

Agilent Poroshell 120 Columns for HPLC and UHPLC

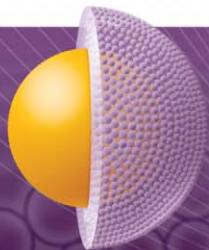
PERFORM RUGGED, FAST LC WITH CONFIDENCE

The Measure of Confidence



Agilent Technologies





Agilent Poroshell 120 columns can make every LC & 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 are from Poroshell 120 users

Poroshell 120 columns provide exceptional efficiency for standard HPLC, and significantly boost the performance you'll get from 600 and 1200 Bar UHPLC instruments.

These next-generation 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 before. Their advanced features include:

- **Extraordinary lot-to-lot reproducibility** – Poroshell 120 columns are manufactured using a proprietary single-step porous shell process that dramatically reduces minute differences between columns and lots
- **Comparable speed and resolution to sub-2 μm columns** with up to 50% less backpressure – take 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 μm frit, and resist plugging with dirty samples
- **Multiple bonded phases** – C18s, C8s, Phenyl-Hexyl, and more! A growing family of bonded phases for method development flexibility; check our web site for up to date information
- **Easy method transfer and scalability** to ZORBAX bonded phases, for highest productivity from lab to lab, around the world
- **UHPLC guard column options** that reduce your operating expenses by extending the life of Poroshell 120 columns



Here is what you'll find in this brochure

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What makes Poroshell 120 columns different?

Unique processes for making our superficially porous particles and bonded phases contribute to the excellent results you can achieve

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A family of bonded phases for flexible selectivity

Poroshell 120 bonded phases let you scale to ZORBAX phases

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Making your HPLC work harder

Yes you can achieve Fast LC performance at HPLC pressures

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Making every LC and LC/MS work harder

Perform high-speed, high-resolution separations on your current instruments

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Increasing the flexibility of your UHPLC methods

Now you can perform very fast, high-efficiency separations under the widest range of separation conditions

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Easy method transfer

Save time and money by moving methods from 5 μm or 3.5 μm columns to Poroshell 120 columns

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How Poroshell 120 columns help you meet the challenges you face every day

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New options for proteins and peptides

Achieve faster peptide mapping and protein separations with Poroshell 120 columns

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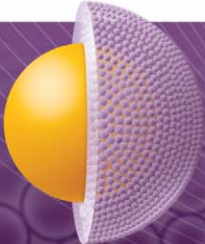
Infinitely better liquid chromatography

Introducing Agilent's 1200 Series Infinity LC systems

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Specifications and ordering information

To learn more about Agilent Poroshell 120 columns, visit
www.agilent.com/chem/poroshell120

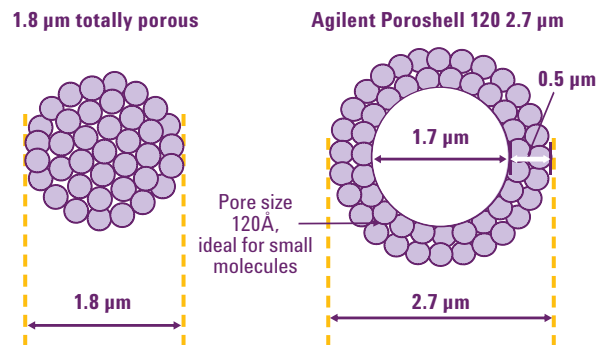


High efficiency at lower pressures Proven column-to-column consistency That's the Poroshell 120 difference



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

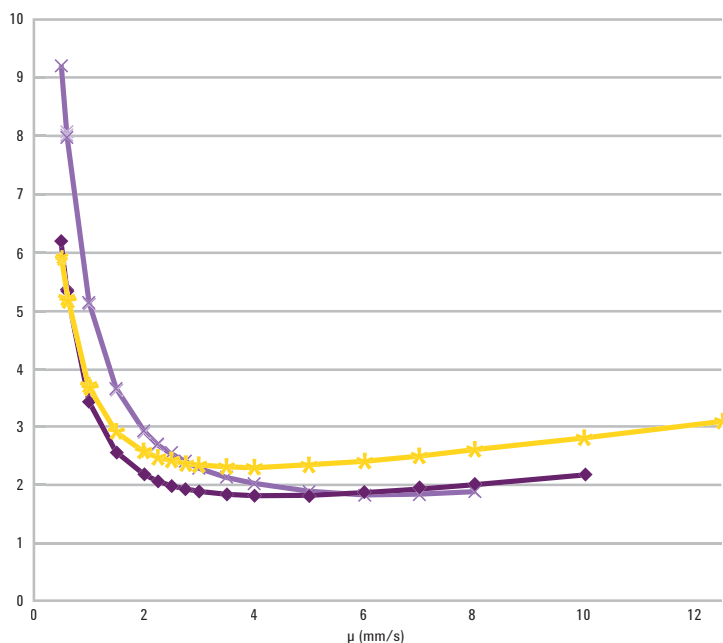
Poroshell 120 particles have a 1.7 μm solid silica core with a 0.5 μm porous outer layer. This unique configuration gives you all the performance advantages of sub-2 μm particles with the backpressure of a sub-3 μm particle.



Superficially porous particles provide similar performance to sub-2 μm particles

This Van Deemter curve shows that Poroshell 120 – a superficially porous, 2.7 μm particle column – can deliver reduced plate heights similar to a 1.8 μm column for similar efficiency.

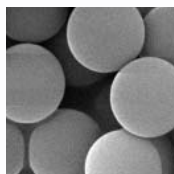
- ◆ Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μm , (USCFX01009) P/N 695975-302
- ✕ Agilent ZORBAX Eclipse Plus C18, 3.0 x 100 mm, 1.8 μm , (USUYB01455) P/N 959964-302
- ✦ Agilent ZORBAX Eclipse Plus C18, 3.0 x 100 mm, 3.5 μm , (USUXV01435) P/N 959961-302



How a Poroshell 120 particle is made

In order to create the best column for small molecule separations, we completely reinvented our superficially porous particle technology. Specifically, we minimized the manufacturing steps involved to ensure maximum particle – and chromatographic – reproducibility.

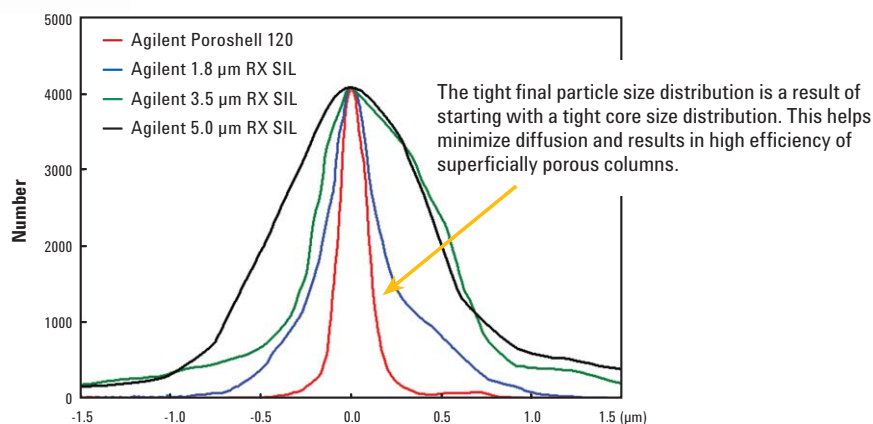
STEP 1 Make the solid core



Poroshell 120 column cores have a very smooth surface and a uniform particle size of 1.7 μm – which contributes 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.

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

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



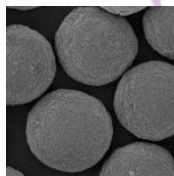
The standard measure of particle size distribution is the 90/10 ratio, which should be below 1.5

As you can see from the chart, 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.

	Agilent Poroshell 120 (2.7 μm) LN B10006	Agilent ZORBAX 1.8 μm	Agilent ZORBAX 3.5 μm	Agilent 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

To learn more about Agilent Poroshell 120 columns, visit www.agilent.com/chem/poroshell120

STEP 2



Apply the porous shell

Some manufacturers create the porous shell by applying layer after layer of particles. At Agilent, however, **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.

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. See the adjoining page for details on all of the Poroshell 120 phases that are available to you.

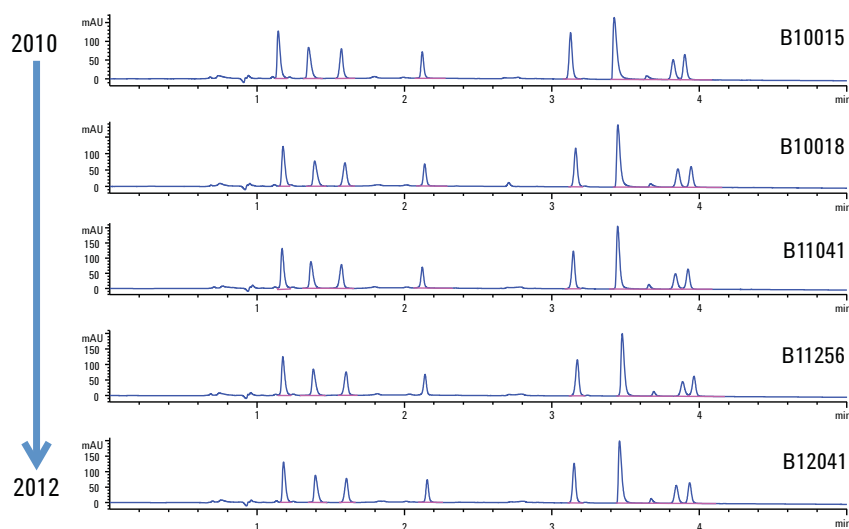
Reproducible performance from lot to lot, year after year

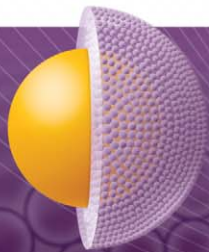
Poroshell 120 particles are made with a proprietary porous particle manufacturing process, invented by Agilent. Instead of traditional multilayering, Poroshell 120 columns are manufactured **using a single-step coacervation process** that produces a more consistent final particle – and more reliable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

Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μ m
P/N 695975-902 – from 5 Different Lots





A variety of bonded phases provides flexible selectivity



Poroshell 120 columns are made at the same facility as Agilent's 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.

