CHROMATOGRAPHY ANALYTICAL CHEMISTRY

Comprehensive Technical Guide to Agilent InfinityLab Poroshell 120 HPLC Columns: Technology, Applications, and Performance Optimization

Published: March 2, 2025 | Index ID: #398

In the evolving landscape of liquid chromatography, the demand for higher resolution, faster analysis times, and improved throughput has driven significant innovations in stationary phase technology. Among these advancements, the **Agilent InfinityLab Poroshell 120**series stands as a definitive benchmark for High-Performance Liquid Chromatography (HPLC) and Ultra-High-Performance Liquid Chromatography (UHPLC) applications. By utilizing superficially porous particle (SPP) technology, these columns bridge the performance gap between traditional 5 µm totally porous particles and sub-2 µm particles, offering UHPLC-like efficiency at significantly lower backpressures.

The Core Technology: Superficially Porous Particles (SPP)

The defining characteristic of the **Poroshell 120** is its particle morphology. Unlike traditional columns packed with totally porous particles (TPP), Poroshell particles consist of a solid silica core surrounded by a porous outer layer. For the standard 2.7 µm particle, this involves a **1.7 µm solid core** and a **0.5 µm porous shell**.

The Van Deemter Equation and Efficiency

To understand why Poroshell 120 columns are revolutionary, one must examine the **Van Deemter Equation**, which describes the relationship between mobile phase linear velocity (u) and height equivalent to a theoretical plate (H):

$$H = A + B/u + C \cdot u$$

- A Term (Eddy Diffusion): In SPP columns, the extremely uniform particle size distribution results in more homogeneous packing. This significantly reduces the A term, as there are fewer variations in the flow paths of analyte molecules through the column bed.
- B Term (Longitudinal Diffusion): This term relates to the diffusion of the analyte in the mobile phase. While not drastically changed by the core-shell structure, it remains a factor at low flow rates.
- C Term (Mass Transfer): This is where the Poroshell 120 excels. In a totally porous particle, molecules must diffuse deep into the center of the particle and back out. In an SPP, the diffusion path is restricted to the 0.5 µm shell. This shorter diffusion path minimizes the resistance to mass transfer, allowing for high flow rates without a significant loss in efficiency (resolution).

Consequently, a 2.7 µm Poroshell column can achieve 80-90% of the efficiency of a sub-2 µm TPP column but at only approximately 40-50% of the backpressure. This allows laboratories to achieve high-resolution separations on standard 400 bar or 600 bar HPLC systems, as well as 1200 bar UHPLC systems.

Phase Chemistries: EC-C18 vs. SB-C18

Agilent offers a wide variety of stationary phases for the Poroshell 120 line to accommodate different selectivity requirements. The two most prominent phases are the **EC-C18** and the **SB-C18**.

1. Poroshell 120 EC-C18 (Endcapped)

The **EC-C18 (Extra Column C18)**is designed as a highly versatile, general-purpose column. It is an ideal starting

point for method development due to its robust nature and broad compatibility.

- Endcapping: This phase is double-endcapped to minimize interactions with residual silanols. This results in excellent peak shapes for basic compounds which might otherwise undergo secondary interactions with the silica surface.
- pH Range: Typically stable between pH 2.0 and 9.0, allowing for flexibility in mobile phase selection.
- Applications: Pharmaceutical impurities, environmental testing, and food safety analysis.

2. Poroshell 120 SB-C18 (StableBond)

The **SB-C18 (StableBond)** phase is engineered specifically for stability at low pH. Unlike most C18 phases, StableBond phases are **not endcapped**.

- Steric Protection: The SB-C18 uses bulky diisopropyl or diisobutyl side groups that sterically protect the siloxane bond from hydrolytic attack at low pH.
- Acidic Stability: It is designed for use at pH 1.0 to 8.0, but it excels specifically in the pH 1-2 range where other columns might experience phase stripping.
- Selectivity: Because it is not endcapped, the SB-C18 offers different selectivity compared to the EC-C18, which can be advantageous for separating closely eluting acidic or neutral isomers.

Technical Specifications and Performance Metrics

The following table provides a comparison of common specifications for Agilent Poroshell 120 columns to assist in selection for specific analytical workflows.

Feature	Poroshell 120 EC-C18	Poroshell 120 SB-C18	Poroshell 120 Preparative
Particle Size	2.7 µm (also 1.9, 4 µm)	2.7 µm	4 µm
Pore Size	120 Å	120 Å	120 Å
Surface Area	~130 m²/g	~130 m²/g	~130 m²/g
pH Range	2.0 - 9.0	1.0 - 8.0	2.0 - 9.0
Endcapped	Yes (Double)	No	Yes
Max Pressure	Up to 1000 bar (Threaded)	Up to 600 bar	Up to 600 bar
Carbon Load	~10%	~8%	~10%

Understanding Column Dimensions

Column ID and length significantly impact sensitivity and solvent consumption. The **Poroshell 120**is available in several configurations:

- Narrow Bore (2.1 mm ID): Ideal for LC/MS applications where low flow rates (0.2–0.5 mL/min) are required to enhance ionization efficiency and reduce solvent waste.
- Standard Bore (4.6 mm ID): The workhorse for routine HPLC. These columns provide high loadability and are less sensitive to extra-column volume (ECV) of the LC system.
- Mid-Bore (3.0 mm ID): A compromise between 4.6 mm and 2.1 mm, offering improved sensitivity over 4.6 mm while maintaining better robustness than 2.1 mm on older systems.

Practical Implementation: Method Development and Optimization

Transitioning from a 5 µm TPP column to a 2.7 µm **Poroshell 120**requires consideration of system volume and flow rates to maximize the efficiency gains.

