

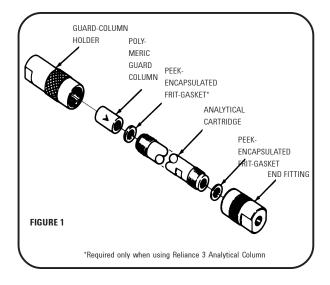
# Agilent Zorbax Reliance Cartridge-Columns

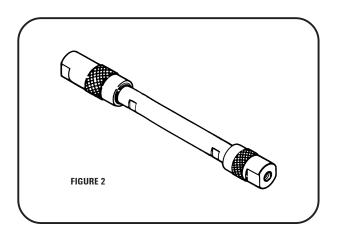
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#### Introduction

The Zorbax Reliance Cartridge-Column Series has been developed to provide convenient, cost-effective, high-speed, liquid chromatographic analyses. The cartridge components assemble quickly and easily to provide a high efficiency, low dead-volume column that seals with PEEK-encapsulated frit-gaskets at pressures up to 350 bar (5000 psi) and temperatures up to 60° C. The reusable cartridge end fittings adapt the cartridge column for connection to standard 1/16" LC fittings. The packings used are Zorbax materials, identical to those used in traditional, high-quality, Zorbax HPLC columns produced with compression fittings. Reliance cartridges are recommended for analyses that require only a moderate number of theoretical plates. These units provide shorter analysis time and savings in column and solvent costs.

Two types of cartridge columns are available: the Reliance 3, which uses 3  $\mu m$  Zorbax packings in a cartridge 6 mm ID x 40 mm long, and the Reliance 5 with 5  $\mu m$  Zorbax packings in a 4 mm ID x 80 mm long cartridge. A column coupler is available that provides a zero-dead-volume coupling of additional cartridges to double or triple total column length and separation efficiency. Prepacked guard-column cartridges also are available with 5  $\mu m$  Zorbax packings.





#### **Product Information**

#### **Zorbax Packings**

Packings used in the cartridge-series columns are the same as those in all other analytical Zorbax columns. Zorbax packings are silica microspheres produced using technology patented by DuPont. The characteristics of Zorbax particles include exceptional structural rigidity and an extremely narrow particle-size distribution, contributing to the formation of efficient, stable, bed structures. These packings consist of exhaustively derivatized, porous-silica microspheres having reproducible, covalently bonded, silane monolayers.

#### Cartridges

The cartridges are made of 316 stainless steel and are available in two column types. The Reliance 3 uses a threaded stainless steel tube, 6 mm ID x 40 mm long, packed with 3  $\mu m$  Zorbax packing. The Reliance 5 is a threaded 4 mm ID x 80 mm tube packed with 5  $\mu m$  Zorbax packing.

The two cartridges are interchangeable; the same thread pattern and the same sealing hardware are used. The packing is retained by stainless steel frits which are pressed into the cartridge. The inlet frit on both the Reliance 3 and Reliance 5 cartridge columns is 2 µm.

The combination of particle size, column diameter and length was chosen to provide similar column plate numbers. Void volumes of the two column configurations are approximately the same (0.5 mL).

#### **Guard Columns**

The guard-column cartridges provide physical (filtration) and chemical (adsorption) protection for the analytical cartridge. The PPS polymeric guard-column tubing is more solvent-resistant than PEEK and is specifically engineered to make leak-tight seals against metal surfaces, without requiring gaskets. The tubes are packed with 5 µm Zorbax packing identical to that in the analytical cartridges to provide the most-effective protection. The 5 µm packing and the close-coupling achieved by the Zorbax Reliance series hardware provides effective protection, typically, with no degradation of separation resolution. The polymeric guardcolumn cartridges consist of unthreaded 4.6 mm ID x 12.5 mm tubes with press-fit 2 µm porosity frits. A packed guard-column cartridge has a void volume of less than 150 μL.

#### **Sealing Hardware**

The Reliance 3 and 5 cartridges are interchangeable; both use the same sealing hardware and gaskets. To eliminate galling of the threads, the sealing hardware and the cartridges are made from two different types of stainless steel. The sealing hardware is Nitronic 60 which is harder than the 316 stainless used for the cartridges. The actual sealing in Reliance-series columns is accomplished with reusable PEEK-encapsulated frit-gaskets compressed at metal-to-metal sealing surfaces (e.g., the column cartridge and the end fitting). Flow rates up to 10 mL/min and pressures up to 350 bar (5000 psi) are permitted.