A range of phases are available to help you perfect your separations:

- **Poroshell 120 EC-C18 and Poroshell 120 EC-C8** (endcapped for the best peak shape): These bonded phases should be your first choice for most separations, including peptide mapping with LC/MS-compatible mobile phases. We recommend that you select the C18 phase first, and use the C8 phase for less retention with a variety of samples.
- **Poroshell 120 SB-C18 and Poroshell 120 SB-C8** (non-endcapped for greater alternate selectivity): Choose these bonded phases for the best performance and longest lifetime at low pH (pH 1-2). Note that the C8 phase enables method transfer from existing ZORBAX SB-C8 columns in many established USP methods.
- **Poroshell 120 Phenyl-Hexyl** (endcapped): Choose this phase for unique selectivity with aromatic compounds when enhancing pi-pi interactions.
- **Poroshell 120 SB-Aq** (non-endcapped): This proprietary phase is ideal for polar compounds, and can be used in highly aqueous conditions.
- **Poroshell 120 Bonus-RP** (endcapped): This alkyl-amide produces orthogonal results for a number of separations, and can be helpful for resolving difficult compounds.
- **Poroshell 120 Hydrophilic Interaction Chromatography (HILIC)**: These columns are rapidly gaining popularity because of their ability to retain and separate small polar analytes. They also deliver high MS sensitivity for LC/MS applications.
- **Poroshell 120 EC-CN**: This cyano phase is very similar to our ZORBAX Eclipse XDB-CN phase – giving you selectivity alternatives for normal and reversed-phase 1D and 2D analysis of polar compounds.
- **And more**: Check our website for the growing list of available bonded phases!

To learn more about Agilent Poroshell 120 columns, visit www.agilent.com/chem/poroshell120

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

Mobile phase: 35% H₂O, 65% CH₃CN

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

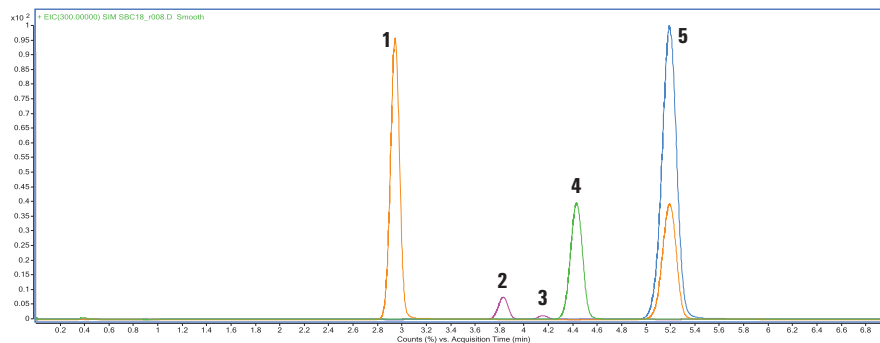
Nebulizer = 40 psi

Capillary = 4000 V

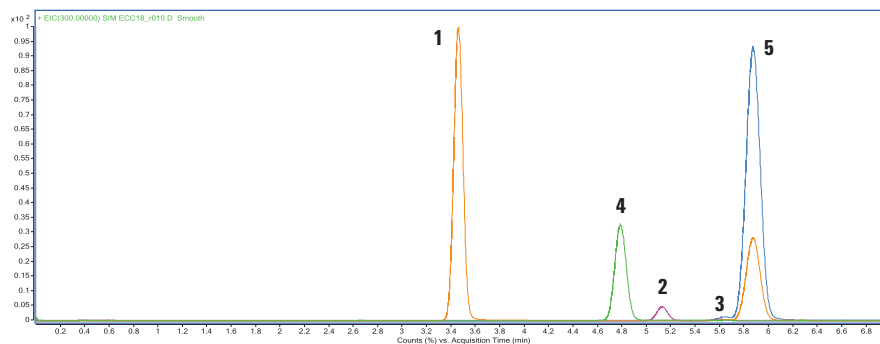
Analytes:

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

Agilent Poroshell 120 SB-C18 3.0 x 100 mm, 2.7 μm
P/N 685975-302



Agilent Poroshell 120 EC-C18 3.0 x 100 mm, 2.7 μm
P/N 695975-302



Poroshell 120 EC-C8 is less retentive for faster analysis of non-polar compounds

Mobile phase: 60% CH₃CN, 40% H₂O

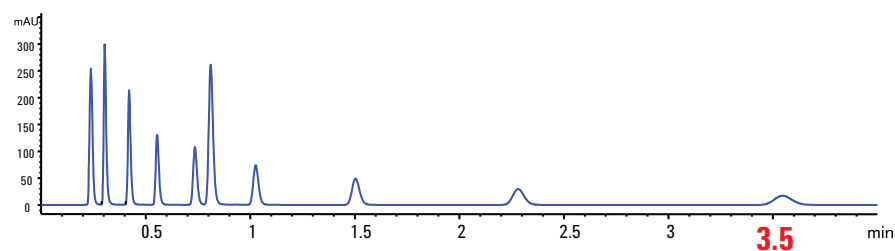
Flow rate: 0.85 mL/min

Temperature: 26 °C

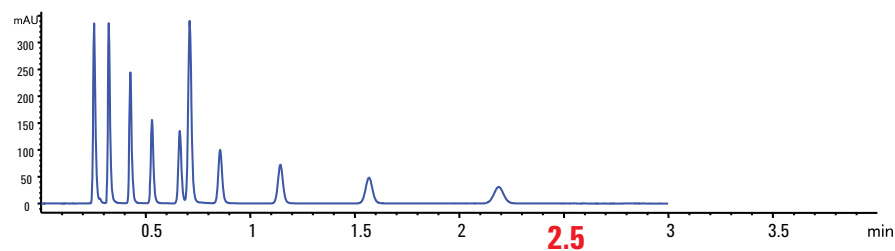
Detection: 254 nm

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

Agilent Poroshell 120 EC-C18, 3 x 50 mm, 2.7 μm
P/N 699975-302



Agilent Poroshell 120 EC-C8, 3 x 50 mm, 2.7 μm
P/N 699975-306



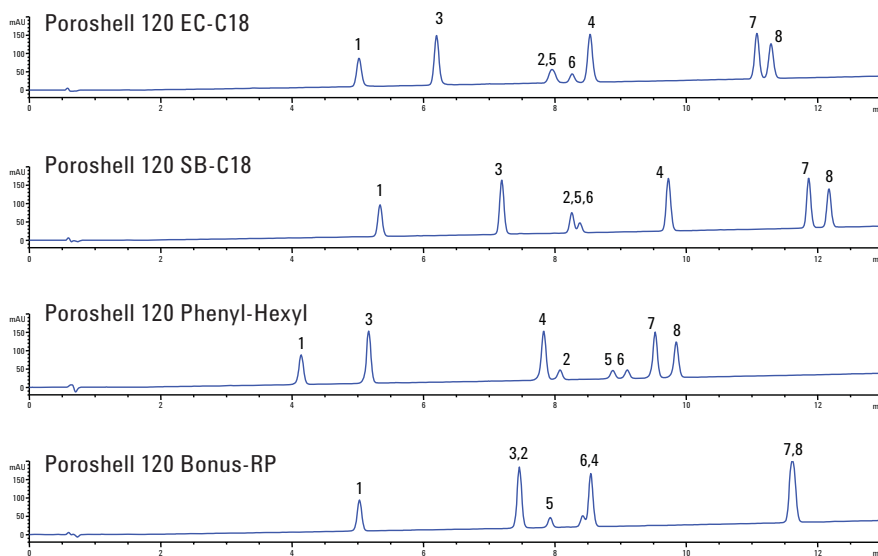
A choice of phases lets you control selectivity

Selectivity changes with 4 different Poroshell 120 phases

This separation of 8 steroids is best accomplished with the Phenyl-Hexyl phase, which provides alternate selectivity to C18 and C8, and more quickly and clearly provides baseline resolution for all 8 compounds.

Columns: Poroshell 120, 2.1 x 100 mm, 2.7 μ m
 Mobile phase: A: 0.1% Formic acid
 B: MeOH + 0.1% formic acid
 Flow rate: 0.4 mL/min
 Temperature: 25 $^{\circ}$ C
 Detection: 260 nm
 Sample: 8 steroids
 Gradient: 40-80% MeOH/14 min

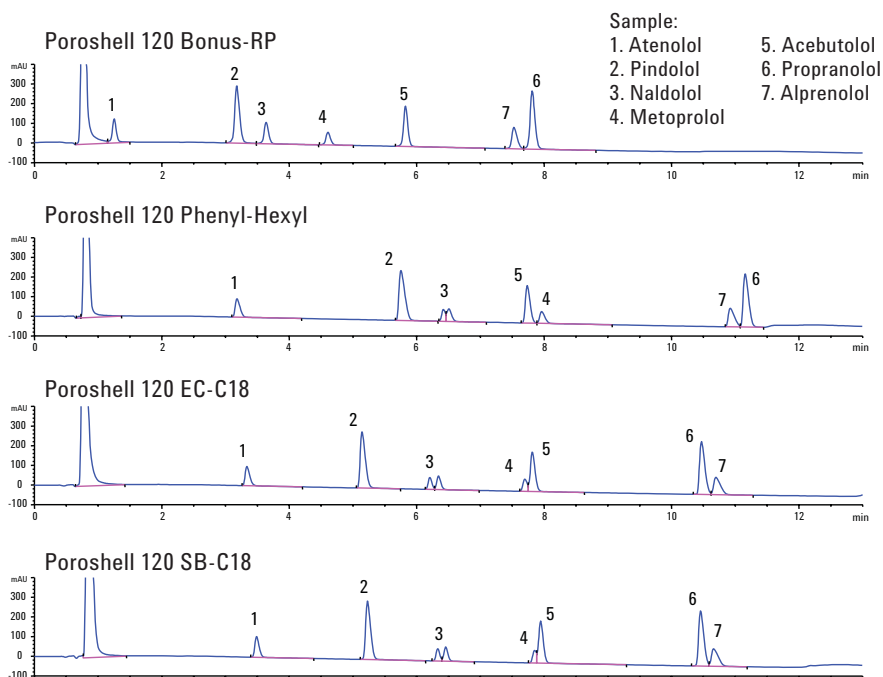
Sample:
 1. Hydrocortisone 4. Testosterone 7. Norethindone acetate
 2. B Estradiole 5. Ethyestradione 8. Progesterone
 3. Androstadiene 3,17 dione 6. Estrone



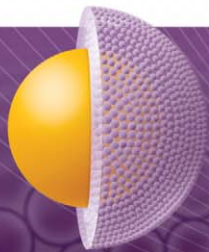
Analysis of Beta Blockers: A comparison of Poroshell 120 phases

This challenging analysis of beta blockers demonstrates how different selectivities produce varying results. Overall, the Bonus-RP phase delivered the best peak shape and resolution; this was especially true for Naldolol, which appeared as a split peak with the C18 and Phenyl-Hexyl phases.

