Capto MMC

Multimodal chromatography

Instructions for Use

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Abstract

Capto™ MMC is a multimodal salt-tolerant BioProcess™ resin for capture and intermediate purification of proteins from large feed volumes by packed bed chromatography.
Capto MMC increases productivity and reduces cost with:

- high dynamic binding capacity at high conductivity
- high volume throughput
- smaller unit operations

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Important

Read these instructions carefully before using the product.

Safety

For use and handling of the product in a safe way, refer to the Safety Data Sheet.

1 BioProcess resins

BioProcess chromatography resins are developed and supported for production-scale chromatography. BioProcess resins are produced with validated methods and are tested to meet manufacturing requirements. Secure ordering and delivery routines give a reliable supply of resins for production-scale. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities. BioProcess resins cover all purification steps from capture to polishing.

2 Properties of Capto MMC

Capto MMC has a ligand with multimodal functionality (Fig 1). The multimodal functionality gives a different selectivity compared to traditional ion exchangers and also provides the possibility of binding proteins at high salt conditions.

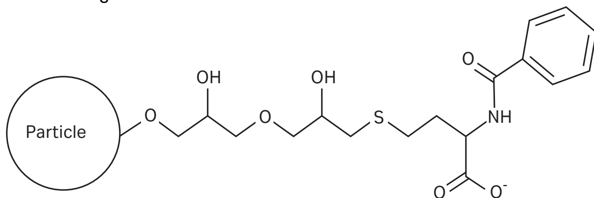

Fig 1. The Capto MMC ligand exhibits many functionalities for interaction with a target molecule. The most pronounced are ionic interaction, hydrogen bonding, and hydrophobic interaction.

Capto MMC is designed to increase speed and throughput in capture and intermediate purification. By offering high capacity at high salt concentrations, and high flow velocities with low backpressure, process cycle times can be reduced and productivity increased.

The resin is based on a high flow agarose matrix. Typical flow velocities at large-scale (1 m column diameter and 20 cm bed height) are 600 cm/h or over, with a backpressure below 3 bar (Fig 2). The highly cross-linked agarose base matrix gives the resin high chemical and physical stability. Characteristics such as capacity, elution behavior, and pressure/flow properties are unaffected by the solutions commonly used in process chromatography (Table 1).

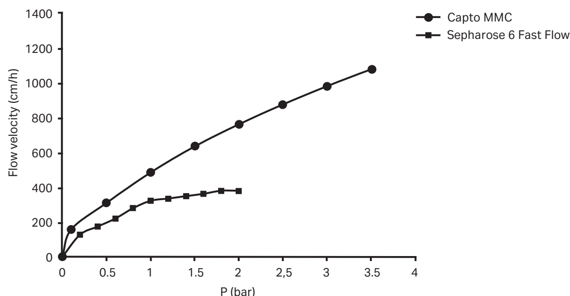

Fig 2. Pressure-flow properties for Capto MMC compared to Sepharose™ 6 Fast Flow. Running conditions: BPG 300 (30 cm i.d.), open bed at settled bed height equal to 20 cm, with water at 20°C.

Table 1. Characteristics of Capto MMC.

Matrix	Highly cross-linked agarose, spherical
Functional group	Multimodal weak cation exchanger
Ionic capacity	0.07 to 0.09 mmol H^+/mL resin
Particle size, d_{50v}[1]	~ 75 µm
Pressure/ flow characteristics	≥ 600 cm/h at ≤ 0.3 MPa in a 1 m diameter column and 20 cm bed height (at 20°C using process buffers with the same viscosity as water)[2]
Dynamic binding capacity, Q_{B10}[3]	≥ 45 mg BSA/mL resin at 30 mS/cm
pH stability, operational[4]	3 to 12
pH stability, CIP[5]	3 to 14
Working temperature[6]	4°C to 30°C
Chemical stability	Stable to commonly used aqueous buffers, 1 M acetic acid, 1.0 M NaOH[7], 8 M Urea, 6 M guanidine hydrochloride, 70% ethanol
Avoid	Oxidizing agents, cationic detergents
Autoclavability	17 min at 121°C in 0.05 M phosphate buffer, pH 7, 10 cycles

[1] Median particle size of the cumulative volume distribution.

[2] The pressure/flow characteristics describes the relationship between pressure and flow under the set circumstances. The pressure given shall not be taken as the maximum pressure of the resin.