## Assembly of a Reliance Cartridge-Column

The standard configuration, (exploded view in Figure 1), requires a guard-column holder, one or two PEEK-encapsulated frit-gaskets, a polymeric guard-column cartridge, an analytical cartridge (either size is interchangeable), and an end fitting. The gaskets are placed at each steel-to-steel interface. The gasket must be placed flat in the fittings. Figure 2 shows an assembled Reliance 5 cartridge column.

To assemble, first place the guard-column holder on the bench top, open-end up. Put the guard cartridge into the holder with the arrow on the cartridge pointing up. (To minimize plugging of the analytical cartridge, flow through the guard column should always be in the direction of the arrow.) If using a Reliance 3 analytical cartridge, a PEEK frit/gasket should then be placed on the outlet of the guard-column cartridge and should be checked for concentric alignment. (Use of a frit/gasket in this position is optional when using Reliance 5 analytical cartridges.) With the guard-column holder still vertical on the bench top, the inlet end of the analytical cartridge column should be screwed in until snug. Next, place the exit end fitting on the bench top, open-end up, and install a frit/gasket. The exit end of the analytical column is then screwed in, with the exit fitting remaining on the bench top. Further wrench-tightening at both ends must be carried out before the column assembly is installed into the LC.

To install the column coupler use an analogous procedure. First, screw one analytical cartridge approximately halfway into the coupler. Then, orient it vertically, open-end up, and install a frit/gasket. Tighten the second cartridge on the gasket before changing the orientation.

If it is desired to operate an analytical cartridge column without a guard column, an end fitting may be used in place of the guard-column holder. A PEEK frit/gasket must be used at both ends to seal metal-tometal surfaces.

# **Column Performance And Testing**

Flow rates of 0.75 and 1.3 mL/min are used for testing the Reliance 5 and Reliance 3 analytical cartridge columns, respectively. The following table summarizes typical efficiency of test solutes (in plate numbers) for the Reliance cartridge columns. Where reversed-phase chromatography is the mode of separation, the table lists the methanol/water ratio of the test mobile phase. For the Zorbax SIL silica column, the test mobile phase is 0.2% water, 8% methanol, and 91.8% chlorobutane. The injection volume for the test should be minimal; we recommend less than 5  $\mu L$ .

#### **Typical Plate Number**

	Reliance 3	Reliance 5	Methanol/Water	Solute
ODS	4200	5800	75/25	Toluene
C8	4000	4200	70/30	Toluene
Phenyl	3700	4000	5/45	p-CI-nitrobenzene
CN	2800	3100	25/75	Phenol
SIL	4600	5600		p-Br-Acetanilide
Rx-C18		5500	65/35	Toluene
Rx-C8		5000	60/40	Toluene
SB-C18		5000	65/35	Toluene
SB-C8		5000	60/40	Toluene
SB-CN		4000	50/50	Naphthalene
SB-Pheny	ıl.	4000	50/50	Naphthalene

The guard-column holder compresses the guard column against the analytical column for a close-coupled connection. Typically, there is no loss of performance with the guard column in place. Similar close coupling results in essentially additive plate counts for coupled cartridges. Therefore, two coupled Reliance 5 ODS columns with a guard cartridge in place should exhibit more than 10,000 plates when tested as described above.

## **Column Protection & Operation Guidelines**

To maintain optimum column performance and long column life, the following guidelines should be observed:

- \* Do not exceed 350 bar (5000 psi) operating pressure.
- \* Do not use a pH outside the range of 2 to 8.
- $^{*}$  Do not exceed an operating temperature of 60° Celsius.
- \* Do not use a cartridge without end fittings installed.