Columns: Poroshell 120, 2.1 x 100 mm, 2.7 μ m
 Mobile phase: A: 10 mM NH_4CO_2 , pH 3.8
 B: MeOH
 Flow rate: 0.4 mL/min
 Temperature: 40 $^{\circ}$ C
 Detection: 260 nm
 Sample: Beta blockers
 Gradient: 10% B to 30% B/12 min



To learn more about Agilent Poroshell 120 columns, visit www.agilent.com/chem/poroshell120



Fast LC/UHPLC performance from a standard HPLC? Agilent Poroshell 120 makes it possible



With Poroshell 120 columns, you can achieve 80-90% or more of the efficiency you would expect from a sub-2 μm Fast LC/UHPLC column – but you can do so at HPLC pressures (below 400 bar).

This ability to perform fast separations at low pressures can dramatically enhance your productivity by allowing you to 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 LC instrument 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 attained by the 1.8 μm column. Note, too, that the pressure on the Poroshell 120 column is about 50% of the pressure on the 1.8 μm column.

Mobile phase: 60% Acetonitrile,
40% water

Flow rate: 0.58 mL/min

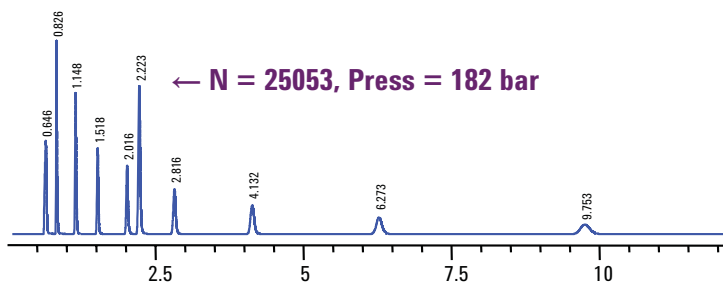
Injection volume: 4 μL

Temperature: 26 °C

Detection: DAD Sig = 254,4 nm
Ref = 360,100 nm

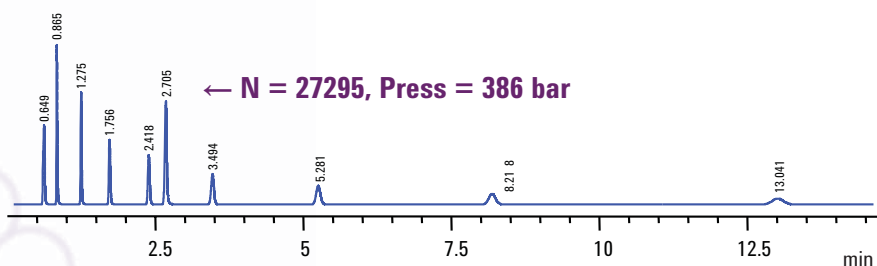
Sample: RRLL checkout sample
(P/N 5188-6529) spiked
w/ 50 μL 2 mg/mL thiourea
in water/acetonitrile (65:35)

Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μm
P/N 695975-302



>90% of the efficiency of 1.8 μm

Agilent Eclipse Plus C18, 3.0 x 100 mm, 1.8 μm
P/N 959964-302



Choose Agilent Poroshell 120 for high efficiency HPLC

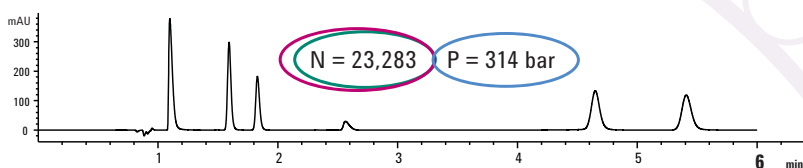
In this analysis of soft drink components, the Poroshell 120 column achieved:

- ▶ >90% of the efficiency of a sub-2 μm column
- ▶ 2x the efficiency of the 3.5 μm column
- ▶ The pressure on the Poroshell 120 column is below 400 bar, while the pressure on the sub-2 μm columns is above 400 bar

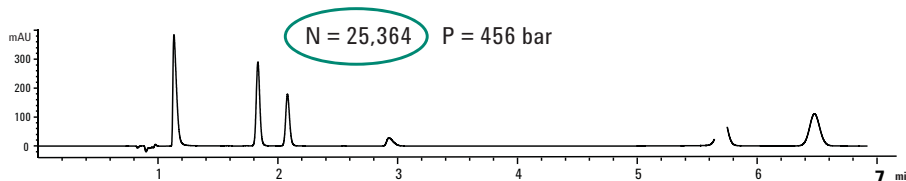
The low backpressure achieved with the methanol mobile phase is especially significant, because methanol generates more pressure than acetonitrile.

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

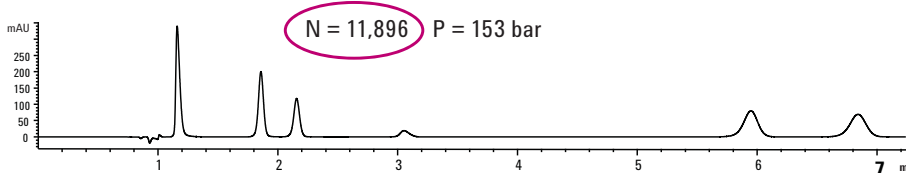
Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μm
P/N 695975-302



Agilent RRHT Eclipse Plus C18, 3.0 x 100 mm, 1.8 μm
P/N 959964-302



Agilent Rapid Resolution Eclipse Plus C18, 3.0 x 100 mm, 3.5 μm
P/N 959961-302



Sample:

- | | |
|--------------------------|-----------------------|
| 1. Saccharin | 4. Aspartame |
| 2. Caffeine | 5. Dehydroacetic acid |
| 3. P-hydroxybenzoic acid | 6. Benzoic acid |

To learn more about Agilent Poroshell 120 columns, visit
www.agilent.com/chem/poroshell120

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 μ L

Temperature: 23 $^{\circ}$ C

Detection: Sig = 254, 4 nm; Ref = Off

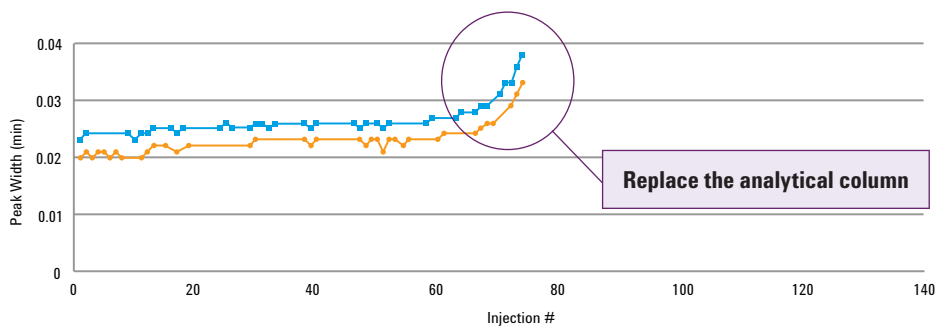
Instrument: Agilent 1200 Series RRLC

Sample preparation: 100 mL Water + 0.333 mL
similac + 1 mL 0.1 mg/mL
sulfachloropyridazine and
sulfamethoxazole



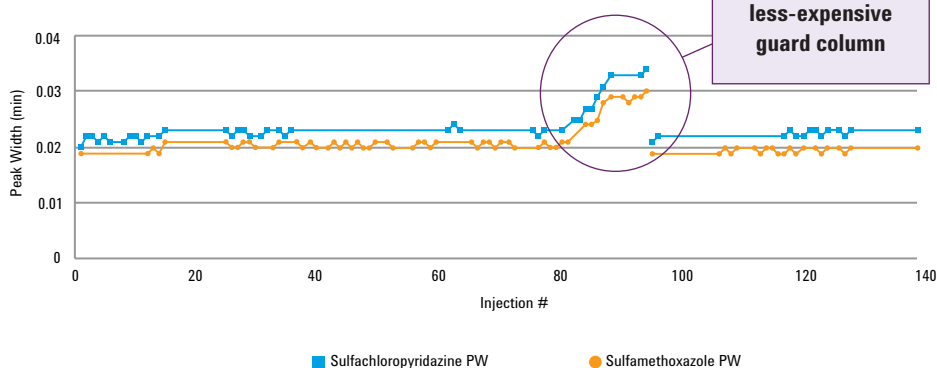
Without a guard column:

Analytical column failure after just 80 injections.



With a guard column:

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

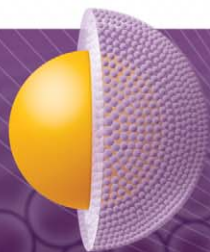


Fast LC applications stay fast with Agilent Fast Guard columns

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 our live video demonstration to learn how easy it is to install Agilent Fast Guard columns: 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 your 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 μm frit which 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 ratio for 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 μm
P/N 695975-302

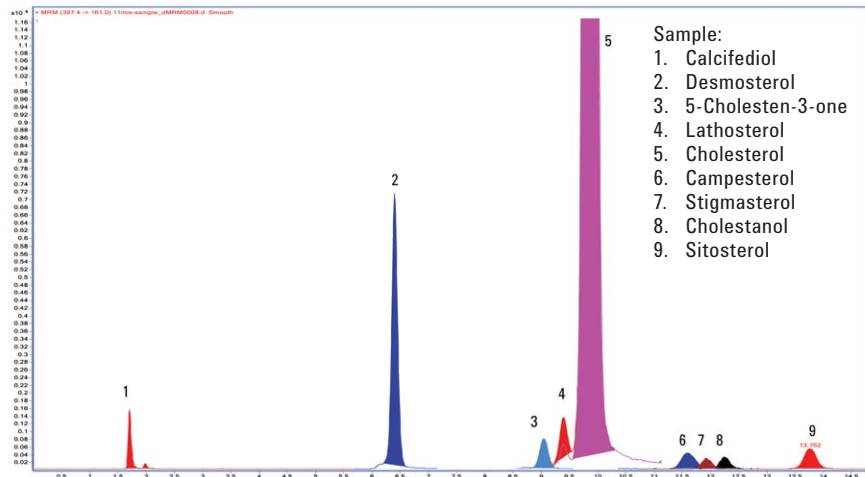
Mobile phase: 80% Acetonitrile,
20% methanol

Flow rate: 0.6 mL/min

Injection volume: 2 μL

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

Detection: APCI, positive ion



To learn more about Agilent Poroshell 120 columns, visit www.agilent.com/chem/poroshell120

1D Separation of Vitamin D2/D3 on Agilent Poroshell 120 EC-C18

Poroshell 120 provides a very fast LC/MS/MS analysis of Vitamin D2/D3 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: H₂O + 0.1% formic acid
B: MeOH + 0.1% formic acid

Flow rate: 0.5 mL/min

Injection volume: 10 µL

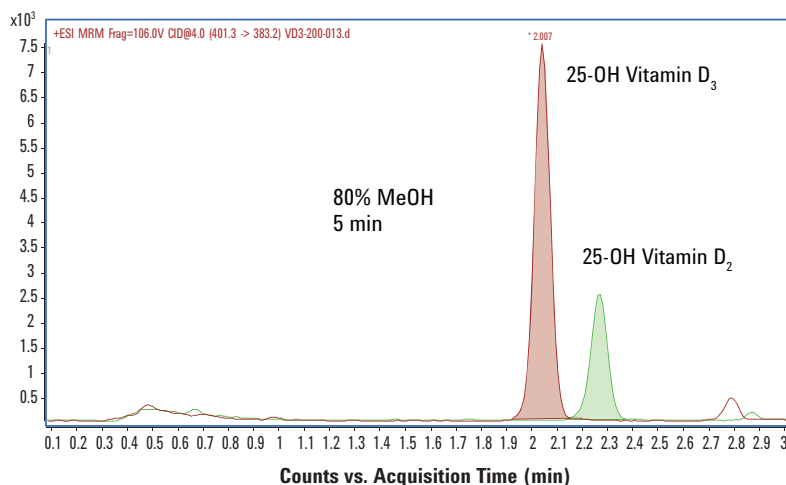
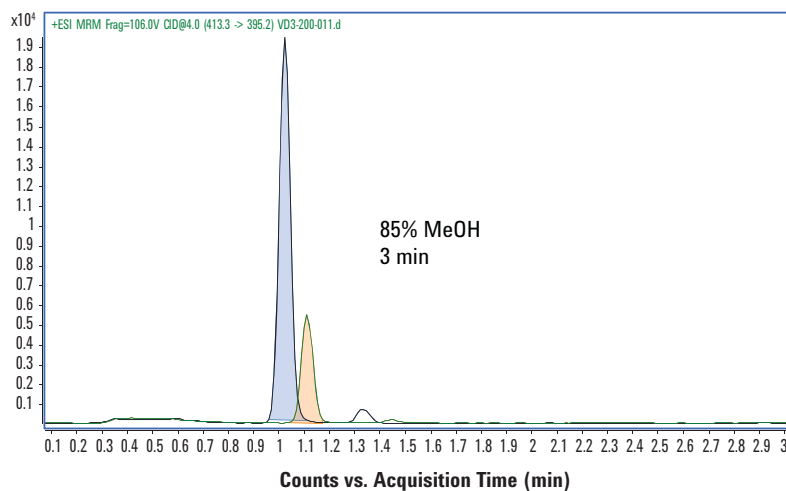
Temperature: 50 °C

Auto sampler temp: 5 °C

Needle wash: Flush port (50:25:25,
IPA: MeOH:H₂O) 5 sec

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

Analysis time: 5 min



Rugged performance – even after 3000 injections

This test confirms the outstanding longevity of Poroshell 120 columns, with little performance degradation after 3000 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	Buprenorphine	0.3
Heroin	0.2	Propoxephine	0.1	Cocaethylene	0.2
Methyl phenidate	0.2	Buprenorphine	0.3	11-nor-9-carboxy-delta9-thc	0

Agilent Poroshell 120 columns help you increase the flexibility of your UHPLC methods

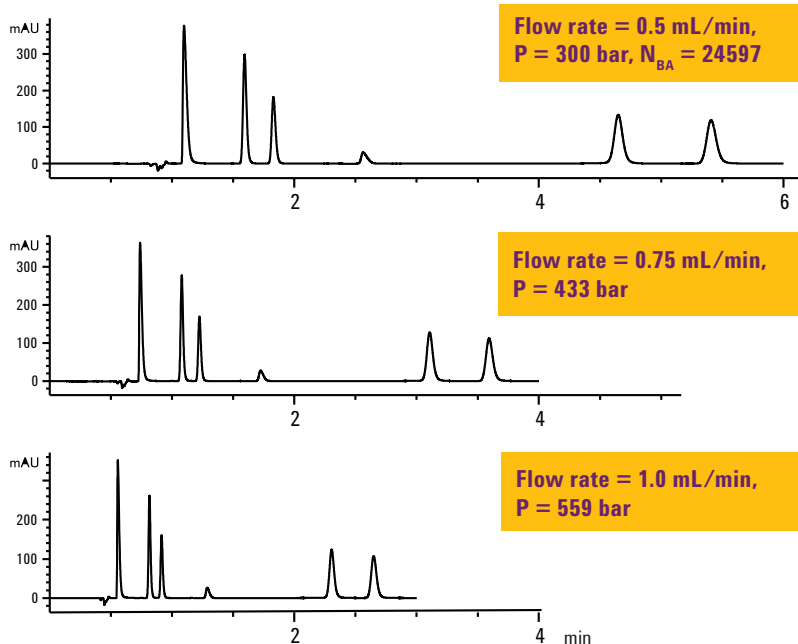


Because Agilent Poroshell 120 columns have a pressure limit of 600 bar, you can successfully apply them to your UHPLC methods – including those that use very long columns, higher flow rates, and viscous solvents.

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 - 560 bar; look to the Agilent 1200 Infinity Series flexible upgrade options to help you take advantage of UHPLC capabilities.



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

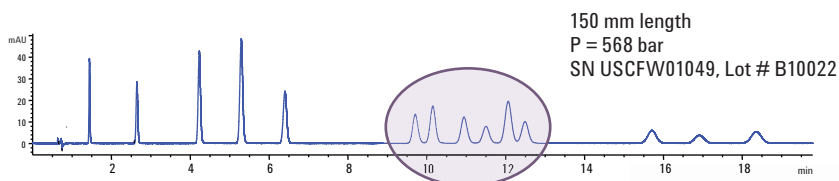
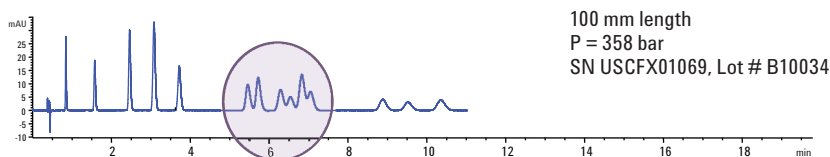
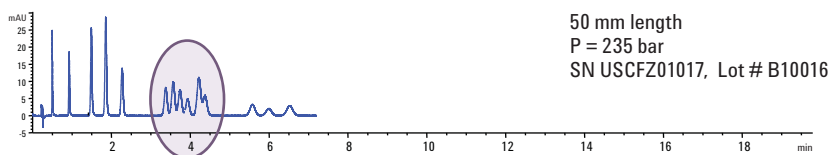
Column:	Agilent Poroshell 120 EC-C18 3.0 x 100 mm, 2.7 μ m P/N 695975-302	Sample:	1. Saccharin 2. Caffeine 3. P-hydroxybenzoic acid 4. Aspartame 5. Dehydroacetic acid 6. Benzoic acid
Mobile phase:	A: 65%, 0.2% Formic acid B: 5% Methanol isocratic		
Flow rate:	Varies		
Injection volume:	1 μ L		
Temperature:	26 °C		
Detection:	Sig = 220, 4 nm, Ref = Off		

To learn more about Agilent Poroshell 120 columns, visit www.agilent.com/chem/poroshell120

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 resolution is impacted by column length – not by the lot 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.