[3] Dynamic binding capacity at 10% breakthrough by frontal analysis at a mobile phase velocity of 300 cm/h in a Tricorn™ 5/100 column at 10 cm bed height (2 min residence time) for BSA in 50 mM sodium acetate, pH 4.75, 250 mM NaCl.

[4] pH range where resin can be operated without significant change in function.

[5] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

[6] Capto MMC can be used under cold-room conditions, but for some proteins the capacity can decrease.

[7] 1.0 M NaOH must only be used for cleaning purposes.

3 Method design and optimization

Aim

The aim of designing and optimizing a method for the separation of biomolecules is to identify conditions that promote binding of the highest amount of target molecule, in the shortest possible time with highest possible product recovery and purity. For optimization of binding conditions, pH, and conductivity (salt concentration) must be screened. Binding at relatively high conductivity can be expected with Capto MMC, but it will depend on the pH. Binding can also be expected at higher pH values compared to traditional ion exchangers.

A pH gradient experiment in which the target molecule is loaded in analytical amounts on Capto MMC and eluted in an increasing pH gradient will help establishing the elution pH. A separate experiment with different salt concentrations in the pH gradient can also be included for better understanding. Knowing the elution pH will be useful when setting up the screening experiments for binding and elution. Establishing the elution pH through a gradient run is more reliable than basing the pH selection on the isoelectric point of the target molecule.

Design of Experiment

The fact that Capto MMC allows capture of proteins at high conductivity in many cases limits the use of increasing salt concentrations as an efficient way of eluting proteins. Optimal elution is often achieved by a combination of changes in pH, buffer concentration, and eluting salt. Design of Experiment (DoE) is an effective tool for investigation of the effect of several parameters on protein recovery in order to establish the optimal elution protocol.

Examples of the DoE approach are described in the application notes *Optimizing elution conditions on Capto MMC using Design of Experiments (*11003548) and *High-throughput screening for elution conditions on Capto MMC using PreDictor™ plates* (28927790). Alternatively, a stepwise elution optimization protocol can be applied. For rapid screening of binding or elution conditions with low sample consumption, PreDictor plates (Instructions 28925834, Data file 28925839) are preferably used.

For further reading, see application note *High-throughput screening and process development for capture of recombinant pro-insulin from E. coli (*28996622) where optimization of a chromatographic capture step for pro-insulin from *E. coli* on Capto MMC is described.

4 Screening of binding and elution conditions

Screening of binding and elution conditions can be done either with traditional column chromatography or in batch format (PreDictor plates). The procedure for the two formats is the same, but by using the latter format screening is quicker and much less sample is consumed, which allows a larger experimental space to be screened. Below, only the plate approach is described, but the procedure is essentially the same for both formats.

5 Optimization of throughput

The dynamic binding capacity (DBC) for the target protein must be determined by frontal analysis using real process feedstock. Since the DBC is a function of the flow velocity applied during sample application, the breakthrough capacity must be defined over a range of different residence times (flow velocities) to show the optimum level of throughput.

Loading conditions

The initial elution pH of the target molecule can be established by performing a pH gradient experiment as described above. PreDictor plates are used to screen for best loading conditions. Vary the salt concentration (typically 0 to 300 mM) and pH (0.5 to 2.5 pH units below the elution pH) and determine the static binding capacity under the different conditions. The screening gives the conditions, that is, the salt concentration and pH at which the capacity is the highest. Before sample loading, adjust the pH and conductivity of the sample to desired loading conditions. This is done by buffer exchange or by direct adjustment of pH and salt concentration. Buffers normally used for ion exchange chromatography can also be used for Capto MMC (Table 2).

Table 2. Recommended buffers

pH interval	Buffer[1]	Concentrations[2]
4 to 5.5	Acetate	20 to 100 mM
3.0 to 6.5	Citrate	20 to 200 mM
5.5 to 6.5	Bis-Tris	20 to 50 mM
6 to 7.5	Phosphate	20 to 200 mM
7.5 to 9.0	Tris	20 to 50 mM
8.8 to 10.6	Glycin-NaOH	20 to 100 mM

[1] The choice of buffer systems and salts can influence performance.

[2] Conductivity can be adjusted by addition of salt or by varying the buffer concentration.

Loading density

The capacity obtained in plates is static binding capacity and not dynamic binding capacity (DBC). The loading density for the elution study must be based on the DBC under optimal binding conditions. How loading density (amount of loaded target molecules per volume resin) is specified varies. Two examples of loading density specifications are:
1) 70% of the dynamic binding capacity at 10% breakthrough, and
2) 80% of the dynamic binding capacity at 1% breakthrough.

