Instructions for Use

BIA SEC Methacrylate Analytical Column (2000 A pores)

BIA-50.7200-2000

SARTORIUS

Contents

1. About These Instructions for Use

These instructions are part of the device. They apply to the device product number indicated on the cover page.

1.1. Accompanying Documents

In addition to these instructions, the following supporting documents may be consulted.

Guideline: Optimisation of LC system for analytical work

2. Safety

⚠**WARNING**
Denotes a hazard that may result in death or severe injury if it is not avoided.

⚠**CAUTION**
Denotes a hazard that may result in moderate or minor injury if it is not avoided.

NOTICE
Denotes a hazard that may result in property damage if it is not avoided.

2.1. Intended Use

BIA SEC Methacrylate Analytical Column is high performance chromatography devices for rapid fractionation of complex biological samples based on size exclusion. The stationary phase is based on spherical porous particles of polyhydroxymethacrylate which is packed in SUS-316 stainless steel housing to allow easy connection to any HPLC system.

The following information is provided to ensure proper product care and optimal product performance.

2.2. Safety Note

Follow the guidelines in this Instructions for Use. Improper use may result in malfunction, personal injury, or damage of the product or material. Follow safety instructions, wear gloves, safety glasses, and a lab coat during operation.

3. Technical Data

Column chemistry	Size Exclusion Chromatography (SEC) Methacrylate
Support matrix	Spherical porous particles of polyhydroxy methacrylate
Column dimensions	Diameter: 8.0 mm; length: 300 mm; bed volume (CV): ~15 mL
Connector	10-32 UNF coned port, 1/16" OD tubing connection
Particle size	10 µm
Pore radius	200 nm
Operating flow rates	0.5 – 1.0 mL/min (max 1.2 mL/min)
Maximum pressure	3 MPa, 30 bar, 435 psi
Operating temperature	4 °C (39 °F) to 70 °C (158 °F)
Chemical stability	see chapter Buffer Selection
Recommended pH	Working range 3–10
Storage conditions	0.02 % Sodium Azide or 20% EtOH
Shelf life	N.D.

4. Installation

Remove the product from its shipping box or crate and place it on a flat surface. Carefully inspect the product for any damage that may have occurred during shipping. Immediately report any such damage to your vendor and the courier. The product is shipped in 0.02 % Sodium Azide at ambient temperature and should be stored upon receiving as stated under Technical Data.

NOTICE
Do not store the product below 0 °C (32 °F).

5. Getting Started

Use the product per these guidelines. Improper use may result in malfunction, personal injury, or damage of the product or material. Follow general safety instructions for laboratory work.

⚠CAUTION
Set the pressure relief valve of the system (pump) to the value indicated in the table Technical Data.

NOTICE

The column should be equilibrated to working temperature for optimal results. Allow sufficient time for the column to reach working temperature.

Setting up the HPLC system is a crucial factor in achieving optimal performance from the product. The following suggestions should be considered:

— *Capillaries:* The inner diameter of the capillaries could strongly affect the peak shape and system pressure. Using the correct capillaries is therefore crucial.
— *Backpressure:* Check the back pressure of the system at a flow rate up to 2 mL/min higher than your working flow rate. Ensure that the back pressure of the system without the column stays at least 10 bar (1 MPa) below the maximum allowed pressure on the column (see Technical Data). Adjust the pressure relief valve accordingly.
— *Detector:* For optimal detector sensitivity set the detector response time to the lowest possible value – for most UV and FLD detectors this value is 0.1 s, for MALS detectors this value is not applicable.
— *Acquisition rate:* The acquisition rate depends on the analysis time. Data acquisition rate of 5 to 10 Hz is recommended.
— *Flow rate:* Typical analysis flow rates are 0.5 – 1.0 mL/min. For flow rate properties of the column see Technical Data.

5.1. General Recommendations

The following are general guidelines to consider when working with SEC chromatography. The guidelines may not apply to specific column chemistry or sample properties.

