



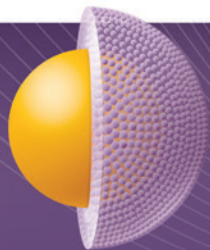
Agilent Poroshell 120 Columns for HPLC and UHPLC

## PERFORM RUGGED, FAST LC WITH CONFIDENCE

The Measure of Confidence



**Agilent Technologies**



## AGILENT POROSHELL 120 COLUMNS CAN MAKE EVERY LC AND LC/MS IN YOUR LAB WORK EVEN HARDER

*"We choose Poroshell 120 because of its rugged performance"*

*"Poroshell 120 provides reliably excellent performance—it's the new 'standard' in our lab"*

*"For complicated samples, which I face most, Poroshell 120 columns save me a lot of time"*

*"Poroshell 120 is my go-to column"*

### QUOTES FROM POROSHELL 120 USERS

Poroshell 120 columns provide exceptional efficiency for standard HPLC, and significantly boost performance from all instruments, whether you have older 400 bar or newer 1300 bar UHPLC systems.

These columns take the technology introduced with our Poroshell 300 columns to the next level—giving you higher throughput and resolution for a wider range of small molecules and peptides than ever. Their advanced features include:

- **Excellent lot-to-lot reproducibility**—Poroshell 120 columns are manufactured using a proprietary, single-step porous shell process that dramatically reduces tiny differences between columns and lots
- **Comparable speed and resolution to sub-2  $\mu\text{m}$  columns** and significant improvements over 5  $\mu\text{m}$  columns with much lower backpressure, taking HPLC and UHPLC performance to a new level of flexibility and efficiency
- **Superior peak shape**—especially at pH 6-7, for faster, more accurate results
- **Long column life**—Poroshell 120 columns use a standard 2  $\mu\text{m}$  frit, and resist plugging with dirty samples
- **Up to TWELVE chemistries**, depending on the particle size, including SB-C18 and SB-C8 for low pH applications and Poroshell HPH-C18 and HPH-C8 for high pH applications
- **Easy method transfer** to ZORBAX bonded phases and within the Poroshell 120 family, for highest productivity from lab to lab, around the world
- **UHPLC guard column options** that reduce your operating costs by extending the life of Poroshell 120 columns
- **Scalability within the Poroshell 120 Family** with column options in 4  $\mu\text{m}$  and 2.7  $\mu\text{m}$  configurations for optimal performance for your method



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Yes, you can achieve Fast LC performance at HPLC pressures **Page 16**

### Making every LC and LC/MS work harder

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Achieve faster peptide mapping and protein separations with Poroshell technology **Page 31**

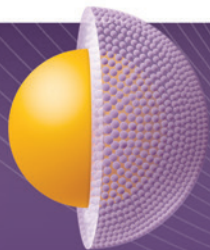
### Infinitely better liquid chromatography

Introducing the Agilent 1290 Infinity II LC **Page 35**

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To learn more about Agilent Poroshell 120 columns, visit  
[www.agilent.com/chem/poroshell120](http://www.agilent.com/chem/poroshell120)



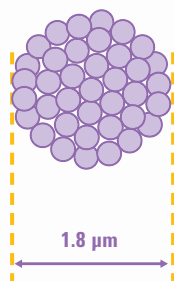


## HIGH EFFICIENCY AT LOWER PRESSURES PROVEN COLUMN-TO-COLUMN CONSISTENCY THAT'S THE POROSHELL 120 DIFFERENCE

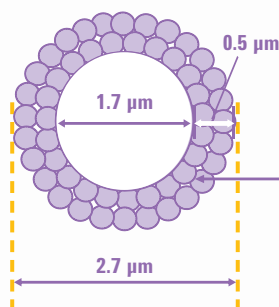
A key feature of Agilent Poroshell 120 columns is their superficially porous microparticulate column packing.

Poroshell 120 columns are available with 4  $\mu\text{m}$  and 2.7  $\mu\text{m}$  particles to provide scalable performance. The particles have a solid silica core with a porous shell. This unique configuration gives you all the performance advantages of smaller totally porous particles, but with lower backpressures.

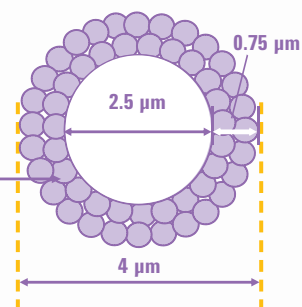
1.8  $\mu\text{m}$  totally porous



Agilent Poroshell 120 2.7  $\mu\text{m}$



Agilent Poroshell 120 4  $\mu\text{m}$

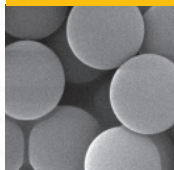


Pore size  
120Å,  
ideal for small  
molecules

### How a Poroshell 120 particle is made

To create the best column for small molecule separations, we completely reinvented our superficially porous particle technology. Specifically, we minimized the number of manufacturing steps to ensure maximum particle—and chromatographic—reproducibility.

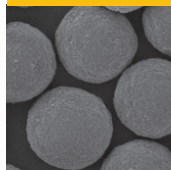
#### STEP 1



#### Make the solid core

Poroshell 120 cores have a very smooth surface and a uniform particle size—which contribute to a tight overall particle size distribution. As a result, you get a more tightly packed column bed and higher efficiency than with totally porous particles.

#### STEP 2



#### Apply the porous shell

At Agilent, we **apply the porous shell in one single step**—similar to the coacervation technique used to make traditional ZORBAX columns. This unique single-step process delivers higher yields and more column-to-column reproducibility than other vendors can.

#### STEP 3

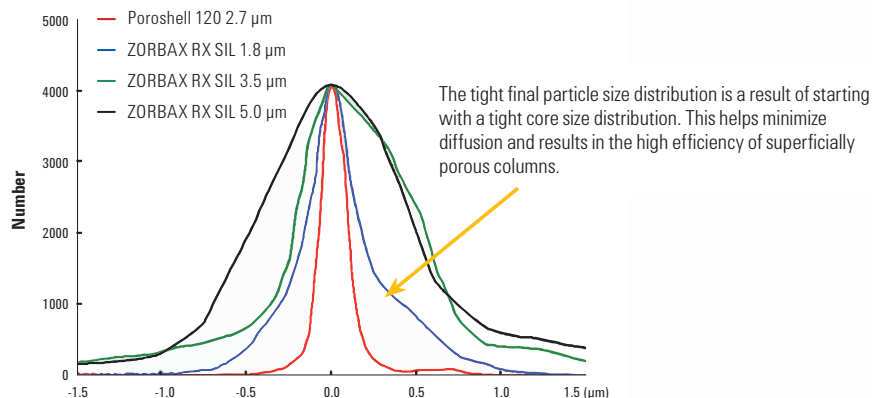
#### Apply the bonded phase

The family of Agilent Poroshell 120 phases is expanding to align with the ZORBAX family for method development flexibility and assured scalability.



## A comparison of particle size distributions between totally porous and Poroshell 120 particles

This graph demonstrates that Poroshell 120 columns have the tightest final particle size distribution—a direct result of starting with a tight core size distribution.



## The standard measure of particle size distribution is the 90/10 ratio, which should be below 1.5

The ZORBAX totally porous particles (1.8 µm, 3.5 µm, and 5.0 µm) all have an acceptable particle size distribution. However, the Poroshell 120 particle has a **25% tighter particle size distribution**, which substantially improves column efficiency.

	Poroshell 120 (2.7 µm) LN B10006	ZORBAX 1.8 µm	ZORBAX 3.5 µm	ZORBAX 5.0 µm
10%	2.40 µm	1.67 µm	3.07 µm	4.59 µm
90%	2.85 µm	2.45 µm	4.44 µm	6.21 µm
90%/10% ratio	1.16	1.47	1.45	1.35

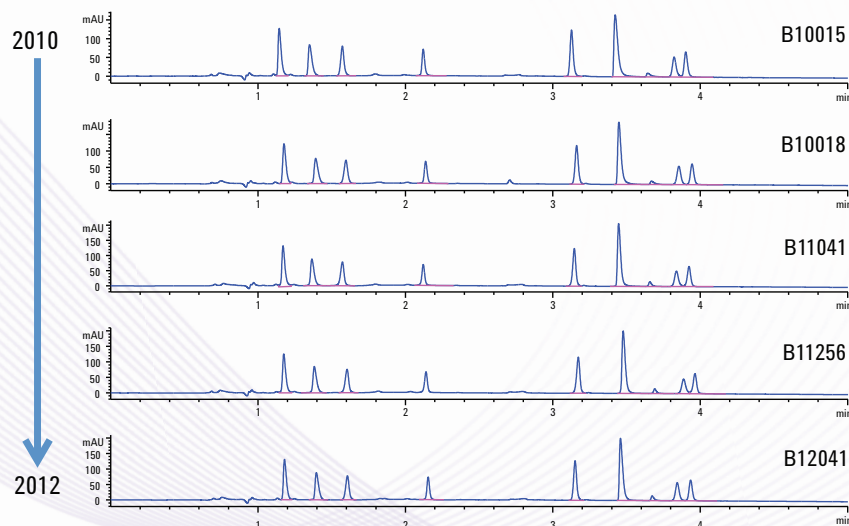
## Reproducible performance from lot to lot, year after year

Poroshell 120 particles are made with a proprietary porous particle manufacturing process, invented by Agilent. Instead of traditional multilayering, Poroshell 120 columns are manufactured **using a single-step coacervation process** that produces a more consistent final particle—and more reliable chromatographic results.

## The simpler the manufacturing process, the more consistent the column

A single-step shell process creates a highly reproducible column, as you can see in this lot-to-lot comparison of five lots.

Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm  
(p/n 695975-902) from five different lots



## A VARIETY OF BONDED PHASES MEANS YOU NEVER HAVE TO COMPROMISE ON SELECTIVITY

Poroshell 120 columns are made at the same facility as the Agilent industry-leading ZORBAX column family. The bonding chemistries used with Poroshell 120 columns mirror those of all ZORBAX columns, giving you the advantages of easier method transfer and assured scalability from lab to lab, around the world.

**All the selectivities you need to perfect your separation**

### AN EXCELLENT FIRST CHOICE

#### **Poroshell 120 EC-C18 (USP L1)\* and EC-C8 (USP L7)\***

You can count on this high-performance phase to deliver excellent peak shape and resolution for acids, bases, and neutrals. The chemistry is very similar to the ZORBAX Eclipse Plus phase, for easy method transferability.

*Exceptional peak shape, efficiency, resolution, and lifetime.*

**Tip:** Select the C18 phase first, and use the C8 phase for less retention with a variety of samples.

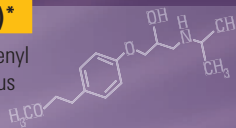
*"A variety of published methods from Agilent use Poroshell 120, allowing for easy method development."*

*"Methods easily convert from [ZORBAX] Eclipse Plus columns to Poroshell 120—we use them for all methods."*

QUOTES FROM **TOXICOLOGY LAB USERS**

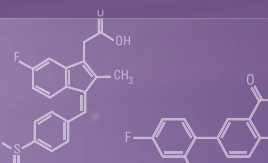
#### Poroshell 120 Phenyl-Hexyl (USP L11)\*

This phase provides alternative selectivity for phenyl groups, and is very similar to ZORBAX Eclipse Plus Phenyl-Hexyl for easy method transfer.



#### Poroshell 120 PFP (USP L43)\*

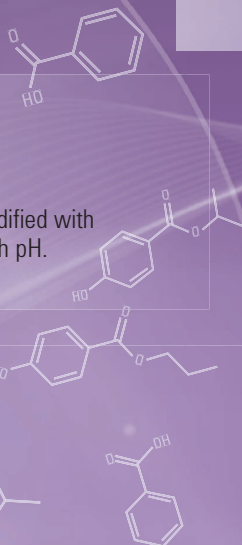
An alternative selectivity for halogenated compounds and polar analytes.



#### HIGH pH APPLICATIONS

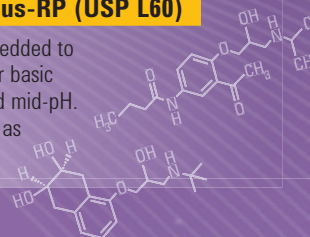
##### Poroshell HPH-C18 (USP L1)\* and HPH-C8 (USP L7)\*

The silica in this special chemistry has been modified with a proprietary process to increase stability at high pH.



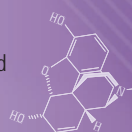
#### Poroshell 120 Bonus-RP (USP L60)

Bonus-RP is polar-embedded to improve peak shape for basic compounds at low- and mid-pH. This phase is the same as ZORBAX Bonus-RP.



#### Poroshell 120 HILIC\*

With its unbonded silica, Poroshell 120 HILIC retains and separates small polar analytes.



#### LOW pH APPLICATIONS

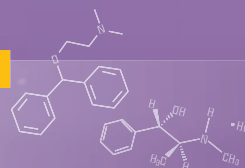
##### StableBond SB-C18 (USP L1) and SB-C8 (USP L7)

StableBond performs well with acids, bases, and neutrals—with superior lifetime at low pH. What's more, these phases transfer readily from ZORBAX SB-C18 and ZORBAX SB-C8 phase chemistries.



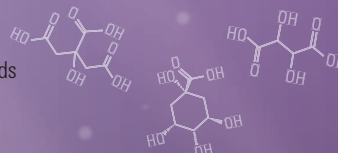
#### Poroshell 120 EC-CN (USP L10)

Similar to ZORBAX Eclipse XDB-CN, this cyano phase simplifies method transfer.



#### Poroshell 120 SB-Aq

This proprietary phase provides an alternate selectivity option, and is ideal for polar compounds and high aqueous conditions. Its chemistry is the same as ZORBAX SB-Aq.



\* Available in 4 µm and 2.7 µm particle sizes.

Visit [www.agilent.com/chem/discoverporoshell](http://www.agilent.com/chem/discoverporoshell) for videos, Application Notes, and more—or to order now



## Agilent Poroshell 120 EC-C18 and Poroshell 120 SB-C18 provide different selectivity for optimizing separations

Mobile phase: 35% H<sub>2</sub>O, 65% CH<sub>3</sub>CN

Flow rate: 1 mL/min

Temperature: 30 °C

MS acquisition: Dynamic MRM

Compound	Precursor ion	Fragmentor voltage
Anandamide	(AEA) 348	135
Palmitoylethanolamide	(PEA) 300	135
2-Arachidonoylglycerol	(2-AG) 379	135
Oleoylethanolamide	(OEA) 326	135

MS Source:

Gas temp: 350 °C

Gas flow: 12 L/min

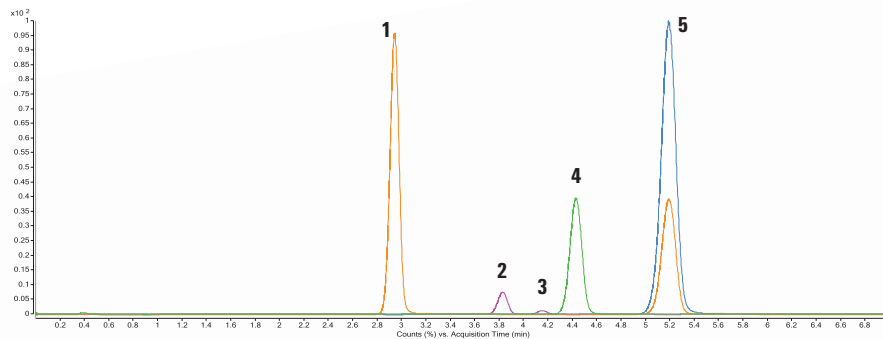
Nebulizer: 40 psi

Capillary: 4,000 V

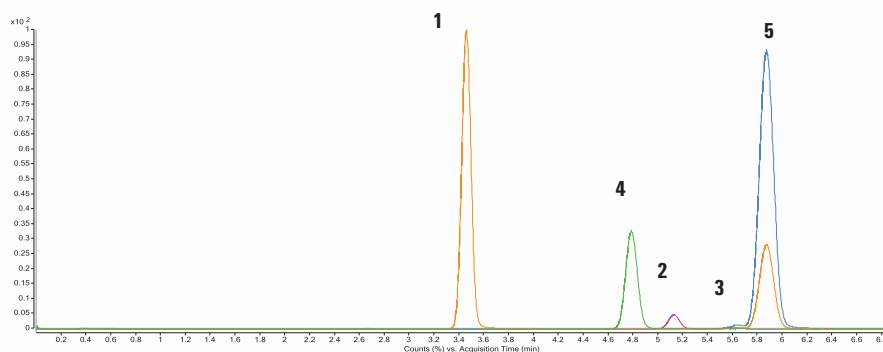
Analytes:

1. Anandamide (AEA)
2. 2-Arachidonoylglycerol
3. Impurity
4. Palmitoylethanolamide (PEA)
5. Oleoylethanolamide (OEA)

Poroshell 120 SB-C18, 3.0 x 100 mm, 2.7 µm  
(p/n 685975-302)



Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm  
(p/n 695975-302)



## Agilent Poroshell 120 EC-C8 is less retentive for faster analysis of nonpolar compounds

Mobile phase: 60% CH<sub>3</sub>CN, 40% H<sub>2</sub>O

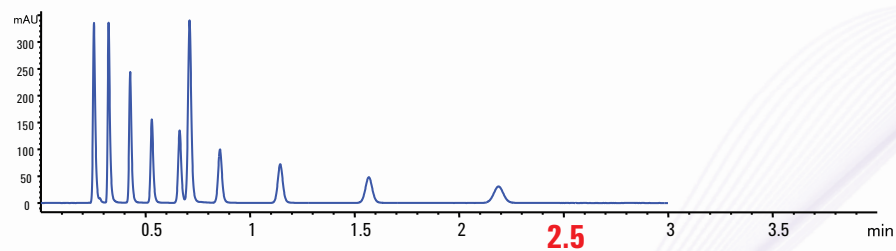
Flow rate: 0.85 mL/min

Temperature: 26 °C

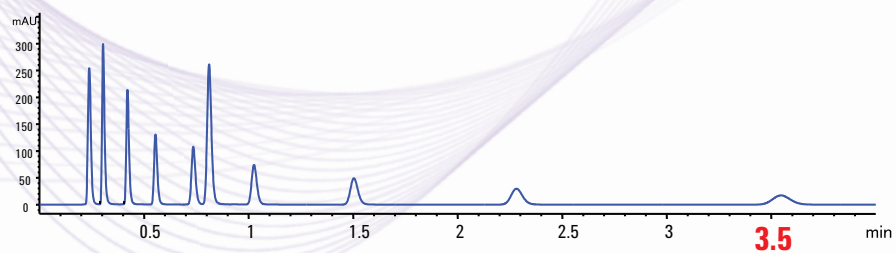
Detection: 254 nm

Sample: 2 µL of RRLC checkout sample  
(p/n 5188-6529), alkylphenones

Poroshell 120 EC-C8, 3.0 x 50 mm, 2.7 µm  
(p/n 699975-306)



Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm  
(p/n 699975-302)



# A choice of particle sizes help you make the best choice for your method development

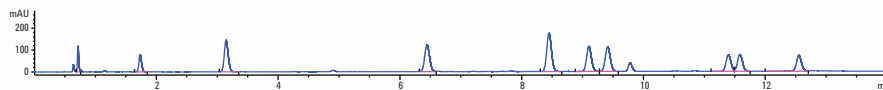
## Easy drop-in replacement for existing 5 $\mu\text{m}$ traditional LC methods

This separation of a phenol mix shows the scalability of selectivity going from 5  $\mu\text{m}$  Eclipse Plus C18 to Poroshell 120 4  $\mu\text{m}$  and 2.7  $\mu\text{m}$  columns. It also demonstrates a significant performance improvement in peak capacity. Additionally, the 4  $\mu\text{m}$  column offers minimal backpressure increases over existing 5  $\mu\text{m}$  columns, and the 2.7  $\mu\text{m}$  offers significantly lower backpressure than sub-2  $\mu\text{m}$  columns.

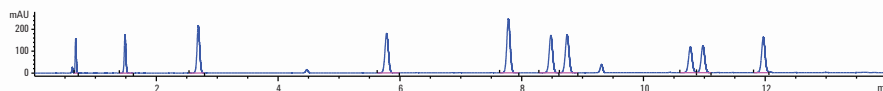
Columns: All columns 4.6 x 100 mm  
 Instrument: Agilent 1260 Infinity LC, pulse damper and mixing column bypassed  
 Mobile phase: A: 0.1% Formic acid  
 B: MeOH + 0.1% formic acid  
 Flow rate: 0.4 mL/min  
 Temperature: 25 °C  
 Detection: 260 nm  
 Sample: Phenol mix  
 Gradient: 40-80% MeOH/14 min

### Phenol mix gradient at 1.5 mL/min

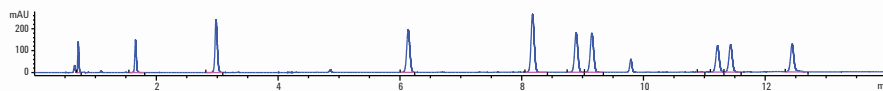
ZORBAX Eclipse Plus C18 5  $\mu\text{m}$



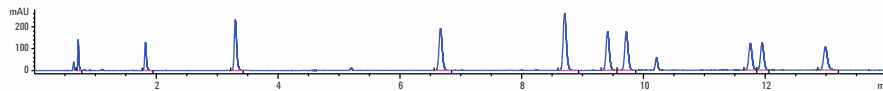
Poroshell 120 EC-C18 4  $\mu\text{m}$



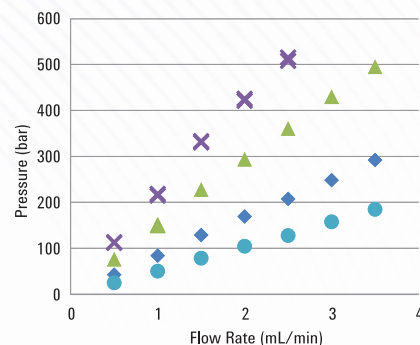
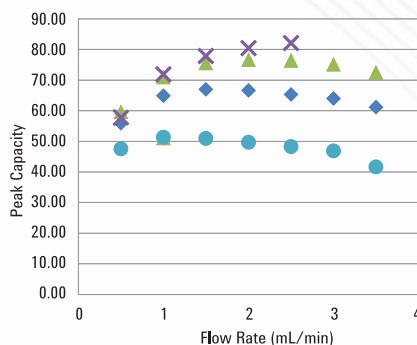
Poroshell 120 EC-C18 2.7  $\mu\text{m}$



ZORBAX Eclipse Plus C18 1.8  $\mu\text{m}$

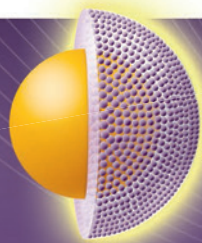


### Phenol mix gradient scaled at flows between 0.5 and 3.5 mL/min



◆ Poroshell 120 EC-C18 4  $\mu\text{m}$       ▲ Poroshell 120 EC-C18 2.7  $\mu\text{m}$   
 ✕ ZORBAX Eclipse Plus C18 1.8  $\mu\text{m}$       ● ZORBAX Eclipse Plus C18 5  $\mu\text{m}$





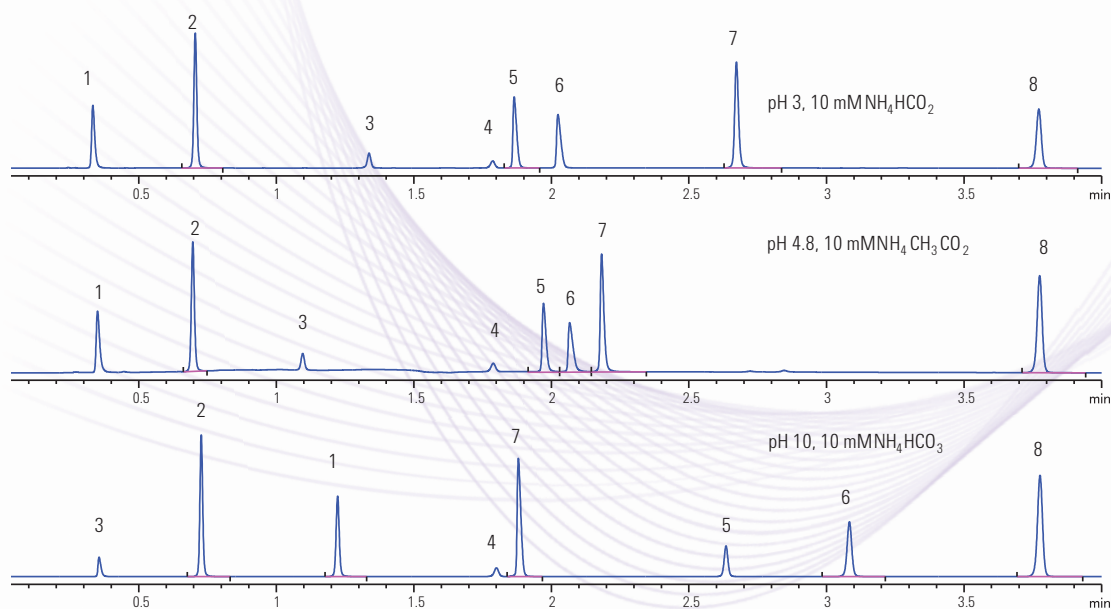
## ENABLE HIGH-PERFORMANCE SCREENING METHODS WITH POROSHELL HPH-C18 AND HPH-C8

A robust method development process is critical to ensuring that your method is long lasting, stable, and reliable. Because the retention and selectivity of ionizable compounds (such as acids and bases) can change significantly with varying pH, it is becoming standard practice to use low, medium, and high pH analyses during method development.

**Poroshell HPH-C18 and HPH-C8** are made by chemically modifying Poroshell particles using proprietary technology to give high pH stability. That means you can use the Poroshell 120 family for *all* your Fast LC method development needs, *regardless of mobile phase pH*.

### Reliable separations for varying pH levels

Here, a method using low, mid, and high pH separates the same mixture of acids, bases, and neutrals. The highest resolution for all compounds was obtained under higher-pH conditions, and so, high pH would be the best choice going forward.



Sample:

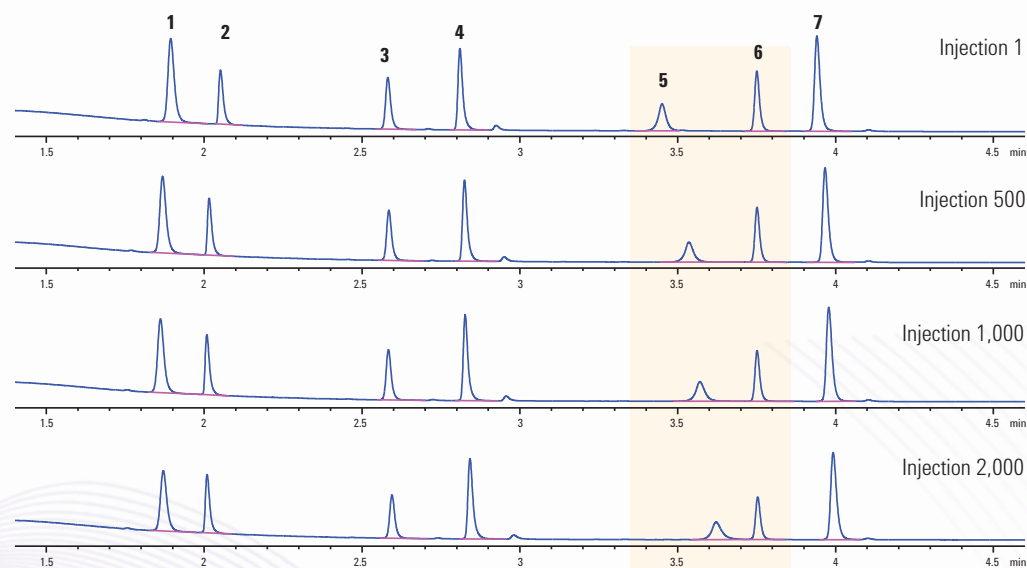
- |                          |                 |
|--------------------------|-----------------|
| 1. Procainamide          | 5. Dipyrimadole |
| 2. Caffeine              | 6. Diltiazem    |
| 3. Acetyl salicylic acid | 7. Diflunisal   |
| 4. Hexanophenone deg.    | 8. Hexanophenon |



## Excellent stability at High pH

Count on Poroshell HPH chemistries for consistent performance and longevity, even when using high-pH mobile phases. Here, 2,000 injections of a separation mixture containing acidic, basic, and neutral compounds were performed under extreme pH 10 conditions on an Agilent Poroshell HPH-C18 and a non-Agilent high pH column. Notice the non-Agilent column's loss of resolution between the Nortryptiline and Heptanophenone while the Poroshell HPH-C18 maintains resolution.

**Column: Agilent Poroshell HPH-C18,** 2.1 x 50 mm, 2.7  $\mu$ m (p/n 699775-702)



Instrument: 1260 Infinity Binary LC

Flow rate:

0.4 mL/min

Sample:

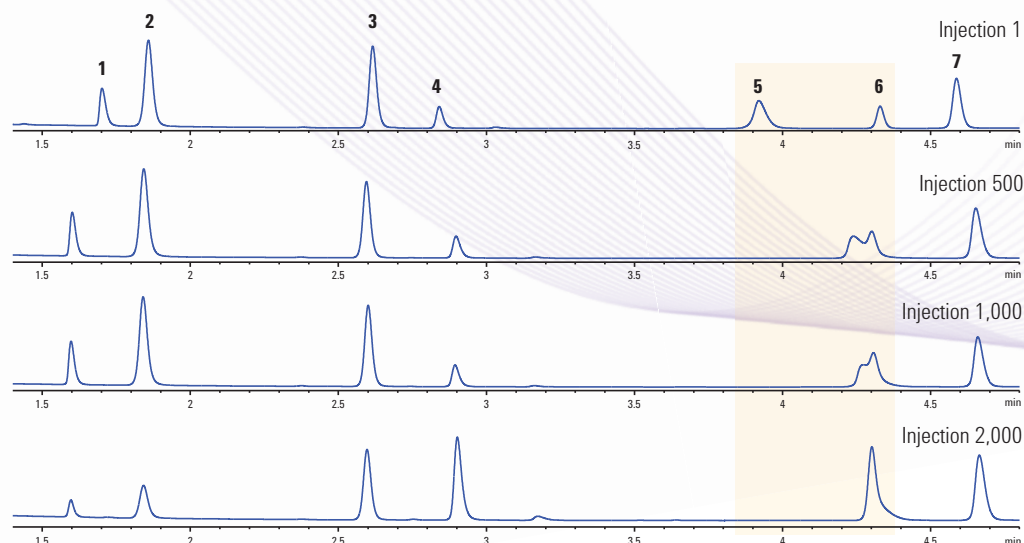
Mobile phase: A: 10 mM Ammonium bicarbonate  
adjusted to pH 10.0 in water  
B: Acetonitrile

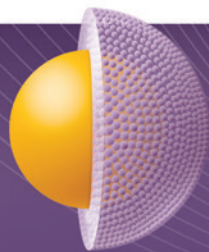
Gradient:

Time	% B
0	5
5	95
5.1	5

1. Methyl salicylate  
2. 4 Chlorocinnamic acid  
3. Acetophenone  
4. Quinine  
5. Nortryptiline  
6. Heptanophenone  
7. Amitriptyline

**Column: other vendor High pH** 2.1 x 50 mm, 3  $\mu$ m





## OPTIMIZE EVERY SEPARATION WITH A CHOICE OF ORTHOGONAL PHASES

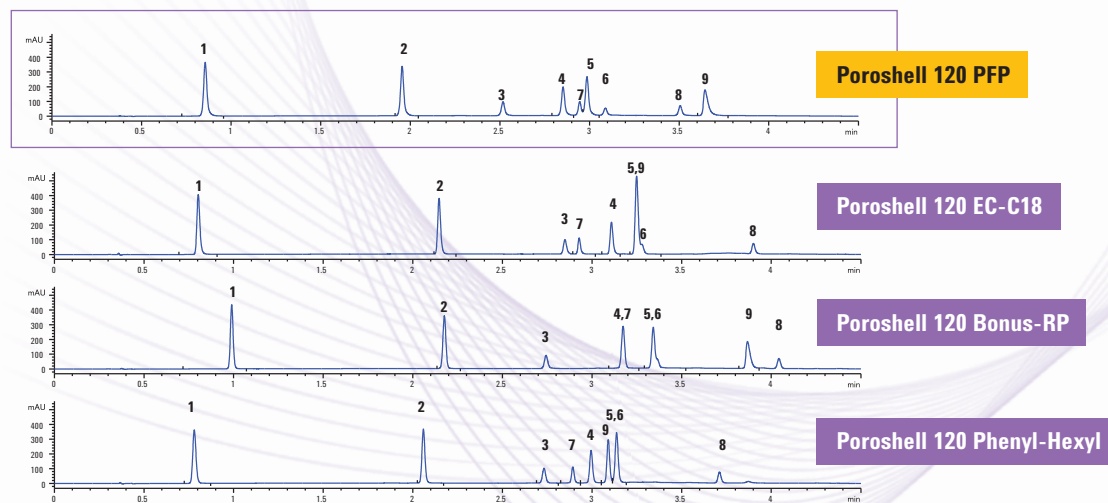
Selectivity is the most powerful tool for optimizing HPLC separations. Poroshell 120 EC-C18 is the best place to start your method development, because of its exceptional flexibility. However, if you are working with challenging analytes, the Poroshell 120 family has many additional chemistries to choose from.

