User Guide

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Agilent ZORBAX 300SB-C18 and SB-C18 Capillary Columns

General description

The Agilent ZORBAX 300SB-C18 is a microparticulate column packing, engineered to have uniform, spherical particles with a controlled pore size of 300 Å. The ZORBAX 300SB-C18 was designed for peptide mapping and separation of synthetic and natural peptides and proteins.

The Agilent ZORBAX SB-C18 is a unique microparticulate C18 packing, engineered to have uniform, spherical particles with a controlled pore size of 80 Å. The ZORBAX SB-C18 is a stable, reversed-phase packing that can be used for basic, neutral, or acidic samples.

Agilent StableBond packing is made by chemically bonding a sterically protected octadecyl stationary phase to a specially prepared, ultrahigh-purity, porous-silica, Agilent ZORBAX microsphere and is well suited to applications using high-sensitivity detectors that require low backgrounds (that is, mass spectrometers). The ZORBAX silica support is designed to reduce or eliminate strong adsorption of basic compounds. The densely covered, sterically protected diisobutyl n-octadecyl stationary phase is chemically stable and gives longer column life. It is particularly well suited for use with aggressive mobile phases (such as mobile phases with pH < 2, high ionic strength, ion-pair additives, TFA, and so on) since the steric protection of the bonded phase resists degradation caused by such mobile phases. This characteristic is particularly important for use in methods that need long-term stability and reproducibility. Columns are loaded to a uniform bed density using a proprietary, high-pressure, slurry-loading technique to give optimum column efficiency.

The column hardware consists of glass-lined tubing with a 3.2 mm outer diameter (od) and a 0.3 or 0.5 mm inner diameter (id). The ends of the tube are reduced to 1.6 mm od so that a special 1/16" zero dead volume union can be attached as a fitting. In this fitting, two stainless steel screens with a nominal mesh size of 2 µm are present to retain the packing.

Operational guidelines

The operational guidelines are as follows:

- The dead volume of the LC system should be reduced as much as possible. It is recommended that polyether ether ketone (PEEK)-coated fused-silica connection capillaries with 0.05 mm id are used. The Capillary, Fittings, and Connectors for HPLC web page is a good starting point for more information.
- The flow rate for optimal column efficiency for capillary HPLC columns is proportionately smaller than that of a standard bore column due to the smaller diameter. The flow rate must be reduced by the square of the diameter ratio of the capillary and standard bore column. Typical flow rates recommended for Agilent capillary HPLC columns are 2 to 20 µL/min (for 0.3 mm id columns) and 6 to 60 µL/min (for 0.5 mm id columns).
- The sample should be dissolved in the initial starting conditions of the mobile phase, or in a solvent that is substantially weaker than the B solvent.
- The volume of sample injected will affect the efficiency of the column. For high efficiency, the sample volume must be kept as small as possible. A good guideline is to inject a volume of 0.05 to 0.1 µL on a 0.3 mm id column, and 0.1 to 0.2 µL on a 0.5 mm id column.
- The narrow-id capillaries used in the system and for column connections may occasionally clog. It is good practice and strongly recommended to monitor and regularly log the column pressure under standard conditions. If there is a deviation of more than 10% from the previously logged values, one of the capillaries may be clogged. In such a case, proceed as described in the "Column care" section.
- The column connection is made with a special hand-tightened screw and ferrule. Move the column connection capillary through the screw and ferrule so that it protrudes by 1 to 2 mm. Then, connect it tightly to the inlet/outlet fitting of the column.
- Due to their small particle size, dry ZORBAX packings are respirable; therefore, they should only be opened in a well-ventilated area.
- Always purge the column with a strong solvent like 100% acetonitrile or methanol before first use.
- A new column contains a mixture of acetonitrile and water. Care should be taken to not pass any mobile phase through the column that might cause a precipitate.
- The direction of flow is not marked on the column. It is recommended that the flow direction is maintained once selected. While it is generally not harmful to the column, reversing the flow should be avoided except to attempt removal of any inlet clogging (see the "Column care" section).
- ZORBAX 300SB-C18 and ZORBAX SB-C18 columns are compatible with water and all common organic solvents.
- The maximum operating pressure for 0.3 and 0.5 mm id columns is 350 bar (5,000 psi).
- The maximum operating temperature is 90 °C.
- Avoid the use of mobile phases below pH 0.8 or above pH 8.0.*
- Columns should not be left in low or elevated pH solvent, or at elevated temperature, when not in use.

***Note**: StableBond columns are designed for high stability at low pH (such as pH < 5). However, all silica-based packings have some solubility in pH > 6 aqueous mobile phases. Therefore, when using silica-based columns under conditions of pH > 6, maximum column lifetime is obtained by operation at low temperatures (< 40 °C) using low buffer concentrations in the range of 0.01 to 0.02 M.

Column care

The capillary columns have screens with a nominal porosity of 2 µm. Samples that contain particulate matter larger than 2 µm may clog the column inlet screen and should be filtered before injection into the column.

If the solvent flow appears to be restricted (high pressure), first check to see that the solvent flow is unobstructed up to the column inlet. If the column has a restriction, there may be particulate matter on the inlet screen. An initial attempt should be made to remove any inlet debris by backflushing the mobile phase through the column. If this fails to return the column back to near its original backpressure, the inlet screens should be changed. To remove the screens, loosen the nut at the column inlet. Then, remove the fitting, taking care not to disturb the column bed. The screens can be flushed out using pressurized air or a high flow rate from an external pump. Install two new screens and carefully tighten the fitting.

Column storage

Columns may be safely stored for short periods in most mobile phases. Long-term storage of silica-based, bonded-phase columns should be in a pure organic solvent–preferably an aprotic liquid such as 100% acetonitrile. If the column has previously been used with a buffered mobile phase, the buffer should first be removed by purging the column with 20 to 30 column volumes of a 50/50 mixture of methanol or acetonitrile and water, followed by 20 to 30 column volumes of the pure solvent. Before storing the column, the end fittings should be tightly capped with end plugs to prevent the stationary phase from drying out.

Ordering information

Part Number	Description
5064-8255	ZORBAX SB-C18, 80 Å, 150 × 0.3 mm, 5 µm
5064-8262	ZORBAX SB-C18, 80 Å, 150 × 0.5 mm, 3.5 µm
5064-8264	ZORBAX 300SB-C18, 300 Å, 150 × 0.5 mm, 5 µm
5064-8267	ZORBAX 300SB-C18, 300 Å, 150 × 0.3 mm, 3.5 µm
5064-8268	ZORBAX 300SB-C18, 300 Å, 150 × 0.5 mm, 3.5 µm
5065-9913	ZORBAX 300SB-C18, 300 Å, 5 × 0.3 mm, 5 µm, 5/pk
5065-4427	Replacement frits (screens) for capillary LC columns, 2 µm, stainless steel, 10/pk

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