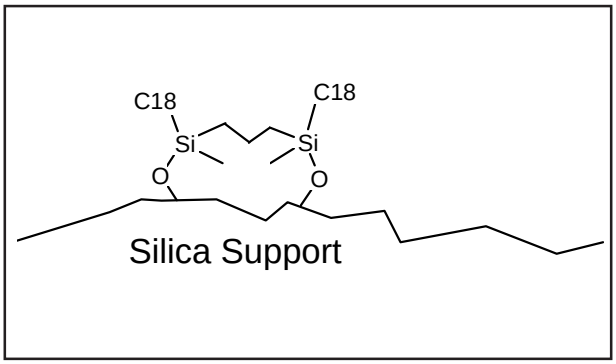
Agilent Zorbax 300Extend-C18

Datasheet

General Description

Zorbax 300Extend-C18 incorporates a patented bidentate organosilane combined with double endcapping to protect its ultra-pure Type B) silica support from dissolution at high pH. 300Extend-C18 is specially designed for stable use with high pH mobile phases and is particularly well-suited for separating highly basic compounds as free bases. In addition, the structure of the unique bidentate-C18 bonded stationary phase also is useful for separations at low and intermediate pH with excellent stability and chromatographic separation properties. 300Extend-C18 packing is made by first chemically bonding a dense monolayer of propylene-bridged bidentate-C18 silane stationary phase to a specially prepared, ultra-high purity (≥99.995% SiO_2) Zorbax 300Rx-SIL porous silica microsphere support. A schematic of the structure of this attached bidentate silane to the silica support is shown below.



The bidentate-C18 bonded phase is double endcapped using proprietary reagents and procedures to obtain maximum deactivation of the silica support surface. The combination of the bidentate-C18 and the exhaustive endcapping produces a unique, highly-hydrophobic stationary phase that greatly reduces the rate of silica dissolution that normally occurs with silica-based column packings at intermediate and high pH. This feature then allows the 300Extend-C18 packing to be used routinely with certain high pH mobile phases for high-efficiency separations. The 300Extend-C18 is especially suited for separating highly basic compounds that produce poor peak shapes on most columns. The uniform, spherical 300Extend-C18 particles are based on ultra-high purity Zorbax 300Rx-SIL that has a nominal surface area of 50 m^2/g and a narrow controlled pore size of 300Å. This special Zorbax silica support (Type B) is designed to eliminate or reduce strong adsorption of basic and highly polar compounds. Columns are loaded to a stable, uniform bed density using a proprietary high-pressure slurry-loading technique to give maximum column efficiency.

Column Characteristics

A typical Quality Control test chromatogram for a 4.6 mm ID x 150 mm (3.5µm) column is shown in Figure 1. The actual QC test and performance of your column is described on the Column Performance Report enclosed with your column.

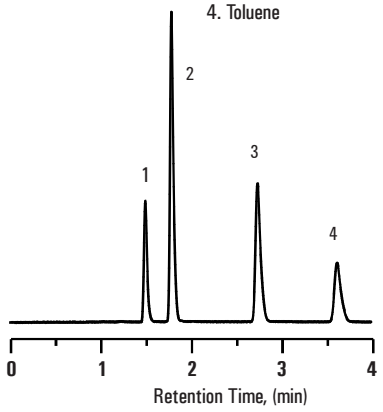
Safety Considerations

- 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 the small particle size, dry Zorbax packings are respirable. Columns should only be opened in a well-ventilated area.

FIGURE 1
300Extend-C18 QC Chromatogram

OPERATING CONDITIONS
Column: 300Extend-C18, 4.6 mm ID x 150 mm (3.5 µm)
Mobile Phase: 70% Methanol/30% Water
Flow Rate: 1.0 mL/min
Temperature: Ambient (23°C)
Detector: UV ABS. (254 nm)
Sample Volume: 2 µL

Peak Identity:		
	1. Uracil	5 µg/mL
	2. Phenol	200 µg/mL
	3. 4-Chloro-nitrobenzene	25 µg/mL
	4. Toluene	850 µg/mL



pH Guidelines

The 300Extend-C18 column consists of porous Zorbax 300Rx-SIL silica with a double-endcapped stationary phase containing about 4% carbon. The bidentate feature results from the covalent bonding of the silane to the silica support with two siloxane groups (see above schematic). The "monofunctionality" of the bonded ligand (one silanol reacted with one equivalent of C18) ensures that the stationary phase can be reproducibly synthesized within narrow limits. This bidentate bonding to the silica support also strongly contributes to the stability of the stationary phase at all pH values; hydrolysis at low pH is inhibited and degradation at intermediate and high pH by silica dissolution is moderated. When using organic buffers, it has been documented that the longevity of such a column at pH 11.0 can exceed 30,000 column volumes without significant separation degradation. This stability is similar to traditional silica-based columns, but only when they are operated at low or intermediate pH. Although this column is primarily proposed for high pH separations, it is highly competitive for low and intermediate pH applications.