Elution conditions

Screening of elution conditions is done in PreDictor plates in a similar manner as screening of binding conditions. The pH (typically 0.5 to 2.5 pH units above the initial elution pH established earlier) and salt concentration (typically 0 to 1 or 2 M) are screened. The loading density is set based on the DBC (above).

An example of an elution screening study is shown in Figure 3 (Application Note 28927790). The Figure compares the results obtained in PreDictor plates with results obtained in columns. For best elution profile a rapid change in pH is desirable. Increasing the ionic

strength of the buffer will facilitate this. The results with NH_4^+ salt illustrates that other salts than NaCl can be more effective.

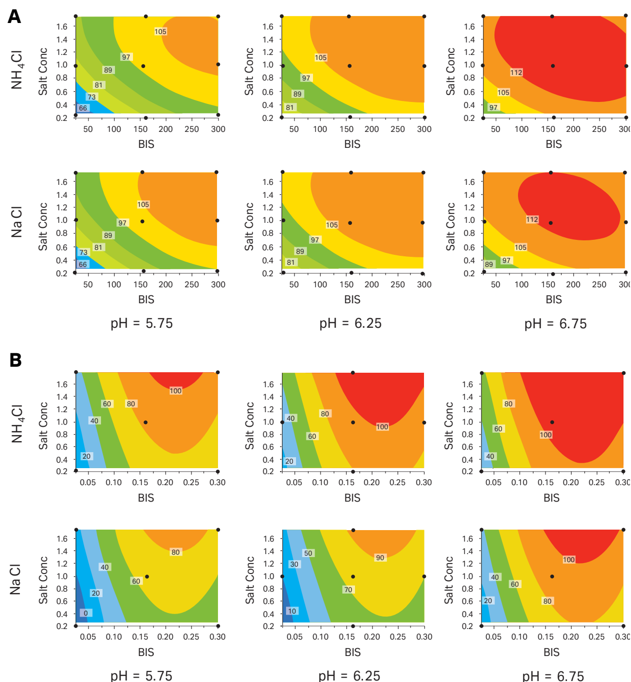

Fig 3. Contour plots for the recovery of BSA in A) PreDictor plates and B) Tricorn column (data from Application Note. *Optimizing elution conditions on Capto MMC using Design of Experiments*. (11003548 AA.). Recovery is plotted as a function of salt concentration and buffer ionic strength (BIS) at three different pH values for the two salt types NaCl and NH_4Cl. Experimental data points are shown as black dots.

The effect of urea and organic modifiers ethanol (EtOH) and isopropanol (IPA) on dynamic binding capacity is shown in Figure 4. The decreased capacity in the presence of urea and organic modifiers suggest that these can be used to improve elution efficiency.

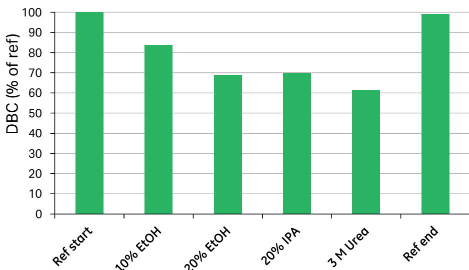

Fig 4. Dynamic binding capacity (DBC) of BSA on Capto MMC. Effect of organic modifiers and urea. DBC is plotted as percent of initial capacity (Ref start).

General purification protocol

- Adjust pH and salt concentration to match optimal conditions found during screening.
- Equilibrate column with loading buffer of the same pH and salt concentration as the sample.
- Apply sample onto the column.
- Wash out unbound material with loading buffer.
- Elute sample under optimal elution conditions established during screening. Gradient (pH or salt) or step-elution can be used.
- Regenerate column to elute bound material.
- Clean-in-place.
- Re-equilibrate.

6 Scaling up

After optimizing the method at laboratory scale, the process can be scaled up, using the following general approach:

1. Select the bed volume according to required binding capacity.
2. Select a column diameter to obtain a bed height of 10 to 45 cm. To utilize the full potential of Capto MMC at large-scale, a typical bed height range is 20 cm or higher.
3. Scale-up is typically done by keeping bed height and flow velocity constant, while increasing bed diameter and volumetric flow rate.

General recommendations for scale-up

However, since optimization is done with small column volumes, in order to save sample and buffer, some parameters like the dynamic binding capacity can be optimized using shorter bed heights than those being used in the final scale. As long as the residence time is constant, the binding capacity for the target molecule remains the same.