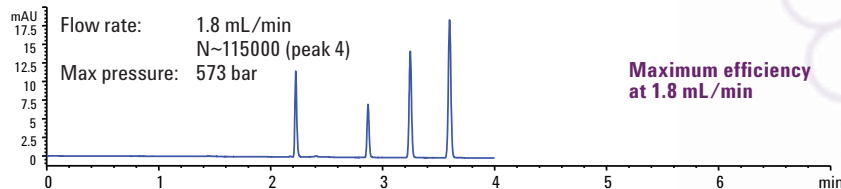
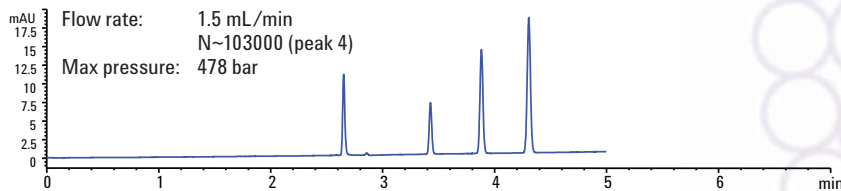
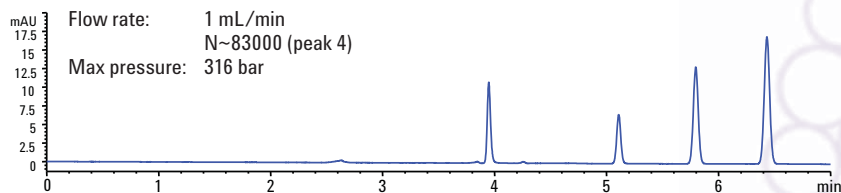
Columns: Poroshell 120 EC-C18, 2.7 μ m
 Mobile phase: 25% Methanol, 75% water
 Flow rate: 1 mL/min
 Temperature: 44 °C

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 #	Compound	Plates	k'
2	Acetophenone	114120	0.29
3	Benzene	109931	0.46
4	Touene	114800	0.65

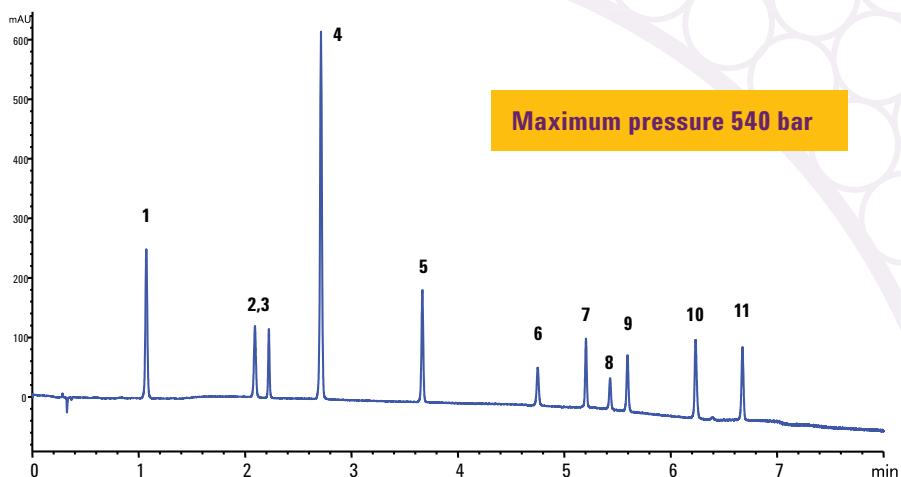
Three Agilent Poroshell 120 EC-C18, 4.6 x 150 mm, 2.7 μ m columns in series for very high efficiency – P/N 693975-902



Maximum efficiency at 1.8 mL/min

Fast analysis on an Agilent Poroshell 120 EC-C18 of 11 common 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 μ m
P/N 695975-902

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

Flow rate: 3.5 mL/min

Injection volume: 5 μ L

Temperature: 40 $^{\circ}$ C

Detection: DAD, 254 nm

Sample:

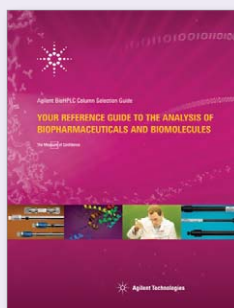
1. Acetaminophen
2. Caffeine
3. 2-Acetamidophenol
4. Acetamide
5. Phenacetin
6. Sulindac
7. Piroxicam
8. Tolmetin
9. Ketoprofen
10. Diflusal
11. Diclofenac

These information-packed selection guides are great resources for your lab



Agilent HPLC Column Selection Guide has been updated and expanded to provide overviews and ordering details for all the small molecule columns in the Agilent columns family. It also includes tips for Fast LC/UHPLC and method development guidance.

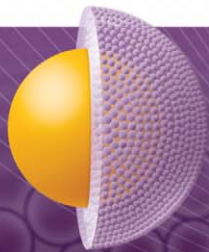
To download now, go to www.agilent.com/chem/getLCGuide



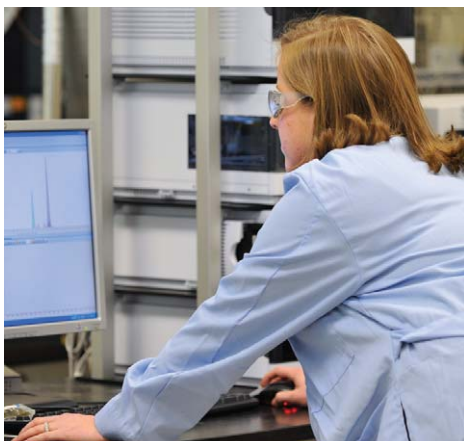
Agilent BioHPLC Column Selection Guide includes advice and tips on method development, solvent choice, mobile phase modification, and optimization – plus abundant examples of biomolecular separations.

Download yours at www.agilent.com/chem/getLCGuide

To learn more about Agilent Poroshell 120 columns, visit www.agilent.com/chem/poroshell120



Complex method transfers, made simple



Many methods developed on longer 5 μm C18 columns can be moved to shorter Poroshell 120 columns quickly and easily. 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.

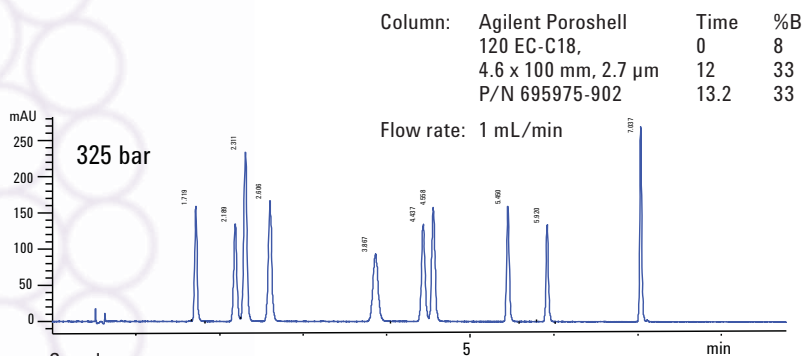
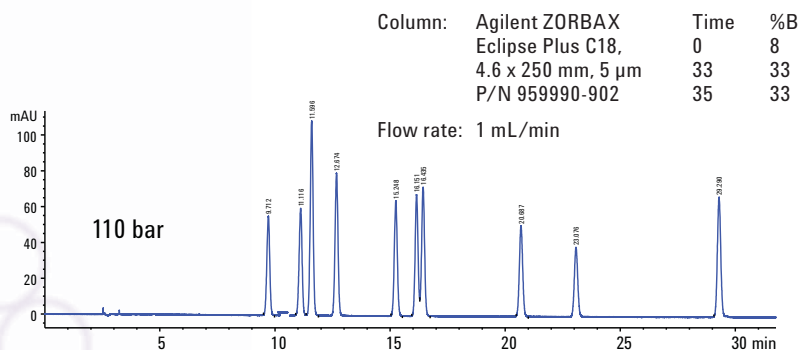
On the following pages, we will show you how five separations, including USP methods, can be repeated on Poroshell 120 columns – and can be completed 3-5 times faster than the same separations on 5 μm columns.

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 μm column to a 100 mm long 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 incredible 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.



Sample:

1. Sulfadiazine
2. Sulfathiazole
3. Sulfapyridine
4. Sulfamerazine
5. Sulfamethazine

6. Sulfamethazole
7. Sulfamethoxy-pyridazine
8. Sulfachloropyridazine
9. Sulfamethoxazole
10. Sulfadimethoxine

Mobile Phase:

- A: 0.1% Formic acid in water
B: 0.1% Formic acid in acetonitrile

USP method for Naproxen tablets – 4.5x faster analysis on Agilent Poroshell 120 at HPLC pressures

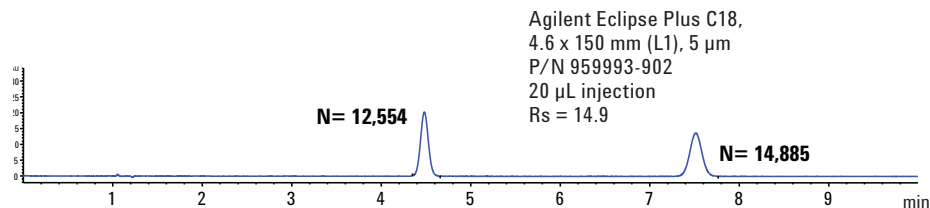
This Naproxen separation demonstrates how easy it can be to convert a method to Poroshell 120 columns *without changing the flow rate or mobile phase*.

The top chromatogram shows a USP analysis on an Agilent Eclipse Plus C18 column, which delivers sharp peaks, three times the needed efficiency, and a resolution of 15.

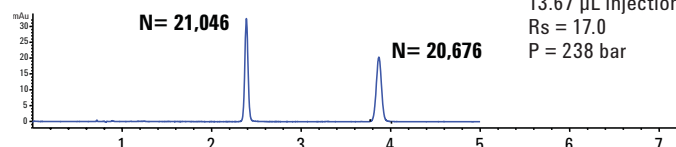
In the middle chromatogram, the Poroshell 120 EC-C18 column (100 mm length) provides greater efficiency and resolution at *twice* the speed of the original method. And because the pressure is only 238 bar, this isocratic method is an excellent HPLC option.

The Poroshell 120 EC-C18 column (50 mm length) on the bottom chromatogram still meets the requirements for efficiency and resolution, but is 4.5 times faster than the 5 µm column. Furthermore, the pressure is only 133 bar, which is very HPLC compatible.

