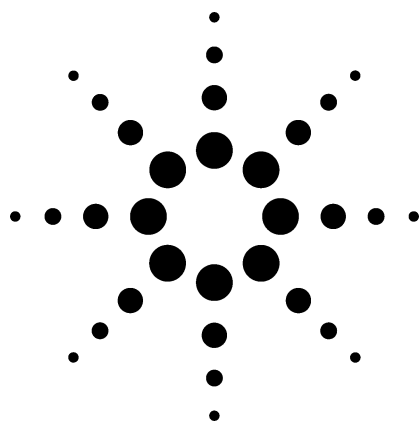


Choosing a ZORBAX Poroshell Phase (C3, C8, or C18) for Fast Separation of Monoclonal Antibodies

Application

Biochemical

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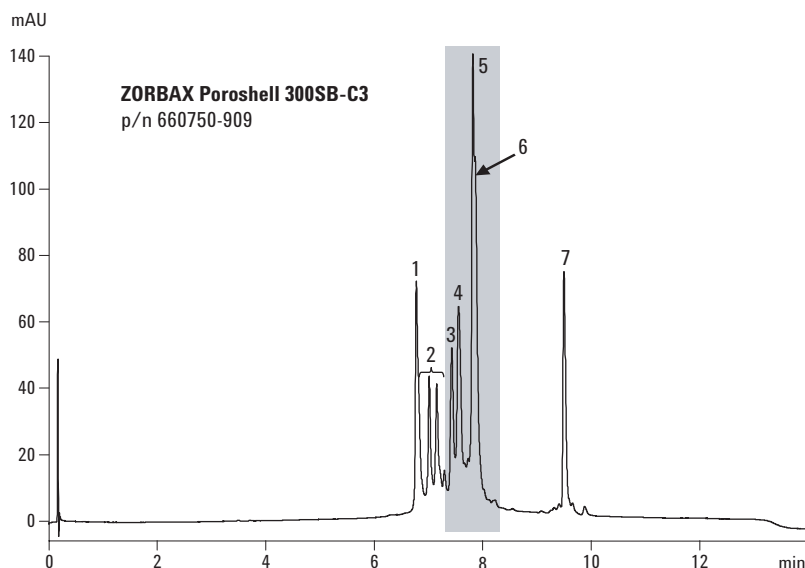


Antibodies represent a class of proteins that are the key to immunological interaction and are of high interest to a biomolecular researcher. They bind to an antigen (protein domains, glycoproteins, DNA, etc.) with extreme specificity. This makes antibodies extremely valuable for use in diagnostics, general research, and therapeutics. IgG is a subset of antibodies having two heavy (50 kDa) and two light chains (25 kDa) that are attached through disulfide bridges. In addition, these molecules are often glycosylated. While a given antibody has a unique amino-acid sequence and is highly specific with regard to its antigen target, the four protein chains of different antibodies have a similar combined mass of approximately 150 kDa. Their large size and similarity in mass can make separations of antibodies difficult. In the examples shown here, ZORBAX Poroshell columns were used for rapid method development and analysis of seven closely related monoclonal IgG antibodies.

The traditional way to separate proteins by reversed phase HPLC is to use a column containing totally porous silica-based particles. This mode of analysis becomes somewhat problematic when the proteins of interest are very large. Large molecules have more complex interactions with the column packing. Large molecules also diffuse too slowly to get quickly into and out of a totally porous HPLC particle. ZORBAX Poroshell technology was designed to facilitate

Highlights

- ZORBAX Poroshell technology provides rapid method development and analysis.
- ZORBAX Poroshell 300SB columns come in a variety of internal diameters and bonded phases.
- The variety of bonded phases provide separation of difficult samples.
- The choice of which ZORBAX Poroshell column will depend on the nature of the sample.



Conditions

Column	ZORBAX Poroshell 300SB-C3 (2.1 × 75 mm, 5 μm) p/n 660750-909
Temperature	70 °C
Flow rate	1.0 mL/min
Detection	UV (210 nm)

Mobile phase

A = H₂O-ACN (90:10)

B = H₂O-ACN (10:90)

Both A and B contain 0.1% TFA and 3 mL/L of PEG 300.

