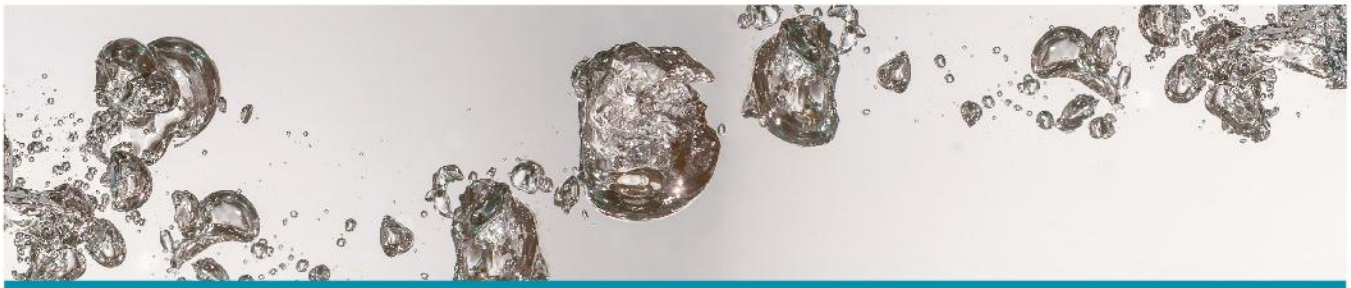
CIM Convective Interaction Media®

INSTRUCTION MANUAL



CIMac™ AAV full/empty - 0.1 Analytical Column (Pores 1.3 µm)

ABOUT CIMac™ ANALYTICAL COLUMNS

CIMac™ Analytical Columns are high-performance monolithic columns offering all the advantages of a special continuous short polymeric bed. These columns are pre-packed in stainless steel housings and allow user friendly connection to HPLC equipment. The stationary phase-matrix consists of a single-piece (monolithic) methacrylate based polymer with a defined morphology (pore size, size distribution, porosity and shape). Due to the monolithic nature (made of one piece) of the stationary phase no previous column packing is needed. The stainless steel housing is designed in an arrow shape predefining a suggested flow direction. The innoviative design of the housing now allows the flow to be applied in both directions, prolonging the lifetime of the column. The housing is designed according to the Sound Engineering Practice guidelines.

Figure 1: CIMac™ Analytical Columns

CIMac™ AAV full/empty-0.1 Analytical Column is intended for in-process and final determination of full and empty AAV vector capsids.

BEFORE YOU BEGIN

Use the product according to guidelines in this Instruction manual. 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.

Unpacking

Carefully unpack the column and inspect it for any damage that may have occurred during shipping. Immediately report any such damage to your vendor and the transport company. CIMac™ AAV full/empty Analytical column is shipped in 20% ethanol at room temperature. Upon arrival store the column between 4°C (39°F) and 25°C (77°F).

WARNING: Never store the column below 0°C (32°F).

Optimising the HPLC system

The set-up of the HPLC system is one of the crucial factors in achieving optimal performance from analytical columns. A low or high pressure gradient HPLC system, connected via capillaries and a mixing chamber to the injection valve or auto-sampler, a filling loop for sample injection, a detector and a recorder or data acquisition system is a prerequisite. Each of these components influences the separation quality when fast analyses are required.

Capillaries: The inner diameter of the capillaries strongly affects the peak shape. Smaller diameter capillaries result in sharper peaks. The use of capillaries with an inner diameter of 0.13 mm or less is recommended.

Back pressure: Check the back pressure of the system at a flow rate up to 2 mL/min higher than your actual flow rate. Ensure that the back pressure of the system without the column stays below 150 bar (15 MPa). Adjust the pressure relief valve accordingly.

Detector: Set the detector response to the lowest possible value – for most UV detectors this value is 0.1 s.

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Acquisition rate: The acquisition rate depends on the analysis time. Typical analysis times in the case of CIMac™ Analytical Columns are less than 15 min. To obtain optimal resolutions for separations of less than 10 minutes, the data acquisition rate should be 5 to 10 Hz.
Flow rate: Can be set up to 3 mL/min (Note: typical analysis flow rates are 0.2-2 mL/min).

General recommendations

To extend the lifetime of the CIMac™ Analytical Column, please consider following guidelines:

- Always use freshly prepared mobile phases (buffers).
- Always filter mobile phases (buffers) and samples through 0.22 µm filter.
- Apply procedures that will prevent precipitation of the sample or impurities in the column.
- If possible, add small amounts of non-ionic and nonadsorbing (at 280 nm) detergents (e.g. 0.1 % Tween 20) in working buffers.
- Regularly check the column performance.
- Utilise the column in both flow directions the analyses in both flow directions of the column.
- Never disassemble the column as it can impair the column's performance.

WARNING: Never let the column dry out!

USING THE CIMac™ AAV full/empty Analytical Column

Buffer selection

Typical buffer for CIMac AAV full/empty columns are 20-100 mM Tris, bis-Trispropane (BTP) with pH in range 8.5-9.5. Elution buffers typically contain a higher salt concentration (for example 200 mM – 2 M NaCl).