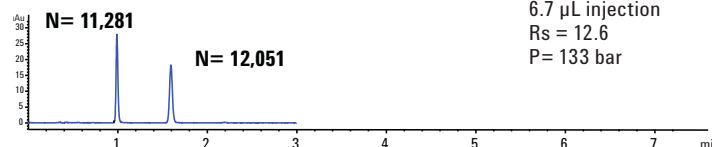
Method Requirement N > 4000, Rs better than 11.5



2x Faster



4.5x Faster



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

Mobile phase: 50:49:1 MeCN:H₂O:acetic acid
Flow rate: 1.2 mL/min

Sample:
1. Naproxen
2. Butyrophenone

Watch our video demonstration 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

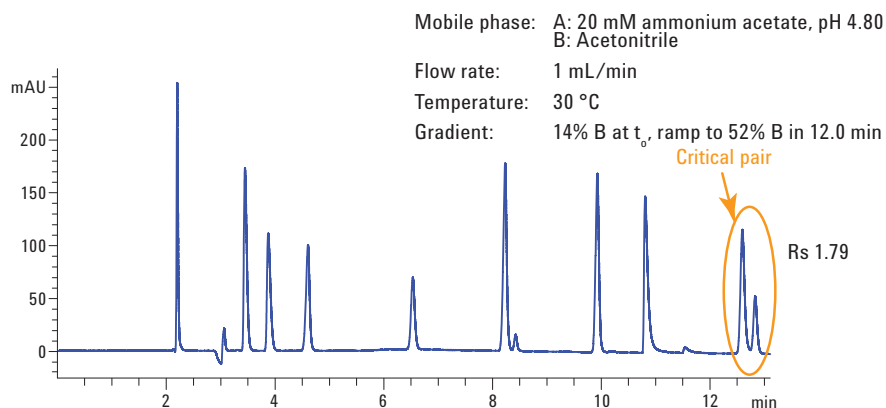


To learn more about Agilent Poroshell 120 columns, visit
www.agilent.com/chem/poroshell120

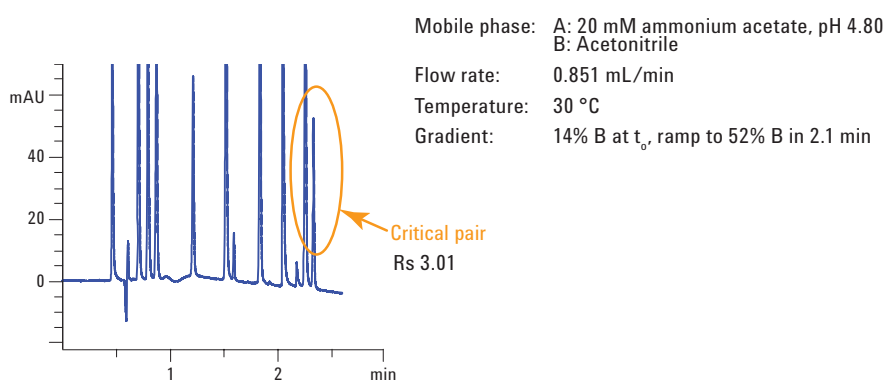
Fast low pressure analysis

Here, a method for analyzing 11 non-nutritive food and beverage additives was transferred from a 5 μm ZORBAX Eclipse Plus C18 column to an Agilent 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.

Agilent ZORBAX Eclipse Plus, 5 μm , $P_{\text{max}} = 120$ bar



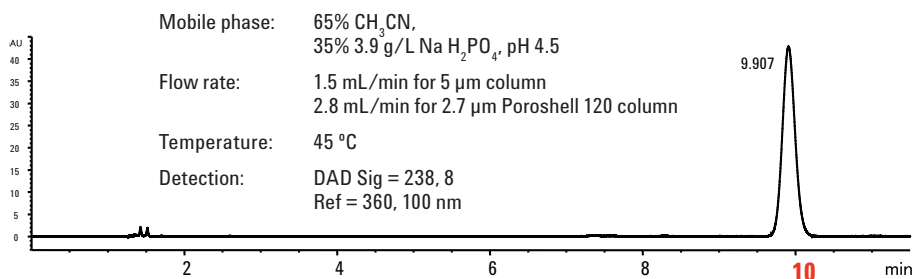
Agilent Poroshell 120, $P_{\text{max}} = 356$ bar



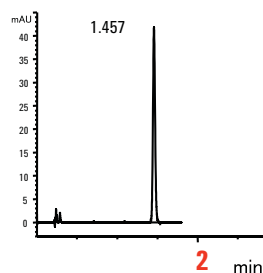
Faster analysis of simvastatin on Poroshell 120

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 75 mm Poroshell 120 EC-C18 column to be substituted for a 250 mm long column *while still being considered a method adjustment*. The Poroshell 120 EC-C18 phase is similar to other USP L1 phases, so the results are similar, but faster.

Agilent ZORBAX Eclipse Plus C18, 4.6 x 250 mm, 5 μm – P/N 959990-902



Agilent Poroshell 120 EC-C18, 4.6 x 75 mm, 2.7 μm – P/N 697975-902



	USP Requirement	5 μm (1.5 mL/min)	2.7 μm (2.8 mL/min)
T_R	n/a	9.907	1.457
k'	> 3.0	5.962	5.122
N	> 4500	16939	14439
T_f	< 2.0	1.09	1.10

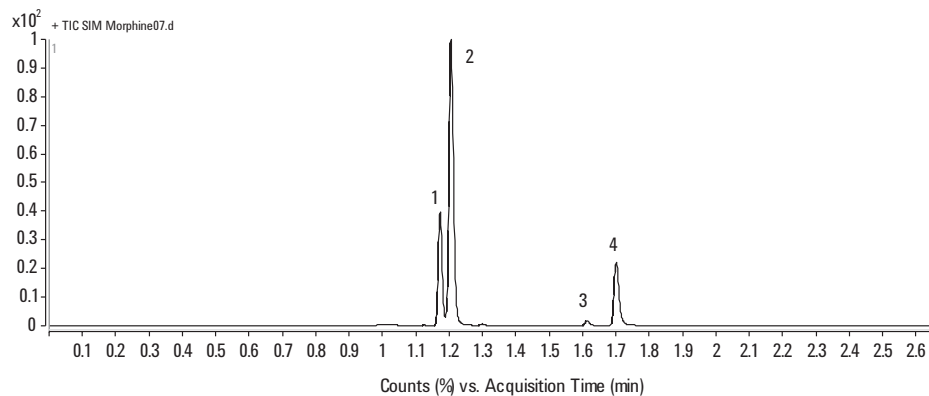
Separation of morphine and metabolites using a Poroshell 120 HILIC column

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, where as a reversed-phase method with high aqueous would have limited retention.

Agilent Poroshell 120 HILIC, 2.1 x 100 mm, 2.7 μ m – P/N 695775-901

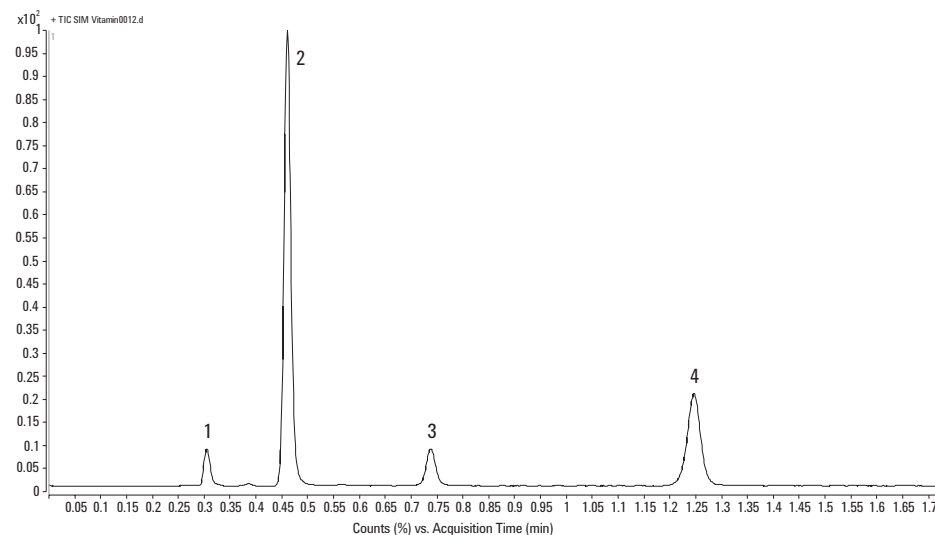


1. Normorphine	Mobile phase: A: 100 mM NH ₄ HCO ₂ , pH 3.2	Time	%B
2. Morphine	B: Acetonitrile 100 mM NH ₄ HCO ₂ , pH 3.2 (9:1)	0	100
3. M6G	Flow rate: 0.8 mL/min	0.44	100
4. M3G	Temperature: 25 °C	1.93	55
	Pressure: 270 to 505 bar		
	System: Agilent 1290 and 6140 QQQ		

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

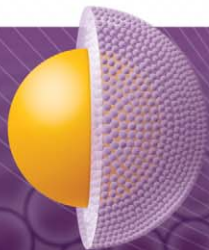
HILIC eliminates the need for ion-pair reagents, such as the hexane sulfonic acid that is typically used in mobile phases for separating B vitamins. It also increases LC/MS compatibility and retention.

Agilent Poroshell 120 HILIC, 2.1 x 100 mm, 2.7 μ m – P/N 695775-901



1. 4 Aminobenzoic acid	Mobile phase: Acetonitrile 100 mM NH ₄ HCO ₂ , pH 3.2 (9:1)
2. Nicotinamide	Flow rate: 0.7 mL/min
3. Riboflavin	Temperature: 25 °C
4. Nicotinic acid	Pressure: 240 bar
	System: Agilent 1290 and 6140 QQQ

To learn more about Agilent Poroshell 120 columns, visit www.agilent.com/chem/poroshell120



Agilent Poroshell 120 columns: **Powerful everyday problem solvers**

A 2 μm column inlet frit stands up to your dirtiest samples

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

Sample loading of basic compounds on Poroshell 120 columns is comparable to sub-2 μm columns

Small, non-porous particles have low surface area available for sample interaction, and are limited in their sample loading capability. Poroshell 120 columns, however, are designed with a larger surface area for greater sample loading capacity. In fact, the loading capacity of Poroshell 120 columns is comparable to 1.8 μm 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 6-7 – when compared to other superficially porous columns.

Agilent 1100 and 1200 Series LC systems can easily be optimized for Poroshell 120 columns

The inherent properties of Poroshell 120 columns make them ideal for most HPLC and UHPLC instruments, including the new 1200 Infinity series LCs. For 1100 and 1200 series LC systems, all that is needed are minor configuration changes (such as flow rate, connector tubing length and id, flow-cell volume, and detector peak-width setting) in order 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 2500 injections.

