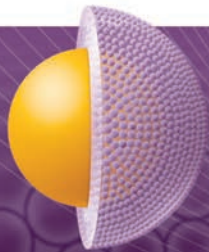Agilent Poroshell 120 Columns for HPLC and UHPLC

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

Here is what you'll find in this brochure

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.

1.8 µm totally porous   **Agilent Poroshell 120 2.7 µm**

0.5 µm

1.7 µm
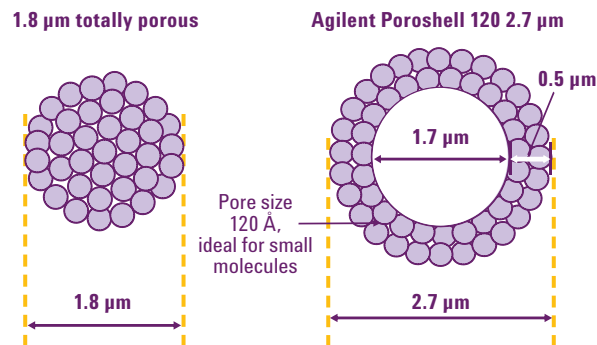
Pore size 120 Å, ideal for small molecules

1.8 µm   **2.7 µm**

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)
   PN 695975-302

✳ Agilent ZORBAX Eclipse Plus C18,
   3.0 x 100 mm, 1.8 µm, (USUYB01455)
   PN 959964-302

✳ Agilent ZORBAX Eclipse Plus C18,
   3.0 x 100 mm, 3.5 µm, (USUXV01435)
   PN 959961-302

μ (mm/s)
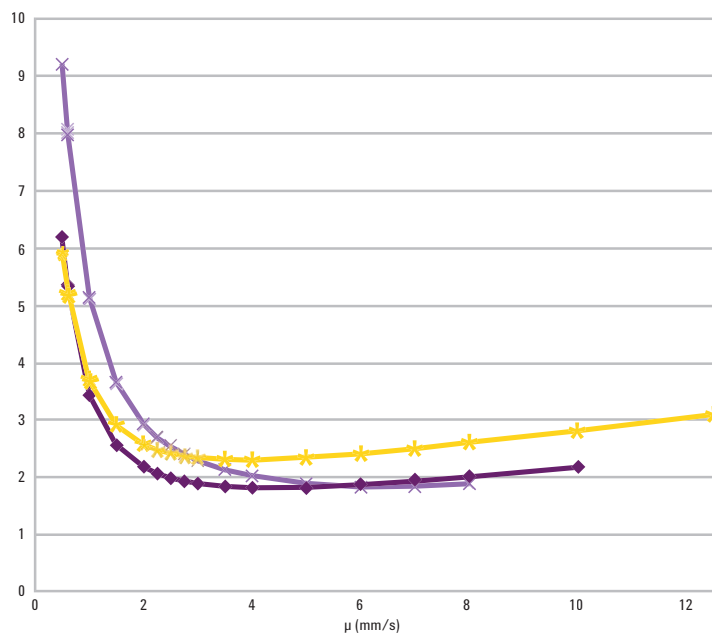
How a Poroshell 120 particle is made

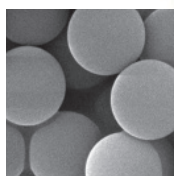In order to create the best column for small molecule separations, we completely reinvented our superficially porous particle technology. Specifically, we minimized the manufacturing steps involved to ensure maximum particle – and chromatographic – reproducibility.
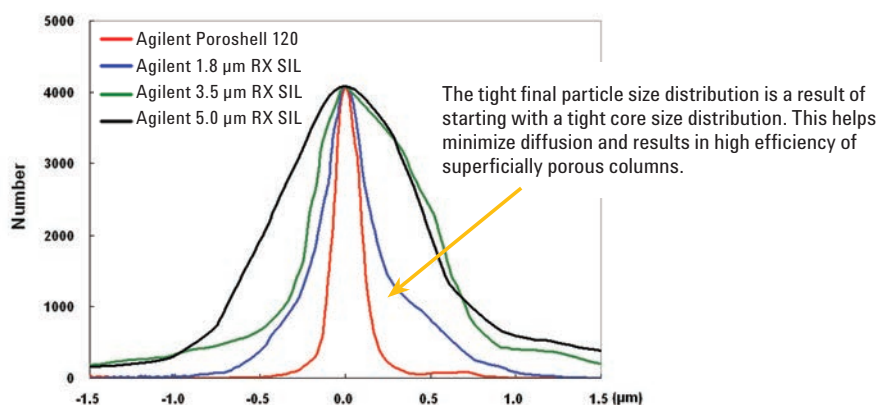
STEP 1   Make the solid core

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 tight final particle size distribution is a result of starting with a tight core size distribution. This helps minimize diffusion and results in high efficiency of superficially porous columns.

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 above, 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
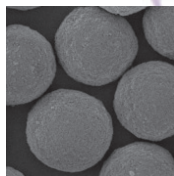
Apply the porous shell

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

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 µm
PN 695975-902 – from 5 Different Lots
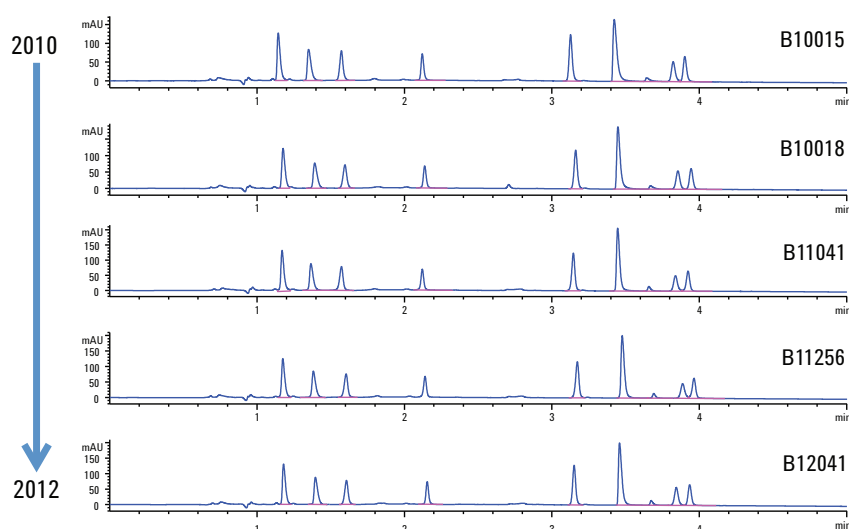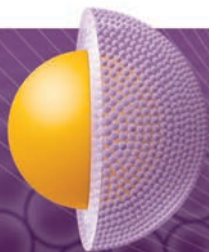
A variety of bonded phases provides
flexible selectivity

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.

- **And more:** Check our website for the growing list of available bonded phases!

*Available summer, 2012

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% H_2O:65% CH_3CN
Flow Rate:	1 mL/min
Temperature:	30 ºC

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:

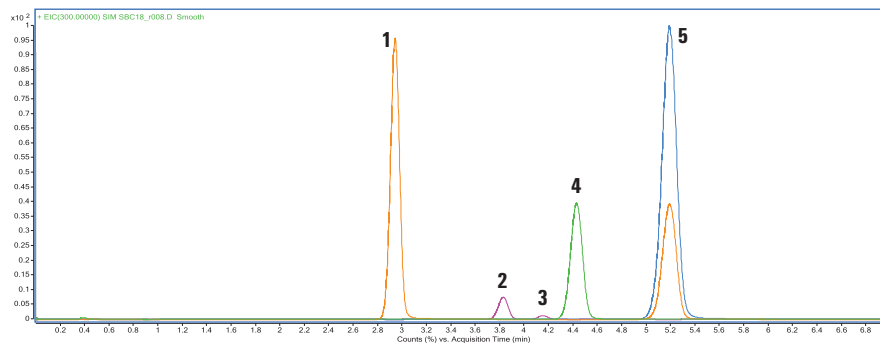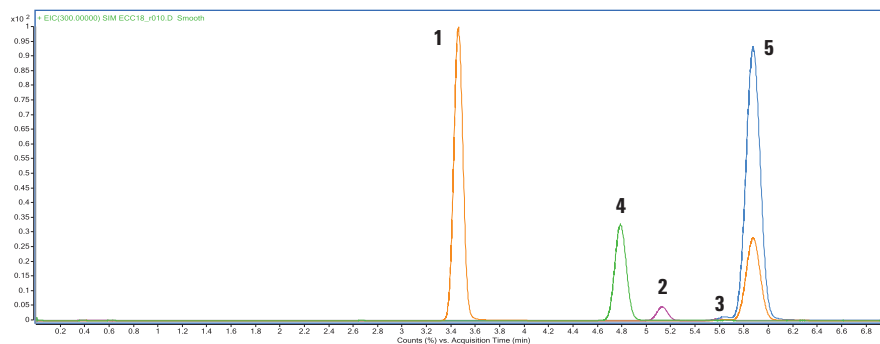| Gas Temp = | 350 ºC |
Gas Flow =	12 L/min
Nebulizer =	40 psi
Capillary =	4000 V