Other factors, such as clearance of critical impurities, can change when column bed height is changed and must be validated using the final bed height. The residence time is approximated as the bed height (cm) divided by the flow velocity (cm/h) applied during sample loading.

7 Packing columns

Recommended columns

Table 3. Recommended columns for Capto MMC

Column	Inner diameter (mm)	Bed volume[1]	Bed height (cm)
Lab-scale			
Tricorn 5/100	5	2 mL	10
Tricorn 10/100	10	8 mL	10
HiScale™ 16/20	16	20 to 40 mL	max 20
HiScale 16/40	16	20 to 70 mL	max 35
HiScale 26/20	26	53 to 106 mL	max 20
HiScale 26/40	26	53 to 186 mL	max 35
HiScale 50/20	50	196 to 393 mL	max 20
HiScale 50/40	50	196 to 687 mL	max 35
Production-scale			
AxiChrom™ [2]	50 to 200	0.2 to 12.5 L	max 40
AxiChrom[2]	300 to 1000	7 to 314 L	max 40
BPG[2,3]	100 to 300	1 to 28 L	max 40
Chromaflow™ standard[3,4]	400 to 800	12 to 51 L	max 30 cm

[1] Bed volume range calculated from 10 cm bed height to maximum bed height.

[2] The pressure rating of BPG 450 is too low to use with Capto resins.

[3] Packing instructions for Capto MMC in process-scale columns are described in Application Note 28925933.

[4] Larger pack stations might be required at larger diameters.

All large-scale columns can be supplied as variable bed height columns. Do not choose large diameter columns if the bed height is low.

Packing Tricorn 5/100 and 10/100 columns

The following instructions are for packing Tricorn 5/100 and Tricorn 10/100 columns with a 10 cm bed height.

For more details about packing Tricorn columns, see the instructions *Tricorn Empty High Performance Columns* (28409488).

Materials needed

Capto MMC

Glass filter funnel

Plastic spoon or spatula

Filtering flask

Measuring cylinder

10 mM NaCl in distilled water

Equipment needed

ÄKTA™ systems, or a stand-alone pump, depending on the flow rate required, can be used for packing. The pump filter unit and the flow restrictor must be removed due to the high flow velocity used in the column packing in order to decrease the system backpressure.

For packing Tricorn 5/100, an additional Tricorn 5/100 tube is used as packing tube which is connected by a Tricorn Packing Connector 5-5.

When packing Tricorn 10/100 column, use a Tricorn Packing Equipment 10/100 which includes the 10-mm packing connector, 100-mm glass tube (to be used as packing tube), and bottom unit with filter holder, cap, and stop plug.

When working with large volumes, real feed or repeated loading, Tricorn coarse filter kits are recommended to reduce the risk of clogging. Use Tricorn 5 Coarse Filter Kit (11001253) or Tricorn 10 Coarse Filter Kit (11001254).

Calculating amount of resin

The amount of resin needed can be calculated by: column cross-sectional area (cm^2) × bed height (cm) × compression factor (sedimented resin bed height/packed resin bed height). The compression factor is approximately 1.1 for Capto MMC.

Washing the resin

Equilibrate all material to room temperature. Capto MMC is delivered in 20% ethanol and must first be washed with the recommended packing solution, 10 mM NaCl. Put the glass filter funnel on the filtering flask. Pour the resin into the funnel and wash in sets with 5 to10 mL of 10 mM NaCl/mL resin. Gently stir with a spatula between additions.

Preparing the packing slurry

Move the washed resin from the funnel into a beaker. Add 10 mM NaCl to obtain a 60% slurry concentration. Measure the slurry concentration after settling overnight in a measuring cylinder. Tricorn columns can also be packed with an excess of resin which can be removed during packing (step 13).

Column packing procedure

To pack the column, use 10 mM NaCl in distilled water as packing solution and proceed as follows:

1. Rinse the column and packing tube in 10 mM NaCl.
2. Insert a bottom filter into the filter holder.
3. Wet the filter and the O-ring on the filter holder by dipping the filter holder into water, buffer, or 20% ethanol.
4. Insert the filter holder into the column tube. Make sure that the "keyed" part of the filter holder fits into the slot on the threaded section on the column tube. Screw the end cap onto the column tube.
5. Screw a suitable Tricorn packing connector onto the top of the column tube. The Tricorn packing connector must be fitted with suitable O-rings (included with the Tricorn packing connector). Screw the Tricorn packing tube into the upper fitting of the Tricorn packing connector.
6. Put the column and packing tube vertically on a lab stand.
7. Pour some packing solution into the tube and make sure that the liquid drips from the column bottom outlet. Insert a stop plug into the bottom unit when approximately 1 cm of packing solution remains.
8. Prime the system and the column inlet capillary with packing solution.