Step 1: Column Selection

Begin with the **EC-C18** for general neutral or basic compounds. If the separation requires highly acidic mobile phases (pH **SB-C18**. For preparative scale-up, ensure you use the 4 µm Poroshell particles to maintain the same selectivity as the analytical 2.7 µm scale.

Step 2: Flow Rate Optimization

Due to the SPP technology, the optimum flow rate (the minimum point on the Van Deemter curve) is higher than that of TPP columns. For a 4.6 x 150 mm, 2.7 µm column, a flow rate of 1.5 to 2.5 mL/min often yields the best resolution without exceeding pressure limits. This allows for significantly shorter run times.

Step 3: Managing Extra-Column Volume (ECV)

Because Poroshell columns produce very narrow peaks, they are highly sensitive to **extra-column volume**. This is the volume in the injector, tubing, and detector cell. To prevent peak broadening:Use 0.12 mm (0.005") ID tubing for 2.1 mm columns.Minimize the distance between the injector and the column.Use a micro flow cell in the detector if possible.

Comparison of Poroshell 120 with Traditional Media

In the following table, we analyze the performance of Poroshell 120 against traditional Totally Porous Particles (TPP) and sub-2 µm UHPLC media.

Metric	5 µm TPP	2.7 µm Poroshell 120 (SPP)	1.8 µm TPP (UHPLC)
Plates per Meter (N)	~100,000	~200,000 - 250,000	~280,000 - 300,000
Backpressure	Low (	Moderate (200 - 600 bar)	Very High (> 800 bar)
Analysis Speed	Slow	Fast	Very Fast
System Requirement	Standard HPLC	HPLC or UHPLC	UHPLC Only
Frits Size	2 µm (Rugged)	2 µm (Rugged)	0.5 µm (Prone to clogging)

A key takeaway is the **frit size**. Because the Poroshell 120 particle is 2.7 µm, Agilent can use 2 µm frits. Sub-2 µm columns require 0.5 µm frits, which clog very easily with sample matrix or mobile phase particulates. This makes Poroshell columns significantly more robust for complex samples like plasma, soil extracts, or food homogenates.

Advanced Features: Threaded Columns and ID Tags

The **InfinityLab Poroshell 120**columns often feature modern hardware enhancements designed for the InfinityLab LC series. These include:

- Threaded Column Fittings: These are designed to withstand pressures up to 1300 bar. They ensure a zero-dead-volume connection, which is critical for maintaining the high efficiency of the core-shell particles.
- Column ID Tags: Many Poroshell columns come with a small electronic tag. When used with an Agilent InfinityLab LC system, the system automatically reads the column type, serial number, and usage history (number of injections, max temperature reached). This is a critical feature for GLP/GMP compliance and prevents the use of the wrong column for a specific method.

Maintenance and Troubleshooting

To maximize the lifespan of a **Poroshell 120 EC-C18** or **SB-C18**column, specific maintenance protocols should be followed.

Handling High Backpressure

If backpressure increases suddenly, it is likely due to particulate accumulation on the inlet frit. **Troubleshooting steps:**Disconnect the column and flush the system to ensure the pressure is not coming from the tubing.Reverse-flush the column (if the manufacturer's instructions allow—most Poroshell 120 columns can be backflushed) into a beaker at a low flow rate to dislodge particulates.Always use a **Fast Guards**or a pre-column filter to protect the analytical column from matrix components.

Peak Tailing and Loss of Resolution

Peak tailing for basic compounds on an **EC-C18** usually indicates silanol activity, suggesting the endcapping may be degrading due to extreme pH. Ensure the mobile phase remains within the pH 2-9 range. For the **SB-C18**, tailing might occur if the sample is basic and the pH is not low enough to suppress silanol ionization, as the phase is non-endcapped.

Equilibration and Storage

Proper equilibration is essential. A Poroshell column typically requires 10-20 column volumes for full equilibration.

For storage, columns should be flushed with a mixture of organic solvent and water (e.g., 50/50 Acetonitrile/Water) to remove salts and buffers, then stored in 100% organic solvent (Acetonitrile or Methanol) to prevent microbial growth and stationary phase hydrolysis.

Case Study: Improving Throughput in Pharmaceutical Assay

Consider a pharmaceutical laboratory running a potency assay for an Active Pharmaceutical Ingredient (API) using a traditional 5 µm, 4.6 x 250 mm C18 column. The run time is 30 minutes, and the resolution between the API and its main degradation product is 2.5.

By switching to an **Agilent Poroshell 120 EC-C18, 4.6 x 100 mm, 2.7 µm** column:

- The run time can be reduced to 6 minutes (an 80% reduction) by increasing the flow rate and utilizing the shorter column length.
- The resolution actually increases to 3.0 because the plate count (N) of the 100 mm Poroshell column is higher than that of the 250 mm 5 µm TPP column.
- Solvent consumption per injection drops from 30 mL to 9 mL, significantly reducing waste disposal costs.

This demonstrates the immediate ROI (Return on Investment) of upgrading to superficially porous technology, even without upgrading the HPLC hardware.

The Impact of Pore Size (120 Å)

The "120" in **Poroshell 120** refers to the 120 Å (Angstrom) pore size. This pore size is optimized for **small molecule analysis** (typically

Conclusion: The Strategic Choice for Modern Laboratories

The **Agilent InfinityLab Poroshell 120** family represents a pinnacle in chromatography media engineering. By meticulously balancing particle size, shell thickness, and chemistry, these columns allow analysts to push the boundaries of what is possible with existing HPLC equipment. Whether it is the robust, general-purpose nature of the **EC-C18** or the specialized low-pH stability of the **SB-C18**, the Poroshell 120 series provides a scalable solution from method development to preparative purification. For laboratories looking to improve data quality while reducing operational costs, the transition to superficially porous particles is no longer an option—it is a technical necessity.