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
PN 685975-302

Agilent Poroshell 120 EC-C18 3.0 x 100 mm, 2.7 µm
PN 695975-302

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

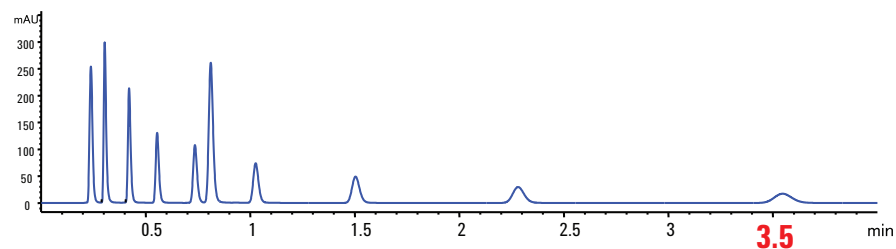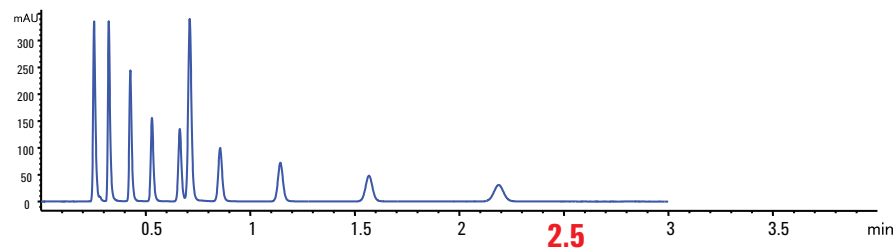| Mobile Phase: | 60% CH_3CN, 40% H_2O |
Flow Rate:	0.85 mL/min
Temperature:	26 ºC
Detection:	254 nm
Sample:	2 µL of RRLC Checkout Sample (PN 5188-6529), alkylphenones

Agilent Poroshell 120 EC-C18, 3 x 50 mm, 2.7 µm
PN 699975-302

Agilent Poroshell 120 EC-C8, 3 x 50 mm, 2.7 µm
PN 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 µm
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:	8 steroids
Gradient:	40-80% MeOH/14 min

Sample:
1. Hydrocortisone
2. B Estradiole
3. Andostadiene 3,17 dione
4. Testosterone
5. Ethyestradione
6. Estrone
7. Norethindone acetate
8. Progestreone
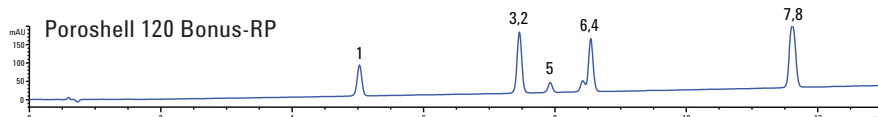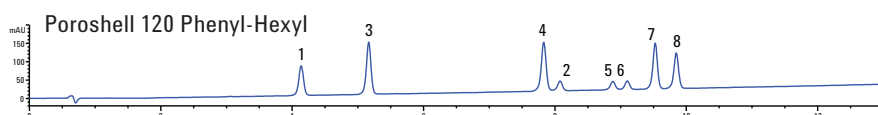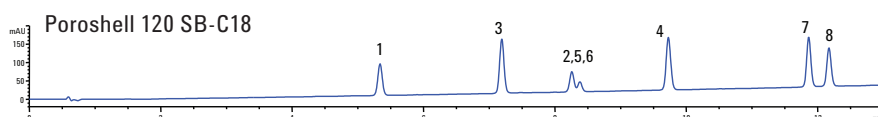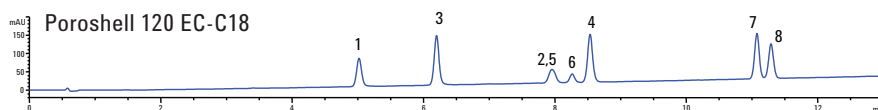
Analysis of Beta Blockers: A comparison of Poroshell 120 phases

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 µm
Mobile Phase:	A: 10 mM NH_4CO_2, pH 3.8 B: MeOH
Flow Rate:	0.4 mL/min
Temperature:	40 °C
Detection:	260 nm
Sample:	Beta Blockers
Gradient:	10% B to 30%B/12 min

Sample:
1. Atenolol
2. Pindolol
3. Naldolol
4. Metoprolol
5. Acebutolol
6. Propranolol
7. Alprenolol
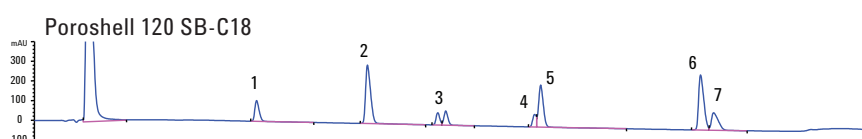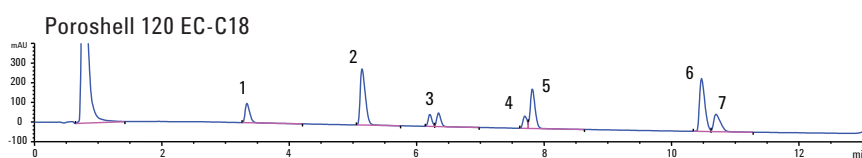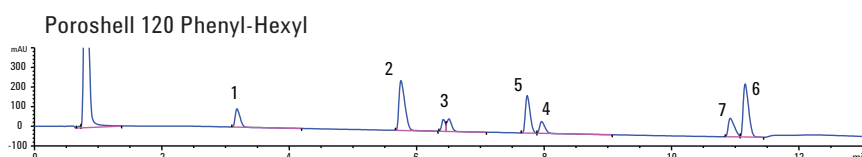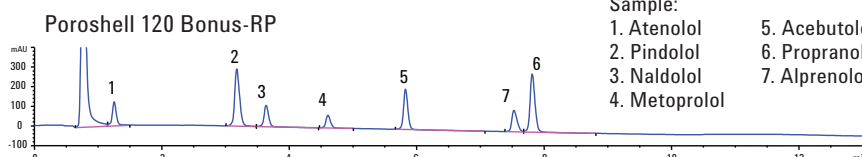
To learn more about Agilent Poroshell 120 columns, visit
www.agilent.com/chem/poroshell120

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.

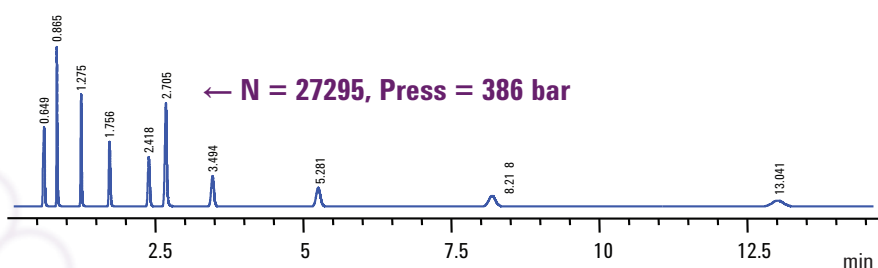|	
Mobile Phase:	60% Acetonitrile: 40% Water
Flow Rate:	0.58 mL/min
Injection Volume:	4 µL
Temperature:	26 ºC
Detection:	DAD Sig = 254,4 nm Ref = 360,100 nm
Sample:	RRLC Checkout Sample (PN 5188-6529) spiked w/ 50 µL 2 mg/mL Thiourea in water/acetonitrile (65:35)

Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm
PN 695975-302

← N = 25053, Press = 182 bar
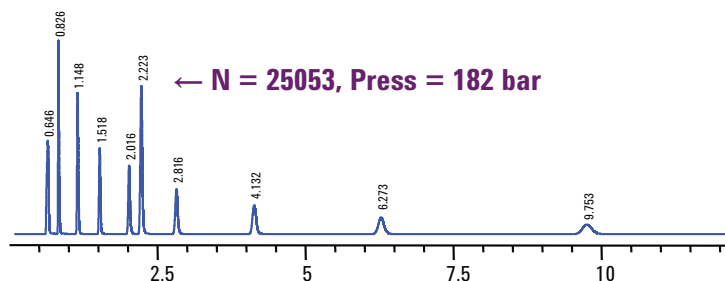
>90% of the efficiency of 1.8 µm

Agilent Eclipse Plus C18, 3.0 x 100 mm, 1.8 µm
PN 959964-302

← N = 27295, Press = 386 bar

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:	65% A: 0.2% Formic Acid: 35% B: Methanol Isocratic
Flow Rate:	0.5 mL/min
Injection Volume:	1 µL
Temperature:	26 °C
Detection:	UV 220 nm

Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm
PN 695975-302

N = 23,283   P = 314 bar
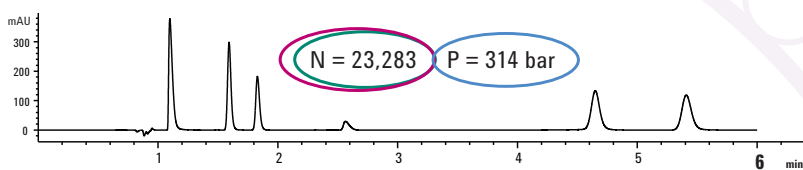
Agilent RRHT Eclipse Plus C18, 3.0 x 100 mm, 1.8 µm
PN 959964-302