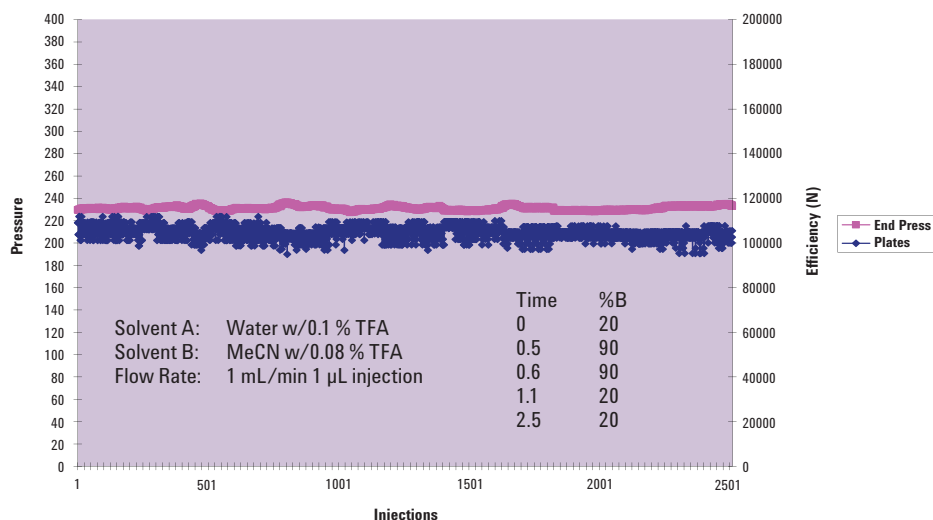
Column: Agilent Poroshell 120 EC-C18,
3.0 x 50 mm, 2.7 µm
P/N 699975-302

Injection volume: 1 µL injections

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

Instrument: Agilent 1200 RRLC (SL)

Diflusalin in plasma

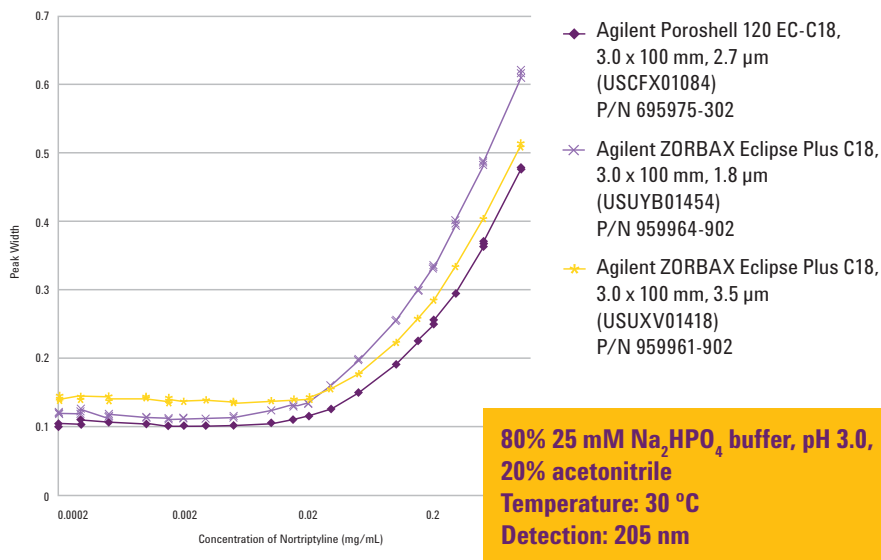


Achieve comparable sample loading to totally porous particles

In this example, we loaded nortriptyline (a basic compound) onto several Agilent and competitive 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 which can compromise resolution.

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

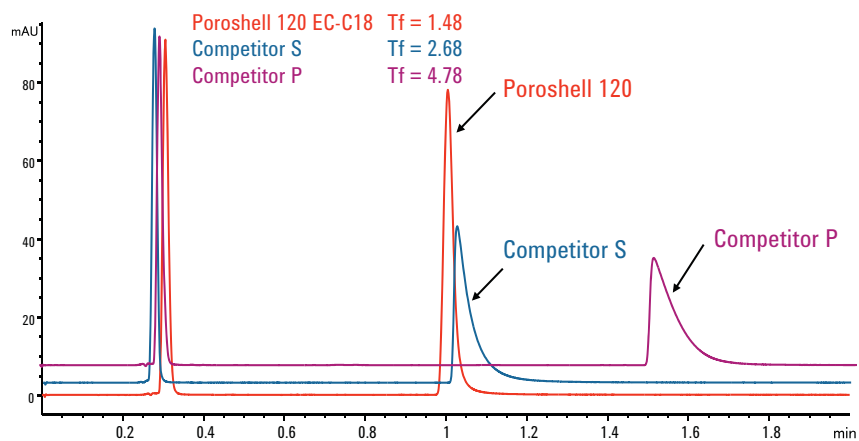
Base loading with nortriptyline



To learn more about Agilent Poroshell 120 columns, visit www.agilent.com/chem/poroshell120

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 competition for challenging analytes.



Columns: 4.6 x 50 mm
P/N 699975-902

Mobile phase: 20 mM 40% Na₂HPO₄, pH 7.00, 60% acetonitrile

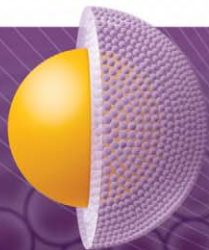
Flow rate: 1.5 mL/min

Temperature: 24 °C

Detector: DAD, 254 nm, 2 µL flow cell

Sample: 2 µL injection of 250 µg/mL amitriptyline, 50 µg/mL uracil in H₂O/CH₃CN (9:1)





Fast, confident separations of proteins and peptides



Agilent Poroshell columns are an excellent choice for separating and characterizing complex bio-molecules, including both *intact* and *digested* proteins. Agilent Poroshell 300 columns are the best choice for fast analysis of intact proteins. Agilent Poroshell 120 columns are well suited for peptide mapping, because they provide high resolution with much shorter analysis times than traditional 5 μm columns. For even greater peptide mapping suitability, check out **AdvanceBio Peptide Mapping columns**, which come pre-tested with a challenging peptide mix to ensure optimal performance for your peptide mapping application.

High flow rates with 2.1 mm id Poroshell 300 for high resolution and fast separations of proteins

Poroshell 300 columns, with their larger pore size and thin shell, are a reliable choice for fast separations of intact proteins. The separation shown here was completed in less than one minute.

With their rapid mass transfer of the superficially porous particle, Poroshell 300 columns are the best columns for high efficiency at higher flow rates for extremely rapid separations of proteins.

Columns: Poroshell 300SB-C18
2.1 x 75 mm, 5 μm
P/N 660750-902

Mobile phase: A: 0.1% TFA
B: 0.07% TFA in ACN

Flow rate: 3.0 mL/min

Temperature: 70 °C

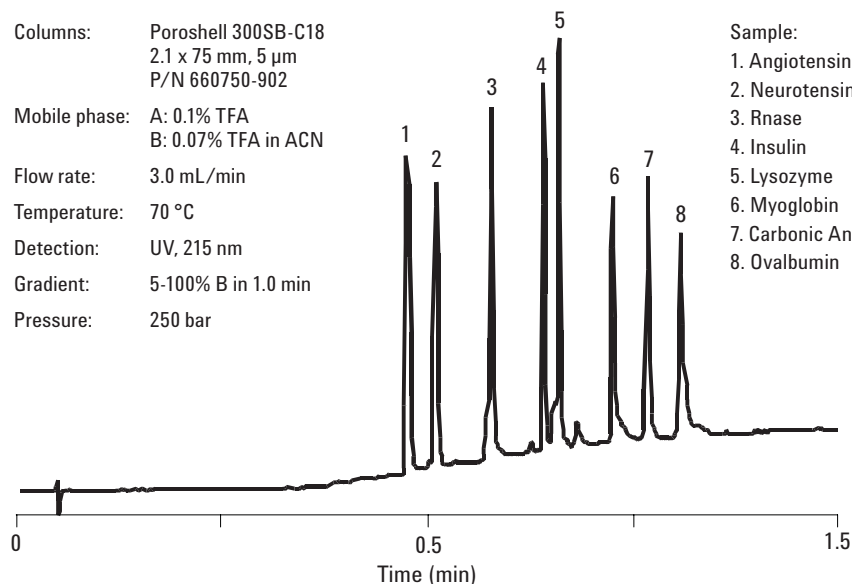
Detection: UV, 215 nm

Gradient: 5-100% B in 1.0 min

Pressure: 250 bar

Sample:

1. Angiotensin II
2. Neurotensin
3. Rnase
4. Insulin
5. Lysozyme
6. Myoglobin
7. Carbonic Anhydrase
8. Ovalbumin



Agilent has one of the broadest families of biocolumns available, including AdvanceBio columns to help you advance accuracy and productivity for bio-separations. Learn more at 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.

QTOF Instrument Parameters

Source – ESI positive

Gas temperature: 325 °C

Drying gas: 10 L/min

Nebulizer: 40 psi

Vcap: 4000 V

Fragmentor: 150 V

Skimmer: 65 V

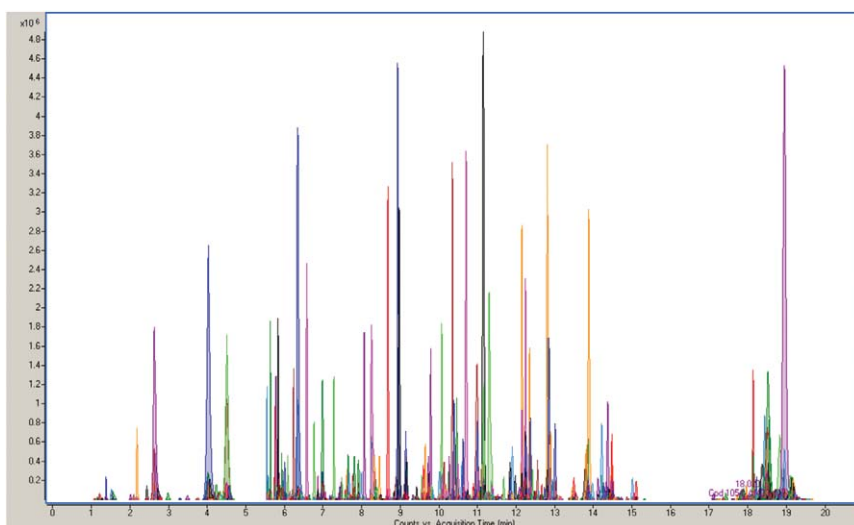
Octapole 1 RF: 750 V

MS: 4 Hz

Mass range: 200-3200 m/z

Reference mass: 922.009798

Acq. mode: Extended dynamic range mode (2 GHz)