- Treat loading material appropriately (e.g. pre-treat, filter, concentrate / dilute, etc.). For more details, please refer to the Guideline ‘Pre-treatment of complex biological samples before column purification and regeneration procedures for columns with increased back pressure’ (biaseparations.com/en/library/guidelines).
- Always use freshly prepared solvents. Solvents stored for a long time may have changed their compositions and may influence elution patterns and/or damage the column.
- Make sure not to let air bubbles enter the column while installing the column. The air bubbles may damage the column. Never allow inside the column to dry. It can damage the column.
- Surfactants can improve recoveries in virus purification. Non-UV-absorbing (at working wavelengths) surfactants will improve the baseline signal.
- Ensure all components of the system used are compatible with the working solutions (e.g. sodium hydroxide, organic solvents, high salt concentrations, etc).
- Use the column within above stated flow rate, pressure, and temperature ranges. Using the column outside the given range may damage the column and lower its performance.

NOTICE

Always ensure mobile phases are compatible before mixing them or applying consecutively on the column. Examples of in-compatible buffers are: magnesium ion-containing buffers and sodium hydroxide (forms precipitate), acetonitrile and sodium hydroxide (forms ammonia and acetate), ammonium acetate and sodium hydroxide (potential formation of explosive atmosphere), ethanol and sodium hydroxide (forms ethoxides). Wash the column with water or another compatible solution when using two incompatible solutions consecutively.

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6. Operating the Column

6.1. System Preparation

Wash the entire HPLC system prior to column installation, including all flow-lines and sample loop by switching the valves, and then replace the washing solution with the eluent to be used. If desired new eluent has low miscibility/solubility to the eluent of previous analysis, first use the eluent that is miscible/soluble to both eluents, and then replace it with the desired eluent.

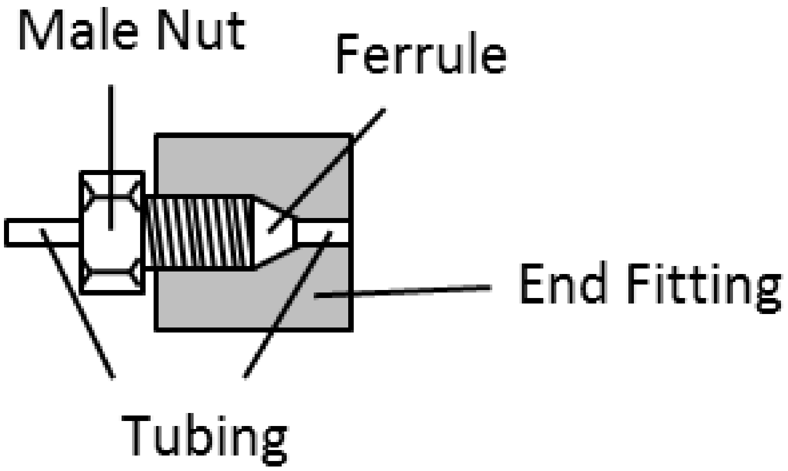
NOTICE
If the eluent left in the HPLC system is not compatible with the column to be used, it may damage the column.

NOTICE
A drastic change in the eluent compositions may remove substances adsorbed on the HPLC system and they may enter and deteriorate the column.

6.2. Connecting the Column

Connect the column to the system in the following order:

1. Connect the column to HPLC system by following the “flow direction arrow” (WRITE TO US) indicated on the column adhesive label. If a guard column is used, position the guard column in front (before the inlet) of the analytical column.
2. Make sure to insert the tubing all the way to the end fitting and secure it with the male nut. It is important that there is no extra space between the tubing and the column side of the end fitting. Presence of an extra space will let the sample to spread out and may result in wide peaks.



3. Set the initial flow rate to less than 0.3 mL/min and start the system. If the column is to be heated during the analysis, keep the low flow rate until the column temperature reaches to the set temperature, and then gradually increase the flow rate to the desired temperature.