9. Fill both column tube and packing tube with slurry. Avoid formation of air bubbles by pouring it along a thin capillary.
10. Attach an extra bottom unit or an adapter unit to the top of the packing tube. Connect the pump to the top of the packing tube and remove the stop plug from the bottom of the column tube.
11. Increase the flow and pack the resin at 540 cm/h (1.8 mL/min in Tricorn 5/100, 7.1 mL/min in Tricorn 10/100). When the liquid above the resin bed is clear, continue packing for 10 min.
12. Pack the resin for an additional 10 min at 3000 cm/h (Tricorn 5/100: 9.8 mL/min, Tricorn 10/100: 39.3 mL/min).
13. Switch off the pump and connect a stop plug into the bottom unit. Remove the packing tube and packing connector (the liquid is allowed to pour down outside the column tube). If necessary, remove excess resin with a Pasteur pipette or spatula by re-suspending the top of the packed bed. Make sure that the resin surface is as even as possible.
14. Add packing solution up to the upper edge of the column tube.
15. Place a pre-wetted filter on top of the fluid in the column.

    Note: *The top coarse filter is inserted by another procedure. See separate instruction included in Tricorn coarse filter kits.*

16. Prepare the adapter unit by screwing the guiding ring, inside the adapter unit, down to its end position so that it is in level with the bottom of the adapter unit.
17. Wet the O-ring on the adapter unit by dipping it in water, packing solution, or 20% ethanol.
18. Screw the guiding ring back 1.5 turns.

19 Attach the top adapter unit onto the column tube, making sure the inner part of the guiding ring fits into the slot on the column tube threads. Make sure that there are no air bubbles.

Note: *The top adapter must be connected but not fully screwed down.*

Note: *Although it is possible to fit the adapter unit on the column tube without keying the inner locking device into the slot on the column tube, the adapter lock will not function. The consequence of this is that the adapter is not locked in position and accidental turning of the adapter is possible.*

20 Connect the pump, remove the stop plug and start a flow (Tricorn 5/100: 1 mL/min, Tricorn 10/100: 5 mL/min)

21 Slowly screw the adapter unit down until the filter meets the bed surface. Make sure that the filter meets the bed horizontally.

22 Increase the flow to 3000 cm/h (Tricorn 5/100: 9.8 mL/min, Tricorn 10/100: 39.3 mL/min).

23 If the resin bed compresses, slowly screw the adapter unit down to the resin surface with maintained flow.

24 Pack the resin for 5 min. If the bed has compressed further, screw the adapter unit down to the resin surface.

25 Stop the flow and connect a stop plug to the bottom unit.

26 Disconnect the pump. Screw the adapter unit down for a further 2/3 turns. Lock the adapter and attach a stop plug. If the adapter lock is correctly attached, it is not be possible to turn the adapter unit.

Testing the packed column

See Chapter *Evaluation of column packing*.

Packing HiScale columns

The following instructions are for packing HiScale 16/20, 16/40, HiScale 26/20, 26/40, and HiScale 50/20, 50/40 with 10, 20, and 35 cm bed heights.

For more details about packing HiScale columns, see instructions *HiScale columns (16, 26, 50) and accessories* (28967470).

Packing preparations

Materials needed

Capto MMC

HiScale column

HiScale packing tube (depending on bed height)

Plastic spoon or spatula

Glass filter G3

Vacuum suction equipment

Filter flask

Measuring cylinder

20% ethanol with 0.4 M NaCl

Equipment

ÄKTA systems, or a stand-alone pump, depending on the flow rate required, can be used for packing.

Equilibrate all materials to room temperature.

Definitions

The bed height of a gravity settled bed differs from the bed height of a bed settled at a given flow (consolidated). Therefore, the compression factor (CF) has to be separated from the packing factor (PF).

$L_{settled}$	Bed height measured after settling by gravity.
L_{cons}	Consolidated bed height Bed height measured after settling the resin at a given flow velocity.
L_{packed}	Packed bed height
CF	Compression factor $CF = L_{settled}/L_{packed}$
PF	Packing factor $PF = L_{cons}/L_{packed}$
A_C	Cross sectional area of the column
V_C	Column volume $V_C = L_{packed} \times A_C$
C_{slurry}	Concentration of the slurry

Preparation of the slurry

To measure the slurry concentration, let the resin settle in 20% ethanol at least overnight in a measuring cylinder or use the method for slurry concentration measurement described in application note 28925932. This method can also be used for HiScale columns.

