Agilent POROSHELL 300SB-C18

Datasheet

General Description

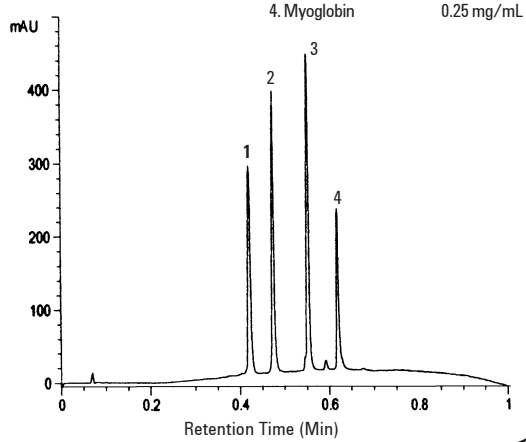
POROSHELL 300SB-C18 is a unique microparticulate superficially-porous C18 column packing. Columns of this material are especially useful for carrying out ultra-fast gradient separations of macromolecules because of the favorable kinetic properties of the special silica support. Very rapid gradient separation of proteins, poly-peptides and related biomacromolecules is attained by operating these columns at high flow rates (mobile phase velocities); rapid mass transfer maintains good column efficiency for excellent separations. This StableBond (SB) packing is made by covalently bonding a patented sterically-protected C18 stationary phase to the specially prepared, high-purity POROSHELL 300 microparticles. The special silica support is designed to reduce or eliminate strong adsorption of basic compounds, and is especially "friendly" towards biomolecules such as proteins. The densely covered, sterically-protected diisobutyl n-octadecylsilane stationary phase allows these particles to be routinely used at low pH (e.g., pH 1) and temperatures to at least 70°C with excellent stability. Therefore, POROSHELL 300SB-C18 is particularly well suited for use with aggressive mobile phases that are widely used for the reversed-phase separation of proteins and other such biomacromolecules (e.g., pH <2, high ionic strength, ion-pair additives, etc.). This packing also is useful for protein, polypeptide, DNA fragment, etc. separations at intermediate pH (e.g., pH 7.5). POROSHELL 300SB-C18 is well suited in applications that use high-sensitivity detectors that require low backgrounds (e.g., mass spectrometers).

The uniform, spherical POROSHELL 300SB-C18 packing is based on ultra-pure, less acidic 5-µm silica particles with a solid core and a 0.25-µm-thick shell of 300Å pores, resulting in a nitrogen surface area of about 4.5 m^2/g. Columns are loaded to a uniform bed density using a proprietary high-pressure slurry-loading technique to give optimum column efficiency.

FIGURE 1
POROSHELL 300SB-C18 QA Chromatogram

OPERATING CONDITIONS
Column: POROSHELL 300SB-C18 2.1 mm ID x 75 mm (5µm)
Mobile Phase A: Water with 0.1% TFA
Mobile Phase B: Acetonitrile with 0.07% TFA
Gradient: 0 Minutes 5%B
1.0 Minutes 100%B
Flow Rate: 2.0 mL/min
Temperature: 70°C
Detector: UV (212nm)
Sample Volume: 2 µL
Peak Identity:

1. Neurotensin	0.25 mg/mL
2. Ribonuclease A	0.25 mg/mL
3. Lysozyme	0.25 mg/mL
4. Myoglobin	0.25 mg/mL



Column Characteristics

A typical Quality Assurance test gradient elution chromatogram of a protein mixture for a 2.1x75 mm column is shown in Figure 1. This column configuration has been found to be a useful compromise to allow a wide range of macromolecular gradient separations.

Safety Considerations

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users of liquid chromatographic equipment should be aware of the toxicity or flammability of their mobile phases.
- Because of its small particle size, dry POROSHELL 300 packings are respirable. Columns should only be opened in a well-ventilated area.

Operational Guidelines

- The direction of flow is marked on the column.
- While generally not harmful to the column, reversed flow should be avoided except to attempt removal of inlet pluggage (see "Column Care" section).
- A new column contains a mixture of methanol and water. Initially, care should be taken not to pass any mobile phase through the column that might cause a precipitate.
- POROSHELL 300SB-C18 is compatible with water and all common organic solvents, including N, N'-dimethylformamide and dimethylsulfoxide.
- Avoid use of this column below pH 0.9 or above pH 8.0.
- Maximum operating pressure is 400 bar (6000 psi).
- Maximum operating temperature at pH <5 is 70°C, for pH 5 -8 is 40° C.