N = 25,364   P = 456 bar
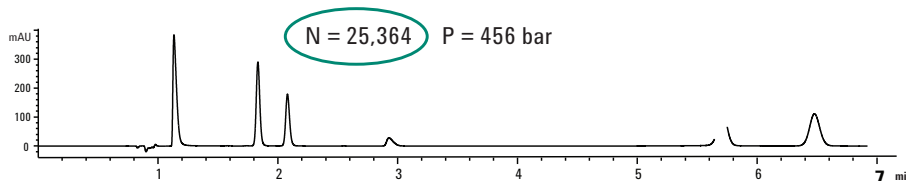
Agilent Rapid Resolution Eclipse Plus C18, 3.0 x 100 mm, 3.5 µm
PN 959961-302
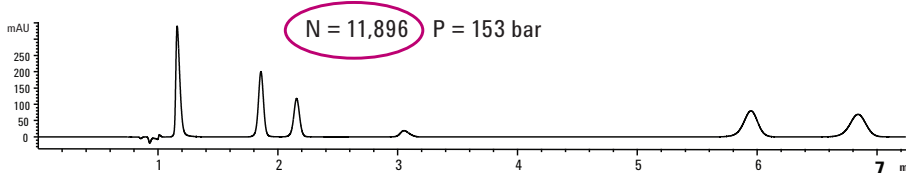
N = 11,896   P = 153 bar

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

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

This HPLC separation of 12 Phenols was performed in just 5 minutes using an Agilent Poroshell 120 EC-C18 column

Importantly, the flow rate was kept to 2.5 mL/min, reducing the amount of mobile phase consumed per analysis to about 15 mL.

Column: Agilent Poroshell 120 EC-C18,
                 4.6 x 50 mm, 2.7 µm
                 PN 699975-902

Mobile Phase: Solvent A: Water
                 with 0.1% Formic Acid
                 Solvent B:wAcetonitrile

Flow Rate: 2.5 mL/min

Gradient: Time   %B
                 0.8   5%
                 6.8   60%
                 1200 SL controlled temperature
                 at 25 ºC 2 mm flow cell

2.5 mL/min, 274 bar

Sample:
1. Hydroquinone
2. Resourcinol
3. Catechol
4. Phenol
5. 4-Nitrophenol
6. p-cresol
7. o-cresol
8. 2-Nitrophenol
9. 3,4 di methyl phenol
10. 2,3 di methyl phenol
11. 2,5 di methyl phenol
12. 1-napthol

Agilent Poroshell 120 gives high efficiency, high resolution separations quickly at HPLC pressures.

Here, the same 12 Phenols were analyzed using a longer (4.6 x 100 mm) Agilent Poroshell 120 EC-C18 column

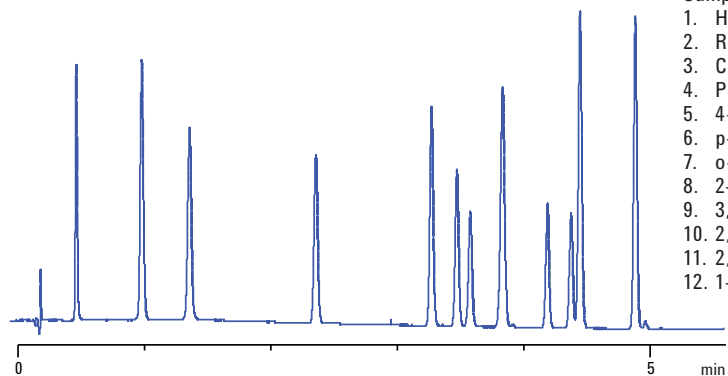By reducing the flow rate to 2.0 mL/min, we kept the pressure less than 400 bar and improved the separation of a late-eluting peak pair (highlighted) with only a minor increase in analysis time. This separation can be achieved using HPLC or, if a higher flow rate is desired, a UHPLC.

Column: Agilent Poroshell 120 EC-C18,
                 4.6 x 100 mm, 2.7 µm
                 PN 695975-902

Mobile Phase: Solvent A: Water
                 with 0.1% Formic Acid
                 Solvent B: Acetonitrile

Flow Rate: 2.0 mL/min

Gradient: Time   %B
                 2.0   5%
                 17   60%
                 1200 RRLC SL controlled
                 temperature at 25 ºC
                 2 mm flow cell

2.0 mL/min, 394 bar
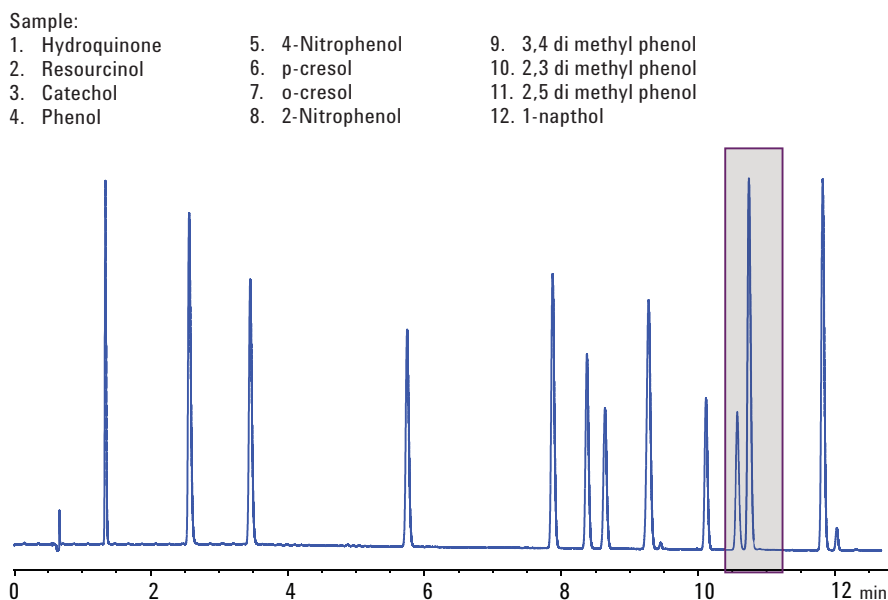
Sample:
1. Hydroquinone
2. Resourcinol
3. Catechol
4. Phenol

5. 4-Nitrophenol
6. p-cresol
7. o-cresol
8. 2-Nitrophenol

9. 3,4 di methyl phenol
10. 2,3 di methyl phenol
11. 2,5 di methyl phenol
12. 1-napthol
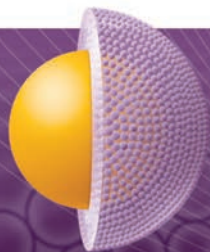
A rugged choice for **high resolution LC/MS and LC/MS/MS**

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
Mobile Phase:	80% ACN/20% Methanol
Flow Rate:	0.6 mL/min
Injection Volume:	2 μL
Temperature:	20 °C
Detection:	APCI, Positive Ion
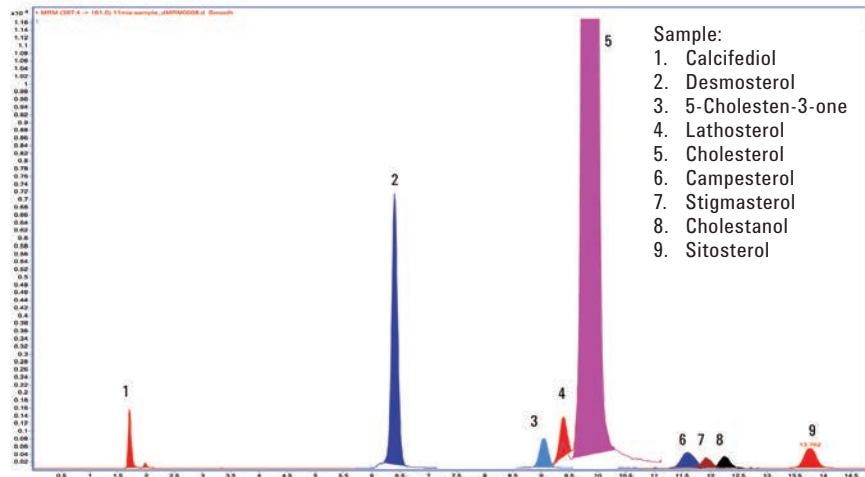
Sample:
1. Calcifediol
2. Desmosterol
3. 5-Cholesten-3-one
4. Lathosterol
5. Cholesterol
6. Campesterol
7. Stigmasterol
8. Cholestanol
9. Sitosterol

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.
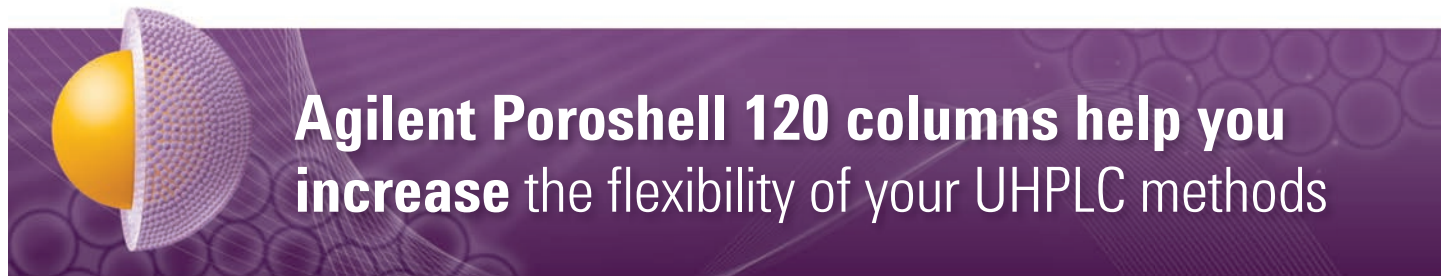
Column:	Poroshell 120 EC-C18, 2.1 x 50 mm, 2.7 µm
Mobile phase:	A: H_2O + 0.1% Formic Acid
	B: MeOH + 0.1% Formic Acid
Flow rate:	0.5 mL/min
Injection volume:	10 µL
Temperature:	50 °C
Auto sampler temp:	5 °C
Needle wash:	flush port (50:25:25, IPA: MeOH:H_2O) 5 seconds
Isocratic Analysis:	A: 20% B: 80%
Analysis Time:	5.0 min

+ESI MRM Frag=106.0V CID@4.0 (413.3 -> 395.2) VD3-200-011.d

85% MeOH
3 min

Counts vs. Acquisition Time (min)

+ESI MRM Frag=106.0V CID@4.0 (401.3 -> 383.2) VD3-200-013.d

* 2.007

25-OH Vitamin D₃

80% MeOH
5 min

25-OH Vitamin D₂

Counts vs. Acquisition Time (min)
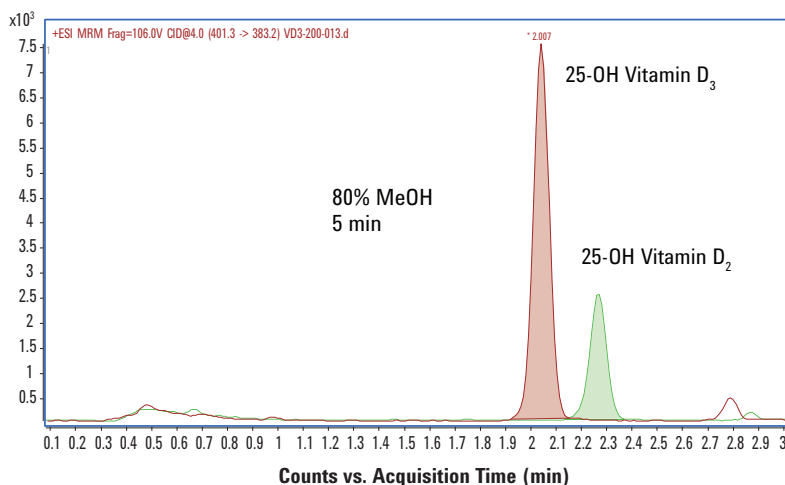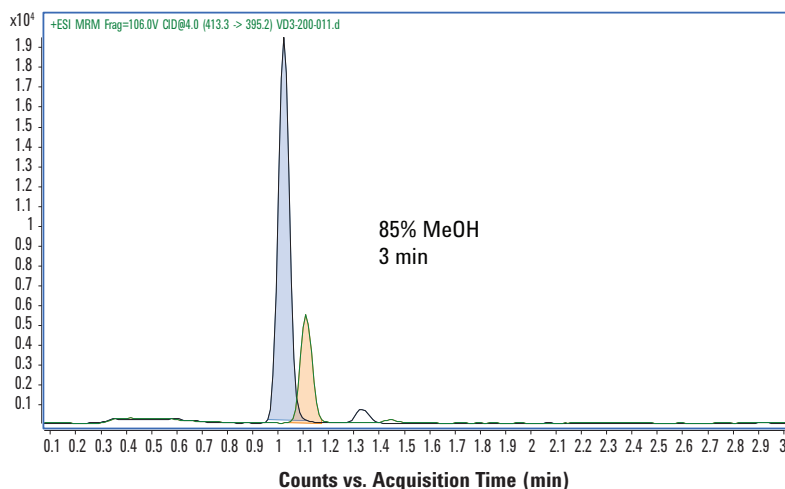
Rugged performance – even after 3000 injections

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.
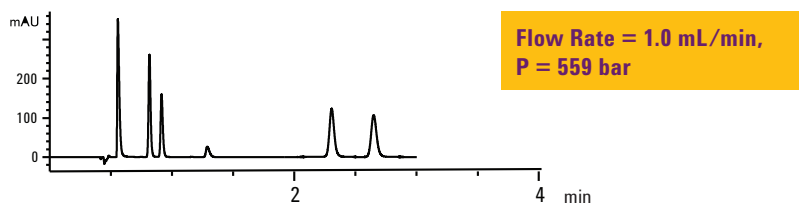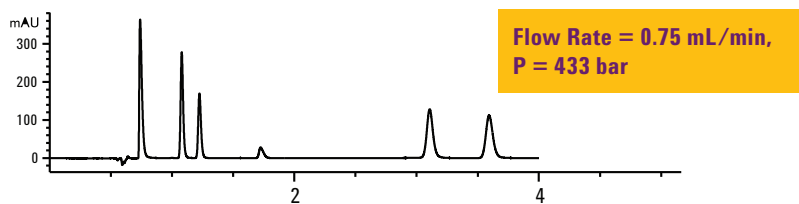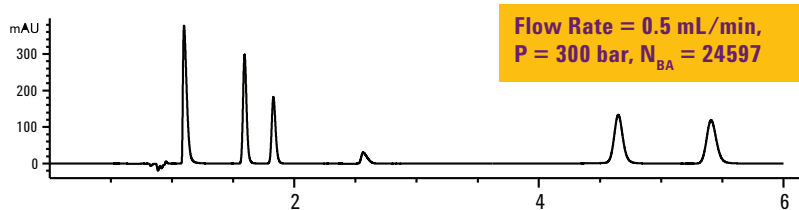
Agilent Poroshell 120 EC-C18 for fast *UHPLC* separations

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.

Flow Rate = 0.5 mL/min, P = 300 bar, N_{BA} = 24597

Flow Rate = 0.75 mL/min, P = 433 bar

Flow Rate = 1.0 mL/min, P = 559 bar

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 µm PN 695975-302	Sample: 1. Saccharin 2. Caffeine 3. P-hydroxybenzoic acid 4. Aspartame 5. Dehydroacetic acid 6. Benzoic acid
Mobile Phase:	65% A: 0.2% Formic Acid: 35% B: Methanol Isocratic	
Flow Rate:	Varies	
Injection Volume:	1 µL	
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.

50 mm length
P = 235 Bar
SN USCFZ01017, Lot # B10016

100 mm length
P = 358 Bar
SN USCFX01069, Lot # B10034

150 mm length
P = 568 Bar
SN USCFW01049, Lot # B10022
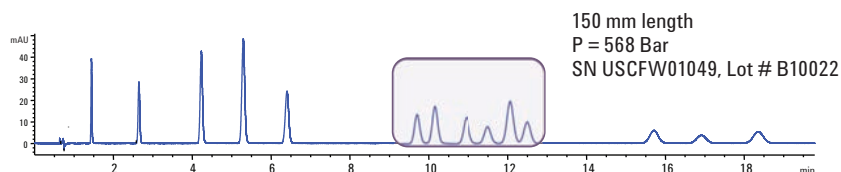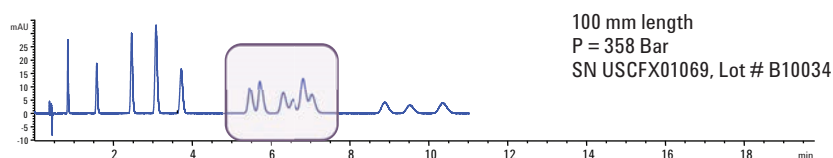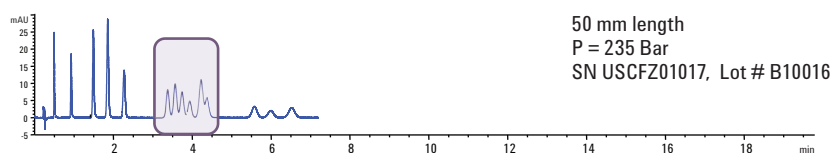
Balancing column length, resolution and analysis time are important for any separation.

Columns:	Poroshell 120 EC-C18, 2.7 µm
Mobile Phase:	25% Methanol: 75% Water
Flow Rate:	1 mL/min
Temperature:	44 ºC

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.

Three Agilent Poroshell 120 EC-C18 , 4.6 x 150 mm, 2.7 µm columns in series for very high efficiency – PN 693975-902

Flow Rate: 1 mL/min
N~83000 (peak 4)
Max Pressure: 316 bar

Flow Rate: 1.5 mL/min
N~103000 (peak 4)
Max Pressure: 478 bar

Flow Rate: 1.8 mL/min
N~115000 (peak 4)
Max Pressure: 573 bar

Maximum efficiency at 1.8 mL/min

Peak #	Compound	Plates	k'
2	Acetophenone	114120	0.29
3	Benzene	109931	0.46
4	Touene	114800	0.65
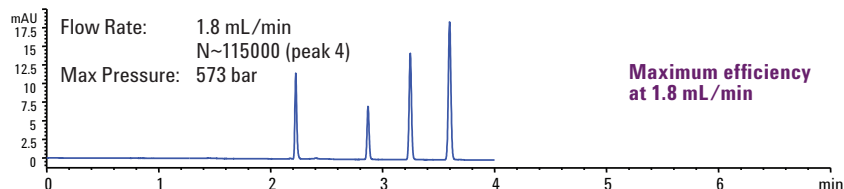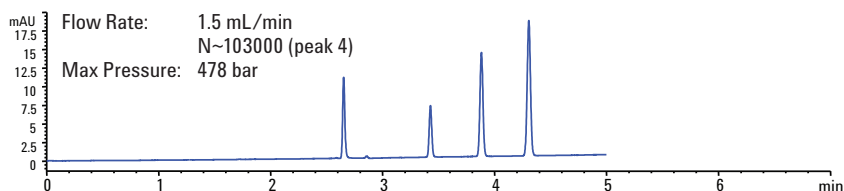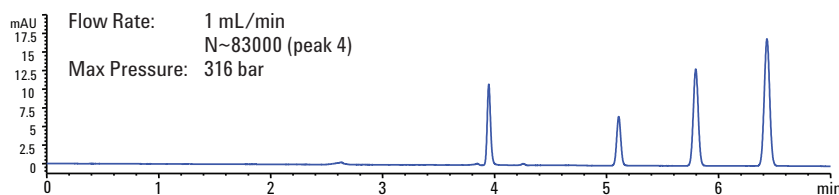
Fast analysis on an Agilent Poroshell 120 EC-C18 of 11 common compounds found in analgesics

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

Maximum Pressure 540 Bar
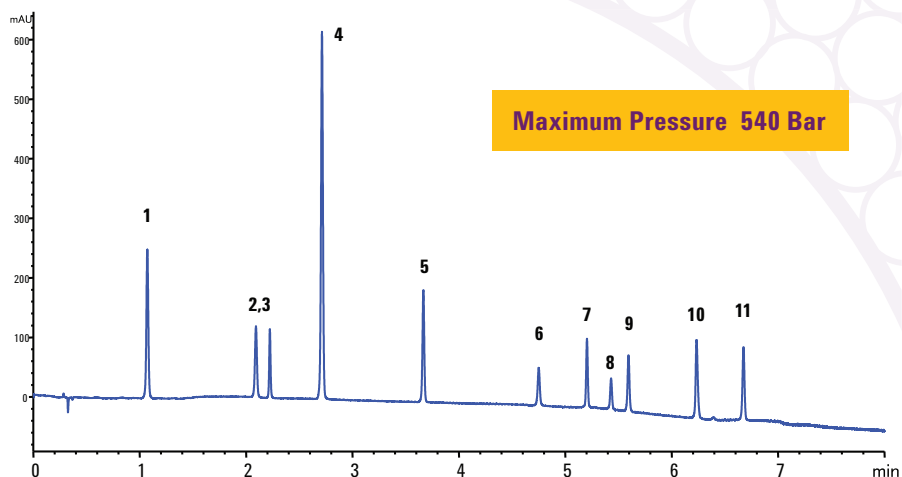
Column:	Poroshell 120 EC-C18, 4.6 x 100 mm, 2.7 µm PN 695975-902
Mobile Phase:	A: Water + 0.1% formic acid B: ACN
Flow Rate:	3.5 mL/min
Injection Volume:	5 µL
Temperature:	40 °C
Detection:	DAD 254 nm

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

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 Aglient 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.

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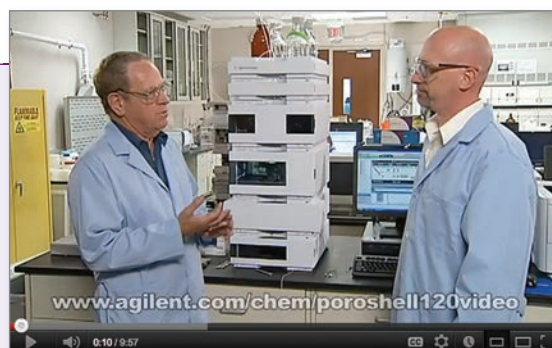
USP method for Naproxen tablets – 4.5X faster analysis on Agilent Poroshell 120 at HPLC pressures

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

Agilent Eclipse Plus C18,
4.6 x 150 mm (L1), 5 μm
PN 959993-902
20 μL injection
Rs = 14.9

N= 12,554   N= 14,885

2X Faster

Agilent Poroshell 120 EC-C18,
4.6 x 100 mm (L1), 2.7 μm
PN 695975-902
13.67 μL injection
Rs = 17.0
P = 238 bar

N= 21,046   N= 20,676

4.5X Faster

Agilent Poroshell 120 EC-C18,
4.6 x 50 mm (L1), 2.7 μm
PN 699975-902
6.7 μL injection
Rs = 12.6
P= 133 bar

N= 11,281   N= 12,051
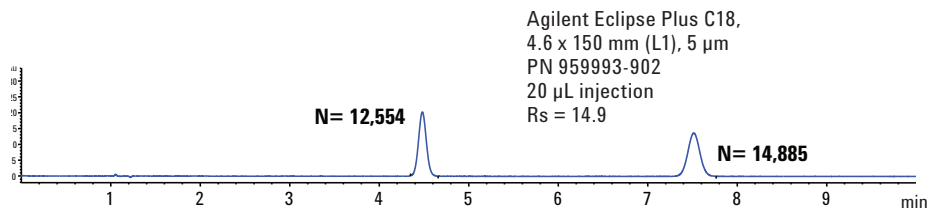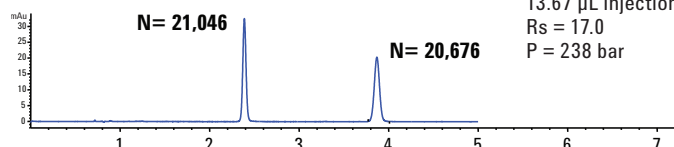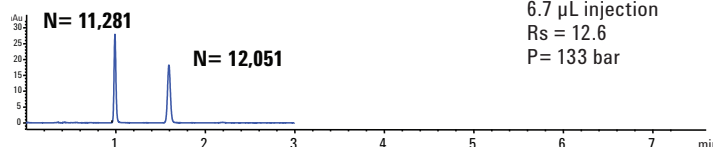
Agilent Poroshell 120 is an excellent choice for faster methods at HPLC pressures.

Mobile Phase: 50:49:1 MeCN:H_2O Acetic Acid

Flow Rate: 1.2 mL/min

Sample:
1. Naproxen
2. Butyrophenone

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.

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

Mobile phase:	A: 20 mM ammonium acetate, pH 4.80
	B: Acetonitrile
Flow rate:	1.000 mL/min
Temperature:	30 °C
Gradient:	14% B at to, ramp to 52% B in 12.0 min

Critical pair
Rs 1.79
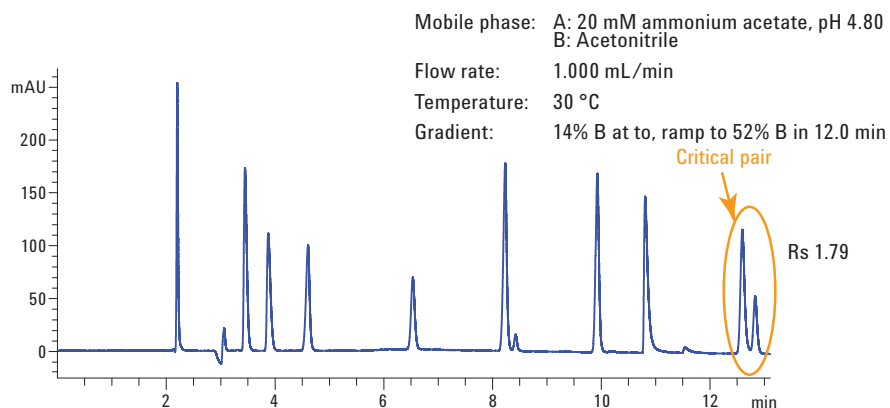
Agilent Poroshell 120, P_{max} = 356 bar

Mobile phase:	A: 20 mM ammonium acetate, pH 4.80
	B: Acetonitrile
Flow rate:	0.851 mL/min
Temperature:	30 °C
Gradient:	14% B at to, ramp to 52% B in 2.1 min

