

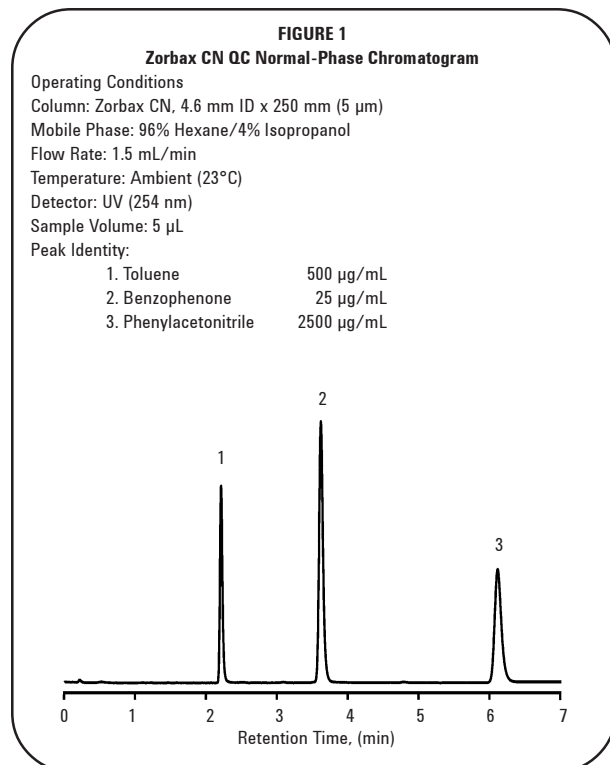
General Description

Agilent Zorbax CN is a polar bonded-phase column packing used for normal or reversed-phase high performance liquid chromatography. This packing is produced by chemically bonding cyanopropylsilane groups to Zorbax SIL particles, followed by an end-capping reaction with trimethylsilane. The cyano bonded phase is a monolayer coating produced by reacting a monofunctional silane with the Zorbax SIL support. A true monolayer bonded phase gives the best lot-to-lot chromatographic reproducibility in columns because a more uniform and controllable surface coverage is achieved.

The uniform, spherical, Zorbax CN particles have a controlled pore size of nominally 60Å. Columns are loaded to a uniform bed density using a proprietary high-pressure slurry-loading technique to give optimum column efficiency.

Column Characteristics

Typical chromatographic performance for a 4.6 mm ID x 250 mm column is shown in Figure 1. The actual performance of your column is described on the enclosed Column Performance Report.



Agilent Zorbax CN

Datasheet

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 Zorbax 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 it is not harmful to the column, reverse flow should be avoided except to attempt removal of inlet pluggage (see "Column Care" section).
- Zorbax CN is compatible with water and all common organic solvents.
- The use of a guard column is recommended to protect the Zorbax CN column and extend its useful lifetime.
- Avoid use of this column below pH 2 or above pH 8.
- Maximum operating pressure for a 1.0 mm to 9.4 mm ID column is 400 bar (6000 psi).
- Maximum operating temperature is 60°C.

NOTE: Zorbax columns are designed for high stability 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 0.01 to 0.02M. Column stability at pH > 6 is also enhanced by avoiding 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 cyanopropyl-bonded stationary phase is relatively polar and is used in the so-called normal-phase mode with relatively non-polar mobile phases such as methylene chloride/hexane or methanol/hexane mixtures. Increasing the amount of the polar component in these mixtures reduces the retention time of the sample. This packing can also be readily used for reversed-phase separations after flushing the column with isopropanol. Highly polar mobile phases such as water/methanol or water/acetonitrile mixtures can then be used. In either mode of separation, gradient-elution techniques are very convenient because of the rapid equilibration of this bonded-phase packing with the mobile phase. Additional information on solvent selection may be found in *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.J. Kirkland, and J.L. Glajch, (John Wiley & Sons, 1997).

Applications

Zorbax CN can be used for separations of compounds ranging from low to high polarity. In the normal-phase mode (low solvent polarity), Zorbax CN is often used to separate compounds that have in the past been separated on silica columns. The rapid equilibration of Zorbax CN with mobile phase often makes it more convenient to use than silica columns, especially for gradient-elution applications. Typical normal-phase applications have been the separation of aromatic alcohols, pesticides, and a broad range of pharmaceuticals, including steroids and analgesics.

Reversed-phase applications parallel those that use C8 or C18 columns. However, the Zorbax CN packing is less retentive (weaker) than alkyl-based packings, requiring a lower concentration of organic mobile phase modifier for proper elution of compounds. In addition, band-spacing differences are often available with the Zorbax CN column that are different from those of conventional C18 or C8 columns, and the Zorbax CN should be considered along with these columns when optimizing a reversed-phase separation. Shorter-chain bonded phases such as Zorbax CN are however, less stable to hydrolysis than are longer-chain phases, especially under low pH conditions. If Zorbax CN reversed-phase separations are found to exhibit peak-retention instability, switching to Zorbax SB-CN should be considered. Zorbax SB-CN columns are highly recommended for reversed-phase cyano applications because of the superior stability of its sterically protected silane chemistry in low pH application.

Column Care

The inlet frit on these columns has a nominal porosity of 2 μm . Samples that contain particulate matter larger than 2 μm may plug the column inlet frit. Zorbax CN guard columns and 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 clean strongly retained materials from the reversed-phase column, flush the column with stronger eluting solvents. When the column is used in the normal-phase mode, solvents such as methanol or dichloromethane should remove most retained compounds. Suggested solvents for cleaning the column after reversed-phase separations include 100% acetonitrile, methanol and 1:1 mixtures of these solvents

with dichloromethane. Dimethyl sulfoxide or dimethylformamide 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/H₂O 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

CN Columns for Normal-Phase (5 μm)

4.6 mm ID x 150 mm	883952-705
4.6 mm ID x 250 mm	880952-705
9.4 mm ID x 250 mm	880952-205

CN Column for Normal-Phase (7 μm)

21.2 mm ID x 250 mm	880952-105
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CN Columns for Reversed-Phase (5 μm)

4.6 mm ID x 150 mm	884950-526
4.6 mm ID x 250 mm	884950-507

Guard Column

4.6 mm ID x 12.5 mm (4 Pack)	820950-905
Guard Column Hardware Kit	820888-901

Preparative Guard Columns

9.4 mm ID x 15 mm (2 pack) ¹	820675-124
9.4 mm ID x 15 mm (2 pack) ²	820675-111
Preparative Guard Column Hardware Kit	840140-901

¹ For reversed-phase operation. Contains 300 Å SB-C3 packing.

² For normal-phase operation. Contains 150 Å Diol packing.

Agilent Part No.



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