Equilibrating

The column should be equilibrated according to the following procedure:

1. Wash the column with at least 1 mL (10 CV) of deionized water, followed by at least 1 mL (10 CV) of the binding mobile phase at one-half of the working flow rate. **Note:** Allow the first 0.2 mL (2 CV) to flow directly into a waste container. This will remove any small particles or air bubbles that may affect the detector cell.
2. Wash the column with at least 1 mL (10 CV) of the eluting mobile phase at one-half of the working flow rate.
3. Finally, wash the column with at least 1 mL (10 CV) of the binding mobile phase again at a working flow rate.

Regeneration Procedure

Regeneration of the column starts with the removal of bound substances from the stationary phase, followed by re-equilibration with the appropriate counter-ion. The following method is recommended:
Wash the column with at least 2 mL (20 CV) of buffer containing 2 M NaCl at the operating flow rate (for details on operating conditions refer to the PSIS). Equilibrate the column according to the procedure described in the section 'Equilibrating CIMmultus™ Advanced Composite Column' above.

Cleaning In Place (CIP) Procedure

In some cases, a simple regeneration of the monolithic column is insufficient. Sample molecules may 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 protocol should be designed according to the type of contaminants present in the sample. An example of a general CIP procedure is presented below:

1. Wash the column with 1 to 2 mL (10 to 20 CV) of a cleaning solution containing 1 M NaOH and 2 M NaCl. **Note**: Use a low flow rate (1 mL/min; 1 CV/min) to ensure exposure of the column to the cleaning solution for several minutes.
2. Wash the column with 1 to 2 mL (10 to 20 CV) of deionised water.
3. Wash the column with 1 to 2 mL (10 to 20 CV) of 1 M ammonium acetate.
4. Wash the column with 1 to 2 mL (10 to 20 CV) of deionised water.

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5. Wash the column with 1 to 2 mL (10 to 20 CV) of a concentrated buffer (e.g. 0.1 M to 0.5 M buffer) to restore the appropriate pH.
6. Re-equilibrate the column with at least 20 mL (20 CV) of the binding mobile phase (buffer).

Sanitisation

CIMac™ Analytical Columns can be sanitized. Pump 1 to 2 mL (10 to 20 CV) of a cleaning solution (1 M NaOH and 2 M NaCl) through the column at low flow rate (1 mL/min; 1 CV/min). Stop the pump and leave the column in contact with the cleaning solution for at least 2 h (up to 12 h) at room temperature. If needed the column can be disconnected from the system, sealed with blind fittings and after sanitization re-connected to the system. After sanitization follow the steps 2. to 6. of the general CIP procedure described above.

WARNING: Ensure that the chromatography system and auxiliary components are compatible with NaOH at the concentrations used. Follow all safety regulations when handling with NaOH solutions.

Storage

When not in use, the column should be washed with at least 1 mL (10 CV) of deionized water and flushed with at least 2 mL (20 CV) of storage solution (20% ethanol) at a flow rate between 0.2 to 0.5 mL/min, sealed with end stoppers, and stored between 4 °C (39°F) to 25 °C (77°F).

Note: do not let the column (monolith) dry out; this could irreversibly impair its chromatographic characteristics.

WARNING: Do not store the CIMac™ AAV full/empty -0.1 Analytical Column below 0 °C (32 °F).

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RETURN OF THE COLUMN

The returned Column should be accompanied with completed Return Form (http://biaseparations.com/terms-conditions).

TROUBLESHOOTING

Problems arising during the analysis are usually related to the column, the sample, the mobile phase, or the instrumentation. It is advisable to use the elimination procedure to exclude the possible causes. The first step is to inject a standard sample to exclude problems with the sample itself. Then, change the mobile phase. After excluding these two parameters the problem is either related to the column or the instrumentation.

Problem	Possible cause	Action
Increased pressure over the column	Blockage in monolithic column, mixing tee, capillaries, in-line high pressure filter or detector cell.	• Clean the detector cell. • Filter mobile phases • Check all the connections (capillaries, fittings) in the system and replace if needed. • Perform the column regeneration and cleaning procedures.
Loss of resolution	• Column not well conditioned. • Clogged column. • Inappropriate linear gradient. • System problems (pumps, mixing, chamber, too wide capillaries, the detector response tie and acquisition rate, blocked injector).	• Extend the equilibration time of the column. • Perform the column regeneration and cleaning procedure. Check the compatibility of your sample with the mobile phases in use (precipitation, coalescence...). • Redesign the linear gradient (shallower gradients will generally result in better separation-resolution). • Perform a system check. • Use a small amount of non-ionic detergent in working buffers.
Poor or no baseline separation	• Elution buffer (pH, ionic strength) is not appropriate. • Inappropriate linear or step gradient (due to the systems' dwell or dead volume). • Hydrophobic sample.	• Check the elution buffer. Check the pH and ionic strength needed to completely elute your sample. • Check the system's parameters. If necessary, increase the time ramp of your linear gradient. • Use a small amount of non-ionic detergent in working buffers.
No binding	• Buffer (pH, ionic strength) is not appropriate.	• Check the binding buffer. Check the pH and ionic strength needed to obtain binding of your sample.

For any additional information please contact us:

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