Washing the resin

Put a glass filter funnel on a filtering flask. Suspend the resin by shaking and pour into the funnel and wash according to the following instructions:

- 5 times with 5 mL 20% ethanol with 0.4 M NaCl/mL resin
- Gently stir with a spatula between additions.
- Move the washed resin from the funnel into a beaker and add 20% ethanol with 0.4 M NaCl to obtain a 50% slurry concentration.

Packing the column

Table 4. Main features of the packing method for HiScale 16/20 and HiScale 16/40

Column	HiScale 16/20	HiScale 16/40	
Bed height (cm)	10	20	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.10	1.10	1.02
Packing velocity (cm/h)	750	750	750
Packing flow rate (mL/min)	25	25	25
Flow condition (cm/h)	750	750	420
Flow condition (mL/min)	25	25	14

Table 5. Main features of the packing method for HiScale 26/20 and HiScale 26/40

Column	HiScale 26/20	HiScale 26/40	
Bed height (cm)	10	20	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.15	1.10	1.03
Packing velocity (cm/h)	750	750	750
Packing flow rate (mL/min)	66	66	66
Flow condition (cm/h)	750	750	420
Flow condition (mL/min)	66	66	37

Table 6. Main features of the packing method for HiScale 50/20 and HiScale 50/40

Column	HiScale 50/20	HiScale 50/40	
Bed height (cm)	10	20	35
Slurry/ packing solution	20% ethanol with 0.4 M NaCl		
Slurry concentration (%)	50	50	50
Packing factor (PF)	1.15	1.15	1.03
Packing velocity (cm/h)	750	750	750
Packing flow rate (mL/min)	250	250	250
Flow condition (cm/h)	750	750	420
Flow condition (mL/min)	250	250	140

Packing procedure

1. Assemble the column according to the column instructions (*HiScale columns (16, 26, 50) and accessories*, 28967470).
2. Put the column tube in a stand.
3. Connect the bottom adapter unit to the pump or a syringe and prime the bottom net with a slow flow of packing solution. This is easiest done if the nets are dry but if air is trapped under the net it can be removed by a light suction with a syringe.
4. Attach the bottom adapter unit in the bottom of the column tube and tighten the O-ring.
5. Fill the column with approximately 1 cm packing liquid using the pump/syringe. Disconnect the pump/syringe and put a stop plug on the outlet.
6. Put the packing tube on top of the column tube.
7. Connect the top adapter to the pump and prime it with a slow downward flow. The net needs to be facing the roof as this is done. If air is trapped under the net it can be removed by a light suction with a syringe.
8. Fill the column with slurry suspended in packing solution. If needed, top up the slurry with extra packing solution so the top adapter dips into the slurry to avoid air under the net.
9. Attach the top adapter unit on top of the packing tube. Tighten the O-ring firmly and remove the bottom stop plug.

10. Start a downward flow with packing velocity according to Table 4, 5, and 6.
11. Let the flow run until the bed has consolidated.
12. Use the scale on the column to measure the bed height. There might be a buildup of resin at the column wall after the bed is consolidated and to easier see where the top of the bed is, a light source can be used.
13. Calculate the final bed height by dividing the consolidated bed height with the desired packing factor.
    $L_{packed} = L_{cons}/PF$
14. Turn off the flow and put a stop plug in the bottom.
15. Dismount the top adapter from the packing tube.
16. Over a beaker or a sink, detach the packing tube from the column.
17. Remount the top adapter in the column tube. Make sure no air is trapped under the net and lower the adapter down to 1 to 2 cm above the bed, making sure the surface is not disturbed.
18. Tighten the O-ring on the adapter. Remove the bottom stop plug and carefully start turning the end cap down. While spilling out liquid through the bottom, proceed turning until the calculated final bed height is reached.
19. Make sure that the pressure peaks that occur during turning the end knob down do not exceed the pressure specifications of the resin.
20. Start a downward flow to flow condition the bed. The flow rate is shown in Table 4, 5, and 6.
21. Let the flow run for about 10 column volumes.
    The column is ready to be tested.

Testing the packed column

See Chapter *Evaluation of column packing*.

8 Evaluation of column packing

Intervals

Test the column efficiency to check the quality of packing. Testing must be done after packing, at regular intervals during the working life of the column, and when separation performance is seen to deteriorate.