For example, our **Poroshell 120 PFP columns** are engineered with a pentafluorophenyl ligand, which provides an orthogonal separation mechanism with traditional reversed-phase columns.<sup>1</sup>

By specifically targeting polar retention mechanisms, PFP phases can separate analytes based on small differences in structure, substitution, and steric access to polar moieties. The resulting selectivity for positional isomers, halogenated compounds, and polar analytes is particularly useful when analyzing complex mixtures and small-molecule pharmaceuticals.

### Comparative analysis of NSAIDs

This separation was completed with four Poroshell 120 chemistries using acetonitrile. Each run was only five minutes long. Only Poroshell 120 PFP resolved all compounds, although both Poroshell 120 EC-C18 and Poroshell 120 Phenyl-Hexyl columns eluted the compounds in the same order. The PFP and Bonus-RP columns had very similar elution orders, with the exception of the last two peaks.



Columns: Poroshell 120 PFP, 4.6 x 50 mm, 2.7  $\mu$ m—(p/n 699975-408)  
 Poroshell 120 EC-C18, 4.6 x 50 mm, 2.7  $\mu$ m—(p/n 699975-902)  
 Poroshell 120 Bonus-RP, 4.6 x 50 mm, 2.7  $\mu$ m—(p/n 699968-901)  
 Poroshell 120 Phenyl-Hexyl, 4.6 x 50 mm, 2.7  $\mu$ m—(p/n 699975-912)

Instrument: 1260 Infinity Binary LC

Mobile phase: A: 20 mM  $\text{NH}_4\text{HCO}_2$ , pH 3.0  
 B: Acetonitrile

Flow rate: 2 mL/min

Detection: UV, 254 nm

Gradient:	Time	% Organic
	0	8
	6	100
	7	100
	8	8

Sample:

1. APAP
2. Phenacetin
3. Piroxicam
4. Tolmetin
5. Ketoprofen
6. Naproxen
7. Sulindac
8. Diclofenac
9. Difunisal

1. "Fluorinated HPLC Phases: Looking Beyond C18 for Reverse-Phase HPLC" M. Przybyciel, LCGC Europe 19(1) pp 19-28, 2006.

## Selectivity—the most powerful tool for optimizing HPLC separations

### Positional isomers (15 compounds)

Mobile phase A, Water (0.1% acetic acid), B, Acetonitrile

Sample:

- |  |                              |
|--|------------------------------|
| 1. 3,4 Dimethoxyphenol                     | 9. 3,5 Dimethylphenol        |
| 2. 2,6 Dimethoxyphenol                     | 10. 2,6 Dimethylphenol       |
| 3. 3,5 Dimethoxyphenol                     | 11. 2,6 Dichlorophenol       |
| 4. 2,6 Difluorophenol                      | 12. 4 Chloro 3 methyl phenol |
| 5. 2,4 Difluorophenol                      | 13. 4 Chloro 2 methyl phenol |
| 6. 2,3 Difluorophenol                      | 14. 3,4 Dichlorophenol       |
| 7. 3,4 Difluorophenol                      | 15. 3,5 Dichlorophenol       |
| 8. Degradation product 2,6 dimethoxyphenol |                              |

Time %B

0 15

13 30

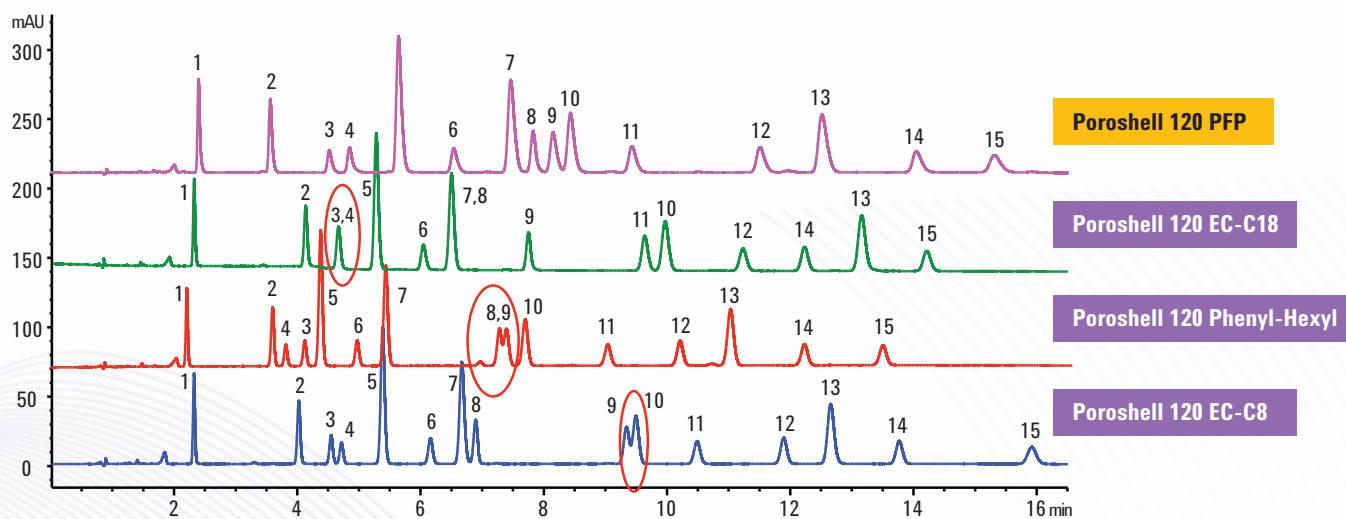
15 30

16 15

Flow rate: 2 mL/min

Detection: 270 nm

Column dimensions: 4.6 x 150 mm

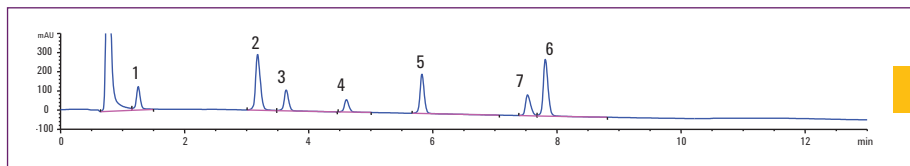


This separation illustrates the benefits of the PFP phase chemistry. Here, 15 positional isomers are analyzed using four different chemistries, with the PFP offering the best resolution.

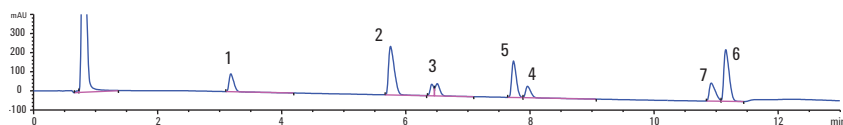


## Analysis of beta blockers: a comparison of Poroshell 120 phases

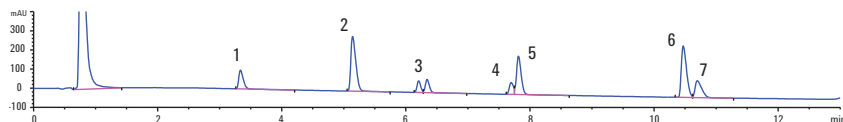
This challenging separation demonstrates how different selectivities produce different results. Overall, the Bonus-RP phase delivered the best peak shape and resolution. This was especially true for nadolol, which appeared as a split peak with the C18 and Phenyl-Hexyl phases.



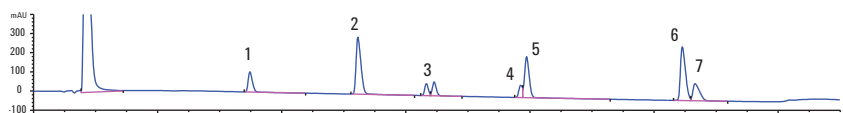
**Poroshell 120 Bonus-RP**



**Poroshell 120 Phenyl-Hexyl**



**Poroshell 120 EC-C18**



**Poroshell 120 SB-C18**

Columns: Poroshell 120 Bonus-RP, 2.1 x 100 mm, 2.7  $\mu$ m (p/n 695768-901)  
 Poroshell 120 Phenyl-Hexyl, 2.1 x 100 mm, 2.7  $\mu$ m (p/n 695775-912)  
 Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7  $\mu$ m (p/n 695775-902)  
 Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7  $\mu$ m (p/n 685775-902)

Instrument: 1260 Infinity Binary LC

Mobile phase: A: 10 mM  $\text{NH}_4\text{HCO}_2$ , pH 3.8  
 B: MeOH

Flow rate: 0.4 mL/min

Temperature: 40 °C

Detection: 260 nm

Gradient: 10% B to 30% B/12 min

Sample:

- |               |                |
|---------------|----------------|
| 1. Atenolol   | 5. Acebutolol  |
| 2. Pindolol   | 6. Propranolol |
| 3. Nadolol    | 7. Alprenolol  |
| 4. Metoprolol |                |

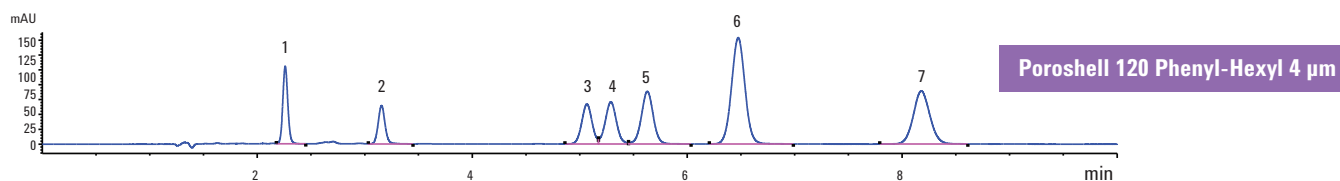
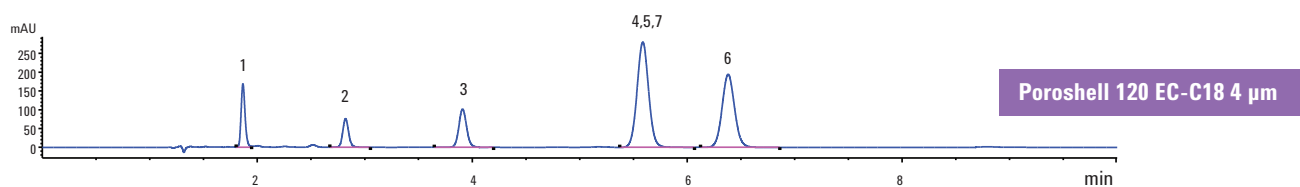
With the Poroshell 120 4  $\mu\text{m}$  columns, you can still take advantage of the flexibility of additional phase chemistries. With five chemistries available, choose the phase that takes advantage of key analyte interactions, such as the pi-pi interactions shown here with a steroid separation.

#### Isocratic Test

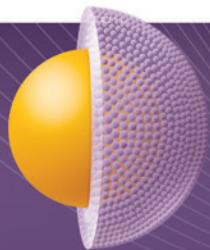
Column: Poroshell 120 18 or PH,  
4.6 x 150 mm, 4  $\mu\text{m}$   
Mobile Phase: 64% MeCN or MeOH  
36% Water w/0.1% acetic acid

Flow rate: 1.2 mL/min  
Temperature: 25 °C  
Detection: 220, 4 nm

Sample:  
1. Triamcinolone 5. DES  
2. Prednisolone 6. Dienestrol  
3. Corticosterone 7. Deoxycorticosterone  
4. Estradiol



Steroids separation on both the Poroshell 120 EC-C18 4  $\mu\text{m}$  and Poroshell 120 Phenyl-Hexyl 4  $\mu\text{m}$  chemistries. You can see that better resolution was achieved on the Poroshell 120 Phenyl-Hexyl due to the pi-pi interactions with the analytes and the stationary phase.



## FAST LC/UHPLC PERFORMANCE FROM A STANDARD HPLC? AGILENT POROSHELL 120 MAKES IT POSSIBLE



With Poroshell 120 columns, you can achieve up to 90% or more of the efficiency you would expect from a sub-2  $\mu\text{m}$  Fast LC/UHPLC column—but at HPLC pressures (below 400 bar).

This ability to perform fast separations at low pressures with both the 4  $\mu\text{m}$  and 2.7  $\mu\text{m}$  column options can dramatically enhance your productivity. Now, you can run more samples in less time—using your lab's existing HPLC systems—as the following examples illustrate. Plus, you'll be ready to transfer your method seamlessly to an Agilent 1200 Infinity Series of your choice when you're ready, for even more productivity.

### UHPLC efficiency with less pressure

For this sample of neutral alkylphenones, the Poroshell 120 column delivered >90% of the efficiency of the 1.8  $\mu\text{m}$  column. Note, too, that the pressure on the Poroshell 120 column is about 50% of the pressure on the 1.8  $\mu\text{m}$  column.

Mobile phase: 60% Acetonitrile,  
40% water

Flow rate: 0.58 mL/min

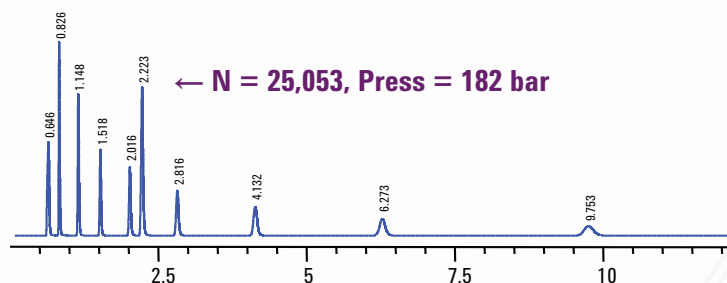
Injection volume: 4  $\mu\text{L}$

Temperature: 26 °C

Detection: DAD Sig = 254.4 nm  
Ref = 360,100 nm

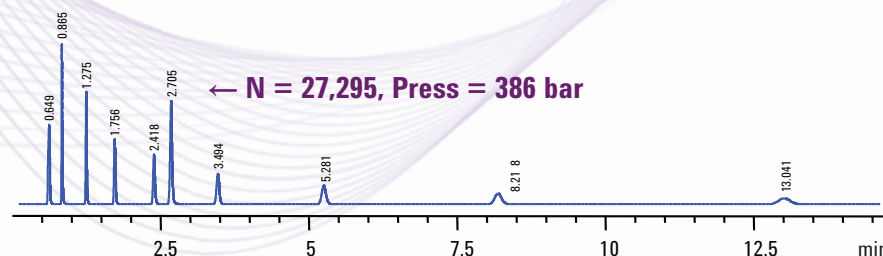
Sample preparation: RRLC checkout sample  
(p/n 5188-6529) spiked  
with 50  $\mu\text{L}$  2 mg/mL thiourea  
in water: acetonitrile (65:35)

Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7  $\mu\text{m}$   
(p/n 695975-302)



>90% of the efficiency of 1.8  $\mu\text{m}$

Eclipse Plus C18, 3.0 x 100 mm, 1.8  $\mu\text{m}$   
(p/n 959964-302)





## Choose Agilent Poroshell 120 for high efficiency HPLC

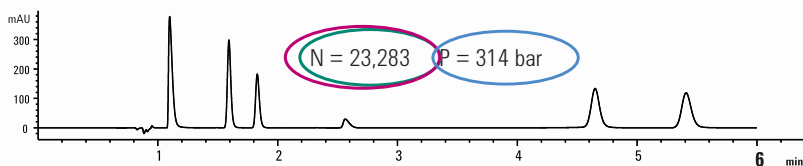
In this analysis of soft drink components, the Poroshell 120 column achieved:

- >90% of the efficiency of a sub-2  $\mu\text{m}$  column
- 2x the efficiency of the 3.5  $\mu\text{m}$  column
- Pressure below 400 bar, while the pressure on sub-2  $\mu\text{m}$  columns is above 400 bar

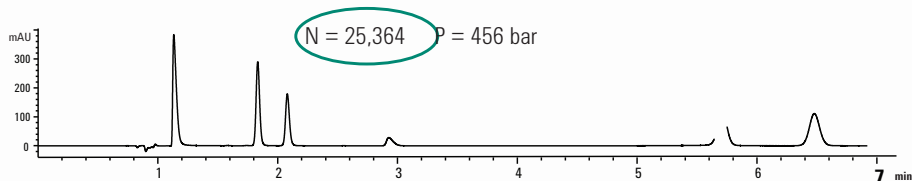
The low backpressure achieved with the methanol mobile phase is especially significant, because methanol generates more pressure than acetonitrile.

