Agilent Poroshell 120 EC-C18 Threaded Column

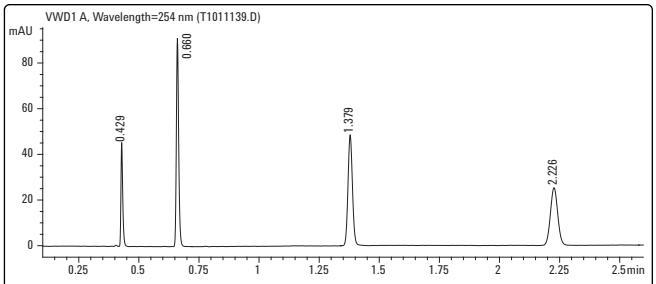
Data Sheet

General Description

Agilent Poroshell 120 EC-C18 is a superficially porous microparticulate column packing. Superficially porous silica particles, such as Poroshell, have a solid silica core and a porous silica outer layer. An EC-C18 bonded phase is applied to the totally porous outer layer for this column. This type of particle provides high efficiency at lower pressures when compared to small, totally porous particles and is ideal for fast or high resolution separations of many types of analytes.

The Poroshell 120 packing has a solid core of 1.7 µm in size with a porous outer layer 0.5 µm thick and a total particle size of 2.7 µm. The particles have a nominal surface area of 120 m^2/g and a controlled pore size of 120Å. The columns can be used up to an operating pressure of 600 bar (9000 psi). The uniform, spherical particles are ultrahigh purity (>99.995% SiO_2) silica. This high purity silica is designed to reduce or eliminate strong adsorption of basic and highly polar compounds.

The EC-C18 bonded phase is made by first chemically bonding a dense monolayer of dimethyl-n-octadecyl silane stationary phase to the porous shell of the Poroshell 120 silica support. The bonded phase packing is then endcapped for using proprietary reagent and procedures to obtain maximum deactivation of the silica surface. Poroshell 120 EC-C18 is a reversed-phase packing that can be used for basic, neutral or acidic samples. The exhaustive endcapping makes it ideal for use with basic compounds, especially those that produce poor peak shapes on other columns. These columns can be used for a wide range of applications over a pH range of 2–9, accommodating most popular mobile phases.



Column:	Agilent Poroshell 120 EC-C18, 4.6 x 100 mm, 2.7 µm
Mobile phase:	60% Acetonitrile/40% H_20
Flow rate:	2.0 mL/min

Figure 1. Agilent Poroshell 120 EC-C18 chromatogram.

Column Characteristics

A typical Quality Control test chromatogram for a Poroshell 120 EC-C18, 2.7 µm, 4.6 mm × 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. The efficiency reported on the Column Performance Report may be higher than the efficiency found in your laboratory. The QC test system may vary from the LC used in your lab and has been modified from a standard system to minimize system volume. This allows a better evaluation of the paced column and assures a more consistent product for the chomatographer.

Safety Considerations

All points of connection in liquid chromatographic systems are potential sources of leaks. Users of LCs and UHPLCs should be aware of the toxicity or flammability of their mobile phases.

These Poroshell 120 columns are mechanically stable and have been tested to very high pressures to assure safe lab operation on a variety of LC and UHPLC instruments. The operating pressure limit for all 2.1-, 3.0- and 4.6-mm id columns is 600 bar (9000 psi). While the 2.1- and 3.0-mm id columns are safe to 1300 bar (20,000 psi) and the 4.6-mm id columns are safe to 1000 bar (16,000 psi), chromatographic performance will be compromised if the 600 bar pressure limit is exceeded and the column may need to be replaced.

Because of its small particle size, dry Poroshell packings are respirable. Columns should only be opened in a well-ventilated area, and opening the column will compromise column performance.

Operational Guidelines

- The direction of flow is marked on the column. The column should only be operated in this direction.

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- These columns are packed and assembled for use up to 600 bar (9000 psi). Disassembling or over-tightening the column will degrade column performance.
- Poroshell 120 EC-C18 is compatible with water and all common organic solvents.
- Avoid use of columns below pH 2 or above pH 9. Poroshell EC-C18 columns are designed for long lifetime over this wide operating range, but like any other silica based column, operation above pH 7 will reduce lifetime. This is especially true at elevated temperatures.
- Poroshell 120 EC-C18 can be used up to 60 °C.

Mobile Phase Selection

The bonded stationary phase is a non-polar C18 for reversed phase use and can be used with typical reversed-phase mobile phases. Typical examples include methanol/water or acetonitrile/water mixtures with or without common additives such as trifluoroacetic acid, formic acid, acetate and phosphate. The column can be used in either isocratic or gradient mode and gradient elution methods with this packing often use 5% acetonitrile or methanol in water as the initial solvent and up to 100% acetonitrile or methanol as the final solvent. Additional information on solvent selection may be found in 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

Poroshell 120 EC-C18 columns are designed for fast and high resolution separations of a wide range of small molecule analytes, including acidic, basic and neutral compounds. The unique, superficially porous particle and 2.7-µm particle size make this column ideal for fast separations at up to 40% to 50% lower pressures than sub 2-µm particles with similar (90% to100%) efficiency. The columns can be used at high flow rates to achieve fast separations.

The 120 Å pore size means these columns are well suited for separations of peptides, such as those from a protein digest. These types of samples can be analyzed efficiently and with mobile phases containing additives such as TFA or formic acid for greater mass spectrometer compatibility.

The Poroshell 120 EC-C18 bonded phase is a good choice for initial analytical method development. Because it can be used across a wide pH range it is good for changing mobile phase pH to optimize selectivity. This is an excellent choice for separating basic compounds with good peak shape because of the complete bonding and endcapping.

Column Care

Samples that contain particulate matter may plug the column inlet frit and should be filtered before injection into the column. The inlet frit size on these columns is nominally 2 µm. Samples that contain particulate matter which is larger than 2 µm will plug the column inlet frit. The column should be operated only in the flow direction marked and should not be backflushed to remove particulates retained on the inlet frit.

To remove strongly retained materials from the column, flush the column with stronger (less polar) solvents. Solvents such as methanol, acetonitrile, isopropanol, or a 95/5 mixture of dichloromethane and methanol should remove most highly retained compounds. When switching between solvents with vastly different polarities it may be necessary to first purge the column with a mutually miscible solvent such as isopropranol.

These columns have a 3/8-inch end nut and 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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