Column efficiency testing

The best method of expressing the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP) and the asymmetry factor (A_S). These values are easily determined by applying a sample such as 1% acetone solution to the column. Sodium chloride can also be used as a test substance. Use a concentration of 0.8 M NaCl in water with 0.4 M NaCl in water as eluent.

For more information about column efficiency testing, consult the application note *Column efficiency testing* (28937207).

Note: *The calculated plate number will vary according to the test conditions and it must only be used as a reference value. It is important that test conditions and equipment are kept constant so that results are comparable. Changes of solute, solvent, eluent, sample volume, flow velocity, liquid pathway, temperature, etc. will influence the results.*

Sample volume and flow velocity

For optimal results, the sample volume must be at maximum 2.5% of the column volume and the liquid velocity 30 cm/h. If an acceptance limit is defined in relation to column performance, the column plate number can be used as one of the acceptance criteria for column use.

Method for measuring HETP and A_s

Calculate HETP and A_S from the UV curve (or conductivity curve) as follows:

$$HETP = \frac{L}{N}$$

$$N = 5.54 \times \left(\frac{V_R}{W_h}\right)^2$$

L = bed height (cm)

N = number of theoretical plates

V_R = volume eluted from the start of sample application to the peak maximum

W_h = peak width measured as the width of the recorded peak at half of the peak height

V_R and W_h are in the same units

The concept of reduced plate height is often used for comparing column performance.

The reduced plate height, h, is calculated as follows:

$$h = \frac{HETP}{d_{50v}}$$

d_{50v} = Median particle size of the cumulative volume distribution (cm)

As a guideline, a value of < 3 is very good.

The peak must be symmetrical, and the asymmetry factor as close to 1 as possible (a typical acceptable range could be $0.8 < A_S < 1.8$).

A change in the shape of the peak is usually the first indication of bed deterioration due to excessive use.

Peak asymmetry factor calculation:

$$A_s = \frac{b}{a}$$

a = ascending part of the peak width at 10% of peak height

b = descending part of the peak width at 10% of peak height

Figure 5 shows a UV trace for acetone in a typical test chromatogram from which the HETP and A_s values are calculated.

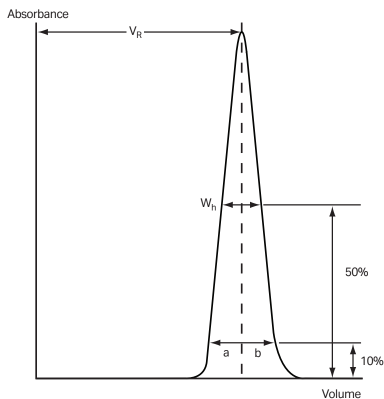

Fig 5. A typical test chromatogram showing the parameters used for HETP and A_s calculations.

9 Maintenance

For the best performance from Capto MMC over a long working life, follow the procedures described below.

Equilibration

After packing, and before a chromatographic run, equilibrate with start buffer by washing with at least 5 bed volumes, or until the column effluent shows stable conductivity and pH values.

Regeneration

After each separation, elute any reversibly bound material with a high ionic strength solution (e.g., 2 M NaCl in buffer) and at the same time increase pH to about 10 to 11. Regenerate the resin by washing with at least 5 bed volumes of buffer, or until the column effluent shows stable conductivity and pH values.

Cleaning-In-Place

Cleaning-In-Place (CIP) is a procedure that removes contaminants such as lipids, endotoxins, and precipitated or denatured proteins that remain in the packed column after regeneration. These types of contamination occur frequently when working with crude feedstock. Regular CIP prevents the buildup of contaminants in the resin bed and helps to maintain the capacity, flow properties, and general performance of Capto MMC.

A specific CIP protocol must be designed for each process according to the type of contaminants present. The frequency of CIP depends on the nature and the condition of the feedstock, but for capture steps CIP is recommended after each cycle.

CIP protocols

Precipitated, hydrophobically bound proteins or lipoproteins	Wash with 1.0 M NaOH at 40 cm/h with reversed flow direction. Contact time 1 to 2 hours, dependent on feed.
Ionically bound proteins	Wash with 0.5 to 2 column volumes of 2 M NaCl with reversed flow direction. Contact time 10 to 15 min.
Lipids and very hydrophobic proteins	Wash with 2 to 4 column volumes of up to 70% ethanol[1], or 1-propanol 1% to 5%, or 30% iso-propanol with reversed flow direction. Contact time 1 to 2 hours, dependent on feed. 1-propanol has higher flash point and might be preferred in an industrial environment. Alternatively, wash with 2 to 4 column volumes of 0.1% nonionic detergent with reversed flow direction. Contact time 1 to 2 hours, dependent on feed.