The 300Extend-C18 column has been used up to pH 11.5 with good results, providing certain operating parameters are followed. For high pH separations, organic buffers should be used and the operating temperature should not exceed 40ºC. Highest column lifetime is obtained at lower temperatures, such as 25ºC. Useful organic buffers and their properties are summarized below:

Organic base	pK_a	Effective pH use range
pyrrolidine	11.3	10.3 - 12.3
triethylamine	10.7	9.7 - 11.7
1-methyl-piperidine	10.3	9.3 - 11.3
glycine	9.8	8.8 - 10.8
TRIS	8.1	7.1 - 9.1
ammonia	9.2	8.2 - 10.2

Other organic buffers such as bis-tris-propane also appear to be acceptable alternatives. All of these buffers can be made by titrating with HCl to the desired pH. Borate buffers apparently also can be used, but less experience is available with these materials. Whenever possible, carbonate and phosphate buffer should be avoided at both intermediate and high pH, since these materials enhance the solubility of the silica support to cause column failure, compared to the organic buffers listed above. Most experience has been obtained with 1-methyl-piperidine buffers, which is useful at pH 11 where most basic compounds are essentially transformed into free bases.

Studies indicate that methanol is a preferred organic modifier for high pH applications, since the rate of silica support dissolution and subsequent column degradation is slower with this solvent than with acetonitrile. Methanol also can produce better peak shapes when the column is used at intermediate pH. No preference for organic modifier has been noted for low pH applications.

1-Methyl-piperidine as supplied by Aldrich Chemical Co., Inc. (M7,260-9) has been successfully used as a buffering material with the 300Extend-C18 column (e. g., see *J. Chromatogr. A*, 797 (1998) 111). While this chemical is reputed to have 99% purity, prolonged use can result in some impurities building up on the inlet, changing column characteristics. Should this occur, a thorough purge of the column with 50% methanol/50% 0.05%trifluoroacetic acid generally restores the column to original performance. 300Extend-C18 guard columns are recommended to protect the analytical column from any contamination source. Ultraviolet transmittance of the organic buffers in the above table is useful down to about 220 nm. The exception is ammonia which can be used at lower wavelengths.

The operating pH of buffers used for separating basic compounds should be at least one pH unit (preferably 1.5 pH units) above the pK_a of the basic compound of interest. (See J. J. Kirkland, *Current Issues in HPLC Technology*, *LC-GC Supplement*, May 1997, S46, Figure 13). Best stability at pH >6 is obtained using organic buffers, and temperatures not exceeding 40ºC, as suggested in recent *J. Chromatogr.* A papers on higher pH separations with silica-based columns [797 (1998) 111; 762 (1997) 97; 728 (1996) 259; 691 (1995) 3] . Phosphate and carbonate buffers should be avoided for best stability of all silica-based columns at intermediate and high pH. Successful operation and good column lifetime at pH 11.5 and 40ºC with a buffer made from Aldrich pyrrolidine also has been demonstrated. Good column stability also has been found with a pH 10.5 mobile phase of ammonia/methanol, which is a useful medium for high pH separations when using a mass spectrometer as a detector. An overview of bidentate chemistry and chromatography was recently published in *Anal. Chem.* 70 (1998) 4344-4352.

Instrument Guidelines

Many HPLC instruments, including Agilent instruments use Vespel rotor seals in their injection valves (both manual and autosampler injection valves). Vespel is recommended for use up to pH 9.5. At pH's above 9.5, Vespel rotor seals will start to degrade, possibly causing plugging of downstream components in the flow path including the HPLC column. Therefore, Vespel rotor seals should be replaced with Tefzel rotor seals, which are stable up to pH 12.5, for applications using pH's above 9.5. The appropriate part numbers are listed below for Agilent instruments:

Agilent Autosampler	Tefzel Rotor Seal P/N
Model 1090	1535-4900
Model 1050	0101-1849
Model 1100	0100-1849

Agilent Manual Injector	Tefzel Rotor Seal P/N
Model 1090 (Rheodyne 7010)	1535-4900
Model 1090 (Rheodyne 7125)	0101-0620
Model 1050 (Rheodyne 7125)	0101-0620
Model 1100 (Rheodyne 7125)	0101-0620

Operational Guidelines

- The direction of flow is marked on the column.
- While it is not harmful to the column, reverse 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.
- 300Extend-C18 is compatible with water and all common organic solvents.
- The use of a 300Extend-C18 guard column is recommended to protect the 300Extend-C18 column and extend its useful lifetime.
- Avoid use of this column below pH 2 or above pH 11.5; optimum operating range is pH 2 - 11.5 (see "pH Guidelines" section).
- Maximum operating pressure for columns up to 9.4 mm ID columns is 400 bar (600 psi).
- Maximum operating temperature is 60ºC.

