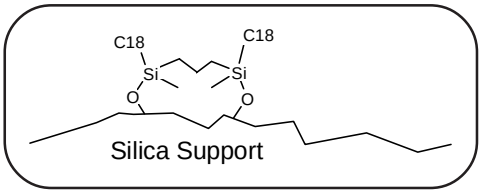
Agilent ZORBAX Extend-C18 RRHT Threaded Column

Datasheet

General Description

Extend-C18 RRHT threaded columns are specially designed for higher pressure operation (up to 600 bar) and are packed with a high performance microparticulate C18 packing for high-speed reversed phase HPLC. ZORBAX Extend-C18 incorporates a patented bidentate organosilane combined with double endcapping to protect its ultra-pure (Type B) silica support from dissolution at high pH. Extend-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. Extend-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 Rx-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 uniform, spherical Extend-C18 particles are based on ultra-high purity ZORBAX Rx-SIL that has a nominal surface area of 180 m^2/g and a narrow controlled pore size of 80Å. 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 1.8 µm Extend-C18 RRHT 4.6-mm ID × 50-mm threaded 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.

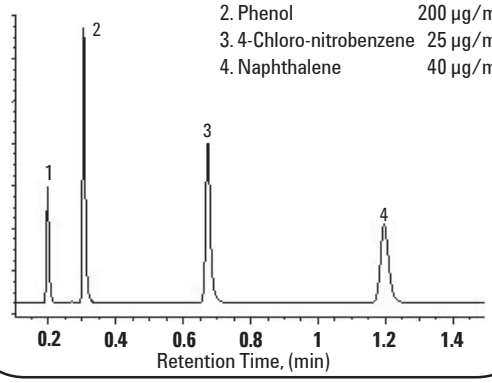
Safety Considerations

- 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.
- These RRHT assembled columns are mechanically stable and have been tested to very high pressures to assure safe lab operation on a variety of LC instruments. The 2.1- and 3.0-mm ID columns will support 20,000 psi (1300 bar) operation and 4.6-mm ID columns will support 16,000 psi (1000 bar) operation. Opening columns may compromise these pressure limits. Chromatographic performance has not been tested above 600 bar.
- Because of the small particle size, dry ZORBAX packings are respirable. Columns should only be opened in a well-ventilated area.

FIGURE 1
Extend-C18 RRHT QC Chromatogram

Opearting Conditions
Column: Extend-C18 RRHT, 4.6 mm ID x 50 mm (1.8 µm) threaded column
Mobile phase: 60% Acetonitrile/40% Water
Flow rate: 2.0 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. Naphthalene	40 µg/mL



pH Guidelines

The bidentate bonding to the silica support 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 Extend-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 40 °C. Highest column lifetime is obtained at lower temperatures, such as 25 °C. 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

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.
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 was 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 Extend-C18 column (for example, 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.
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 40 °C, as suggested in *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 40 °C 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 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).
- These columns are packed and assembled for high pressure (up to 600 bar) use. Disassembling the column will degrade column performance.
- Extend-C18 is compatible with water and all common organic solvents.
- 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 is 600 bar (9000 psi).
- Maximum operating temperature is 60 °C.

  NOTE: Extend-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 40 °C.

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.
Useful 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).

Column Care

Samples that contain particulate matter may plug the column inlet frit and should be filtered before injection into the column.

If solvent flow appears to be restricted (unusually 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 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 column should be replaced.

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.

Since columns have 3/8-inch end nuts, a short 3/8-inch wrench should be used to attach the columns to the instrument to avoid any additional tightening of the end fittings. Over tightening the end fittings will cause damage and require column 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 was 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 (for example, 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.

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