BIOshell™
U/HPLC Columns

This Data Sheet Contains Important Information About The Product.

Care & Use Sheet for 3.4 µm BIOshell A400 Protein C4 Columns

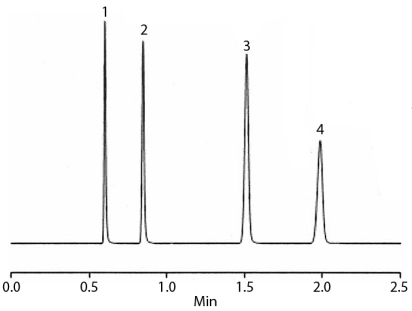
Description

BIOshell A400 Protein C4 columns are high speed, high performance liquid chromatography columns based on a new wide-pore (400 Å) Fused-Core® particle design. The Fused-Core particle provides a thin porous shell of high purity silica surrounding a solid silica core. This particle design exhibits very high column efficiency due to the shallow diffusion paths in the 0.2 µm thin porous shell and the small overall particle size of 3.4 micron. The densely bonded, extensively endcapped dimethylbutylsilane stationary phase of BIOshell A400 Protein C4 provides a stable, reversed phase packing that can be used for separating high molecular weight compounds such as proteins and large peptides.

Column Characteristics

Figure 1 shows a sample Quality Control test chromatogram for a 10 cm x 4.6 mm column. A printed report including the actual QC chromatogram and performance results is enclosed with every column. Also included with each column is a QA test report for the specific batch of packing contained in the column. The Fused-Core particle has a surface area of ~ 15 m^2/g and an average pore size of 400 Å.

Figure 1. QC Chromatogram



1.	Uracil	30 µg/mL
2.	Phenol	500 µg/mL
3.	Propiophenone	120 µg/mL
4.	1-Cl-4-nitrobenzene	150 µg/mL

Test Conditions

column:	10 cm x 4.6 mm
mobile phase:	25:75, acetonitrile:water
flow rate:	1.5mL/min
temp.	ambient (~24 °C)
det.:	UV @ 254 nm
sample volume	1 µL

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Operation Guidelines

- The preferred direction of flow is marked on the column label.
- Operation of the column in reversed flow mode may be used to attempt removal of inlet blockage or contamination.
- A new column contains acetonitrile. Initial care should be taken to avoid mobile phases that are immiscible with this or could cause a precipitate.
- Water and all common organic solvents are compatible with BIOshell A400 Protein C4 columns
- BIOshell A400 Protein C4 columns are best used at temperatures below 90 ºC for maximum column life.
- Mobile phase pH for BIOshell A400 Protein C4 columns is best maintained in the pH range of 2-9 range for maximum column stability.
- BIOshell A400 Protein C4 columns are stable to operating pressures up to 600 bar (9000 psi).

Column Care

To maximize column life, ensure that samples and mobile phases are particle free. The use of guard columns or an in-line filter with 0.5 µm porosity between the sample injector and the column is highly recommended. The 2 µm porosity frits on BIOshell A400 Protein C4 columns are less subject to pluggage than are the 0.5 µm frits typically used with other small particle columns. Should the operating pressure of the column suddenly increase beyond normal levels, reversing the flow direction of the column may be attempted to remove debris on the inlet frit.

To remove strongly retained materials from the column, flush the column in the reverse direction with very strong solvents such as 100% of the organic component of the mobile phase in use. A mixture (95/5 v/v) of dichloromethane and methanol is often effective at this task. Extreme cases may require the use of very strong solvents such as dimethylformamide (DMF) or dimethylsulfoxide (DMSO).

Column Storage

Long-term storage of silica-based, reversed-phase columns is best in 100% acetonitrile. Columns may be safely stored for 3 or 4 days in most common mobile phases. However, when using buffers, it is best to protect both the column and the HPLC equipment and remove the salts by flushing the column with the same mobile phase without the buffer (e.g., when using 60/40 ACN/buffer, flush the column with 60/40 ACN/H_2O) to eliminate any danger from corrosion from the salts while providing rapid re-equilibration of the column with the original mobile phase.

Before storing the column, the end-fittings should be tightly sealed with the end-plugs that came with the column to prevent the packing from drying.

Safety

- **HPLC columns are for laboratory use only. Not for drug, household, or other use.**
- Users of HPLC columns should be aware of the toxicity or flammability of the mobile phases chosen for use with the columns. Precautions should be taken to avoid contact and leaks.
- HPLC columns should be used in well-ventilated environments to minimize concentration of solvent fumes.

Applications

The BIOshell A400 Protein C4 bonded phase is nonpolar in nature. It is best utilized with mobile phases that are mixtures of acetonitrile and water or methanol and water. Higher levels of the organic solvent component will typically reduce the retention of the sample compounds. Operation at elevated temperatures (e.g., 40-60 ºC) will reduce the viscosity of the mobile phase, lower column pressure, and allow the use of faster flow rates for higher sample throughput. Gradient elution techniques using 5 -10% organic component as the initial mobile phase and increasing to 100% organic component as the final mobile phase often can affect separations of complex sample mixtures in minimal time.

BIOshell A400 Protein C4 columns are eminently suited for the reversed-phase separation of high molecular weight compounds such as proteins with MW of 400 to 500 KDa. The use of 20-50 mM buffers is always recommended for optimum results and long-term stability when separating ionizable compounds. Additional information on solvent selection and separation techniques can be found in Chapters 6-8 of Practical HPLC Method Development, Second Edition, L.R. Snyder, J.L. Glajch, and J.J. Kirkland, (John Wiley & Sons, 1997).

Guidelines for Low-Volume Columns

High performance columns with small internal volumes (shorter lengths, internal diameters < 3 mm) are being increasingly used for high speed separations, especially with specialty detection systems such as mass spectrometers. These low volume columns generate peaks having considerably less volume than those eluting from columns of larger dimensions (e.g., 15 cm x 4.6 mm). The efficiency of separations performed in low volume columns is highly dependent on the HPLC system having components designed to minimize band spreading. All low volume columns perform best when used with proper attention to the following factors:

- **Detector:** Flow cells should be of low-volume design (preferably <2 µL). To properly sense and integrate the often very fast peaks that elute from low volume columns, the detector response time should be set to (~ 0.1 second or less) and the integration software should sample the detector signal at least 20 points per second.
- **Injector:** The injection system should be of a low volume design (e.g., Rheodyne® Model 8125). Autosamplers will often cause band spreading with low volume columns but may be used for convenience with the expectation of some loss in column efficiency.
- **Connecting Tubing:** The shortest possible lengths of connecting tubing with narrow internal diameters (at most 0.005 inch, 0.12 mm ID) should be used to connect the column to the injector and the detector cell. The tubing must have flat ends and should bottom out inside all fittings. ZDV (zero dead volume) fittings should always be used where required.
- **Peak Retention:** As retention is increased, the volume of a peak increases, decreasing the effects on band spreading caused by components of the instrument.
- **Sample Solvent:** For isocratic separations, the sample should be dissolved in the mobile phase or in a solvent that is weaker (more polar) than the mobile phase. For gradient separations, the sample should be dissolved in the initial mobile phase or in a solvent substantially weaker than the final mobile phase.
- **Injection Volume:** For isocratic separations, the volume of sample injected should be kept as small as possible (typically 2 µL or less). Sample volumes are less critical for gradient separations, especially if the sample is dissolved in a weak solvent.

Ordering Information

For ordering information or for technical support on this product, visit the Sigma-Aldrich website at **sigmaaldrich.com** or contact the Sigma-Aldrich office or a designated distributor in your country.

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