  NOTE: 300Extend-C18 columns are designed for stability over a wide pH range. When using these silica-based columns under conditions of pH >6, maximum column lifetime is obtained by operation at temperatures not exceeding 40ºC.

Mobile Phase Selection

The bonded stationary phase is nonpolar in nature and is best used with mobile phases such as methanol/water or acetonitrile/water mixtures. Increasing the amount of organic component usually reduces the retention time of the sample.

Due to the relatively high viscosity of recommended mobile phases, increased efficiency can be achieved with the use of column temperatures in the range of 40-60°C; however, best column lifetime is achieved with operation at ≤40°C. Gradient-elution techniques for this packing often use 5% methanol or acetonitrile as the initial solvent and 100% methanol or acetonitrile as the final solvent. Additional information on solvent selection may be found in Chapters Six and Seven, *Introduction to Modern Liquid Chromatography,* Second Edition, L.R. Snyder and J.J. Kirkland, (John Wiley & Sons, 1979), and Chapters Six, Seven and Eight, *Practical HPLC Method Development*, Second Edition, L.R. Snyder, J.L. Glajch, and J.J. Kirkland, (John Wiley & Sons, 1997).

Applications

300Extend-C18 HPLC columns are specifically designed for reversed-phase separation of synthetic and natural peptides and proteins, and peptide fragments from enzymatic digests (peptide mapping). The wide-pore (300Å) packing is highly recommended for solutes with molecular sizes greater than 4,000 daltons. 300Extend-C18 can be used with basic, neutral or acidic compounds. Ionizable compounds (basic, acidic) often can be satisfactorily separated at pH ~3 with this column. The 300Extend-C18 column also can be used with intermediate pH (5 - 8) mobile phases for compounds that are not stable at low pH, or for separations that have band spacing problems. However, 300Extend-C18 is especially suited for separating basic compounds at high pH (9 - 11.5) to obtain stable separations with excellent peak shapes and column efficiency. For optimum results and long-term stability and reproducibility, the use of 10 - 50 mM buffers is always recommended when separating ionizable compounds. For separations at low or intermediate pH, basic modifiers such as triethylamine or dimethyloctylamine usually are not required to achieve efficient separations with symmetrical peaks. In rare cases, 10 -20 mM of triethylamine or 5 -10 mM dimethyloctylamine might be needed in the low or intermediate pH separation of certain highly basic compounds. No basic modifiers are required when high pH separations are performed as suggested.

Column Care

The inlet frit on these columns has a nominal porosity of 2 µm. Samples that contain particulate matter which is larger than 2 µm will plug the column inlet frit and should be filtered before injection into the column. Zorbax guard columns and a hardware kit are recommended for use with such samples (see Part Numbers).

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 25-30 mL of mobile phase through the column. If this fails to return the column to near its original back-pressure, the inlet frit should be changed. To remove the frit, carefully loosen the nut at the inlet, taking care not to turn the end fitting itself. Then carefully remove the fitting taking care not to disturb the column bed. The frit should drop out when the fitting is tapped sharply on a hard surface. Install a new frit and carefully tighten the fitting.

To remove strongly retained materials from the column, flush the column with stronger (less polar) solvents. Solvents such as methanol, acetonitrile, or a 95/5 mixture of dichloromethane and methanol should remove most highly retained compounds. In extreme cases, dimethyl sulfoxide or dimethylformamide at low flow rates may also be used for this purpose. When switching between solvents with vastly different polarities, it may be necessary to first purge the column with a mutually miscible solvent such as isopropanol.

Since columns have 1/16" terminations, a short 1/4" wrench should be used in assembling fittings to prevent overtightening the ferrules. Overtightening the fittings can damage the fitting and necessitate replacement.

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.
300Extend-C18 Columns (3.5 µm)	
2.1 mm ID x 50 mm	765750-902
2.1 mm ID x 150 mm	763750-902
4.6 mm ID x 50 mm	765973-902
4.6 mm ID x 150 mm	763973-902
300Extend-C18 Columns (5 µm)	
4.6 mm ID x 150 mm	773995-902
4.6 mm ID x 250 mm	770995-902
Guard Columns	
2.1 mm ID x 12.5 mm (4 pack)	821125-932
4.6 mm ID x 12.5 mm (4 pack)	820950-932
Guard Column Hardware Kit	820888-901

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