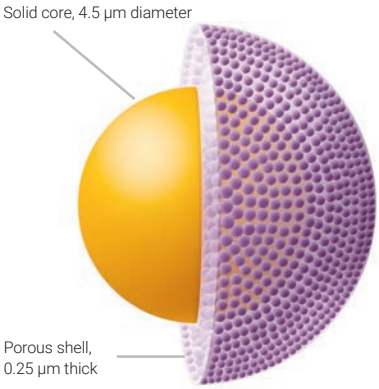
Poroshell 300 AB

- High speed separations of biomolecules with superficially porous particles
- 300 Å pores provide high efficiency and recovery with proteins (up to 1,000 kDa)
- Achieve long lifetime at low pH with Poroshell 300SB and at high pH with 300Extend-C18
- Optimize recovery and selectivity with four different bonded phases—300SB-C18, 300SB-C8, 300SB-C3, and 300Extend-C18



Poroshell 300 columns are ideal for fast separations of proteins and peptides because the 5 µm diameter superficially porous particle allows for fast flow rates to be used while maintaining sharp, efficient peaks. Poroshell columns with StableBond bonded phases provide excellent stability and selectivity choices with TFA and formic acid mobile phases. The Poroshell 300Extend-C18 column can be used from pH 2-11 for unique separations. These columns can also be used for analytical protein separations and LC/MS separations.

Peptides and proteins are typically separated slowly to reduce the potential peak broadening of these slow diffusing analytes. However, Poroshell columns use a superficially porous particle made with a thin layer of porous silica, 0.25 µm thick, on a solid core of silica. This reduces the diffusion distance for proteins, making rapid HPLC separations of peptides and proteins up to 500-1,000 kDa possible with 400/600 bar HPLC systems, including the 1260 Infinity II bio-inert LC.

UHPLC Column Specifications

Bonded Phase	Pore Size	Temp Limits*	pH Range*	Endcapped
Poroshell 300SB-C18, C8, C 3	300 Å	90 °C	1.0–8.0	No
Poroshell 300Extend-C18	300 Å	40 °C above pH 8 60 °C above pH 8	2.0–11.0	Yes

Specifications represent typical values only

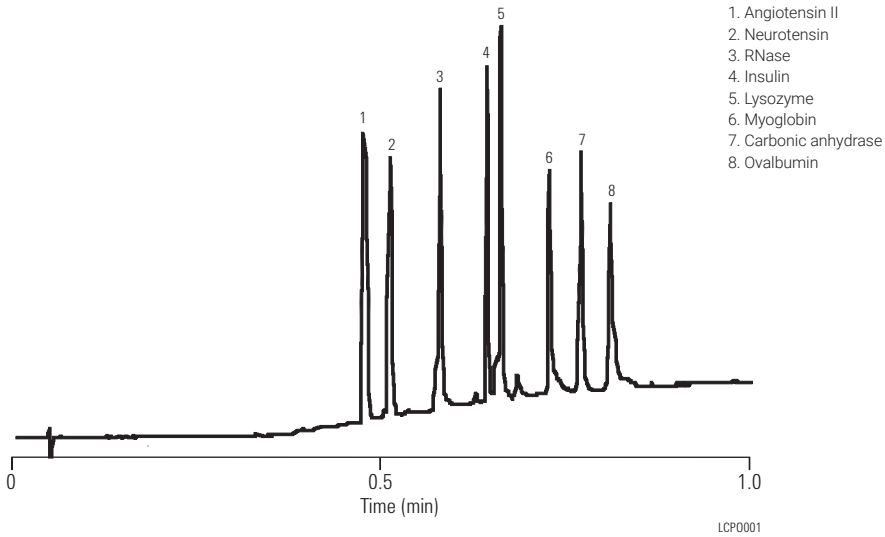
* 300StableBond columns are designed for optimal use at low pH. At pH 6–8, the highest column stability for all silica-based columns is obtained by operating at temperatures < 40 °C and using low buffer concentrations in the range of 0.01–0.02 M. At mid or high pH, 300Extend-C18 is recommended.

Poroshell 300 columns

AB Part of the AdvanceBio family

Poroshell 300 columns separate proteins and peptides in seconds

Column:	**Poroshell 300SB-C18** **660750-902** **2.1 x 75 mm, 5 µm**
Mobile phase:	A: 0.1% TFA in H_2O B: 0.07% TFA in ACN
Flow rate:	0.3 mL/min
Gradient:	5–100% B in 1.0 min
Temperature:	70 °C, 260 bar
Detector:	UV, 215 nm
Sample:	Proteins and peptides



This separation of eight polypeptides and proteins is completed in less than 60 seconds. Each peak is sharp and efficient.

Tips and tools

Further information can be found in:

Characterization of Glycosylation in the Fc Region of Therapeutic Recombinant Monoclonal Antibody (publication **5991-2323EN**)

Using the High-pH Stability of ZORBAX Poroshell 300Extend-C18 to Increase Signal-to-Noise in LC/MS (publication **5989-0683EN**)

www.agilent.com/search

Rapid high resolution analysis of fragmented IgG

Column: **Poroshell 300SB-C3**
660750-909
2.1 x 75 mm, 5 µm

Mobile phase: A: Water (5% AcOH, 1.0% FA, 0.05% TFA)
B: 70/20/10 IPA:ACN:water (5% AcOH, 1.0% FA, 0.05% TFA)

Flow rate: 1.0 mL/min

Injection volume: 2 µL

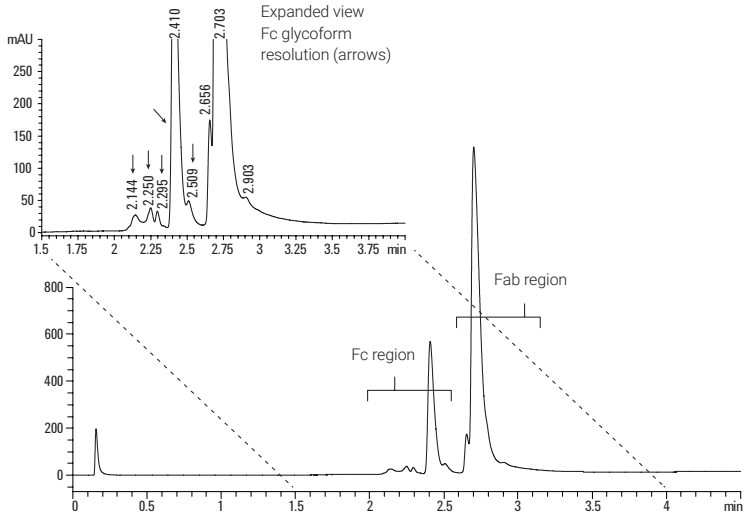
Gradient: Segmented

Time (min)	% B
0	20
4	45
8	45
9	90
10	20

Temperature: 80 °C

Detector: UV, 280 nm

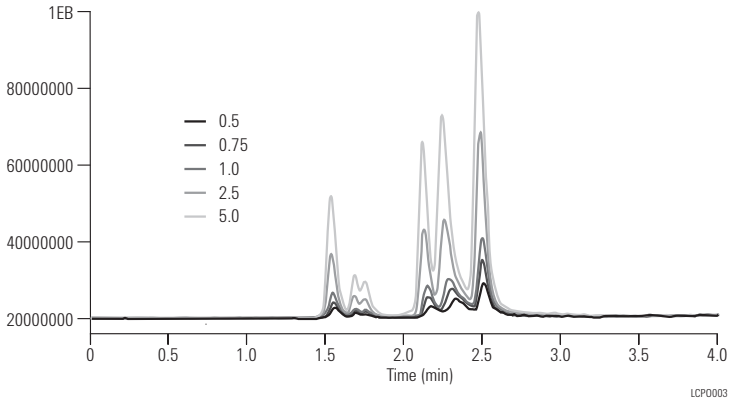
Instrument: 1200 Infinity series with high performance autosampler, binary pump, thermostatted column compartment (TCC), and diode array detector (DAD) coupled to a 6224 Accurate-Mass TOF LC/MS



Reversed-phase separation of IgG1 after papain digestion showing two primary peaks of the Fc and Fab fragments. The inset details partially resolved peaks representing variants of the Fc and Fab fragments (arrows).

MicroBore Poroshell 300 columns provide maximum sensitivity for LC/MS

Column:	**Poroshell 300SB-C18 661750-902 1.0 x 75 mm, 5 µm**	
Mobile phase:	A: Water + 0.1% formic acid B: ACN + 0.1% formic acid	
Flow rate:	600 µL/min	
Gradient:	20–100% B in 5.5 min	
Temperature:	80 °C	
MS conditions:	LC/MS:	Pos. ion ESI; Vcap 6,000 V
	Drying gas flow:	12 L/min
	Drying gas temperature:	350 °C
	Nebulizer:	45 psi
	Fragmentor voltage:	140 V
	Scan:	600-2,500
	Stepsize:	0.15 amu
	Peak width:	0.06 min
Sample:	1 µL	



With narrow bore diameters of 2.1 mm, 1.0 mm, and 0.5 mm, Poroshell columns make an ideal LC/MS partner. When the sample is very limited, the 1.0 mm or 0.5 mm id Poroshell columns are an excellent choice for high sensitivity LC/MS analyses. Sensitive MS molecular weight determinations are possible with as little as 0.5 to 5 pmole of protein on Poroshell columns. The columns have also been used for rapid MS identification of intact proteins, even in the presence of stabilizers and tissue culture media.

Tips and tools

Agilent offers an extensive selection of vials and sample containment solutions including polypropylene and deactivated and siliconized glass. To see the full range, see publication **5990-9022EN**.

www.agilent.com/chem/vials-productivity

Protein elution patterns

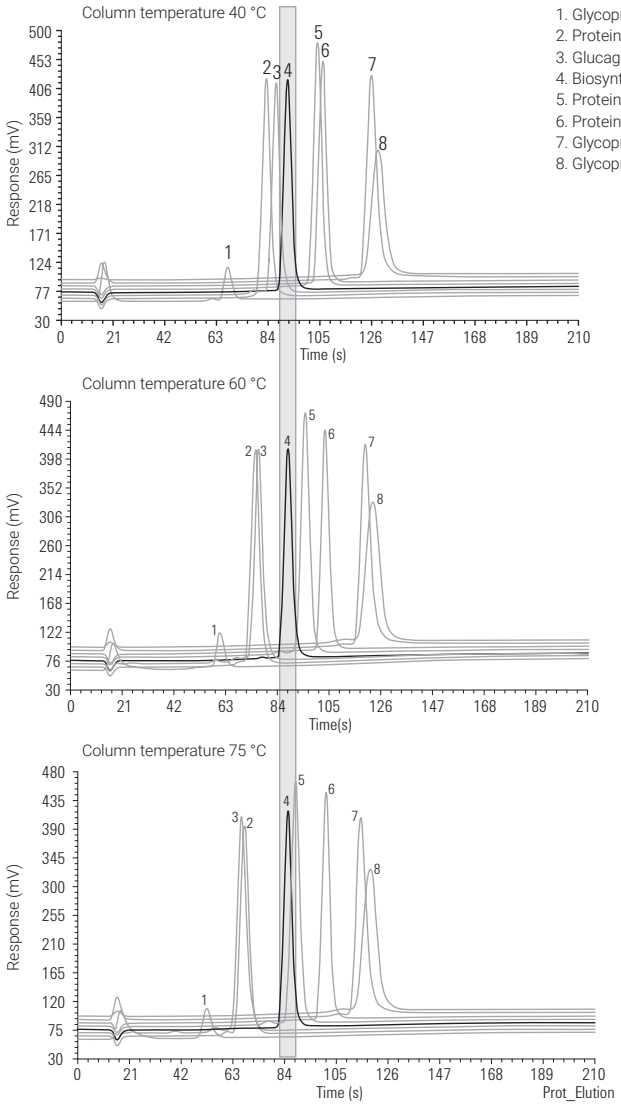
Column: **Poroshell 300SB-C8 660750-906 2.1 x 75 mm, 5 µm**

Mobile phase: A: 0.1% TFA in H_2O
B: 0.1% TFA in ACN

Flow rate: 1.0 µL/min

Gradient: 20 to 70% B in 3 min

Detector: UV, 214 nm



1. Glycoprotein X, mol wt ~22 kDa
2. Protein I, mol wt ~4 kDa
3. Glucagon, mol wt ~3.5 kDa
4. Biosynthetic human insulin, mol wt ~6 kDa
5. Protein J, mol wt ~3 kDa
6. Protein K, mol wt ~6 kDa
7. Glycoprotein Y, mol wt ~45 kDa
8. Glycoprotein Z, mol wt ~30 kDa

Poroshell 300

Description	Size (mm)	Partical Size (µm)	Poroshell 300SB-C18	Poroshell 300SB-C8	Poroshell 300SB-C3	Poroshell 300Extend-C18
Narrow Bore	2.1 x 75	5	660750-902	660750-906	660750-909	670750-902
MicroBore	1.0 x 75	5	661750-902	661750-906	661750-909	671750-902
Capillary	0.5 x 75	5		5065-4468		
Guard cartridge, 4/pk	2.1 x 12.5	5	821075-920	821075-918	821075-924	
Guard hardware kit			820999-901	820999-901	820999-901	
MicroBore guard, 3/pk	1.0 x 17	5	5185-5968	5185-5968	5185-5968	5185-5968