Column: 3.0 x 100 mm, 2.7  $\mu\text{m}$   
 Mobile phase: A: 65%, 0.2% Formic acid  
 B: 35% Methanol isocratic  
 Flow rate: 0.5 mL/min  
 Injection volume: 1  $\mu\text{L}$   
 Temperature: 26  $^{\circ}\text{C}$   
 Detection: UV, 220 nm

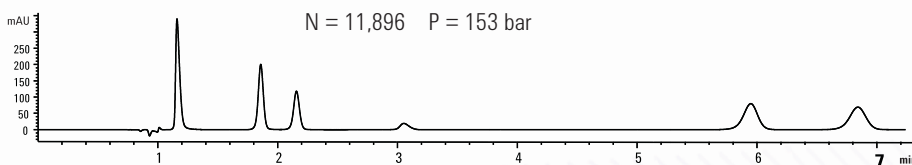
Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7  $\mu\text{m}$   
 (p/n 695975-302)



ZORBAX RRHT Eclipse Plus C18, 3.0 x 100 mm, 1.8  $\mu\text{m}$   
 (p/n 959964-302)



ZORBAX Rapid Resolution Eclipse Plus C18, 3.0 x 100 mm, 3.5  $\mu\text{m}$   
 (p/n 959961-302)



Sample:  
 1. Saccharin  
 2. Caffeine  
 3. p-Hydroxybenzoic acid  
 4. Aspartame  
 5. Dehydroacetic acid  
 6. Benzoic acid

## Why use a guard column?

Simply put, guard columns can save your lab money by extending the life of your analytical column.

Installing a less expensive guard column, especially when analyzing dirty samples, prevents damage caused by particulate matter and strongly adsorbed material. As a guide, you should replace your guard column when the plate number, pressure, or resolution changes by more than 10%. However, you will need to make an exact determination based on your application.

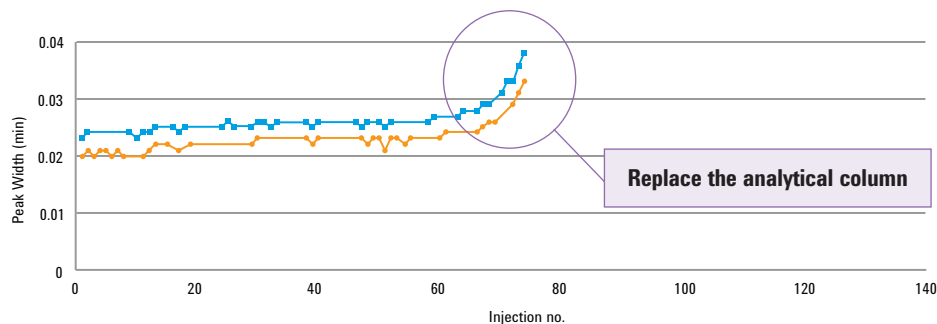
The example here is an **accelerated lifetime test** with 300:1 water:similac with 0.1 mg sulfachloropyridazine and sulfamethoxazole.

Mobile phase: A: 0.1% Formic acid in water  
B: Acetonitrile  
Flow rate: 0.65 mL/min  
Gradient: Hold 10% B for 2 min,  
ramp to 45% B in 2 min  
Injection volume: 10  $\mu$ L  
Temperature: 23  $^{\circ}$ C  
Detection: Sig = 254, 4 nm; Ref = Off  
Instrument: 1200 Infinity Series  
Sample preparation: 100 mL Water + 0.333 mL  
similac + 1 mL 0.1 mg/mL  
sulfachloropyridazine and  
sulfamethoxazole



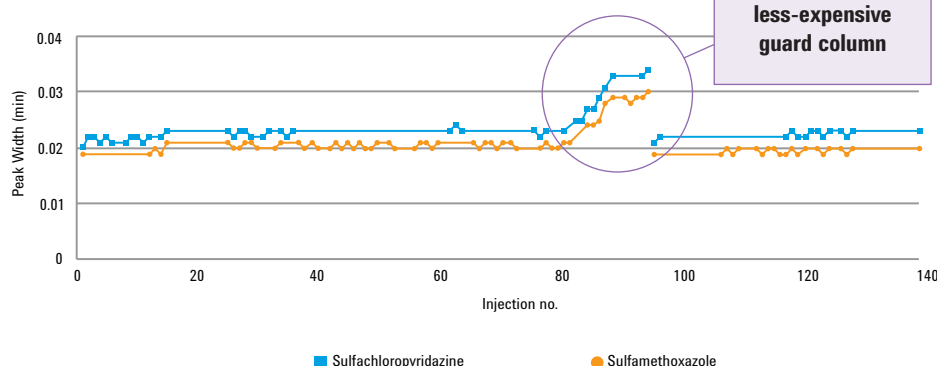
### Without a guard column:

Analytical column failure after just 80 injections.



### With a guard column:

The guard column fails after 80 injections—preserving the analytical column.



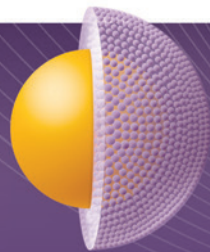
## Fast LC applications stay fast with Agilent Fast Guard columns

Agilent Fast Guard columns for UHPLC are rugged and reliable at high pressures, and are fully compatible with Agilent Fast LC and UHPLC columns. They can also be installed without any special tools.

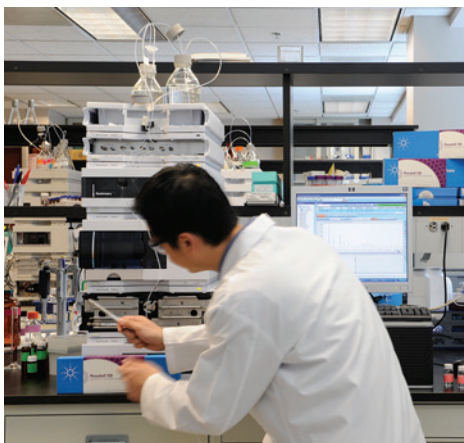
Watch the video to learn how easy it is to install

Agilent Fast Guard columns: [www.agilent.com/chem/poroshell120](http://www.agilent.com/chem/poroshell120)





## A RUGGED CHOICE FOR HIGH RESOLUTION LC/MS AND LC/MS/MS



Agilent Poroshell 120 columns can make your LC/MS and LC/MS/MS systems work even harder. Their porous outer layer and solid core limit diffusion distance and improve separation speed, while their narrow particle size distribution improves efficiency and resolution. Other advantages include:

- Quick and efficient resolution of critical isobaric compounds
- Better resolution of closely eluting peaks
- More compounds resolved in a single analysis
- Improved LC/MS accuracy and identification
- A standard 2  $\mu\text{m}$  frit that resists plugging with dirty samples

### Separation of cholesterol and other sterols using Poroshell 120 EC-C18 columns with LC/MS/MS

Note that adequate resolution was obtained, even at the 2000:1 cholesterol:lathosterol. This is critical for effective quantitation, because the two compounds have the same molecular weight.

Column: Poroshell 120 EC-C18,  
3.0 x 100 mm, 2.7  $\mu\text{m}$   
(p/n 695975-302)

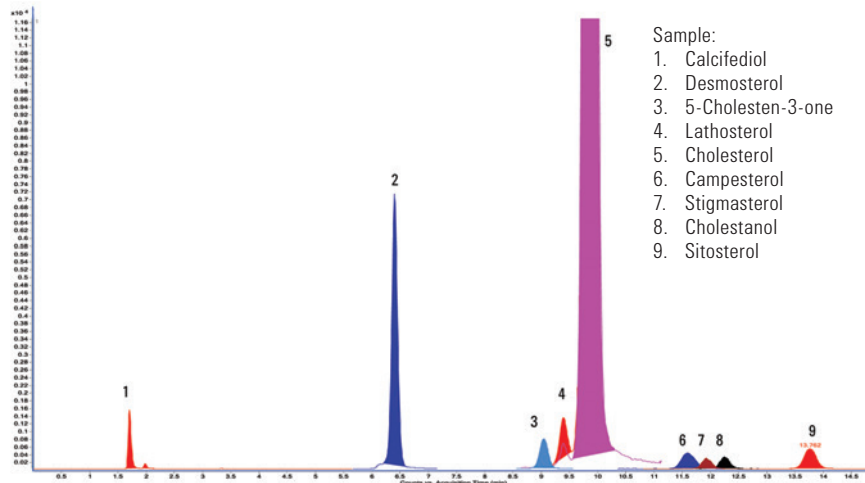
Mobile phase: 80% Acetonitrile,  
20% methanol

Flow rate: 0.6 mL/min

Injection  
volume: 2  $\mu\text{L}$

Temperature: 20  $^{\circ}\text{C}$

Detection: APCI, positive ion



## 1D-Separation of Vitamin D<sub>2</sub>/D<sub>3</sub> on Agilent Poroshell 120 EC-C18

Poroshell 120 provides a very fast LC/MS/MS analysis of vitamin D<sub>2</sub>/D<sub>3</sub> in plasma. Isocratic conditions were varied to compare speed of separation with chromatographic resolution.

Column: Poroshell 120 EC-C18,  
2.1 x 50 mm, 2.7 µm  
(p/n 699775-902)

Mobile phase: A: H<sub>2</sub>O + 0.1% formic acid  
B: MeOH + 0.1% formic acid

Flow rate: 0.5 mL/min

Injection volume: 10 µL

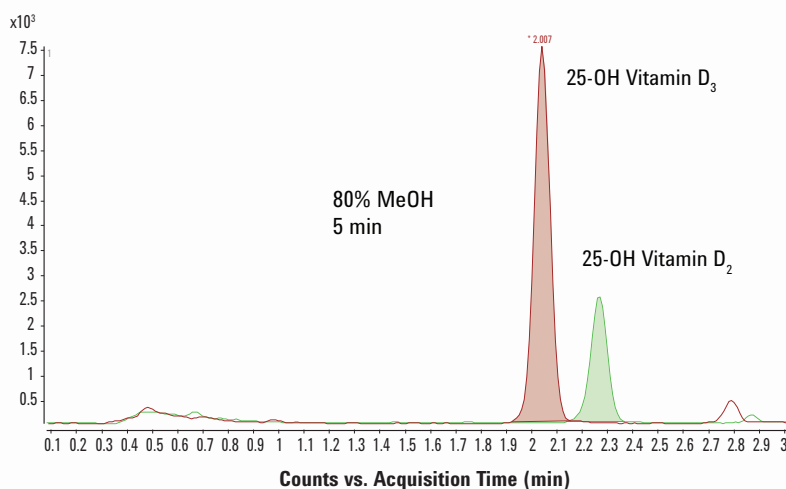
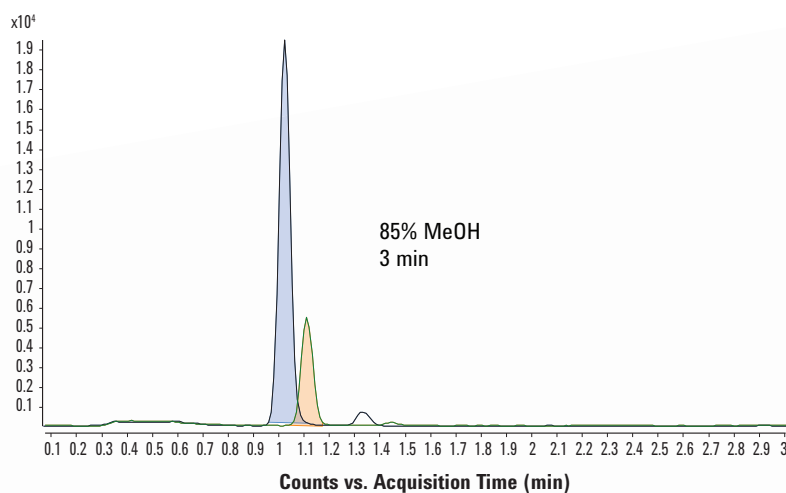
Temperature: 50 °C

Autosampler temp: 5 °C

Needle wash: Flush port (50:25:25,  
IPA: MeOH:H<sub>2</sub>O) 5

Isocratic analysis: A: 20%, B: 80%

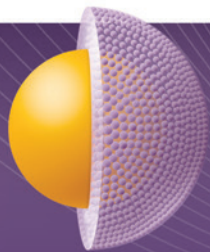
Analysis time: 5 min



## Rugged performance—even after 3,000 injections

This test confirms the outstanding longevity of Poroshell 120 columns, with little performance degradation after 3,000 injections. Stability is expressed in the consistency of the retention times (%RSD).

Analyte	%RSD (RT)	Analyte	%RSD (RT)	Analyte	%RSD (RT)
Morphine	0.7	Meperidine	0.4	Triazolam	0
Codeine	0.4	Zolpidem	0.3	Naltrexone	0.1
Hydrocodone	0.4	Fentanyl	0.1	Chlordiazepoxide	0.1
MDMA	0.3	EDDP	0.1	Desmethyl diazepam	0.1
Norfentanyl	0.2	Nitrazepam	0.1	Cocaethylene	0.2
Heroin	0.2	Propoxyphine	0.1	11-nor-9-carboxy-delta-9-THC	0
Methylphenidate	0.2	Buprenorphine	0.3		



## AGILENT POROSHELL 120 COLUMNS HELP YOU INCREASE THE FLEXIBILITY OF YOUR UHPLC METHODS

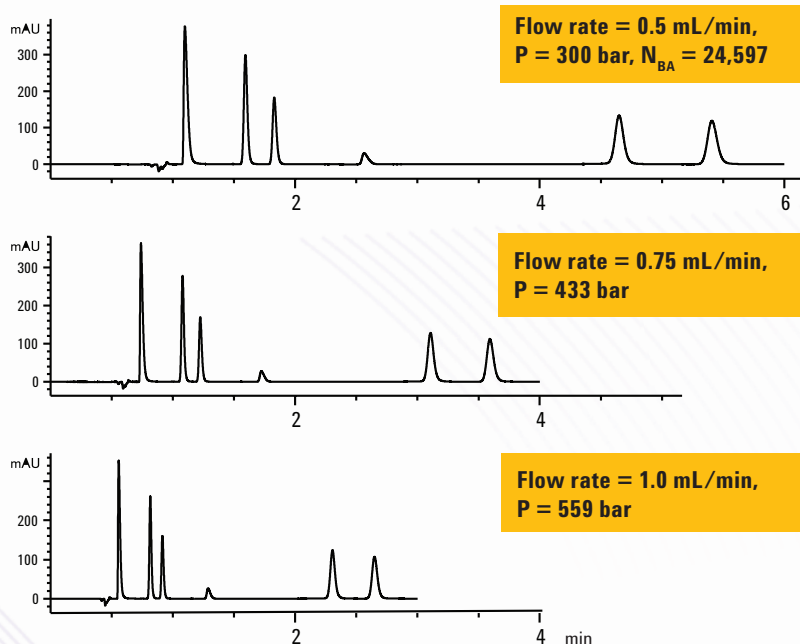


### Agilent Poroshell 120 EC-C18 for fast UHPLC separations

This example shows a fast separation using a mobile phase that generates higher pressures. In the top chromatogram, a 3.0 mm id column was used, with a flow rate of 0.5 mL/min and a pressure below 400 bar—making this a typical LC separation.