In addition, we recommend that the chromatographer:

- \* Flush new cartridge columns with 20 column volumes (about 10 mL) of methanol, then 20 milliliters of mobile phase before use. This will avoid any equilibration problem, especially with ion-pair mobile phases, and will ensure reproducible selectivity with new columns.
- Use the column-test mobile phase or 100% acetonitrile for long-term storage.
- \* Store at room temperature.
- \* Use a guard column to protect the analytical cartridge.
- \* Flush a minimum of 20 column volumes of mobile phase through the column assembly after installing a new guard column to ensure re-equilibration before resuming sample injections.
- \* Replace the guard column when the system pressure has increased by 10% from normal or after 100 to 200 injections.
- \* Minimize the system dead volume to maintain effi-
- Pump mobile phase through the column in the recommended direction.

#### **Columns For Reversed-Phase:**

# ODS, C8, CN, Phenyl, Rx-C18, Rx-C8, SB-C18, SB-C8, SB-CN, SB-Phenyl

For reversed-phase chromatography, a nonpolar, bonded, stationary phase is used with polar mobile phases such as water/methanol or water/acetonitrile mixtures. Increasing the amount of organic component usually reduces sample elution time. Mobile-phase gradients with the organic component increasing as a function of time are also useful. Since the mobile phases have high viscosities, elevated column temperatures (<60° C) are often used to increase column efficiency. Additional information on solvent selection may be found in references 1 and 2.

lon-pair chromatography may also be performed on reversed-phase columns using a suitable counter-ion (e.g., tetra-n-butylammonium nitrate for acidic compounds or sodium hexanesulfonate for basic compounds).

Ten types of Zorbax packings-ODS, C8, Rx-C18, Rx-C8, Phenyl, CN, SB-C18, SB-C8, SB-CN, and SB-Phenyl-are available in Reliance cartridges for reversed-phase chromatography. These packings differ in stationary-phase strength and selectivity. The stationary-phase strength decreases in the following order: ODS (C18) >C8 >Phenyl >CN. Less-retentive packings require a smaller proportion of organic in the mobile phase to obtain the same retention (k'). The selectivity of the packings for specific samples cannot be predicted. Changing to a more-polar reversed-phase packing may improve a difficult separation.

Zorbax Reliance ODS columns contain very-nonpolar C18 ligands for reversed-phase HPLC. They are produced by chemically bonding monofunctional imethyloctadecylsilane groups to the surface of

Zorbax SIL particles. This reaction is followed by end-capping with trimethylchlorosilane.

Zorbax Reliance C8 columns consist of a nonpolar C8 bonded-phase packing for reversed-phase HPLC. This packing is produced by chemically bonding octyldi-methylsilane groups to the silica surface of Zorbax SIL particles. After the primary bonding, end-capping with trimethylchlorosilane is performed. Zorbax Reliance Phenyl columns contain a nonpolar aromatic bonded-phase for reversed-phase HPLC. This packing is produced by bonding a monolayer of dimethyl-2-phenylethyl silane groups to the silica surface. This reaction is followed by end-capping with trimethylchlorosilane.

Zorbax Reliance CN columns contain a weakly polar bonded phase used for either reversed-phase or normal-phase chromatography. A dimethylcyanopropysilane reagent is used for surface derivatization, followed by end-capping with trimethylchlorosilane.

Zorbax Rx-C18 and Zorbax Rx-C8 columns contain reversed-phase packings for HPLC of basic compounds. Monomeric C18 and C8 silanes are chemically bonded to a high-purity, "deactivated", Zorbax Rx silica to provide improved peak symmetry for amine-containing compounds. These columns are not end-capped. Zorbax StableBond stationary phases (SB-C18, Rx/SB-C8, SB-CN, SB-Phenyl) are produced with a patented bonding process that stabilizes the bonded phase. These monomeric phases provide improved stability and reproducibility when operating with mobile phases having a pH less than 3. These StableBond phases are particularly useful for the analysis of polar or basic compounds, since they are also produced from "deactivated" Zorbax Rx (Type B) silica.

# **Columns For Normal-Phase:**

#### SIL CN

In normal-phase chromatography, the stationary phase is more polar than the mobile phase. Mobile phases are usually totally organic, consisting of a less-polar organic solvent (e.g., hexane) and a more-polar organic solvent (e.g., methylene chloride). Increasing the percentage of the more-polar solvent decreases the retention. The elutropic solvent series described in Snyder and Kirkland<sup>1-2</sup> is helpful in selecting mobile phases and designing gradients.