Multisegment timetable

Time (min)	% Solvent B
0.00	19
12.00	41
12.10	19
14.00	19

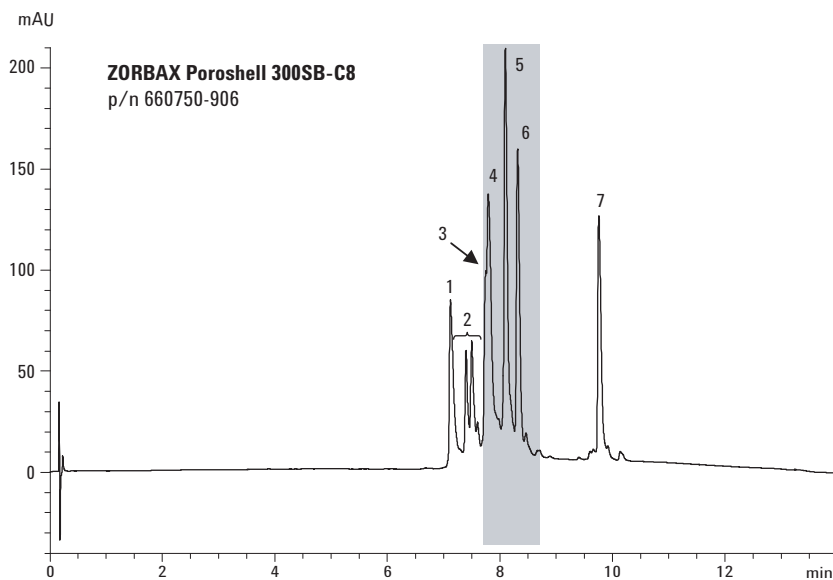


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fast, high-resolution HPLC separations of large molecules (antibodies and other proteins). This is made possible through its superficially porous surface and solid silica core. Molecules diffuse through a short porous layer and cannot penetrate the core; this allows the flow rate to be greatly increased and separation time to be reduced, while resolution stays constant. In addition to providing rapid separations, ZORBAX Poroshell packing is manufactured with a variety of bonding chemistries, such as SB-C18, SB-C8, and SB-C3. As will be shown, change from one bonded phase to another is useful in achieving separation of difficult samples.

The seven antibodies shown in the chromatograms are very similar in molecular weight and are quite large (approximately 150 kDa). As mentioned, this can make for difficult reverse-phase HPLC separations. All seven antibodies are known to be glycosylated, further complicating the analysis. In the first chromatogram, a rapid multisegment gradient was developed using an optimized Agilent 1100 system and a ZORBAX Poroshell 300SB-C3 column; all but antibodies in peaks 5 and 6 could be separated in as little as 14 minutes. (Refer to Agilent publication number 5988-9998EN for an explanation of how to get optimum performance from both the Agilent 1100 and the Agilent ZORBAX Poroshell used together.) The method was then transferred directly to a ZORBAX Poroshell 300SB-C8 column. By changing only the column bonded phase, the method was now suitable for separation of peaks 5 and 6. This second method was achieved at the loss of separation between peaks 3 and 4; however, by using both methods, all seven antibodies can be separated in as little as 28 min. Duplicate runs also serve to confirm quantitative data. Ultimately, change of bonded-phase type (in this case, SB-C3 to SB-C8) is one of the quickest ways to achieve a desired separation. Note that the second antibody elutes as a doublet (two peaks) in both methods.

In summary, use of different bonded phases can be crucial to successful separation of a particular protein sample, especially if these proteins are very large and heterogeneous. Bonded-phase choice can improve peak shape and recovery, as well as achieve desired selectivity. ZORBAX Poroshell often enhances peak shape and recovery through its simplified interaction with large proteins. The structure of ZORBAX Poroshell particles allows very short analysis times that can facilitate rapid method development as well as high throughput analyses.



Conditions

Column	ZORBAX Poroshell 300SB-C8 (2.1 × 75 mm, 5 μm) p/n 660750-906
Temperature	70 °C
Flow rate	1.0 mL/min
Detection	UV (210 nm)

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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Data courtesy of: Novartis Pharma, Biotechnology, Basel; Dr. Kurt Forrer and Patrik Röethlisberger

Mobile phase

Same as first chromatogram.

Multisegment timetable

Same as first chromatogram.

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