Although the top separation was fast (just under 6 minutes), the middle and bottom chromatograms show that you can reduce run times to **under 3 minutes** by increasing the flow rate. These faster analyses will take your pressure to 400 to 560 bar. Explore the Agilent 1200 Infinity Series flexible upgrade options to help you take advantage of UHPLC capabilities.

Because Poroshell 120 columns have a pressure limit of 600 bar, you can successfully apply them to your UHPLC methods—including those that use very long columns, higher flow rates, and viscous solvents.



More viscous solvents like methanol can be used at HPLC or UHPLC pressures.

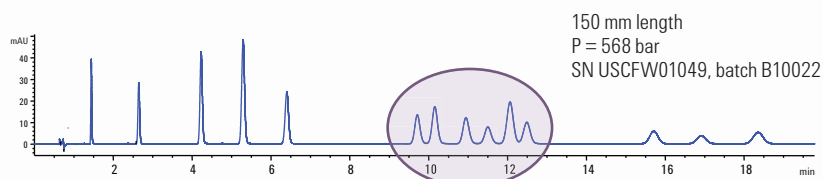
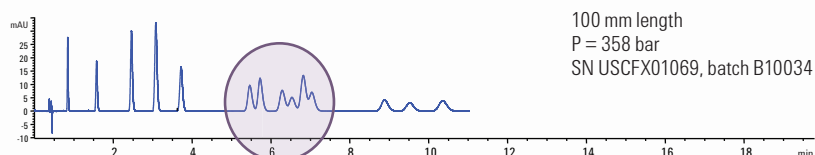
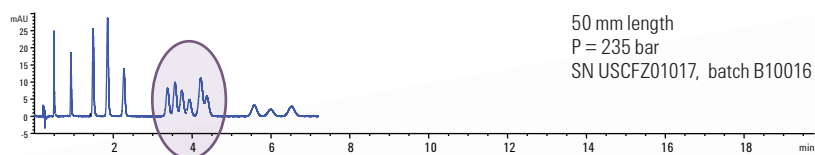
Column:	Poroshell 120 EC-C18 3.0 x 100 mm, 2.7 $\mu$ m (p/n 695975-302)	Sample:
Mobile phase:	A: 65%, 0.2% Formic acid B: 5% Methanol isocratic	1. Saccharin
Flow rate:	see chromatograms	2. Caffeine
Injection volume:	1 $\mu$ L	3. p-Hydroxybenzoic acid
Temperature:	26 °C	4. Aspartame
Detection:	Sig = 220, 4 nm, Ref = Off	5. Dehydroacetic acid
		6. Benzoic acid



## Agilent Poroshell 120 for HPLC and UHPLC comparison of EPA 8330 separation on short and long columns

Poroshell 120 columns give you the flexibility to choose longer columns for higher resolution. Here, you can see that as the column gets longer, resolution improves and pressure increases (up to UHPLC pressures for the longest column).

Note that column length affects resolution—not by the batch of material used in the column—proving that Poroshell 120 columns deliver reproducible performance.



Balancing column length, resolution, and analysis time are important for any separation.

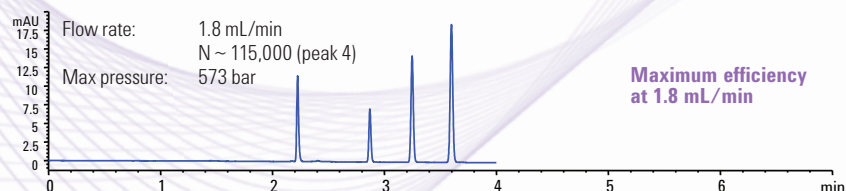
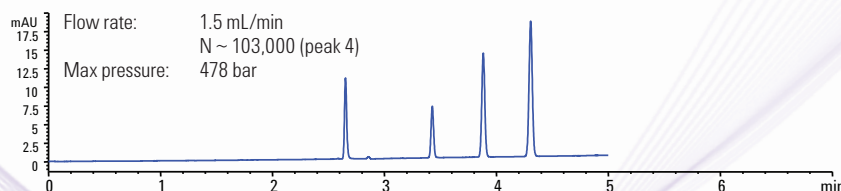
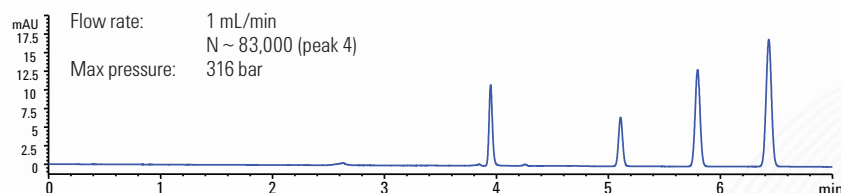
Column: Poroshell 120 EC-C18, 2.7  $\mu$ m  
Mobile phase: 25% Methanol, 75% water  
Flow rate: 1 mL/min  
Temperature: 44 °C

## Agilent Poroshell 120 columns in series deliver the highest efficiency at HPLC and UHPLC pressures

Because low backpressure is one of the advantages of Poroshell 120 columns, you can couple several columns in series to achieve the highest separation power per unit time. This enables better separation of complex samples.

Peak no.	Compound	Plates	k'
2	Acetophenone	114,120	0.29
3	Benzene	109,931	0.46
4	Toluene	114,800	0.65

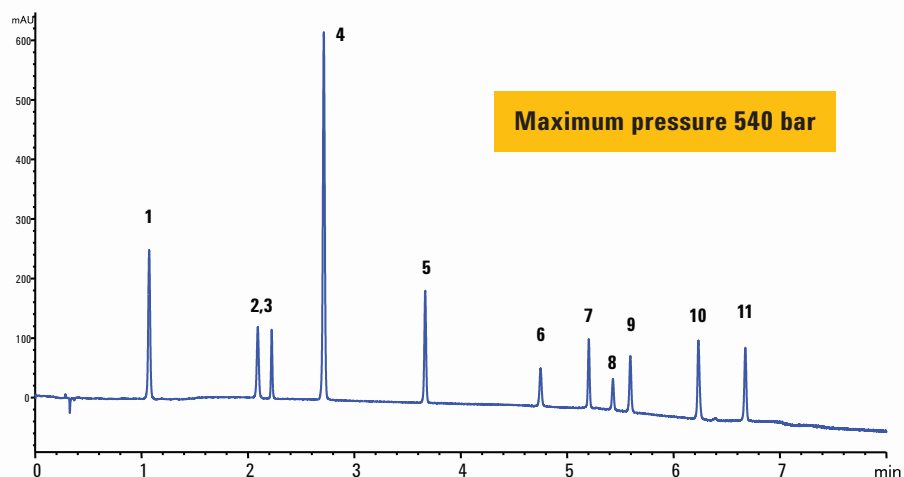
Three Poroshell 120 EC-C18, 4.6 x 150 mm, 2.7  $\mu$ m (p/n 693975-902) columns in series for very high efficiency.



Maximum efficiency at 1.8 mL/min

## Fast analysis on an Agilent Poroshell 120 EC-C18 of 11 compounds found in analgesics

Here, we used a high flow rate to speed up the separation of 11 common analgesic compounds using a Poroshell 120 column.



Column: Poroshell 120 EC-C18,  
4.6 x 100 mm, 2.7 µm  
(p/n 695975-902)

Mobile phase: A: Water + 0.1% formic acid  
B: Acetonitrile

Flow rate: 3.5 mL/min

Injection volume: 5 µL

Temperature: 40 °C

Detection: DAD, 254 nm

Sample:

1. Acetaminophen
2. Caffeine
3. 2-Acetamidophenol
4. Acetamide
5. Phenacetin
6. Sulindac
7. Piroxicam
8. Tolmetin
9. Ketoprofen
10. Diflusal
11. Diflunisal



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# COMPLEX METHOD TRANSFERS MADE SIMPLE



Many methods developed on longer 5  $\mu\text{m}$  C18 columns can be moved to Poroshell 120 columns quickly and easily, especially with the newly available Poroshell 120 4  $\mu\text{m}$  columns. New changes to the USP regulations are making it easier to transfer conventional methods to newer technologies like Agilent Poroshell 120. This enables chromatographers to significantly increase throughput and reduce costs.

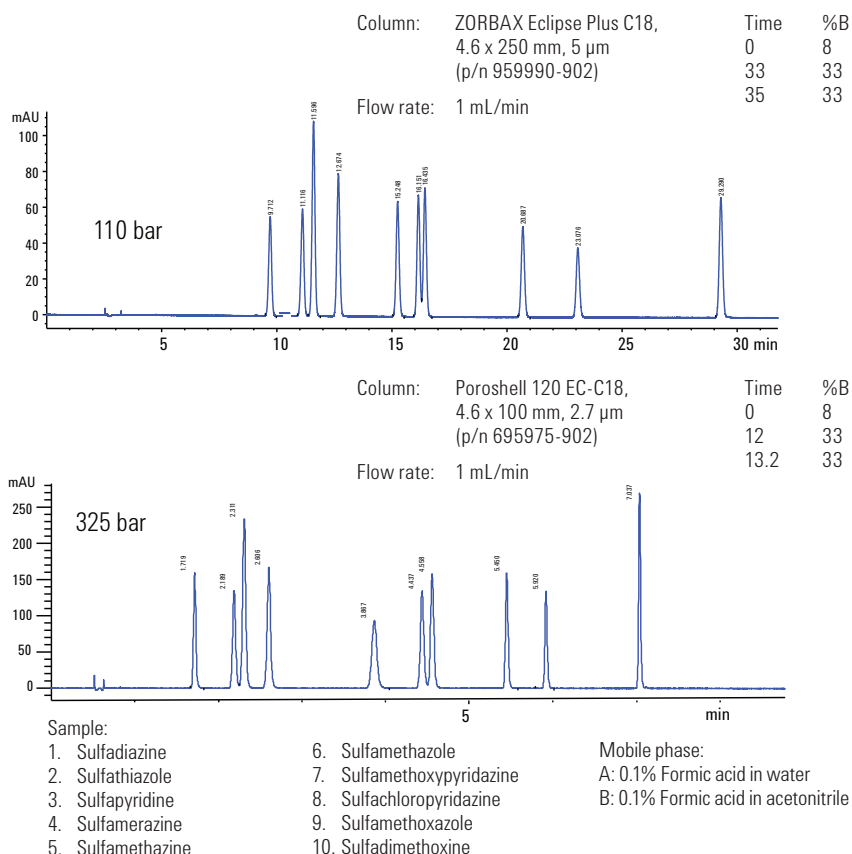
On the following pages, we will show you how five separations, including USP methods, can be repeated on Poroshell 120 columns—and can be completed 3-5 times faster than the same separations on 5  $\mu\text{m}$  columns.

## Transfer methods between Agilent Poroshell 120 and ZORBAX for time savings or scalability

In this example, a complex method was transferred from a ZORBAX Eclipse Plus C18 250 mm, 5  $\mu\text{m}$  column to a 100 mm Poroshell 120 EC-C18 column. All conditions were kept the same, except for the gradient time, which was adjusted for the shorter column.

As you can see, both separations are the same. However, the bottom chromatogram was generated in just over 7 minutes instead of 30 minutes for the top chromatogram—an excellent productivity improvement.

Keep in mind that both separations were run on an older Agilent 1100 Series instrument—proving that even gradient methods can be transferred while keeping the pressure below 400 bar.



## The Agilent Poroshell 120, 4 $\mu$ m column expansion

This addition provides the Poroshell 120 platform with a scalable solution for chromatographers and method developers. This robust platform extension, initially consisting of the EC-C18, EC-C8, Phenyl-Hexyl, PFP, and HILIC chemistries, helps you enter the Poroshell 120 family in a very simple manner with the ease

of drop-in method replacements. Column pressures are 50% less than the 2.7  $\mu$ m Poroshell 120, and efficiencies are nearly double those of traditionally totally porous 5  $\mu$ m columns. Chromatographers looking for moderate performance increases can adopt the 4  $\mu$ m Poroshell 120 columns into their method with simplicity.

### USP method for naproxen tablets—4.5x faster analysis on Agilent Poroshell 120 at HPLC pressures

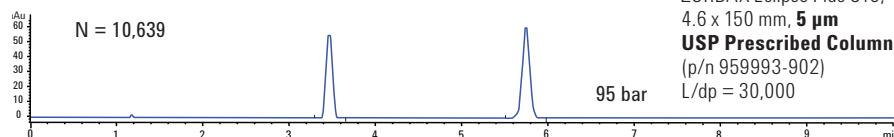
This naproxen separation demonstrates how easy it can be to convert a method to Poroshell 120 columns *without changing the flow rate or mobile phase*.

The 1st chromatogram shows a USP analysis on an Agilent ZORBAX Eclipse Plus C18 column, which delivers sharp peaks, three times the needed efficiency, and a resolution of  $\sim 14$ .

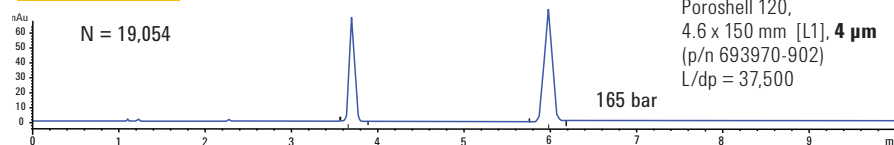
In the 2nd and 3rd chromatograms, the Poroshell 120 EC-C18 4  $\mu$ m columns (150 mm and 100 mm) provide greater efficiency and speed of the original method as easy, drop-in replacements. And because the pressure is only 165 bar for the 150 mm column and 98 bar for the 100 mm column, this isocratic method is an excellent HPLC option.

In the 4th chromatogram, the Poroshell 120 EC-C18 2.7  $\mu$ m column (100 mm) provides greater efficiency and resolution at nearly 2x the speed of the original method. The Poroshell 120 EC-C18 column (50 mm), in the 5th chromatogram still meets the requirements for efficiency and resolution, but is 4.5 times faster than the 5  $\mu$ m column.

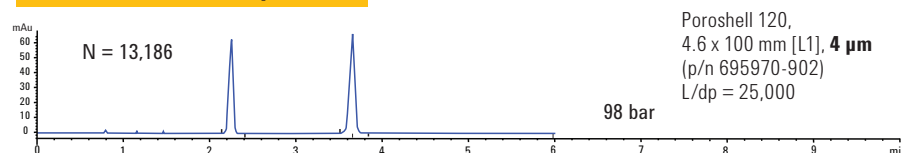
System suitability method requirement:  $N > 4,000$ ,  $R_s > 11.5$



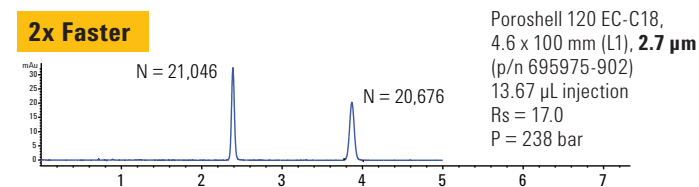
2x Efficiency



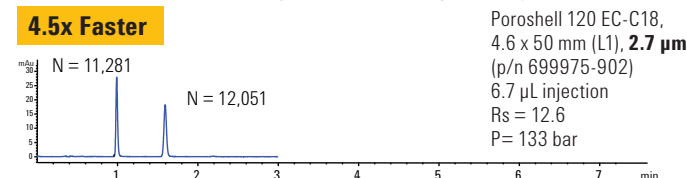
2x Faster, same backpressure



2x Faster



4.5x Faster



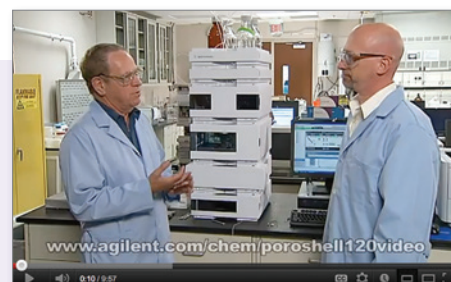
Poroshell 120 is an excellent choice for faster methods at HPLC pressures.

Mobile phase: 50:49:1 MeCN:H<sub>2</sub>O:acetic acid  
Flow rate: 1.2 mL/min

Sample:  
1. Naproxen  
2. Butyrophenone

Watch the video to learn how to transfer a naproxen method to Poroshell 120 columns, and optimize your LC system for the best results.

