Instructions for Use

CIMac SDVB 0.1 mL Analytical Column (2 µm channels)

CIM Convective Interaction Media®
BIA-110.9001-2

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

CIMac Analytical Monoliths are high performance chromatography devices for rapid high-resolution fractionation of complex biological samples. The stationary phase is polymerised as a monolith with homogeneous channel size and surface chemistry. Each unit is mounted in a precision engineered stainless steel housing to allow easy connection to any HPLC system.

The backbone of SDVB column is highly hydrophobic with a strong affinity for hydrophobic or less polar compounds. It enables fast separation of hydrophobic analytes under reverse phase conditions. The following information are guidelines for column use, and should be modified as needed.

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	SDVB (reverse phase; poly(styrene-co-divinylbenzene))
Channel radius	1050 nm (950 nm – 1150 nm)
Support matrix	poly(styrene-co-divinylbenzene)
Monolith dimensions	Diameter: 5.2 mm; length: 4.95 mm; bed volume (CV): 0.1 mL
Connector	10-32 UNF coned port, 1/16'' OD tubing connection
Ligand density	N.D.
Operating flow rates	0.2 – 3 mL/min (1 – 15 cm/min; 2 – 30 CV/min)
Maximum pressure	15 MPa, 150 bar, 2175 psi
Operating temperature	4 °C (39 °F) to 60 °C (140 °F)
Chemical stability	The PS-DVB monolith is highly stable accross the entire pH range. It can tolerate common organic solvents (acetonitrile, methanol, ethanol). Exposure to aqueous mobile phases should be kept to a minimum. Avoid rapid changes from aqueous to organic mobile phase. A linear gradient or shallow steps should be used.
Recommended pH	Working range 2–13, cleaning in place 1–14
Storage conditions	2 °C (36 °F) to 25 °C (77 °F); 20 % ethanol
Shelf life	2 years

4. Installation

Remove the product from its shipping box or crate and place 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 the designated storage solution 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 CIMac™ Analytical Columns. The following suggestions should be considered:

Capillaries: The inner diameter of the capillaries strongly affects the peak shape. Using capillaries with smaller diameter will result in sharper peaks.

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 detectors this value is 0.1 s.

Acquisition rate: The acquisition rate depends on the analysis time. A typical analysis time in the case of CIMac™ Analytical Columns is less than 15 min. Data acquisition rate of 5 to 10 Hz is recommended.

Flow rate: Typical analysis flow rates are 0.2–2 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 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 mobile phases, filtered through 0.2 µm filter, compatible with mobile phases.
- Air bubbles will not disturb the stationary phase and can be washed out of the column. However, drying the monolith risks damaging the stationary phase.
- Surfactants can improve recoveries in virus purification. Non-ionic surfactants will not interact with ion exchange chromatography media. 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).

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.

5.2. Buffer Selection

For optimal operation and column lifetime, 10 % (v/v) of organic modifier is recommended in all mobile phases. Exposure to aqueous mobile phases without organic modifier should be kept to a minimum. Consider compatibility between the aqueous (buffering species) and organic (commonly acetonitrile or methanol) portion of the mobile phase, as well as compatibility with the cleaning solution.

Outgassing can occur when organic solvents are combined with aqueous mobile phase. While bubbles do not affect the monolith stationary phase, outgassing could impact the detectors. If the LC system used does not have inline degassing, the mobile phase can be degassed before use. In addition, pre-mixed solutions can be used instead of mixing neat solutions with the LC. For example, to run a gradient from 10 % acetonitrile to 50 % acetonitrile, pre-mix the solutions and run the gradient from 100% A to 100 % B instead of connecting water to inlet A and acetonitrile to inlet B.

The surface of reverse-phase columns are highly hydrophobic, thus excluding water from their surface. To ensure the surface of the stationary phase is suitably "wetted", at least 1 % organic solution should be in the mobile phases used (10 % organic solution recommended in mobile phases).

NOTICE
Organic solvents cause swelling of the stationary phase. Step methods should be avoided, and the concentration of organic solvent should be increased gradually over several column volumes.

NOTICE
Limit contact with aprotic organic solvents (e.g. acetonitrile, DCM), to the method run (gradient). Short (overnight) and long term storage in the designated storage solution. See table Characteristics of the monolith.

NOTICE
Never add acetonitrile directly to alkaline solutions (e.g. NaOH). Acetonitrile hydrolyses to ammonia and acetate.

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6. Operating the Column

6.1. Connecting the Column

Connect the column to the system in the following order:

1. Carefully remove the blind fitting on one side and connect the inlet tubing to the column.
2. Carefully remove the blind fitting on the opposite side and connect the outlet tubing to the column.