Critical pair
Rs 3.01
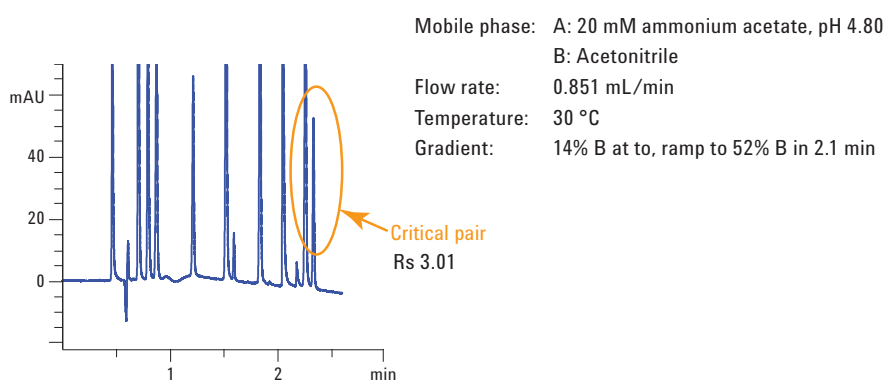
Faster analysis of Simvastatin on Poroshell 120

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 – PN 959990-902

Mobile Phase:	65% CH_3CN,
	35% 3.9 g/L N aH_2PO_4 (pH 4.5)
Flow Rate:	1.5 mL/min for 5 µm column
	2.8 mL/min for 2.7 µm
	Poroshell 120 column
Temperature:	45 °C
Detection:	DAD Sig = 238, 8
	Ref = 360, 100 nm
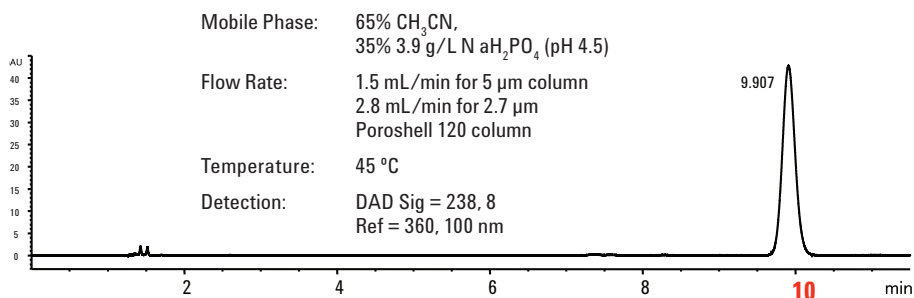
9.907

Agilent Poroshell 120 EC-C18, 4.6 x 75 mm, 2.7 µm – PN 697975-902
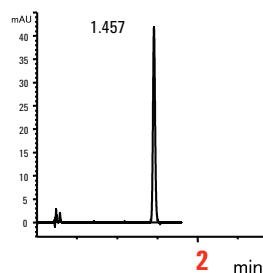
1.457

	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

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.

Column:	Agilent ZORBAX	Time	%B
	Eclipse Plus C18	0	8
	4.6 x 250 mm, 5 µm	33	33
	PN 959990-902	35	33

Flow Rate: 1 mL/min

Column:	Agilent Poroshell	Time	%B
	120 EC-C18,	0	8
	4.6 x 100 mm, 2.7 µm	12	33
	PN 695975-902	13.2	33

Flow Rate: 1 mL/min

Sample:
1. Sulfadiazine
2. Sulfathiazole
3. Sulfapyridine
4. Sulfamerazine
5. Sulfamethazine
6. Sulfamethazole
7. Sulfamethoxypyridazine
8. Sulfachloropyridazine
9. Sulfamethoxazole
10. Sulfadimethoxine

Mobile Phase:
A: 0.1% formic acid in Water
B: 0.1% formic acid in ACN

Insulin analysis: Transfer from 5 µm ZORBAX Eclipse Plus C18 to a Poroshell 120 column for increased efficiency

The Chinese Pharmacopoeia method for insulin can be easily transferred between the traditional 5 µm Eclipse Plus C18 column and the Poroshell 120 EC-C18 column for greater efficiency, resolution, and sensitivity.

Agilent ZORBAX Eclipse Plus C18, 4.6 x 150 mm, 5 µm
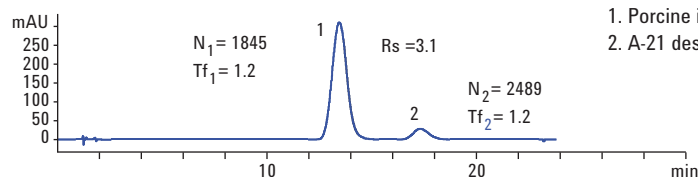
1. Porcine insulin
2. A-21 desamido insulin

Agilent Poroshell 120 EC C18, 4.6 x 100 mm, 2.7 µm
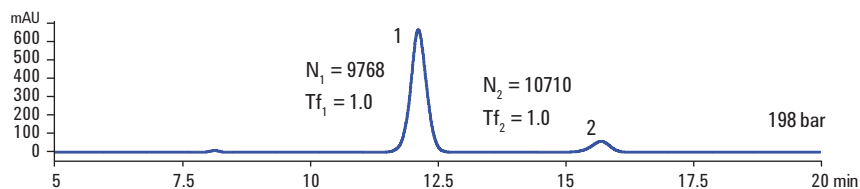
To learn more about Agilent Poroshell 120 columns, visit
www.agilent.com/chem/poroshell120
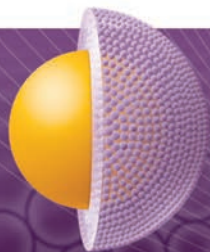
Agilent Poroshell 120 columns:
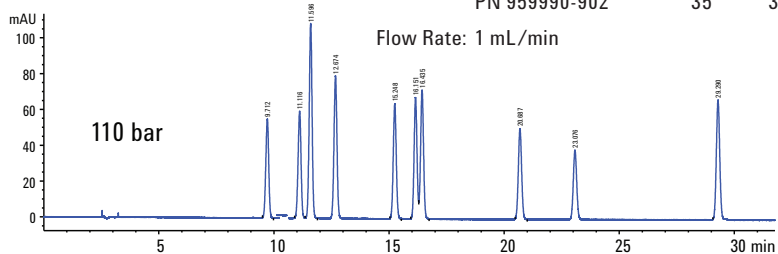# Powerful everyday problem solvers

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.
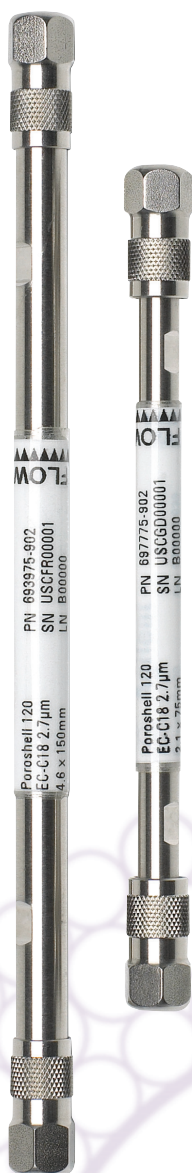
Agilent Poroshell 120 resists plugging with 2 µm frit

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 PN 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 Diflusinal in 50/50 Water-MeCN 10 µg/mL (Final concentration Diflusinal 1 µg/mL) Shaken and allowed to settle 10 minutes **Not Centrifuged/Not Filtered**
Instrument:	Agilent 1200 RRLC (SL)

Diflusinal in Plasma

Solvent A: Water w/0.1 % TFA
Solvent B: MeCN w/0.08 % TFA
Flow Rate: 1 mL/min 1 µL injection

Time	%B
0	20
0.5	90
0.6	90
1.1	20
2.5	20
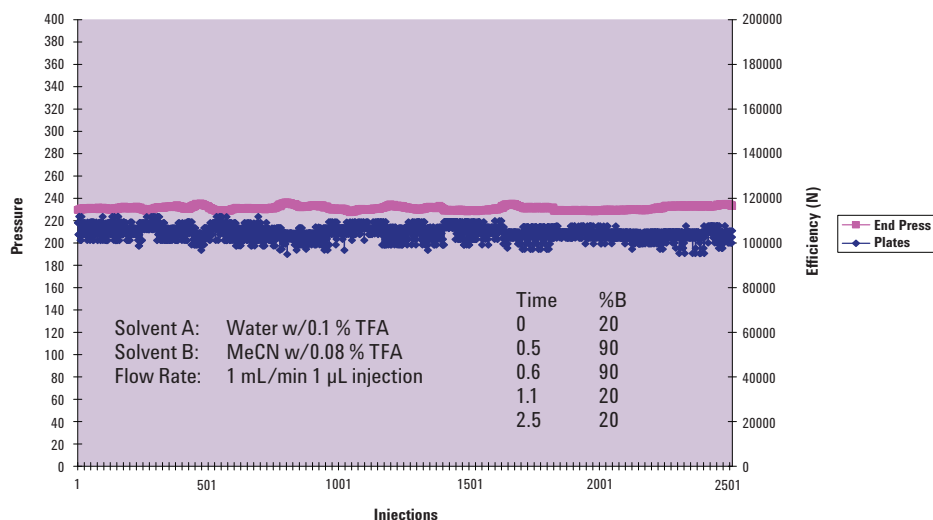
Achieve comparable sample loading to totally porous particles