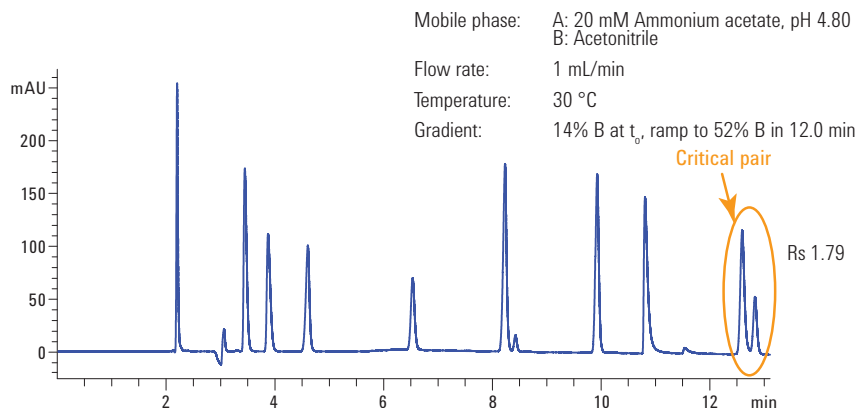
Go to [www.agilent.com/chem/poroshell120video](http://www.agilent.com/chem/poroshell120video)



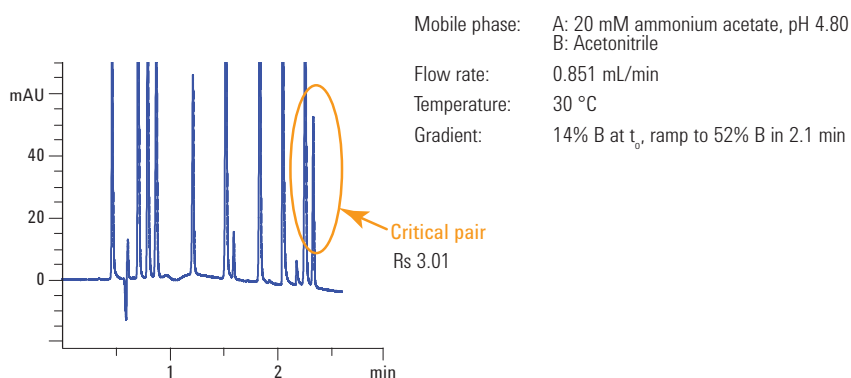
## Fast low pressure analysis

Here, a method for analyzing 11 non-nutritive food and beverage additives was transferred from a 5  $\mu\text{m}$  ZORBAX Eclipse Plus C18 column to a Poroshell 120 EC-C18 column, reducing the analysis time from over 13 minutes to less than 3 minutes. Solvent consumption was reduced by more than 80% and resolution of the critical pair improved from 1.8 to 3.0.

ZORBAX Eclipse Plus, 5  $\mu\text{m}$ ,  $P_{\text{max}} = 120$  bar



Poroshell 120 EC-C18, 2.7  $\mu\text{m}$

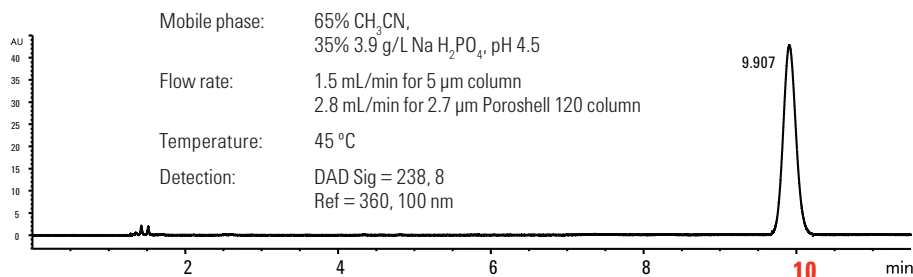


## Faster analysis of simvastatin on Poroshell 120

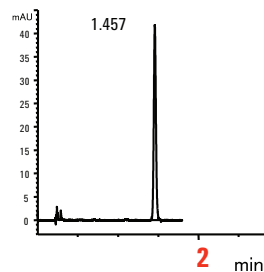
Here, a 10-minute USP method for simvastatin tablets was easily transferred to a Poroshell 120 column, with 5x faster results. Note that we reduced the column length by 70%, allowing a Poroshell 120 EC-C18, 75 mm column to be substituted for a 250 mm column *while still being considered a method adjustment*.

The Poroshell 120 EC-C18 phase is similar to other USP L1 phases, and so the results are similar, but faster.

ZORBAX Eclipse Plus C18, 4.6 x 250 mm, 5  $\mu\text{m}$  – (p/n 959990-902)



Poroshell 120 EC-C18, 4.6 x 75 mm, 2.7  $\mu\text{m}$  – (p/n 697975-902)



	USP Requirement	5 $\mu\text{m}$ (1.5 mL/min)	2.7 $\mu\text{m}$ (2.8 mL/min)
$T_R$	n/a	9.907	1.457
$k'$	> 3.0	5.962	5.122
N	> 4500	16,939	14,439
$T_f$	< 2.0	1.09	1.10



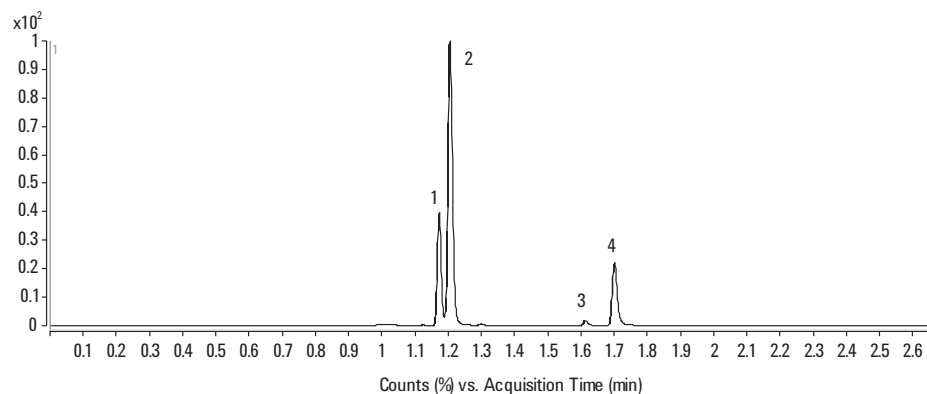
## Separation of morphine and metabolites using a Poroshell 120 HILIC column

An increasing number of labs are using HILIC early in drug discovery and development for several reasons:

- To achieve MS compatibility
- To improve retention of polar compounds and their *more* polar degradation products
- To increase LC/MS sensitivity

The separation of morphine and metabolites is one example of a fast, efficient HILIC LC/MS method. Here, you can see that these polar compounds are completely resolved in under 2 minutes with excellent peak shape and efficiency on the Poroshell 120 HILIC column. A reversed-phase method with high aqueous would have limited retention.

Poroshell 120 HILIC, 2.1 x 100 mm, 2.7  $\mu$ m – (p/n 695775-901)

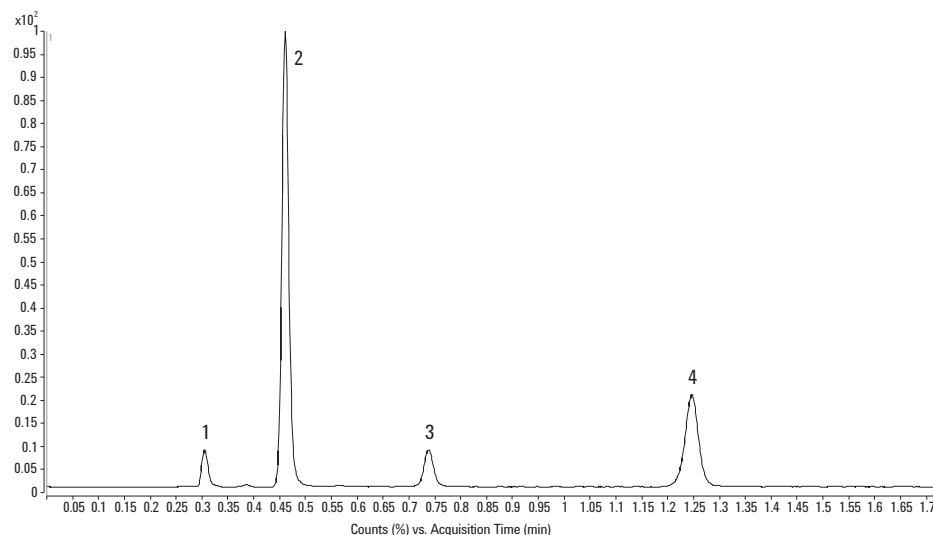


Sample	Mobile phase:	A: 100 mM $\text{NH}_4\text{HCO}_2$ , pH 3.2	Time	%B
1. Normorphine		B: Acetonitrile: 100 mM $\text{NH}_4\text{HCO}_2$ , pH 3.2 (9:1)	0	100
2. Morphine	Flow rate:	0.8 mL/min	0.44	100
3. M6G	Temperature:	25 °C	1.93	55
4. M3G	Pressure:	270 to 505 bar		
	System:	1290 Infinity LC and 6410 Triple Quadrupole LC/MS		

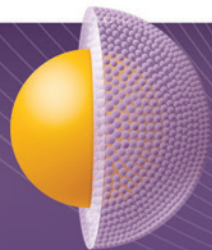
## Analysis of vitamin B and related compounds using a Poroshell 120 HILIC, 2.1 x 100 mm, 2.7 $\mu$ m column

HILIC eliminates the need for ion-pair reagents, such as the hexane sulfonic acid that is typically used in mobile phases for separating B vitamins. It also increases LC/MS compatibility and retention.

Poroshell 120 HILIC, 2.1 x 100 mm, 2.7  $\mu$ m – (p/n 695775-901)



Sample	Mobile phase:	Acetonitrile: 100 mM $\text{NH}_4\text{HCO}_2$ , pH 3.2 (9:1)
1. 4 Aminobenzoic acid	Flow rate:	0.7 mL/min
2. Nicotinamide	Temperature:	25 °C
3. Riboflavin	Pressure:	240 bar
4. Nicotinic acid	System:	1290 Infinity LC and 6410 Triple Quadrupole LC/MS



## AGILENT POROSHELL 120 COLUMNS: POWERFUL EVERYDAY PROBLEM SOLVERS

### A 2 $\mu\text{m}$ column inlet frit stands up to your dirtiest samples

Sub-2  $\mu\text{m}$  particles offer significant speed and resolution advantages, but are susceptible to clogging with dirty samples because a 0.5  $\mu\text{m}$  frit must be used at the column inlet. Poroshell 120 columns solve this problem with a standard 2  $\mu\text{m}$  frit that resists plugging with dirty samples—including unfiltered plasma.

### Sample loading of basic compounds on Poroshell 120 columns is comparable to sub-2 $\mu\text{m}$ columns

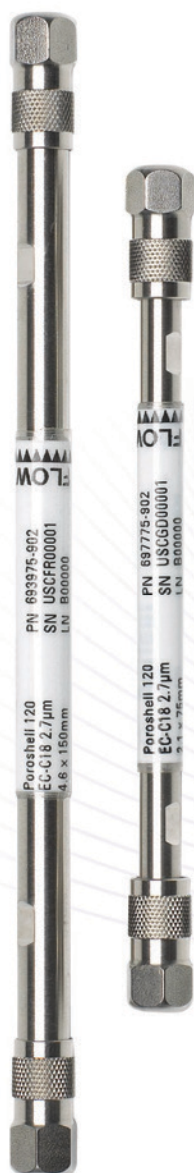
Small, non-porous particles have low surface area available for sample interaction, and are limited in their sample loading capability. However, Poroshell 120 columns are designed with a larger surface area for greater sample loading. In fact, the loading capacity of Poroshell 120 columns is comparable to 1.8  $\mu\text{m}$  columns—even for the most difficult basic compounds.

### The peak shape you need for your most accurate results

Poroshell 120 columns provide exceptional peak shape—especially at pH 6 to 7—when compared to other superficially porous columns.

### Agilent 1100 and 1200 Infinity Series are easily optimized for Poroshell 120 columns

The inherent properties of Poroshell 120 columns make them ideal for most HPLC and UHPLC instruments, including the new 1290 Infinity II. For 1100 and 1200 Infinity Series LC systems, all you need are minor configuration changes (such as flow rate, connector tubing length and id, flow-cell volume, and detector peak-width setting) to achieve superior results with lower pressures and higher efficiencies.



## Agilent Poroshell 120 resists plugging with 2 µm frit

Even with “dirty” samples, such as unfiltered plasma, Poroshell 120 columns show great resistance to plugging. Here, we precipitated the proteins, but did not centrifuge or filter the sample. Even under these conditions, there was no pressure increase, even after 2,500 injections.

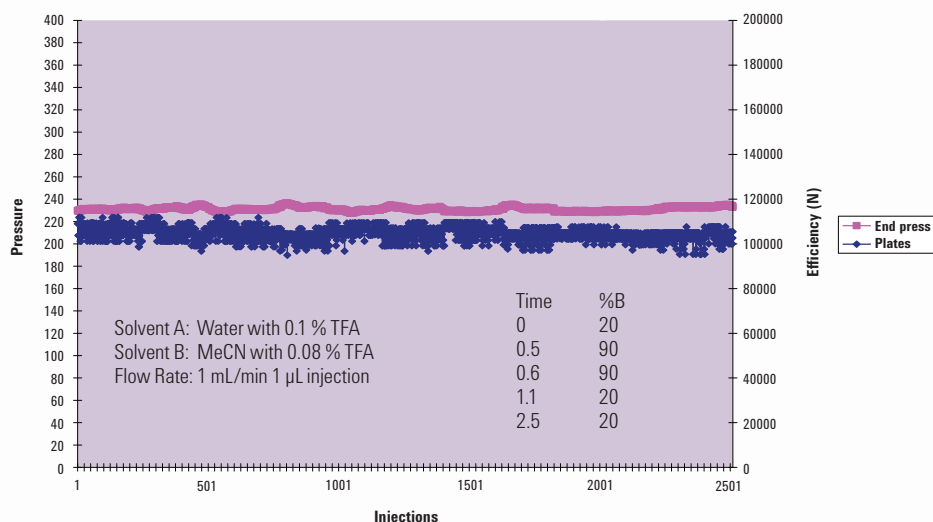
Column: Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm (p/n 699975-302)

Injection volume: 1 µL injections

Sample: Precipitated plasma: 2 parts plasma, 7 parts 20:80 water:MeCN with 0.1% formic acid with 1 part diflunisal in 50:50 water:MeCN 10 µg/mL (final concentration diflunisal 1 µg/mL) shaken and allowed to settle 10 minutes  
**Not centrifuged and not filtered**

Instrument: 1200 Infinity RRCL (SL)

### Diflunisal in plasma

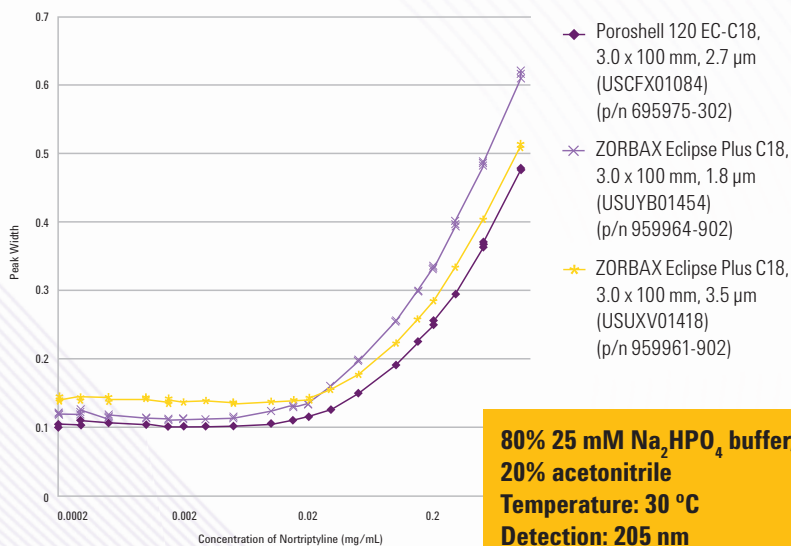


## Achieve comparable sample loading to totally porous particles

In this example, we loaded nortriptyline (a basic compound) onto several Agilent columns. Note that the Poroshell 120 2.7 µm column has the same loading capacity as the 1.8 µm column, and that the 3.5 µm column has a broader starting peak width that can compromise resolution.