NOTICE
The flow path is defined on the column label and must not be reversed at high flow rates.

NOTICE

It is recommended to apply flow in reverse direction during column cleaning to displace any debris or particles accumulated on the frit of the column.

NOTICE
It is recommended to set the pump limiter to avoid exceeding the maximum pressure.

NOTICE
Verify that there is no solvent leak. The solvent leak may cause electronic leakage, rust, and/or chemical injury.

NOTICE
When restarting the system after column installation or after holding the eluent flow, start the system at less than 0.3 mL/min flow rate. A rapid increase in pressure can damage the column.

NOTICE
If the column was heated during the analysis, lower the flow rate to less than 0.3 mL/min at the end of analysis. Then, turn off the column oven to let the column temperature return to room temperature before stopping the pump. This is to prevent creating an empty space in the column, which deteriorates the column.

NOTICE
If the pump was stopped while the eluent inside the column is still hot, the eluent volume decreases and creates an empty space when the eluent temperature decreases.

6.3. Equilibration

The column should be equilibrated with eluent. Use system detectors as indication of successful equilibration. Conductivity and pH at the outlet should match buffer specifications. To replace the solvent, set the system at less than 0.3 mL/min. Recommended solvent volume to introduce at each step is 3 to 5 times of the column volume.

NOTICE
Check compatibility of the desired new solvent and the solvent currently filled in the column. If needed wash the column with 3 - 5 CV of water to prevent mixing of incompatible buffers.

NOTICE
Frequent solvent replacement deteriorates the column, and is thus not recommended.

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7. Buffer Selection

The following are general guidelines to consider when working with the column:

- Degas the eluent fully to prevent the formation of air bubbles.

– Buffers and aqueous solutions of different salts can be used separately or together. Usable buffers include phosphate, acetate, citrate, and Tris. Usable aqueous salt solutions include sodium chloride, sodium sulfate, potassium sulfate, and ammonium sulfate. Their recommended concentration ranges are 0.05 to 0.3 M. Please keep total concentration of salts under 0.5 M.
– Salts such as lithium bromide can be added to DMF and DMSO. Their recommended concentration ranges are 10 - 50 mM.
– Protein denaturants such as urea and guanidine hydrochloride can also be used. However, their required concentration are usually high, which may damage the column during the solvent replacement. Thus, it is recommended to dedicate the column for these specific uses.
– Surfactants such as SDS and Brij-35 can also be added to the eluent. However, since surfactants tend to remain on the column, solvent replacement after their use takes a longer time than replacement from general solvents. The replacement time can be shortened by using 30 to 50 % (v/v) methanol.
– The maximum usable solvent percentage of methanol and acetonitrile is 75%, of DMF and DMSO 100%.
– When adding chloride ions, make sure the eluent pH is 6 or higher.
– Always ensure mobile phases are compatible before mixing them or applying consecutively on the column. Examples of in-compatible buffers are: magnesium ion-containing buffers and sodium hydroxide (forms precipitate), acetonitrile and sodium hydroxide (forms ammonia and acetate), ammonium acetate and sodium hydroxide (potential formation of explosive atmosphere), ethanol and sodium hydroxide (forms ethoxides). Wash the column with water or another compatible solution when using two incompatible solutions consecutively.
– When using a mixture of buffer (or aqueous solution of salt) and organic solvent, make sure there is no precipitation of salt.
– Solvents stored for a long time may have changed their compositions and may influence elution patterns and/or damage the column.
– When using highly corrosive salts such as sodium chloride, wash out the salts at the end of analysis. The metal parts of the devices and/or the columns may rust.

NOTICE
Do not use borate buffer as it forms a complex with diol group of the packing material.

NOTICE
Column pressure is influenced by eluent composition, flow rate, and column temperature. When changing the eluent compositions, adjust the flow rate and column temperature so that the column pressure remains below the usable maximum pressure.