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
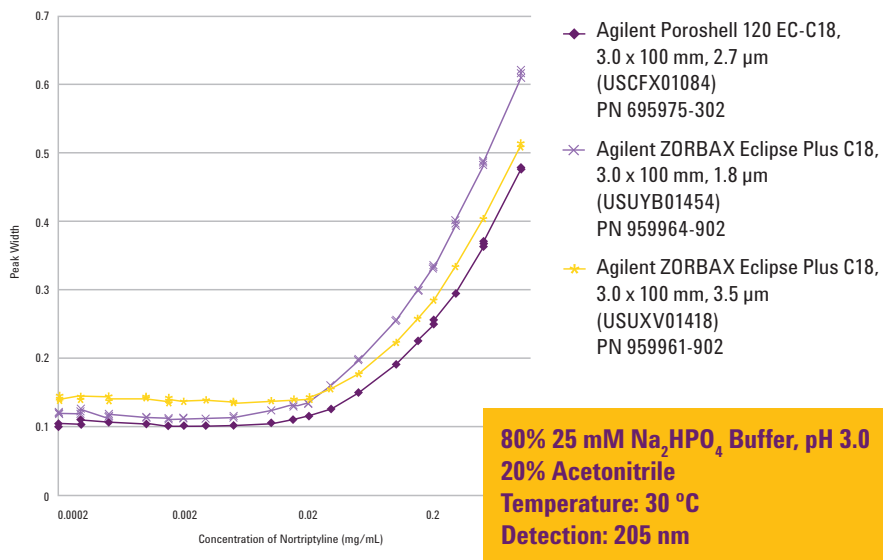
◆ Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm (USCFX01084) PN 695975-302

✕ Agilent ZORBAX Eclipse Plus C18, 3.0 x 100 mm, 1.8 µm (USUYB01454) PN 959964-902

✴ Agilent ZORBAX Eclipse Plus C18, 3.0 x 100 mm, 3.5 µm (USUXV01418) PN 959961-902

80% 25 mM Na$_2$HPO$_4$ Buffer, pH 3.0
20% Acetonitrile
Temperature: 30 ºC
Detection: 205 nm

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.

Poroshell 120 EC-C18	Tf = 1.48	
Competitor S	Tf = 2.68	
Competitor P	Tf = 4.78	
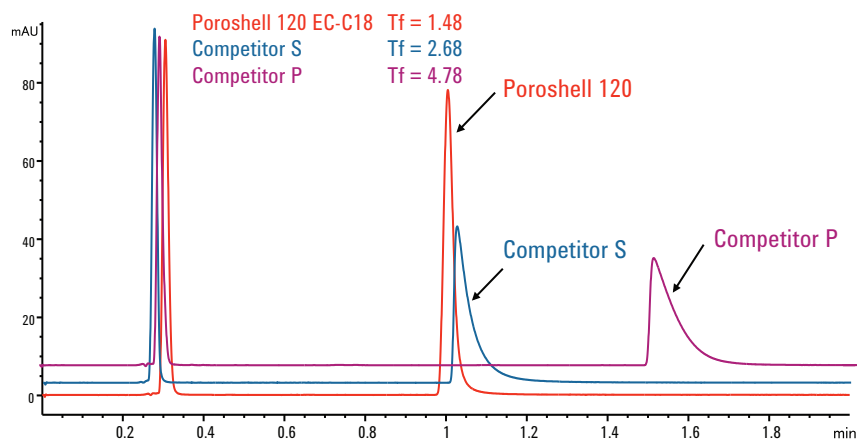
Columns:	4.6 x 50 mm PN 699975-902
Mobile Phase:	20 mM 40% Na_2HPO_4, pH 7.00 60% Acetonitrile
Flow Rate:	1.5 mL/min
Temperature:	24 °C
Detector:	DAD 254 nm, 2 µL flow cell
Sample:	2 µL injection of 250 µg/mL amitriptyline, 50 µg/mL uracil in H_2O/CH_3CN (9:1)

24
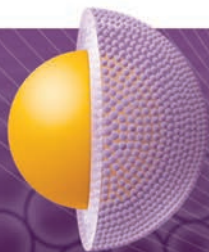
Fast, confident separations
of proteins and peptides

Agilent Poroshell columns are the ideal 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.

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 PN 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
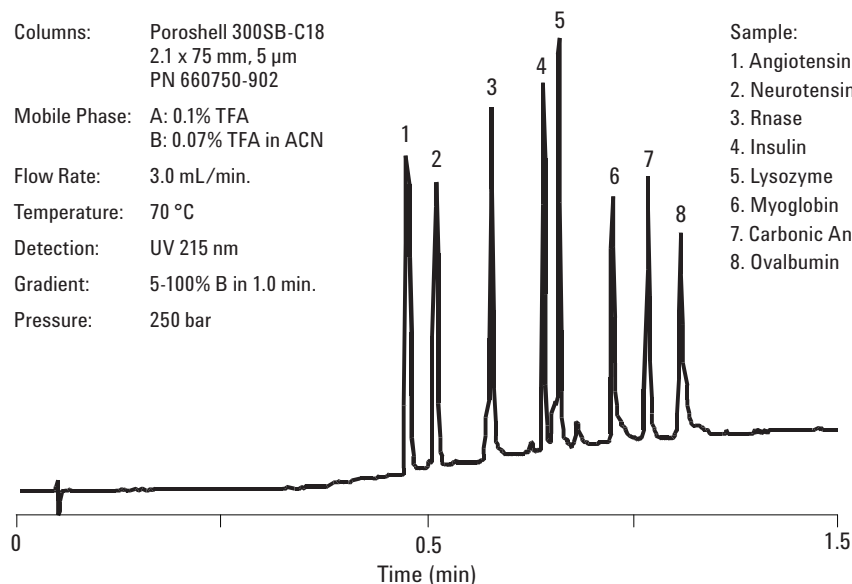8. Ovalbumin

Time (min)

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

5 replicate runs of mAb tryptic peptide map on Agilent Poroshell 120 column

In this example, the peptide map of a digested monoclonal antibody was generated using a Poroshell 120 column. Note the high resolution and reproducible results that were achieved.
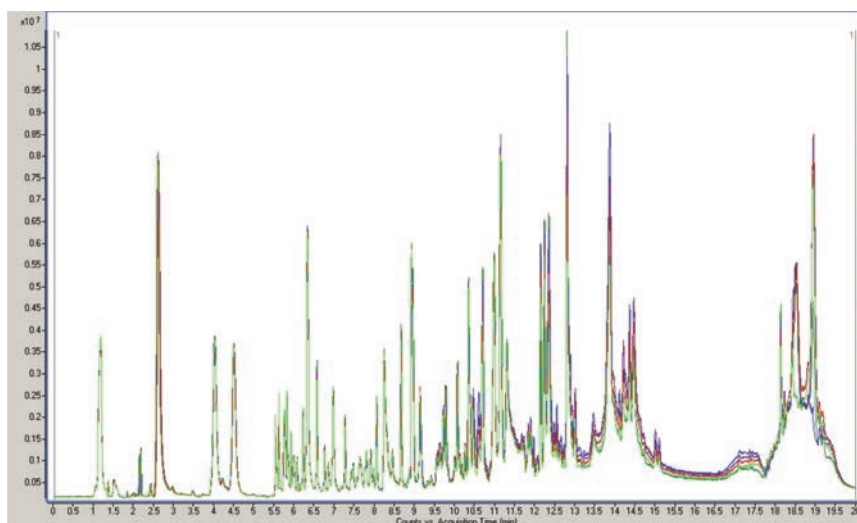
BioConfirm Molecular Feature Extractor of Stratagene mAb trypsin peptide map

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 ºC
Drying Gas:	10L/min
Nebulizer:	40psi
Vcap:	4000V
Fragmentor:	150V
Skimmer:	65V
Octapole 1 RF:	750V
MS:	4 Hz
Mass Range:	200-3200 m/z
Reference Mass:	922.009798
Acq. Mode:	Extended Dynamic Range Mode (2GHz)
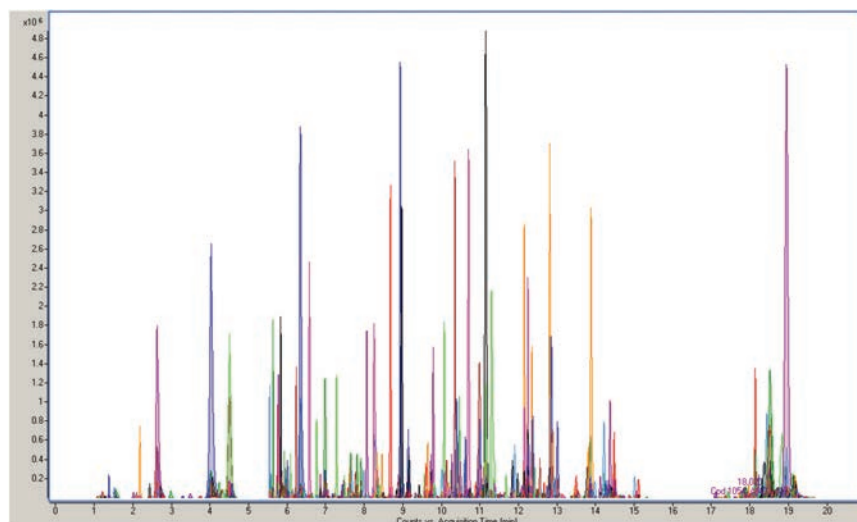
Column:	Poroshell 120 SB-C18, 3.0 x 150 mm, 2.7 µm PN 683975-302
Mobile phase:	A: Water, 0.1% Formic Acid
	B: ACN, 0.1% Formic Acid
Flow rate:	0.3 mL/min
Tempurature:	40 °C
Detection:	QTOF, ESI Positive
Gradient:	Shown in table

Time	%B		Time	%B
0	2		15.1	90
3	2		17	90
13	45		18	2
15	65			

More peaks resolved using Poroshell 120 columns: Protein digest analysis

The 120Å pore size is well suited for the fast, high-resolution analysis of small hydrophilic peptides and peptide fragments in protein digests.