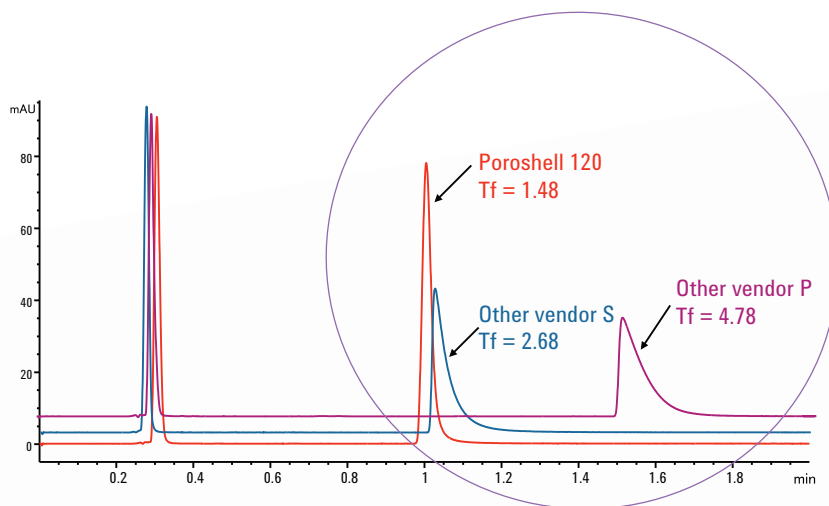
The loads on these columns are typical, proving that Poroshell 120 columns can be used with confidence in basic separations.

### Base loading with nortriptyline



## Agilent Poroshell 120 columns deliver superior peak shape for better results with basic compounds

Here is another basic compound separation, proving how Poroshell 120 columns outperform the other vendors for challenging analytes.



Column: Poroshell 120 EC-C18,  
4.6 x 50 mm, 2.7  $\mu$ m  
(p/n 699975-902)

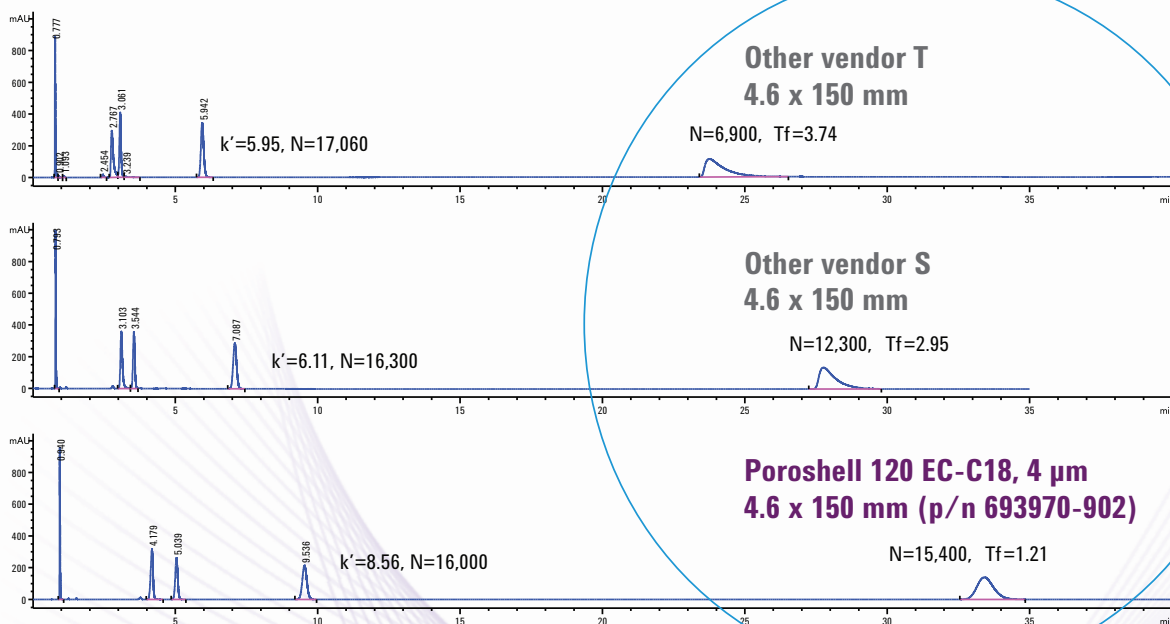
Mobile phase: 20 mM 40%  $\text{Na}_2\text{HPO}_4$ ,  
pH 7, 60% acetonitrile

Flow rate: 1.5 mL/min

Temperature: 24  $^{\circ}\text{C}$

Detector: DAD, 254 nm, 2  $\mu$ L flow cell

Sample: 2  $\mu$ L injection of  
250  $\mu\text{g/mL}$  amitriptyline,  
50  $\mu\text{g/mL}$  uracil in  $\text{H}_2\text{O}:\text{CH}_3\text{CN}$  (9:1)



Column: Poroshell 120 EC-C18,  
4.6 x 150 mm, 4  $\mu$ m

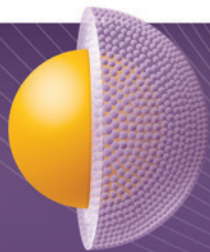
Mobile phase: 20 mM 40%  $\text{K}_2\text{HPO}_4 / \text{KH}_2\text{PO}_4$ ,  
pH 7, 60% methanol

Flow rate: 1.2 mL/min

Sample:

1. Uracil
2. Propranolol
3. Butyl Paraben
4. Dipropylphthalate
5. Amitriptyline





## FAST, CONFIDENT SEPARATIONS OF PROTEINS AND PEPTIDES



### High speed, high-resolution separation of monoclonal antibodies

Large biomolecules such as monoclonal antibodies are typically separated slowly to reduce potential peak broadening of these slow-diffusing analytes. However, the Poroshell technology used in AdvanceBio RP-mAb columns reduces the diffusion distance, thus allowing higher flow rates and steeper gradients—even on 600 bar systems.

The wide 450Å diameter of the pores in the thin layer provides full access to the bonded phase by large monoclonal antibody molecules, ensuring the best possible chromatography. The choice of robust bonded phases designed for monoclonal antibody separations, C4, SB-C8, and a unique diphenyl, provide a range of selectivities that allow resolution to be optimized.

Agilent Poroshell technology is an excellent choice for separating and characterizing complex biomolecules, including both *intact* and *digested* proteins. **Agilent AdvanceBio RP-mAb columns** focus on the unique challenges of monoclonal antibody characterization. For mapping peptides in protein digests, use **AdvanceBio Peptide Mapping columns** which are pretested with a challenging peptide mix to ensure optimal performance for your peptide mapping application.

Column: AdvanceBio RP-mAb C4  
2.1 x 100 mm, 3.5 µm (p/n 795775-904)

Mobile phase: A: 0.1% TFA in water:IPA (98:2)  
B: IPA:ACN:mobile phase A (70:20:10)

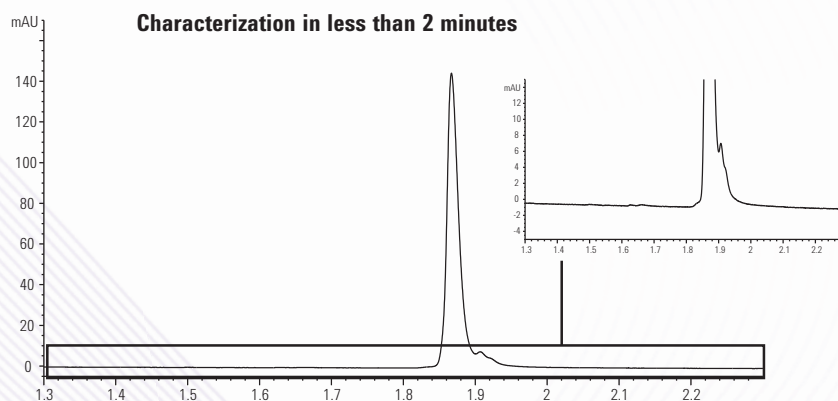
Flow rate: 1.0 mL/min

Temperature: 80 °C

Detector: UV, 254 nm

Gradient: 10-58% B in 4 min, 1 min wash at 95% B, 1 min reequilibration at 10% B

Sample: 5 µL injection of humanized recombinant Herceptin variant IgG1 intact from Creative Biolabs (1 mg/mL)



Agilent has one of the broadest families of biocolumns available, including AdvanceBio columns to help you advance accuracy and productivity for bioseparations. Learn more at [www.agilent.com/chem/advancebio](http://www.agilent.com/chem/advancebio)



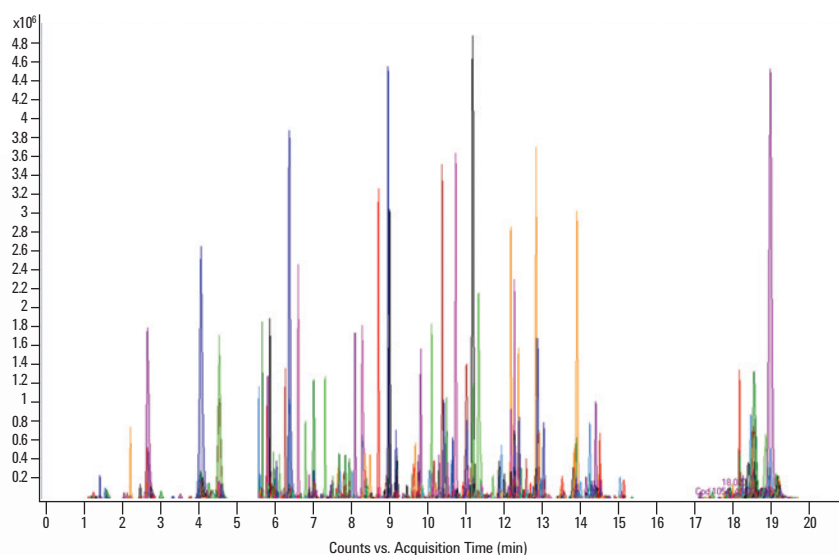
## BioConfirm Molecular Feature Extractor of Stratagene mAb trypsin peptide map

Using the BioConfirm Molecular Feature Extractor, we can demonstrate 100% sequence coverage on both the *light* and *heavy chains* of the same monoclonal antibody.

### Q-TOF Instrument Parameters

Source—ESI positive

Gas temperature: 325 °C  
Drying gas: 10 L/min  
Nebulizer: 40 psi  
Vcap: 4,000 V  
Fragmentor: 150 V  
Skimmer: 65 V  
Octapole 1 RF: 750 V  
MS: 4 Hz  
Mass range: 200-3200 m/z  
Reference mass: 922.009798  
Acq. mode: Extended dynamic range mode (2 GHz)



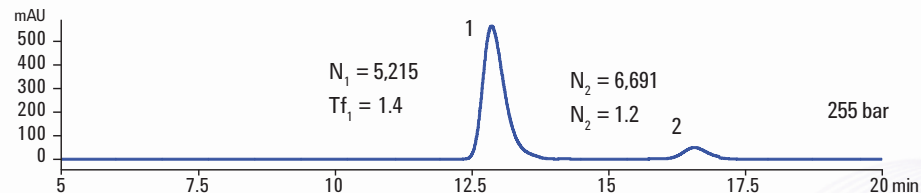
Column:	Poroshell 120 EC-C18, 3.0 x 150 mm, 2.7 µm (p/n 683975-302)	Detection:	Q-TOF, ESI positive		
		Gradient:	Shown in table		
Mobile phase:	A: Water, 0.1% formic acid B: ACN, 0.1% formic acid	Time	%B	Time	%B
		0	2	15.1	90
		3	2	17	90
Flow rate:	0.3 mL/min	13	45	18	2
Temperature:	40 °C	15	65		

## Insulin analysis: transfer from a 1.8 µm ZORBAX StableBond column to a Poroshell 120 column for increased efficiency

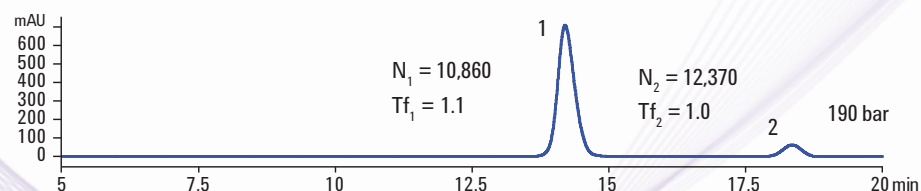
The Poroshell 120 SB-C18 column provided double the efficiency of the ZORBAX RRHD SB-C18 80Å due to the larger pore size and more rapid diffusion in the 120Å pores. Poroshell 120 columns are ideal for small protein insulin or other peptides, providing higher efficiency at lower pressure.

ZORBAX SB-C18, 4.6 x 100 mm, 1.8 µm—(p/n 828975-902)

1. Porcine insulin
2. A-21 desamido insulin

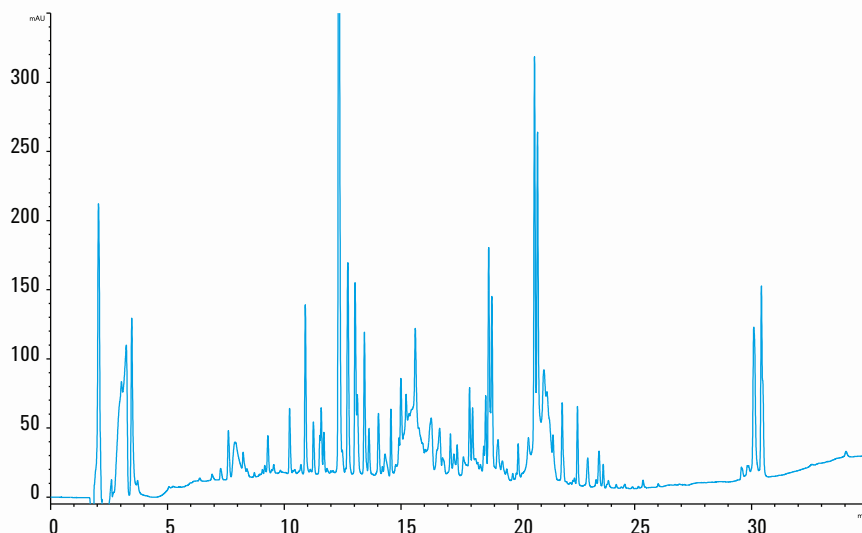


Poroshell 120 SB-C18, 4.6 x 100 mm, 2.7 µm—(p/n 685975-902)

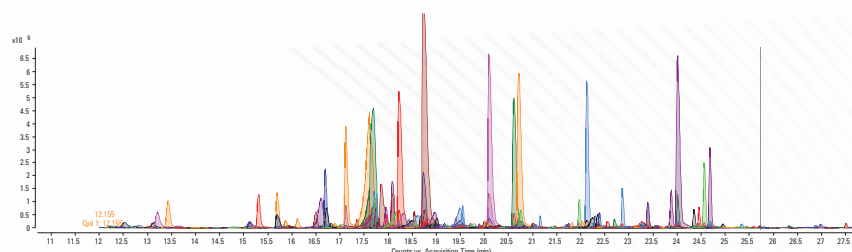


## Peptide map of a biosimilar EPO

The **top** chromatogram shows a peptide map of a highly glycosylated EPO from a biosimilar. Note the excellent resolution achieved for small peptide fragments using UV detection. The **bottom** chromatogram shows the same separation using mass spectroscopy to determine the sequence coverage (100%). UV detection is used for comparing peptide maps, while MS is ideal for identifying amino acid substitutions and modifications. So, you can easily confirm protein identity, and identify any posttranslational modifications, using the AdvanceBio Peptide Mapping column.