The column can be disconnected from the system by reversing the above steps.

Note: The flow path inside the housing is symmetrical, and analysis can be performed in both directions.
Note: 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.

6.2. Equilibration

The column should be equilibrated before use. The equilibration procedure should avoid steep transitions between mobile phases containing low percent of organic modifier to mobile phases containing high percent of organic modifier. The binding mobile phase should be of similar composition to the loading sample, and it is recommended to use a binding mobile phase which contains at least low percent of organic solvent (e.g. 1 % acetonitrile).

1. Starting with a column in storage solution, wash the column with at least 20 CV of binding mobile phase. **Note**: It is useful to flow the first few CV directly into waste without going through the detector cell. This will remove any air bubbles that may affect the detector cells.
2. Run a linear gradient to 100 % elution mobile phase over 10 CV.
3. Hold at 100% elution mobile phase for 20 CV. A reduced flow rate will ensure extended contact time with elution mobile phase (containing organic solvent) and improve equilibration. Alternatively, a static hold in elution buffer can be used (without flow).
4. Run a linear gradient over 10 CV back to the starting mobile phase.
5. Repeat steps 1–4 until a stable reproducible baseline is achieved or perform several blank runs (injecting binding mobile phase in place of sample) until the baseline is stable and reproducible.

7. 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.

7.1. Cleaning in Place (CIP)

Sample molecules may bind to the column strongly and not completely elute from the column or may even precipitate on the column. This build-up of contaminants on the monolithic column may cause loss of resolution and binding capacity, increased back pressure, or a complete blockage of the column. A specific CIP procedure should be considered for the type of contaminants present in the sample. An example of a general CIP procedure is presented below.

⚠**CAUTION**
In case of pressure increase during cleaning, adjust flow rate to remain below the maximum pressure allowed over the column.

Perform the following procedure at up to half the maximum operating flow rate. This will ensure sufficient contact time between the monolith and cleaning solution.

1. Wash the column with 10 CV of binding mobile phase (low % of organic modifier)
2. Wash the column with 10 CV of eluting mobile phase (high % of organic modifier)
3. Wash the column with 10 CV of binding mobile phase (low % of organic modifier)
4. Equilibrate the column.

Note: If CIP does not restore column performance completely, consider extending contact time of cleaning solution.

8. Storage

Clean and equilibrate the column before storage. It is recommended to store the column in the designated storage solution both overnight and for long-term storage.

NOTICE
Limit contact with aprotic organic solvents (e.g. acetonitrile, DCM), to the method run (gradient). Short (overnight) and long term storage in the designated storage solution. See table Technical Data.

NOTICE
NaOH-ethanol mixtures at any concentration form ethoxide anions that are highly destructive to biomolecules, and ligands on chromatography media. Neutralise the column environment before introducing ethanol.

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1. Wash the column with 15 CV storage solution. **Note:** Apply organic solvents using linear gradient of 10 CV or in shallow steps of 10 %. Avoid rapid change from aqueous to organic mobile phase.
2. Seal the column with blind fittings and store at the temperature specified in Technical Data. If there is a possibility of biological contamination from the sample it is recommended to store the column between 2 °C (36 °F) and 8 °C (46 °F).

Note: Reduce the flow rate when using viscous solvents (such as ethanol) to avoid pressure increase.
Note: Never combine acetonitrile and alkaline solutions (e.g. NaOH). Acetonitrile hydrolyses to ammonia and acetate.

9. 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).

10. 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.

11. Ordering Information

Transferring the workflow to a different scale or format (analytical, screening) is simple with CIM®. Contact your local support to find the appropriate products.

Purification Scale Products

Catalog number	Product name
311.9001-2	CIMmultus® SDVB 1 mL Monolithic Column (2 µm channels)
414.9001-2	CIMmultus® SDVB 4 mL Monolithic Column (2 µm channels)
411.9001-2	CIMmultus® SDVB 8 mL Monolithic Column (2 µm channels)
614.9001-2	CIMmultus® SDVB 40 mL Monolithic Column (2 µm channels)
611.9001-2	CIMmultus® SDVB 80 mL Monolithic Column (2 µm channels)
814.9001-2	CIMmultus® SDVB 400 mL Monolithic Column (2 µm channels)
811.9001-2	CIMmultus® SDVB 800 mL Monolithic Column (2 µm channels)
1014.9001-2	CIMmultus® SDVB 4000 mL Monolithic Column (2 µm channels)
1011.9001-2	CIMmultus® SDVB 8000 mL Monolithic Column (2 µm channels)

Screening Solutions

Catalog number	Product name
BIA-122.9001-2	CIM® SDVB 0.05 mL Monolithic 96-well Plate (2 µm channels)

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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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