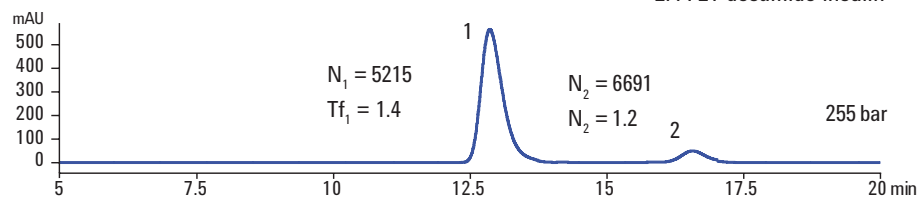
Column:	Poroshell 120 EC-C18, 3.0 x 150 mm, 2.7 µm P/N 683975-302	Detection:	QTOF, ESI positive
		Gradient:	Shown in table
Mobile phase:	A: Water, 0.1% formic acid B: ACN, 0.1% formic acid	Time	%B
		0	2
		3	2
Flow rate:	0.3 mL/min	13	45
Temperature:	40 °C	15	65
		Time	%B
		15.1	90
		17	90
		18	2

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

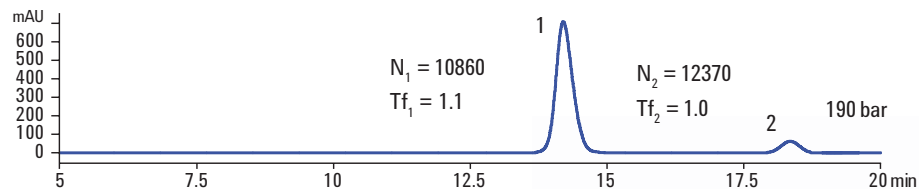
Poroshell 120 SB-C18 column provided double the efficiency of the ZORBAX RRHD SB-C18 80Å due to the larger pore size and more rapid diffusion in the 120Å pores. The Poroshell 120 columns are ideal for the small protein insulin or other peptides, providing higher efficiency at lower pressure.

Agilent ZORBAX SB-C18, 4.6 x 100 mm, 1.8 µm

1. Porcine insulin
2. A-21 desamido insulin

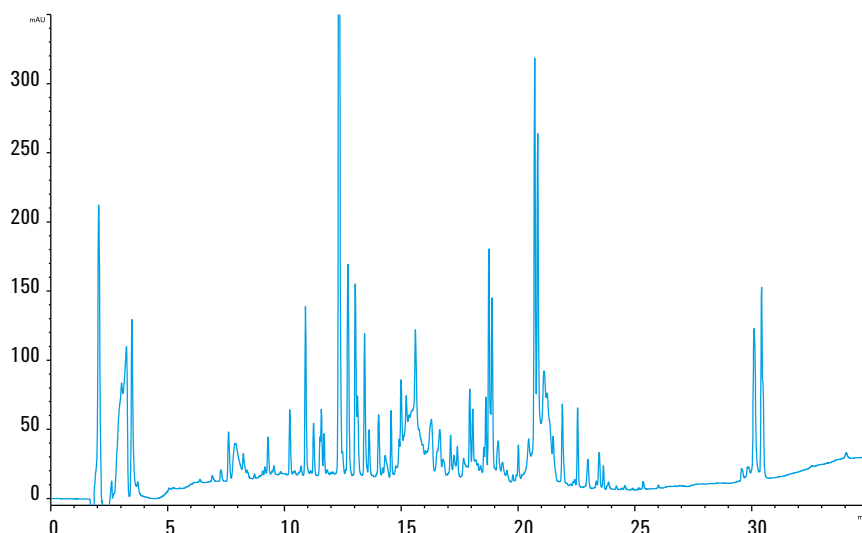


Agilent Poroshell 120 SB-C18, 4.6 x 100 mm, 2.7 µm – P/N 685975-902

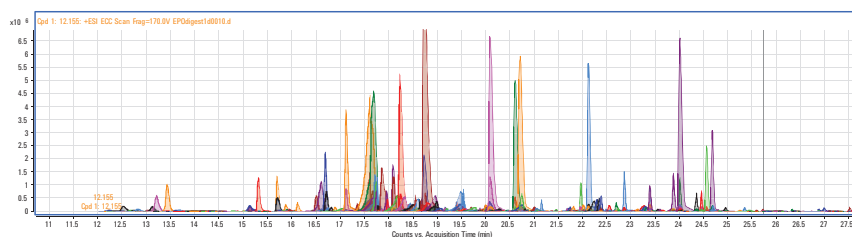


Peptide Map of a Biosimilar EPO

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 post-translational modifications, using the AdvanceBio Peptide Mapping column.



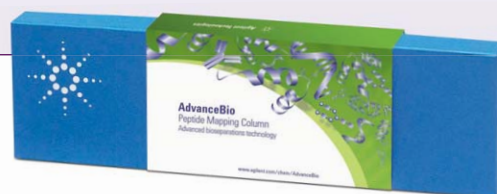
Column:	AdvanceBio Peptide Mapping, 2.1 x 250 mm, 2.7 μ m, p/n 651750-902	Detection:	220 nm
Flow Rate:	0.4 mL/min	Gradient:	A, water (0.1% FA); B, ACN (0.1% FA); 0-28 min, 3-45% B; 28-33 min, 45-60% B; 33-34 min, 60-95% B
Injection:	5 μ L (2.0 mg/mL)		
Temp:	55 $^{\circ}$ C		



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
or request Publication No. 5991-1696EN.



To learn more about Agilent Poroshell 120 columns, visit
www.agilent.com/chem/poroshell120

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 μm (stable to 1200 bar) and ZORBAX Rapid Resolution High Throughput (RRHT), 1.8 μm (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 2. ZORBAX RRHD 1.8 μm	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 1200s, Agilent 1100s (400 bar) as well as the 1220 Infinity LC or 1260 Infinity LC (600 bar)	1. Poroshell 120 2. ZORBAX Eclipse Plus 3.5 μm and 5 μm	With Poroshell 120, 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 can't transfer, the ZORBAX Eclipse Plus column will provide 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 LC)	1. ZORBAX RRHD 1.8 μm 2. Poroshell 120	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.

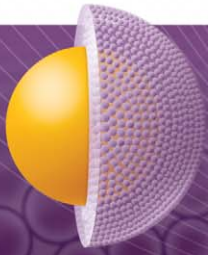
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Specifications and ordering information

Agilent Poroshell 120 ordering information (2.7 µm)

Size (mm)	EC-C18	EC-C8	Phenyl-Hexyl	SB-C18	SB-C8	SB-Aq	Bonus-RP	HILIC	EC-CN
4.6 x 150	693975-902	693975-906	693975-912	683975-902	683975-906	683975-914	693968-901	693975-901	693975-905
4.6 x 100	695975-902	695975-906	695975-912	685975-902	685975-906	685975-914	695968-901	695975-901	695975-905
4.6 x 75	697975-902	697975-906		687975-902					
4.6 x 50	699975-902	699975-906	699975-912	689975-902	689975-906	689975-914	699968-901	699975-901	699975-905
4.6 x 30	691975-902	691975-906		681975-902					
3.0 x 150	693975-302	693975-306	693975-312	683975-302	683975-306	683975-314	693968-301	693975-301	693975-305
3.0 x 100	695975-302	695975-306	695975-312	685975-302	685975-306	685975-314	695968-301	695975-301	695975-305
3.0 x 75	697975-302	697975-306		687975-302					
3.0 x 50	699975-302	699975-306	699975-312	689975-302	689975-306	689975-314	699968-301	699975-301	699975-305
3.0 x 30	691975-302	691975-306		681975-302					
2.1 x 150	693775-902	693775-906	693775-912	683775-902	683775-906	683775-914	693768-901	693775-901	693775-905
2.1 x 100	695775-902	695775-906	695775-912	685775-902	685775-906	685775-914	695768-901	695775-901	695775-905
2.1 x 75	697775-902	697775-906		687775-902					
2.1 x 50	699775-902	699775-906	699775-912	689775-902	689775-906	689775-914	699768-901	699775-901	699775-905
2.1 x 30	691775-902	691775-906		681775-902					

Note: Poroshell 120 columns have a 600 bar/9000 psi pressure limit.

Agilent Poroshell 120 Fast Guards for UHPLC

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



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 ² /g
EC-C8	120Å	60 °C	2.0-8.0	Double	5%	130 m ² /g
Phenyl-Hexyl	120Å	60 °C	2.0-8.0	Double	9%	130 m ² /g
SB-C18	120Å	90 °C	1.0-8.0	No	8%	130 m ² /g
SB-C8	120Å	80 °C	1.0-8.0	No	5.5%	130 m ² /g
SB-Aq	120Å	80 °C	1.0-8.0	No	Proprietary	130 m ² /g
Bonus-RP	120Å	60 °C	2.0-9.0	Triple	9.5%	130 m ² /g
HILIC	120Å	60 °C	0.0-8.0	No	N/A	130 m ² /g
EC-CN	120Å	60 °C	2.0-8.0	Double	3.5%	130 m ² /g

Specifications represent typical values only.

Agilent Poroshell 300 ordering information (5 µm)

Description	Size (mm)	Agilent Poroshell 300SB-C18	Agilent Poroshell 300SB-C8	Agilent Poroshell 300SB-C3	Agilent Poroshell 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/6000 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 only.

Agilent AdvanceBio Peptide Mapping columns ordering information

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.



To learn more about Agilent Poroshell 120 columns, visit
www.agilent.com/chem/poroshell120



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Agilent Poroshell 120 is part of a continuing tradition of performance, expertise and value for small molecule and biomolecule chromatography. We offer alternate selectivities and choices for fast LC separations, including 1200 bar ZORBAX RRHD columns, 1.8 μm , as well as a range of columns for characterization of biomolecules using size exclusion and ion-exchange.

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