[1] Specific regulations can apply when using 70% ethanol since it can require the use of explosion-proof areas and equipment.

Sanitization

To reduce microbial contamination in the packed column, sanitization using 0.5 to 1.0 M NaOH with a contact time of 1 hour is recommended. The CIP protocol for precipitated, hydrophobic bound proteins or lipoproteins removes bound contaminants and sanitizes the resin effectively.

Storage

Store unused resin in the container at a temperature of 4°C to 30°C. Make sure that the screw top is fully tightened. Packed columns must be equilibrated in 20% ethanol to prevent microbial growth. After storage, equilibrate with at least 5 bed volumes of starting buffer before use.

10 Ordering information

Product	Pack size	Product code
Capto MMC	25 mL	17531710
	100 mL	17531702
	1 L	17531703
	5 L	17531704
	10 L	17531705
	60 L[1]	17531760

1 Pack sizes available upon request

All bulk resins products are supplied in suspension in 20% ethanol. For additional information, including data file, contact your local Cytiva representative.

Related product	Quantity	Product code
HiTrap™ Capto MMC	5×1 mL	11003273
HiTrap Capto MMC	5×5 mL	11003275
HiTrap Capto IEX Selection Kit	5×1 mL	28934388
PreDictor Capto MMC, 6 µL	4×96 well filter plates	28925814
PreDictor Capto MMC, 20µL	4×96 well filter plates	28925815
PreDictor Capto MMC, 50 µL	4×96 well filter plates	28925816
PreDictor RoboColumn Capto MMC, 200 µL	one row of eight columns	28986084
PreDictor RoboColumn Capto MMC, 600 µL	one row of eight columns	28986178
HiScreen™ Capto MMC	1×4.7 mL	28926980
HiTrap Capto MMC	5×1 mL	11003273
HiTrap Capto MMC	5×5 mL	11003275
PreDictor Capto MMC, 6 µL	4 x96-well filter plates	28925814

Related product	Quantity	Product code
PreDictor Capto MMC, 20 µL	4 x96-well filter plates	28925815
PreDictor Capto MMC, 2, 4, 6, 8, 20 and 50 µL	4x96-well filter plates	28943281
ReadyToProcess™ Capto MMC	1 L	28951118
ReadyToProcess Capto MMC	2.5 L	28929120
ReadyToProcess Capto MMC	5 L	29146145
ReadyToProcess Capto MMC	10 L	28929121
ReadyToProcess Capto MMC	20 L	28929122
Tricorn 5/20 column	1	28406408
Tricorn 5/50 column	1	28406409
Tricorn 5/100 column	1	28406410
Tricorn 5/150 column	1	28406411
Tricorn 5/200 column	1	28406412
Tricorn 10/20 column	1	28406413
Tricorn 10/50 column	1	28406414
Tricorn 10/100 column	1	28406415
Tricorn 10/150 column	1	28406416
Tricorn 10/200 column	1	28406417
Tricorn 10/300 column	1	28406418
Tricorn 10/600 column	1	28406419
HiScale 16/20	1	28964441
HiScale 16/40	1	28964424
HiScale 26/20	1	28964514
HiScale 26/40	1	28964513

Related literature		Product code
Handbook	Multimodal Chromatography Handbook	29054808
Data files	Capto MMC	11003545
	HiScreen prepacked columns	28930581
	PreDictor 96-well filter plates and Assist Software	28925839
Application notes	Methods for packing Capto MMC in production-scale columns	28925933
	Optimizing elution conditions on Capto MMC using Design of Experiment	11003548
	High-throughput screening and process development for capture of recombinant pro-insulin from E. coli	28996622
	Efficient purification of the pertussis antigens toxin, filamentous haemagglutinin, and pertactin in chromatography workflows	29227789
	High-throughput screening for elution conditions on Capto MMC using PreDictor plates	28927790
Instructions	Tricorn Empty High Performance Columns	28409488
	HiScale columns (16, 26, 50) and accessories	28967470
	Determine the compression factor and slurry concentration. Method description and practical example	29001351
	Determine the compression factor and slurry concentration: a brief overview	29001794
	Packing Capto adhere and Capto MMC using verified methods	28925933

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