Effect of Flow Rate on Ultrafast Separation of Proteins – Comparison of ZORBAX Poroshell Technology to Totally Porous Columns

Application

Biochemical

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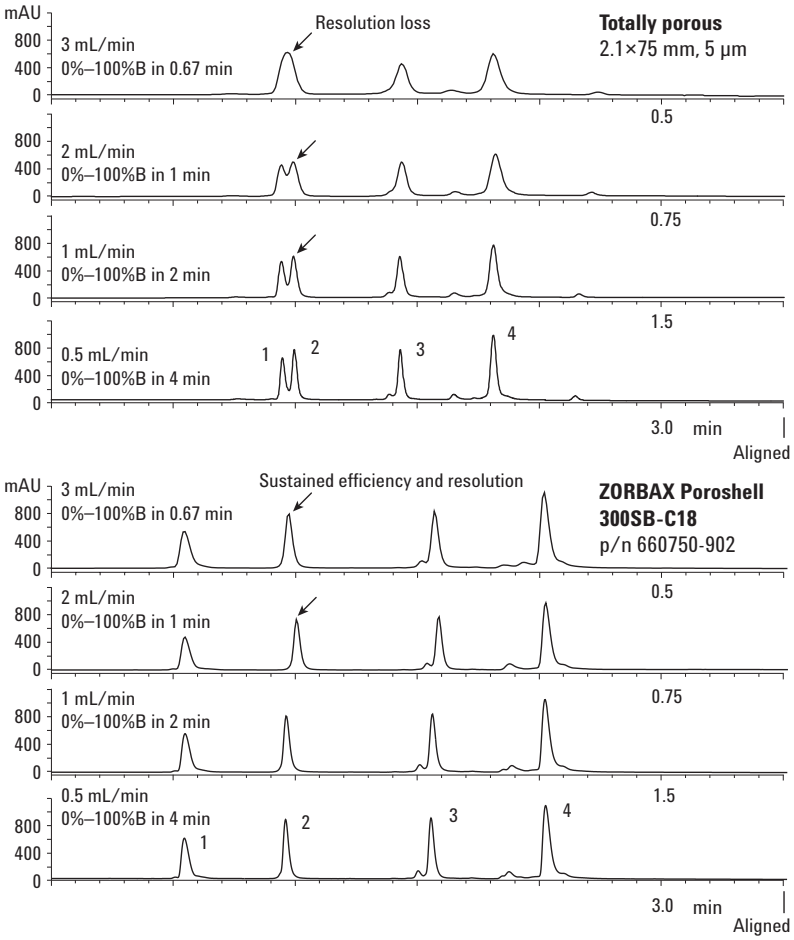
In the modern protein laboratory, speed, resolution, and sensitivity are sometimes competing goals. Frequently the analyst is working under conditions of limited sample size, many samples, and a need for resolution of contaminants in the shortest possible time. ZORBAX Poroshell column technology addreses these goals in a unique way, providing the analyst with a powerful tool for the rapid separation and analysis of proteins.



Highlights

- ZORBAX Poroshell 300SB-C18 allows the use of higher mobile phase linear velocities, with no loss in resolution, resulting in shorter run times.
- Because of the thin superficially porous layer, ZORBAX Poroshell 300SB-C18 allows rapid equilibration of slowly diffusing large molecules.
- ZORBAX Poroshell 300SB-C18 uses proven StableBond technology to maintain stability at low pH and elevated temperatures.
- ZORBAX Poroshell 300SB-C18 columns are ideally suited to the rapid LC/MS analysis of intact proteins. LC/MS is now a standard technique used in protein chemistry laboratories.

Sample peak	Protein name
1	Neurotensin
2	RNAse A
3	Lysozyme
4	Myoglobin

Mobile phase

A = 95% H_2O, 5% ACN with 0.1% TFA
B = 5% H_2O, 95% ACN with 0.07% TFA

Conditions

Instrument	Agilent 1100 WPS with AutoBypass
Column	**ZORBAX Poroshell 300SB-C18** (2.1 × 75 mm, 5 µm) p/n 660750-902
Temperature	70 °C
Flow rate	As indicated
Detection	UV (215 nm)

When analyzing small molecules by HPLC, increase of flow rate can be of great advantage in achieving faster separations. If the absolute flow rate becomes too great, the column diameter and flow can be reduced while the same relative flow rate (linear velocity) is maintained. Small molecules have high diffusion constants and can move into and out of the pores of HPLC particles very quickly. These molecules tend to elute in with the same peak width even as flow is increased. The result is extremely fast, high-resolution separations with typical flow and solvent consumption.

For bio-macromolecules, such as proteins, attempts to shorten analysis time by increasing the flow rate can lead to band broadening. This occurs as a result of smaller diffusion constants for large molecules. As mobile phase moves faster and faster past the HPLC particles, these large molecules cannot move into and out of porous particles before significant mobile phase moves past (top chromatogram). The result is increasingly broad peaks as flow rate (linear velocity) is increased.

ZORBAX Poroshell technology facilitates rapid analysis and method development of large molecules by use of its shortened diffusion path and its tolerance of high linear velocity (high relative flow rate). ZORBAX Poroshell particles consist of a solid silica core covered by a thin totally porous crust. Large molecules, which diffuse very slowly compared to small molecules, can move into and out of the thin crust in a very short time. Flow rate can now be increased to decrease run time, without significant peak broadening (bottom chromatogram). ZORBAX Poroshell columns are typically used at flow rates five to ten times those used with a column of the same dimensions but containing totally porous particles. The result is run times that are five to ten times shorter than that typically possible. Finally, the short length and 5-µm particle size of ZORBAX Poroshell columns helps keep back pressures within desirable limits.

Proteins analyzed by HPLC are almost always separated using a mobile-phase gradient. To produce the top and bottom chromatograms shown here, gradient time was adjusted to correspond with flow rate. In general, keeping gradient volume (flow rate multiplied by the gradient time) constant results in chromatograms with the same relative elution pattern. The separation looks the same, but occurs within shorter run times as flow rate is increased. Protein separations on ZORBAX Poroshell often benefit from increasing flow rate alone (with no adjustment to gradient time). This is because increasing gradient volume (flow rate multiplied by gradient time) decreases the gradient slope, thereby increasing relative retention (k') and resolution (Rs). The resolution of a 5 min run is achieved in 0.5 min!

The additional benefit of using a rapid, high-resolution separation in initial method development should not be overlooked. A series of scouting runs may be made in one-fifth to one-tenth the usual time. This allows the analyst to get through the method development and into the actual analyses much more quickly. This speed in method development is especially useful when using mass spectrometry for detection.

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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