BIOshell™
U/HPLC Columns

This Data Sheet Contains Important Information About The Product.

Care & Use Sheet for 2.7 µm BIOshell A160 Peptide C18 Columns

Description

BIOshell A160 Peptide C18 is a high speed, high performance liquid chromatography column based on a new 160 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 for high MW solutes (up to 20 kDa) due to the shallow diffusion paths in the 0.5 micron thick porous shell and the small overall particle size of 2.7 microns. The non-endcapped, sterically protected di-isobutyl-octadecylsilane bonded phase of BIOshell A160 Peptide C18 provides a stable, reversed phase packing with a pore structure and pore size that is optimized for reversed phase HPLC separations of peptides and polypeptides, using typical acidic mobile phases favored for protein structure-function and proteomic applications.

Column Characteristics

Figure 1 shows a sample Quality Control test chromatogram for a 4.6 mm x 5 cm column. A printed report including the actual QC test chromatogram and a Batch Quality Assurance Test are enclosed with every column.

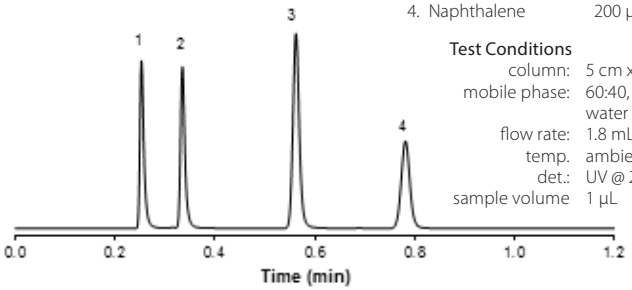
The fused core particle has a surface area of ~80 m^2/g and an average pore size of 160 Å. The fused core particles are 30% to 50% heavier than commercially available totally porous particles due to the density of the solid cores. The effective surface area per column is similar to columns packed with totally porous particles having surface areas in the range of 120 - 150 m^2/g.

Figure 1. QC Chromatogram

1.	Uracil	30 µg/mL
2.	Phenol	500 µg/mL
3.	1-Cl-4-nitrobenzene	120 µg/mL
4.	Naphthalene	200 µg/mL

Test Conditions

column:	5 cm x 4.6 mm
mobile phase:	60:40, acetonitrile: water
flow rate:	1.8 mL/min
temp.	ambient (30 °C)
det.:	UV @ 254 nm
sample volume	1 µL



Operation Guidelines

- The direction of flow is marked on the column label.
- Reversed flow may be used to attempt removal of inlet blockage or contamination.
- A new column contains a mixture of acetonitrile and water. Initial care should be taken to avoid mobile phases that are immiscible with this mixture or could cause a precipitate.
- BIOshell A160 Peptide C18 columns have been application tested using low pH mobile phases (pH 1 - 2.5) at operational temperatures up to 100 °C, and have been qualified to exhibit very long column life at 60 °C at intermediate pH (up to pH 6). The maximum recommended temperature for operation at pH 6 - 8 is 40 °C for long column life.
- BIOshell A160 Peptide C18 columns are stable to operating pressures up to 600 bar (9000 psi).
- Separations of complex peptide samples on BIOshell A160 Peptide C18 columns, such as tryptic digests of proteins, are typically accomplished with gradient elution from 2% to 50% acetonitrile in the mobile phase. Although the column is compatible with 100% aqueous mobile phases, it is always advisable to check retention reproducibility of poorly retained (early eluting) sample components, when using very low organic content (<2%) at the start of a gradient.

Column Care

To maximize column life, ensure that samples and mobile phases are particle free. The use of guard columns or an inline filter with 0.5 micron porosity between the sample injector and the column is highly recommended. The 2 micron porosity frits on BIOshell A160 Peptide C18 columns are less subject to plugging than are the 0.5 micron 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 removing lipidic contaminants and certain detergents. 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 short periods (up to 3 or 4 days) in most common mobile phases. However, when using buffers, it is best to remove the salts to protect both the column and the HPLC equipment by flushing the column with the same mobile phase without the buffer. Before storing the column, the end-fittings should be tightly sealed with the end-plugs that came with the column.

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

BIOshell A160 Peptide C18 columns are commonly operated by running a gradient from low (5%) to high percent (95%) acetonitrile at low pH. The use of elevated temperature improves sample throughput. The sterically protected C18 bonded phase in BIOshell A160 Peptide C18 columns allows long term operation of the column at low pH and elevated temperature. Peptide separations are efficiently conducted using low pH mobile phase modifiers, often at 0.01-0.1% concentration, most popularly employing trifluoroacetic acid (TFA), and the related perfluorocarboxylic acids, pentafluoropropionic acid (PFPA) and heptafluorobutyric acid (HFBA). These acids exhibit desirable low UV transparency, volatility, and peptide ion pairing properties. Additional opportunities for low pH operation include the normal short chain carboxylic acids, formic acid and acetic acid, as well mineral acids, such as phosphoric acid (0.001-0.02 M). Additional information on solvent selection and separation techniques can be found in Chapters Six, Seven, Eight, and Eleven in 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 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 cell volumes should be <2 µL. To properly sense and integrate the very fast peaks that can elute from low volume columns, the detector response time should be set to the fastest level (~0.1 second) to allow integration of signal by software of at least 20 points across the narrowest peak.
- **Injector:** The injection system should be of a low volume design (e.g., Rheodyne® Model 8125). Autosamplers will often cause band spreading, 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 small internal diameters (≤0.005 inch, 0.12 mm I.D.) must be used to connect the column to the injector and the detector cell.
- **Peak Retention:** As retention is increased, the peak volume increases, decreasing extra column band spreading caused by components of the instrument.
- **Sample Solvent:** For isocratic separations, the volume of sample injected should be kept as small as possible (≤2 µL) in a solvent weaker than the mobile phase. Sample volumes are less critical for gradient separations, and a larger volume is possible 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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