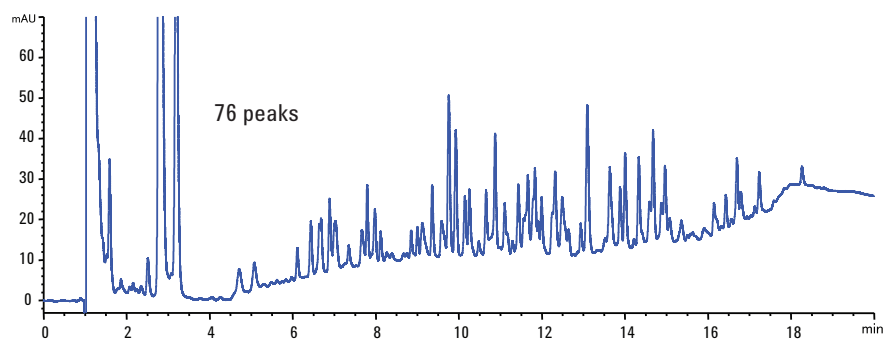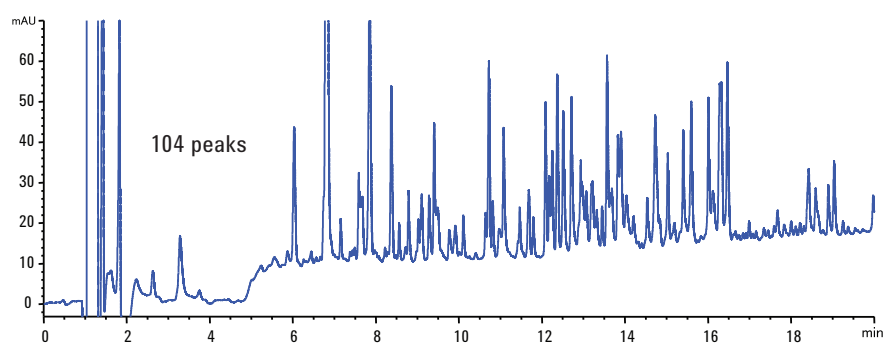|	
Mobile Phase:	A: Water (0.1% TFA) B: ACN (0.08% TFA)
Flow Rate:	0.30 mL/min
Injection Volume:	10 µl
Tempurature:	40 ºC
Sample:	BSA tryptic digest (vendor)
Gradient:	hold 3% B, 3 min., 35-65% B; 30min. 4 minute post run time
DAD:	215 nm

Phenomenex Aeris Widepore, XB-C18, 2.1 x 150 mm, 3.6 µm

76 peaks

Poroshell 120 EC-C18, 2.1 x 150 mm, 2.7 µm

104 peaks

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

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

$N_1 = 5215$
$Tf_1 = 1.4$

$N_2 = 6691$
$N_2 = 1.2$

255 bar
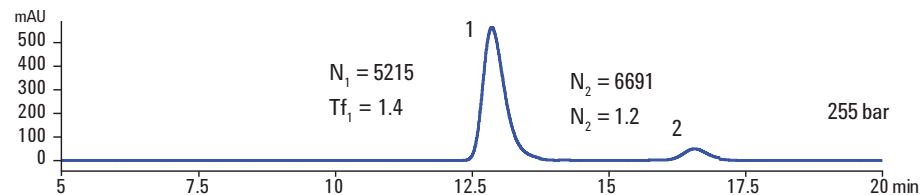
Agilent Poroshell 120 SB C18, 4.6 x 100 mm, 2.7 µm
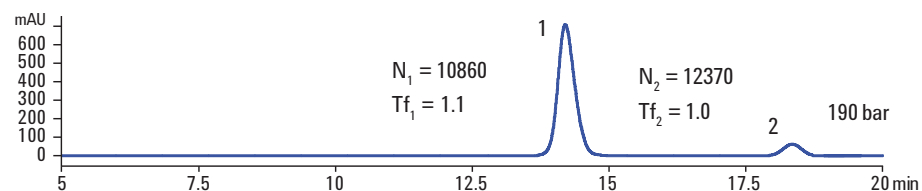
$N_1 = 10860$
$Tf_1 = 1.1$

$N_2 = 12370$
$Tf_2 = 1.0$

190 bar
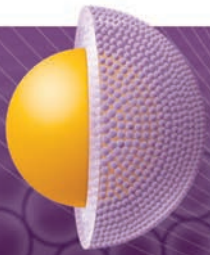
Push your UHPLC performance to *infinite* limits and run your conventional methods with confidence

Whether you need a "workhorse" LC system for routine analysis or the most sophisticated, high-resolution LC/MS system, the Agilent 1200 Infinity Series has what you're looking for.

Together with Poroshell 120 columns, our 1200 Infinity Series LC systems deliver ultimate resolution and sensitivity, while helping you boost your separation power per time. They also ensure easy method transferability between systems – without redevelopment or revalidation.

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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 Throughout (RRHT), 1.8 µm (stable to 600 bar). We bond all these columns with similar stationary phases for assured scalability. With all these choices, you have flexibility in creating 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 these instruments. Poroshell 120 can be used on the 600 bar instruments to optimize their performance.

Agilent Poroshell 120 ordering information (2.7 µm)

Size (mm)	EC-C18	EC-C8	Phenyl-Hexyl	SB-C18	SB-C8*	SB-Aq	Bonus-RP*
4.6 x 150	693975-902	693975-906	693975-912	683975-902	683975-906	683975-914	693968-901
4.6 x 100	695975-902	695975-906	695975-912	685975-902	685975-906	685975-914	695968-901
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
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
3.0 x 100	695975-302	695975-306	695975-312	685975-302	685975-306	685975-314	695968-301
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
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
2.1 x 100	695775-902	695775-906	695775-912	685775-902	685775-906	685775-914	695768-901
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
2.1 x 30	691775-902	691775-906		681775-902			

*Available summer 2012
Note: Poroshell 120 columns have a 600 bar/9000 psi pressure limit.

Agilent Poroshell 120 bonded phase specifications

Bonded Phase	Pore Size	Temp. Limits	pH Range	Endcapped	Carbon Load
EC-C18	120Å	60 ºC	2.0-8.0	Yes	8%
EC-C8	120Å	60 ºC	2.0-8.0	Yes	5%
Phenyl-Hexyl	120Å	60 ºC	2.0-8.0	Yes	8%
SB-C18	120Å	90 ºC	1.0-8.0	No	7.5%
SB-C8	120Å	80 ºC	1.0-8.0	No	4.5%
SB-Aq	120Å	80 ºC	1.0-8.0	No	Proprietary
Bonus-RP	120Å	60 ºC	2.0-9.0	Triple	5%

Specifications represent typical values only.
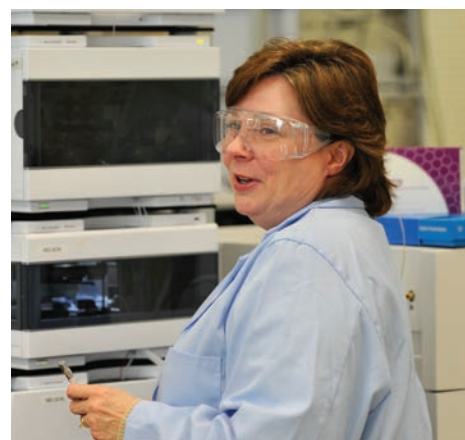
Agilent Poroshell 300 ordering information (5 µm)

| 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	970750-902
MicroBore	1.0 x 75	661750-902	661750-906	661750-909	971750-902
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 ºC	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.

To learn more about Agilent Poroshell 120 columns, visit
www.agilent.com/chem/poroshell120
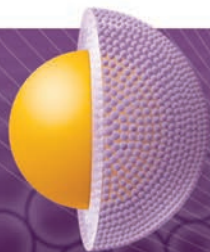
Agilent Chemistries:
Keeping you in command of your analyses

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.

Agilent Bond Elut Silica and Polymeric SPE and Captiva Filtration families of sample prep products offer the widest range of solutions to help you increase throughput and improve the quality of your data.

Agilent's meticulous production oversight ensures column and sample prep consistency and performance. With more than 40 years of experience in producing polymers and silica chemistries, our team is committed to continuously developing new advances that make you most productive.

For more information

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

In the U.S. and Canada, call toll free: **1-800-227-9770**,
option 3, then option 3 again

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