Two types of Zorbax packings (SIL and CN) are available in Reliance cartridges for normal-phase chromatography. These packings differ in retentivity and selectivity. SIL is more retentive than CN. The CN packing requires a less-polar mobile phase to obtain the same retention. The selectivity of columns for specific samples cannot be predicted. Switching to a different packing may improve a difficult separation.

Zorbax Reliance SIL columns are used primarily for normal-phase adsorption chromatography. For maximum reproducibility the water content on the silica surface must be constant. The use of 50% water-saturated mobile phases or the addition of 0.2% alcohol to the mobile phase is recommended. Equilibration can be slow on silicas. At least 20 column volumes of

solvent should be allowed to flow through the column for equilibration.

Zorbax SIL behaves chromatographically much like silica particles used in thin-layer chromatography but with significantly better resolution. SIL columns can be used to separate a wide variety of nonionic organic compounds, ranging from hexane-soluble to alcohol-soluble. SIL columns can be used for many of the same compounds separated by bonded-phase chromatography, but generally show different peak spacing.

The Zorbax Reliance CN column is a weakly polar, bonded phase that has been used for either reversed-phase or normal-phase chromatography. In normal-phase mode, the CN is less retentive than the SIL; therefore, less of the polar mobile-phase component is required to obtain the same retention. The CN column, which equilibrates more rapidly than unbonded silica, is much less sensitive to changes in the trace quantities of water in the mobile phase.

# Safety

Safety precautions must be observed while operating any HPLC column, including the Zorbax Reliance cartridge column. Considerations for safe operation are primarily concerned with chemical exposure. Prior to using any chemical, hazards should be assessed. Precautions should be taken to prevent exposure to potential hazards both under normal operating conditions, and in case of spills, leaks, and other accidents. The small particles in the column packing are respirable; therefore, cartridges should not be opened.

Ordering Information Zorbax 5 µm Cartridge-Columns: 4 mm ID x 80 mm	Agilent Part No.
ODS (C18)	820662-902
ODS (3 pack)	820662-932
C8	820662-906
C8 (3 pack)	820662-936
Rx-C18	820967-902
Rx-C8	820967-901
Phenyl	820662-912
Phenyl (3 pack)	820662-942
CN	820662-905
CN (3 pack)	820662-935
SB-C18	820975-902
SB-C8	820975-906
SB-CN	820975-905
SB-Phenyl	820975-912
SIL	820662-901
SIL (3 pack)	820662-931

#### Zorbax 3 µm Cartridge-Columns:

6 mm ID x 40 mm	
ODS (C18)	820668-902
ODS (3 pack)	820668-932
C8	820668-906
C8 (3 pack)	820668-936
Phenyl	820668-912
Phenyl (3 pack)	820668-942
CN	820668-905
CN (3 pack)	820668-935
SIL	820668-901
SIL (3 pack)	820668-931

## **Analytical Cartridge-Column Hardware**

Reliance cartridge-column fittings are available in a kit or parts can be ordered separately.

Cartridge Column Fitting Kit	820678-901
Guard Column Holder	820565-001
Column Coupler	820617-001
End Fitting	820669-001
PEEK Frit/Gaskets, 2 μm,	
(5 pack)	280960-901

# Polymeric Guard-Column Cartridges: 4.6 mm ID x 12.5 mm (4 pack)

ODC (C10)

ODS (C18)	820950-902
SAX	820950-903
SCX	820950-904
CN	820950-905
C8	820950-906
NH <sub>2</sub>	820950-908
Diol	820950-911
Phenyl	820950-912
Rx-C8	820950-913
Rx-C18	820950-914
SB-C8	820950-915
SB-CN	820950-916
SB-Phenyl	820950-917
300SB-C8	820950-918
Rx-SIL	820950-919
SB-C18	820950-920
300SB-C18	820950-921
SB-C3	820950-922
300SB-CN	820950-923
300SB-C3	820950-924
XDB-C18	820950-925
XDB-C8	820950-926
XDB-Phenyl	820950-927
Bonus-RP	820950-928
Extend-C18	820950-930
Guard-Column Hardware	

Guard-column hardware for polymeric guard columns is available as a kit.

Guard Column Hardware Kit 820888-901



820950-901

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