NOTICE
Shear degradation occurs more likely in larger molecular weight compounds. The result of shear degradation may appear as lower molecular weight measurement than the actual value and/or low reproducibility. If shear degradation is suspected, use a lower flow rate.

NOTICE
Whenever water is required, use ultra-pure water freshly generated by a water purification system or water from a newly opened HPLC grade distilled water bottle. Use of HPLC grade organic solvents of guaranteed quality, which can be used without problems in HPLC is recommended. If organic solvents with different grades are used together, make sure that their qualities are all suitable for the analysis prior to the use. Solvents left in opened bottles for a long time should not be used. The content may have been changed, absorbed moisture, or has been contaminated.

8. Sample Preparation

During sample preparation consider next recommendations:
— If possible, use the eluent for analysis to dissolve or dilute samples. If this is difficult, use a solvent which has a composition that is as close as possible to the eluent composition and which fully dissolves or dilutes the sample.
— Suggested injection volume is 50 to 100 μL.

NOTICE
When a sample is dissolved in a solvent other than the eluent and if the sample matrix contains components which do not dissolve in the eluent fully, precipitates may form and clog the column.

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9. Cleaning | Maintenance

Cleaning and maintenance of the column may improve its lifetime and increase reproducibility. Sample properties should be taken into account for column cleaning.

9.1. Cleaning in Place (CIP)

We recommend to clean the column after one day of analysis or after observing decreased column performance:
— Problems in peak shapes and elution time changes or elevated column pressure are often caused by the deposition of insoluble or adsorbing components from the sample/flow-line inside the column.
— During the column cleaning, disconnect the detector and collect the washing solution directly from the column outlet into a waste container (i.e., do not let the solution go through the detector).

Cleaning procedure:
— Insoluble components that block the column inlet may be removed by reversing the flow direction, i.e., introducing the eluent from the column outlet, with flow rate at less than half of the recommended flow rate.
— Follow below cleaning steps for adsorbing components. For an efficient cleaning, reverse the flow direction. Set the flow rate at less than 0.5 mL/min. Recommended solvent volume to introduce is 5 to 10 times of the column volume.
  - Adsorption of hydrophobic compounds (when using aqueous eluent): introduce the eluent with a high polar organic solvent (acetonitrile or methanol).
  - Adsorption of ionic compounds: introduce the eluent with a higher salt concentration.

NOTICE
Keep the organic solvent and salt concentrations within the concentrations stated in Chapter 7.

NOTICE
Keeping the washing solution in the column for a long time will lead to column deterioration. Please replace the washing solution with the eluent immediately after cleaning.

NOTICE
If the column performance does not improve (recover) after performing the column cleaning, please replace the

column with a new one.

⚠CAUTION
Remain below the maximum pressure specified in Technical Data.

⚠CAUTION
Ensure compatibility between the current column solution and cleaning solutions (see examples in General Recommendations).

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10. Storage

Remove the column from HPLC system after replacing the in-column solvent with the initial shipping solvent or 20% EtOH. Securely tighten the end caps and store the column at a location with stable temperature (a cool and dark space is recommended). Refer to section Equilibration for how to replace the eluent.

11. Troubleshooting

Problems arising during the analysis are usually related to the column, sample, mobile phase, or the instrumentation. It is advisable to use an elimination approach to exclude possible causes. Please refer to our troubleshooting guide (biaseparations.com/en/library/guidelines).

12. Decommissioning | Transportation

If there is reason to return the product, complete a Return Form (biaseparations.com/en/terms-conditions) and contact help.bia@sartorius.com.

Contaminated samples used during the process that could cause biological or chemical hazards are potentially hazardous substances. If the product has come into contact with hazardous substances, steps must be taken to ensure proper decontamination and declaration.

Procedure
Decontaminate the product. The operator of the product is responsible for adhering to local government regulations on the proper decontamination and declaration for transport and disposal.

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The information and figures contained in these instructions correspond to the version date specified below. Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice. Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote the other gender as well.

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