cm/h. When running at higher flow velocities, the asymmetry factor and reduced plate height continue to behave linearly, indicating that the efficiency test can be run at any flow velocity. However, the expectation of the result needs to be adjusted based on the test flow velocity used.

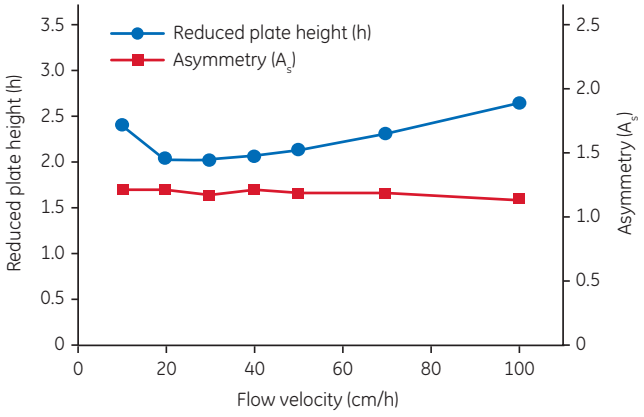

Fig 8. Reduced plate height and asymmetry values at different flow velocities for Capto S ImpAct packed to a bed height of 20 cm in a Chromaflow 600 column (*CF* = 1.10). These values should be regarded as examples. However, similar behavior can be expected for packed beds in other columns such as AxiChrom and BPG.

Conclusions

This procedure describes packing of Capto S ImpAct in AxiChrom columns using the custom mode feature of Intelligent Packing. Packing of Capto S ImpAct in BPG 100 and 300 and Chromaflow 600 columns is also described.

Capto S ImpAct medium can be packed in AxiChrom columns to bed heights between 10 and 40 cm. The flexibility of column diameters and bed heights enables full utilization of the flow capacity of Capto S ImpAct, allowing processes with higher bed heights for improved resolution, increased residence time, or if floor space is limited; or lower bed heights and larger diameters to decrease process time.

Each packing method described is related to a specific packing solution. Deviation from use of the packing solutions described can have significant impact on the *PF* and subsequently on the packing result. To utilize the full flow potential of Capto S ImpAct medium, AxiChrom columns are recommended.

Ordering information

Product	Quantity	Code number
Capto S ImpAct	25 mL	17-3717-01
Capto S ImpAct	100 mL	17-3717-02
Capto S ImpAct	1 L	17-3717-03
Capto S ImpAct	5 L	17-3717-04
Capto S ImpAct	10 L	17-3717-05
Capto S ImpAct	60 L	17-3717-60
Slurry Concentration Kit	1	29-0961-00

For more information about AxiChrom, BPG, and Chromaflow columns as well as Chromaflow Packing Stations, visit www.gelifesciences.com

Data files

Capto S ImpAct	28-9259-32
AxiChrom columns	28-9290-41
BPG columns 100, 140, 200, 300, and 450 series	18-1115-23
Chromaflow columns	18-1138-92
Media Wand	28-9331-01

Application notes

Column efficiency testing	28-9372-07

Instructions for use

Capto S ImpAct medium	29-0925-01
BPG columns	18-1170-70
Chromaflow columns	56-3193-25
Chromaflow Packing Stations	29-0462-28
AxiChrom 50, 70 and 100 columns	28-9331-08
AxiChrom 140 and 200 columns	28-9431-23
AxiChrom 300-1600 columns	28-9562-90

www.gelifesciences.com/captosimpact

GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK
GE Healthcare Europe, GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Bio-Sciences Corp., 800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA
GE Healthcare Japan Corporation, Sanken Bldg., 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan
For local office contact information, visit www.gelifesciences.com/contact

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GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden