High Speed and *ultra* High Speed Peptide Mapping of Human Monoclonal IgG on ZORBAX Poroshell 300SB-C18, C8, and C3

Application

Biochemical

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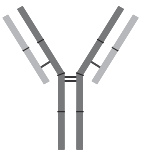

Antibodies are a group of proteins that are the key to directed immunological interaction. They can bind to an antigen (protein, glycoprotein, DNA, etc.) with extreme specificity. This property makes antibodies very valuable for use in diagnostics, general research, and for therapeutics. Treatment of intact antibodies with various chemicals and enzymes allows the specific separation of the heavy and light chains, removal of sugar moieties, and/or cleavage of the polypeptide chains. Separation of the peptide fragments (mapping) after cleavage with a proteolytic enzyme of high specificity, such as Lys-C, gives a characteristic and reproducible pattern of peaks which can be collected, sequenced, and run through a mass spectrometer (MS).

This application note demonstrates the utility of using superficially porous chromatographic media (Poroshell) to achieve substantial improvements in analysis turnaround times when running high-resolution peptide maps. Figure 1 shows comparative peptide maps of a human monoclonal antibody, Lys-C digest. Note the time scales of the separations. The Poroshell maps take one-sixth of the turnaround time and show essentially the same number of peaks. See Table 1.

Highlights

- High speed peptide separations using Poroshell technology result in high-resolution analysis in one-fifth the time.
- Method development is more rapid, since run times are shortened using Poroshell technology.
- Poroshell 300SB columns come in a variety of internal diameters and bonded phases. This gives a wide variety of choices for the optimal fast separation of proteins and peptides.

A Poroshell column is shown above with an Agilent 1100 HPLC system.

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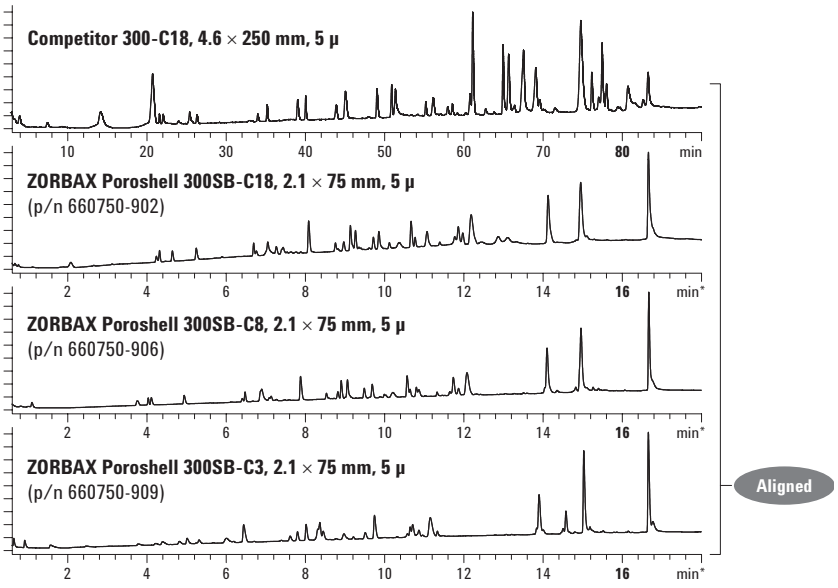


Competitor Column Conditions

Temperature:	Ambient
Detection:	UV, 210 nm
Injection:	50 µL
Sample:	Lys-C digest of human monoclonal antibody
Flow:	0.3 mL/min

Poroshell Column Conditions

Temperature:	70 °C
Detection:	UV, 210 nm
Injection:	10 µL
Sample:	Lys-C digest of human monoclonal antibody
Flow:	1.0 mL/min

Figure 1. High speed peptide maps of a human monoclonal antibody, Lys-C digest, using three different ZORBAX Poroshell columns and one competitive column. Note the time scales required for the separations. 16 vs 80 min respectively.

The speed and resolution of Poroshell technology require a little more explanation to fully appreciate their impact. Note that the turnaround time in Figure 1 is only 21.5 min, with all peaks eluting in less than 17 min., a reasonably fast analysis. We are also achieving the resolution observed for a typical 120 min run. This increased speed results from the high flow rate used, relative to column id. [A flow rate of 1 mL/min on a 2.1-mm id column is equivalent to 5 mL/min on a 4.6 mm id column – five-fold a typical flow rate.] Higher flow increases the volume of the gradient delivered over the same 20.5-min run time.

Simply increasing the gradient volume reduces the gradient slope, increases relative retention (k'), and results in increased resolution, that is, the resolution of a 120-min run is achieved in 20.5 min!

Competitor

Mobile phase

A = 0.1% TFA in water
B = 0.1% TFA in ACN

Gradient timetable

Time (min)	% Solvent B
1.00	00.0
10.00	00.0
110.00	50.0
120.00	70.0
125.00	00.0
135.00	00.0

Poroshell

Mobile phase

A = 0.1% TFA in water
B = 0.1% TFA in ACN

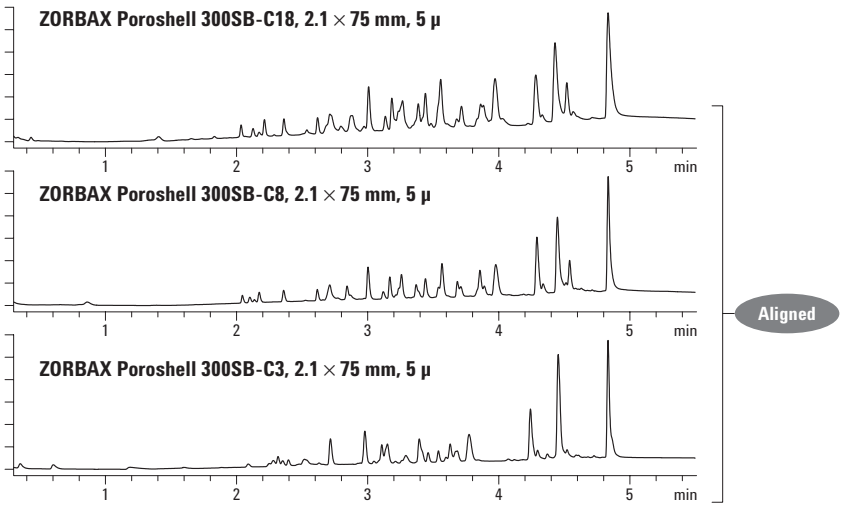
Gradient timetable

Time (min)	% Solvent B
0.00	00.0
20.00	50.0
20.50	100.0
21.50	100.0

Table 1. Number of Peaks Recognized Versus Column Type

Column	No. of peaks recognized (120 min run)	No. of peaks recognized (20.5 min runs)	No. of peaks recognized (5.6 min runs)
Competitor C18	57	–	–
Poroshell-C18	–	55	46
Poroshell-C8	–	58	48
Poroshell-C3	–	54	47

For *ultra* high speed mapping the run time can be cut even further, to 5.6 min, as seen in Figure 2. Some resolution loss occurs (~18%–19%) which does not impact the search for tryptophan-containing peptides (Table 1). The productivity increase is simply enormous >20-fold compared, to the standard 120-min runs. For those needing really high throughput, this is the way to go. Poroshell's unique properties allow runs at *ultra* high speeds with relative impunity, particulary when using MS as the detector.



Poroshell

Mobile phase

A = 0.1% TFA in water
B = 0.1% TFA in ACN

Gradient timetable

Time (min)	% Solvent B
0.0	00
5.5	55
5.6	55
7.0	00

Poroshell Column Conditions

Temperature:	70 °C
Detection:	UV, 210 nm
Injection:	10 µL
Sample:	Lys-C digest of human monoclonal antibody
Flow:	1.0 mL/min

Figure 2. *Ultra* high speed peptide maps of a human monoclonal antibody, Lys-C digest, using three different ZORBAX Poroshell columns.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem.
Search “Poroshell”.

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