NOTE: StableBond columns are designed for high stablity at low pH (e.g., 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 10 to 20 mM. Column stability at pH > 6 is also enhanced by using organic buffers rather than phosphate and carbonate buffers [ref.: H.A. Claessens, M.A. van Straten, and J.J. Kirkland, J. Chromatogr. (A), 728 (1996) 259].

Mobile Phase Selection

The nonpolar bonded stationary phase is best used with mobile phases such as acetonitrile/water or methanol/water mixtures. Increasing the concentration of the organic modifier generally reduces the retention of the sample. Initial gradient elution separations typically use 5% aqueous acetonitrile or methanol as the initial solvent, and 100% organic as the final solvent. Separations of polypeptides, proteins, DNA fragments, etc. often use trifluoroacetic or formic acid modifier for pH control and/or as an ion-pairing additive for desired retention and selectivity. Organic buffers such as TRIS are especially useful for intermediate pH applications. Additional information on solvent selection may be found in Chapters six, seven and eight of *Practical HPLC Method Development, Second Edition*, L. R. Snyder, J. J. Kirkland and J. L. Glajch (John Wiley and Sons, New York, 1997).

Applications

The POROSHELL 300SB-C18 column is especially suited for gradient separations performed at low pH, with mobile phases such as the acetonitrile/aqueous trifluoroacetic acid combinations widely used for protein and peptide reversed-phase separations. However, protein separations also have been successfully performed at pH 7.5 with TRIS buffer. DNA fragments and similar materials also can be gradient-separated with acetonitrile/aqueous trifluoroacetic acid mobile phases. The preferred 2.1x75 mm column configuration used for POROSHELL 300SB-C18 permits high flow rates to be used for very fast separations. Flow rates of 1-2 mL/min have been found useful for this configuration, but up to 3 mL/min at 70°C have been used (pressure: < 300 bar) for long periods without difficulty.

For optimum performance, HPLC equipment used with this column should have the capability to operate effectively for rapid, low-volume peaks with a minimum of extra-column dead volume that would degrade separation resolution. Satisfactory results have been obtained with an Agilent Technologies Model 1100 liquid chromatograph fitted with a microcell and a microsampling valve. To accurately define ultra-fast peaks, detectors and data handling devices should have a data rate of greater than 10 points/sec. Conventional autosamplers generally do not provide optimum sampling because of conditions that can lead to extra-column band broadening and reduced resolution.

The amount of sample that can be loaded on the 2.1mm ID column in gradient separations is a function of solute molecular weight. About 2 - 3 nanomoles of a typical protein/polypeptide can be separated without significant band broadening. For example, this relates to about 50 µg of ovalbumin (MW=45,000) and about 2 µg of angiotensin II (MW=1046).

Column Care

After use, unwanted biologically-active contaminants can be eliminated from the column by purging with strong acid (e.g., formic). Strongly alkaline solutions should not be used, as is the case for all silica-based column packings. The column may be purged with very strong solvents (e.g., 1:1 methanol/dichloromethane) to eliminate unwanted highly-retained contaminants. In difficult cases, mobile phases containing dimethylsulfoxide or N,N'-dimethylformamide at low flow rates overnight may also be used for this purpose.

The inlet frit on these columns have a nominal porosity of 2 µm. Samples that contain particulate matter which is larger than 2 µm will plug the column inlet frit.

If solvent flow appears to be restricted (high column back-pressure), check first to see that solvent flow is unobstructed up to the column inlet. If the column has the restriction, there may be particulate matter on the inlet frit. An initial attempt should be made to remove any inlet debris by back-flushing 10-15 mL of mobile phase through the column.

Storage Recommendations

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 been previously used with a buffered mobile phase, the buffer should first be removed by purging the column with 20-30 column volumes of a 50/50 mixture of methanol or acetonitrile and water, followed by 20-30 column volumes of the pure solvent. Before storing the column, the end-fittings should be tightly capped with end-plugs to prevent the packing from drying out.

Columns may be safely stored for short periods in most mobile phases. However, to protect equipment, it is desirable to remove salts from the instrument and column by purging the column with the same mobile phase without the buffer (e.g. using 60/40 ACN/H_2O to remove a 60/40 ACN/0.02 M phosphate buffered mobile phase). Re-equilibration is rapid with the original mobile phase when using this approach, and any danger of corrosion from the salts is eliminated.

Ordering Information	Agilent Part No.
POROSHELL 300SB-C18 Column (5 µm)	
2.1 mm ID x 75 mm	660750-902
1.0 mm ID x 75 mm	661750-902
POROSHELL 300SB-C18 Guard Column (5 µm)	
2.1 mm ID x 12.5 mm (4/pk)	821075-920
Guard Column Hardware Kit	820888-901

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