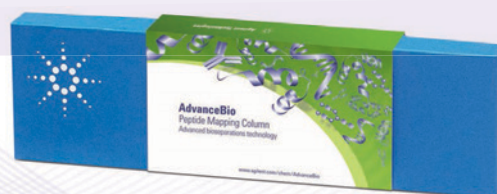
Column:	AdvanceBio Peptide Mapping, 2.1 x 250 mm, 2.7 $\mu$ m (p/n 651750-902)	Detection:	220 nm
Flow Rate:	0.4 mL/min	Gradient:	A: water (0.1% FA); B: ACN (0.1% FA); 0-28 min, 3-45% B; 28-33 min, 45-60% B; 33-34 min, 60-95% B
Injection:	5 $\mu$ L (2.0 mg/mL)		
Temp:	55 $^{\circ}$ C		



EPO digest, LC/MS TOF  
100% sequence coverage achieved using MassHunter Workstation software

**Agilent AdvanceBio Peptide Mapping columns** offer the same Fast LC advantages of Poroshell 120 columns, and are batch-tested with a rigorous peptide mix to ensure suitability and reproducibility. AdvanceBio Peptide Mapping column choices include the new 250 mm length for maximum resolution of the most complex peptide maps.

Learn more at [www.agilent.com/chem/advancebio](http://www.agilent.com/chem/advancebio)  
or request Publication No. 5991-1696EN.



## Which Fast LC column is best for you?

Agilent offers the widest range of Fast LC columns, including Poroshell 120, ZORBAX Rapid Resolution High Definition (RRHD) columns, 1.8  $\mu\text{m}$  (stable to 1200 bar) and ZORBAX Rapid Resolution High Throughput (RRHT), 1.8  $\mu\text{m}$  (stable to 600 bar). We bond all of these columns with similar stationary phases for assured scalability. With all of these choices, you have flexibility in creating a method to optimize your situation.

Your Lab Situation	Agilent Recommends	Rationale
Both UHPLC (1000+ bar) and HPLC instruments (e.g. Agilent 1290 Infinity LC and 1260 Infinity LC—600 bar)	1. Poroshell 120, 4 $\mu\text{m}$ and 2.7 $\mu\text{m}$ 2. ZORBAX RRHD 1.8 $\mu\text{m}$	Poroshell 120 is an easy column to use on both instrument types. ZORBAX RRHD will help you optimize the capabilities of the 1290 Infinity LC for UHPLC.
Only 400-600 bar HPLCs—Agilent 1200 Infinity Series, Agilent 1100 (400 bar) as well as the 1220 Infinity LC or 1260 Infinity LC (600 bar)	1. Poroshell 120, 4 $\mu\text{m}$ and 2.7 $\mu\text{m}$ 2. ZORBAX Eclipse Plus 3.5 $\mu\text{m}$ and 5 $\mu\text{m}$	With Poroshell 120 4 $\mu\text{m}$ and 2.7 $\mu\text{m}$ columns, you can enhance the performance of older 400 bar instruments, and also get even better performance from newer 600 bar UHPLC instruments. For established methods that you cannot transfer, the ZORBAX Eclipse Plus column provides exceptional peak shape and performance.
A mix of UHPLC instruments (Agilent 1290 Infinity LC, other 1000+ bar instruments) and some HPLC instruments (e.g. 1200 Infinity Series)	1. ZORBAX RRHD 1.8 $\mu\text{m}$ 2. Poroshell 120, 2.7 $\mu\text{m}$	ZORBAX RRHD can deliver optimum performance on all of these instruments. Poroshell 120 can be used on the 600 bar instruments to optimize their performance.

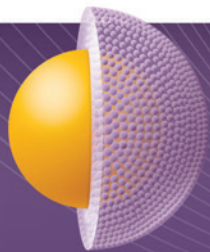


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## Agilent Poroshell 120 4 µm columns

NEW

Size (mm)	EC-C18	EC-C8	PFP	Phenyl-Hexyl	HILIC	HPH-C18	HPH-C8
4.6 x 250	690970-902	690970-906	690970-408	690970-912	690970-901	690970-702	690970-706
4.6 x 150	693970-902	693970-906	693970-408	693970-912	693970-901	693970-702	693970-706
4.6 x 100	695970-902	695970-906	695970-408	695970-912	695970-901	695970-702	695970-706
4.6 x 50	699970-902	699970-906	699970-408	699970-912	699970-901	699970-702	699970-706
3.0 x 250	690970-302	690970-306	690970-308	690970-312	690970-301	690970-502	690970-506
3.0 x 150	693970-302	693970-306	693970-308	693970-312	693970-301	693970-502	693970-506
3.0 x 100	695970-302	695970-306	695970-308	695970-312	695970-301	695970-502	695970-506
3.0 x 50	699970-302	699970-306	699970-308	699970-312	699970-301	699970-502	699970-506
2.1 x 250	650750-902	650750-906	650750-408	650750-912	650750-901	690770-702	690770-706
2.1 x 150	693770-902	693770-906	693770-408	693770-912	693770-901	693770-702	693770-706
2.1 x 100	695770-902	695770-906	695770-408	695770-912	695770-901	695770-702	695770-706
2.1 x 50	699770-902	699770-906	699770-408	699770-912	699770-901	699770-702	699770-706

## Guard columns for 4 µm columns

NEW

Size (mm)	EC-C18	HPH-C18	HPH-C8
4.6 x 5	820750-916	820750-930	820750-929
3.0 x 5	823750-916	823750-930	823750-929
2.1 x 5	821725-916	821725-930	821725-929

## Agilent Poroshell 120 2.7 µm columns

Size (mm)	EC-C18	EC-C8	SB-C18	SB-C8	HPH-C18	HPH-C8
4.6 x 150	693975-902	693975-906	683975-902	683975-906	693975-702	693975-706
4.6 x 100	695975-902	695975-906	685975-902	685975-906	695975-702	695975-706
4.6 x 75	697975-902	697975-906	687975-902			
4.6 x 50	699975-902	699975-906	689975-902	689975-906	699975-702	699975-706
4.6 x 30	691975-902	691975-906	681975-902			
3.0 x 150	693975-302	693975-306	683975-302	683975-306	693975-502	693975-506
3.0 x 100	695975-302	695975-306	685975-302	685975-306	695975-502	695975-506
3.0 x 75	697975-302	697975-306	687975-302			
3.0 x 50	699975-302	699975-306	689975-302	689975-306	699975-502	699975-506
3.0 x 30	691975-302	691975-306	681975-302			
2.1 x 150	693775-902	693775-906	683775-902	683775-906	693775-702	693775-706
2.1 x 100	695775-902	695775-906	685775-902	685775-906	695775-702	695775-706
2.1 x 75	697775-902	697775-906	687775-902			
2.1 x 50	699775-902	699775-906	689775-902	689775-906	699775-702	699775-706
2.1 x 30	691775-902	691775-906	681775-902			

## Agilent Poroshell 120 2.7 µm columns (continued)

Size (mm)	Phenyl-Hexyl	SB-Aq	Bonus-RP	HILIC	EC-CN	PFP
4.6 x 150	693975-912	683975-914	693968-901	693975-901	693975-905	693975-408
4.6 x 100	695975-912	685975-914	695968-901	695975-901	695975-905	695975-408
4.6 x 50	699975-912	689975-914	699968-901	699975-901	699975-905	699975-408
3.0 x 150	693975-312	683975-314	693968-301	693975-301	693975-305	693975-308
3.0 x 100	695975-312	685975-314	695968-301	695975-301	695975-305	695975-308
3.0 x 50	699975-312	689975-314	699968-301	699975-301	699975-305	699975-308
2.1 x 150	693775-912	683775-914	693768-901	693775-901	693775-905	693775-408
2.1 x 100	695775-912	685775-914	695768-901	695775-901	695775-905	695775-408
2.1 x 50	699775-912	689775-914	699768-901	699775-901	699775-905	699775-408

Note: Poroshell 120 columns have a 600 bar/9,000 psi pressure limit.

## Agilent Poroshell 120 Fast Guards for UHPLC

Size (mm)	EC-C18	EC-C8	SB-C18	Phenyl-Hexyl	PFP	HPH-C18
4.6 x 5	820750-911	820750-913	820750-912	820750-914	820750-915	820750-921
3.0 x 5	823750-911	823750-913	823750-912	823750-914	823750-915	823750-921
2.1 x 5	821725-911	821725-913	821725-912	821725-914	821725-915	821725-921

Size (mm)	HPH-C8	SB-C8	SB-Aq	Bonus-RP	HILIC	EC-CN
4.6 x 5	820750-922	820750-923	820750-924	820750-925	820750-926	820750-927
3.0 x 5	823750-922	823750-923	823750-924	823750-925	823750-926	823750-927
2.1 x 5	821725-922	821725-923	821725-924	821725-925	821725-926	821725-927

Note: Guards supplied as 3/pk.

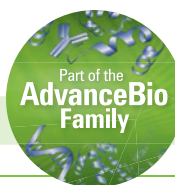


## Agilent Poroshell 120 bonded phase specifications

Bonded Phase	Pore Size	Temp. Limits	pH Range	Endcapped	Carbon Load	Surface Area
EC-C18	120Å	60 °C	2.0-8.0	Double	10%	130 m <sup>2</sup> /g
EC-C8	120Å	60 °C	2.0-8.0	Double	5%	130 m <sup>2</sup> /g
SB-C18	120Å	90 °C	1.0-8.0	No	9%	130 m <sup>2</sup> /g
SB-C8	120Å	80 °C	1.0-8.0	No	5.5%	130 m <sup>2</sup> /g
HPH-C18	100Å	60 °C	3.0-11.0	Double	Proprietary	95 m <sup>2</sup> /g
HPH-C8	100Å	60 °C	3.0-11.0	Double	Proprietary	95 m <sup>2</sup> /g
Phenyl-Hexyl	120Å	60 °C	2.0-8.0	Double	9%	130 m <sup>2</sup> /g
SB-Aq	120Å	80 °C	1.0-8.0	No	Proprietary	130 m <sup>2</sup> /g
Bonus-RP	120Å	60 °C	2.0-9.0	Triple	9.5%	130 m <sup>2</sup> /g
HILIC	120Å	60 °C	0.0-8.0	No	N/A	130 m <sup>2</sup> /g
EC-CN	120Å	60 °C	2.0-8.0	Double	3.5%	130 m <sup>2</sup> /g
PFP	120Å	60 °C	2.0-8.0	Yes	5.1%	130 m <sup>2</sup> /g

Specifications represent typical values.

Unique chemistries  
increase high-pH  
stability



## Agilent Poroshell 300 5 µm columns

Description	Size (mm)	300SB-C18	300SB-C8	300SB-C3	300Extend-C18
Narrow Bore	2.1 x 75	660750-902	660750-906	660750-909	670750-902
MicroBore	1.0 x 75	661750-902	661750-906	661750-909	671750-902
Capillary	0.5 x 75		5065-4468		
Guard Cartridge, 4/pk	2.1 x 12.5	821075-920	821075-918	821075-924	
Guard Hardware Kit		820888-901	820888-901	820888-901	
MicroBore Guard, 3/pk	1.0 x 17	5185-5968	5185-5968	5185-5968	5185-5968

Note: Poroshell 300 columns have a 400 bar/6,000 psi operating pressure limit.

## Agilent Poroshell 300 bonded phase specifications

Bonded Phase	Pore Size	Temp. Limits	pH Range	Endcapped
Poroshell 300SB-C18, C8, C3	300Å	90 °C	1.0-8.0	No
Poroshell 300Extend	300Å	40 °C above pH 8 60 °C below pH 8	2.0-11.0	Yes

Specifications represent typical values.

## Agilent AdvanceBio RP-mAb columns

Description	Part Number
C4, 4.6 x 150 mm, 3.5 µm	793975-904
C4, 4.6 x 100 mm, 3.5 µm	795975-904
C4, 4.6 x 50 mm, 3.5 µm	799975-904
C4, 2.1 x 150 mm, 3.5 µm	793775-904
C4, 2.1 x 100 mm, 3.5 µm	795775-904
C4, 2.1 x 75 mm, 3.5 µm	797775-904
C4, 2.1 x 50 mm, 3.5 µm	799775-904
SB-C8, 4.6 x 150 mm, 3.5 µm	783975-906
SB-C8, 4.6 x 100 mm, 3.5 µm	785975-906
SB-C8, 4.6 x 50 mm, 3.5 µm	789975-906
SB-C8, 2.1 x 150 mm, 3.5 µm	783775-906
SB-C8, 2.1 x 100 mm, 3.5 µm	785775-906
SB-C8, 2.1 x 75 mm, 3.5 µm	787775-906
SB-C8, 2.1 x 50 mm, 3.5 µm	789775-906
Diphenyl, 4.6 x 150 mm, 3.5 µm	793975-944
Diphenyl, 4.6 x 100 mm, 3.5 µm	795975-944
Diphenyl, 4.6 x 50 mm, 3.5 µm	799975-944
Diphenyl, 2.1 x 150 mm, 3.5 µm	793775-944
Diphenyl, 2.1 x 100 mm, 3.5 µm	795775-944
Diphenyl, 2.1 x 75 mm, 3.5 µm	797775-944
Diphenyl, 2.1 x 50 mm, 3.5 µm	799775-944

## Agilent AdvanceBio RP-mAb specifications

Bonded Phase	Pore Size	Temp. Limits	pH Range*	Endcapped
AdvanceBio RP-mAb C4	450Å	90 °C	1.0-8.0	Yes
AdvanceBio RP-mAb SB-C8	450Å	90 °C	1.0-8.0	No
AdvanceBio RP-mAb Diphenyl	450Å	90 °C	1.0-8.0	Yes

Specifications represent typical values.

\*Columns are designed for optimal use at low pH. At pH 6-8, highest column stability for all silica-based columns is obtained by operating at temperatures <40 °C and using low buffer concentrations in the range of 0.01-0.02 M.

## Agilent AdvanceBio Peptide Mapping columns

Description	Part Number
4.6 x 150 mm, 2.7 µm	653950-902
3.0 x 150 mm, 2.7 µm	653950-302
2.1 x 250 mm, 2.7 µm	651750-902
2.1 x 150 mm, 2.7 µm	653750-902
2.1 x 100 mm, 2.7 µm	655750-902
4.6 mm Fast Guard*	850750-911
3.0 mm Fast Guard*	853750-911
2.1 mm Fast Guard*	851725-911

\*Fast Guards extend column lifetime without slowing down the separation or affecting resolution.

## Agilent AdvanceBio Peptide Mapping specifications

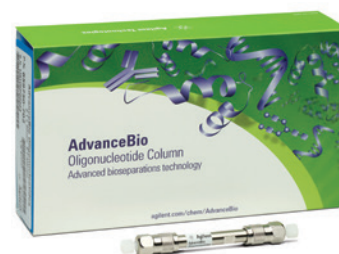
Bonded Phase	Pore Size	Temp. Limits	pH Range*	Endcapped
C18	120Å	60 °C	2.0-8.0	Double

Specifications represent typical values only.



## Agilent AdvanceBio Oligonucleotide columns

Description	Part Number
2.1 x 50 mm, 2.7 µm	659750-702
2.1 x 100 mm, 2.7 µm	655750-702
2.1 x 150 mm, 2.7 µm	653750-702
2.1 mm Fast Guard	821725-921
4.6 x 50 mm, 2.7 µm	659950-702
4.6 x 100 mm, 2.7 µm	655950-702
4.6 x 150 mm, 2.7 µm	653950-702
4.6 mm Fast Guard	820750-921
Oligonucleotide Resolution Standard	5190-9028
Oligonucleotide Ladder Standard	5190-9029



## Agilent AdvanceBio Oligonucleotide specifications

Bonded Phase	Pore Size	Temp. Limits	pH Range*	Endcapped
C18	100Å	65 °C	3.0-11.0	Double

**Agilent innovation:** The first high-pH stable superficially porous particle LC column for oligonucleotide analysis

## Agilent AdvanceBio Glycan Mapping columns

Description	Part Number
4.6 x 250 mm, 2.7 µm	680975-913
4.6 x 150 mm, 2.7 µm	683975-913
4.6 x 100 mm, 2.7 µm	685975-913
2.1 x 250 mm, 2.7 µm	651750-913
2.1 x 150 mm, 2.7 µm	683775-913
2.1 x 100 mm, 2.7 µm	685775-913
2.1 mm, 2.7 µm, Fast Guard	821725-906



## Agilent AdvanceBio Glycan Mapping specifications

Bonded Phase	ID (mm)	Particle Size (µm)	Endcapped	pH Stability	Operating Temperature	Pressure limit
Amide HILIC	2.1 and 4.6	1.8, fully porous	No	2-7	60 °C	1200 bar
Amide HILIC	2.1 and 4.6	2.7, superficially porous	No	2-7	60 °C	600 bar



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