Analyzing Post-Translational Modifications of Intact IgG using Aeris™ WIDEPORE Core-Shell HPLC/UHPLC and BioSep™ GFC/SEC Columns

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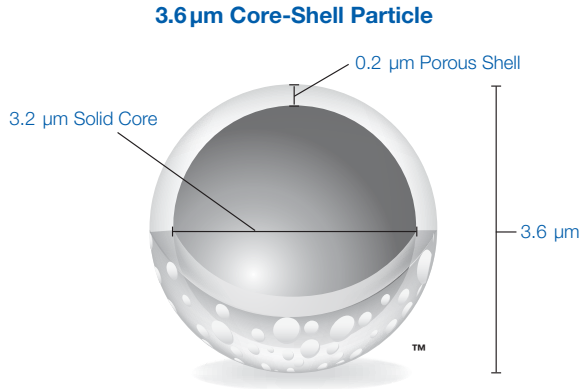
For the analysis of large, intact proteins like recombinant IgG therapeutics, Phenomenex offers two bioseparation product lines (Aeris and BioSep) for improved solutions in identifying low-level post-translational modifications. Aeris WIDEPORE is a reversed phase core-shell HPLC/UHPLC column that features improved resolution and recovery versus traditional fully porous 300 Å media. BioSep columns offer high performance gel filtration separations that offer improved resolution and inertness versus other aqueous GFC columns.

Introduction

The ever growing interest in protein therapeutics has found researchers looking for improved solutions for identifying post-translational modifications of proteins. Two specific areas of interest include developing better reversed phase solutions for intact protein analysis as well as new solutions for aggregate analysis using gel filtration chromatography. Recent technological advances in HPLC/UHPLC column technology offer improved solutions for both areas: large pore core-shell Aeris WIDEPORE for reversed phase intact protein separation and BioSep-SEC-S gel filtration columns for protein aggregate analysis.

Aeris 3.6 µm WIDEPORE core-shell particle columns feature high permeability, low hydrophobicity, and an inert surface for high recovery, improved peak shape, and better resolution for even the most hydrophobic of proteins. This core-shell technology changes the face of ultra-high performance bioseparations, negating the need for high backpressures to achieve increased column efficiency. Aeris core-shell technology is specifically designed to accommodate the slower diffusion of proteins into porous particles. A graphic representation of the Aeris particle is shown in **Figure 1**.

Figure 1.



Graphical representation of an Aeris WIDEPORE 3.6 µm particle. A 0.2 µm porous shell surrounds a 3.2 µm solid core. This particle geometry is specifically designed to narrow the peak width and improve resolution for proteins and other large molecules.

BioSep gel filtration columns feature a hydrophilic ligand bonded to a high pore volume rigid silica particle. BioSep features reduced protein adsorption leading to more accurate protein and aggregate recoveries versus other gel filtration media. BioSep also features a narrow particle size distribution which provides significant improvements in protein peak efficiency and resolution. These features deliver chromatographers more accurate, rugged, and reproducible methods for quantitating low-level aggregates in protein samples. This technical note features examples of the use of each column for analyzing recombinant IgG.

Materials and Methods

All chemicals, standards and antibodies were obtained from Sigma Chemical (St. Louis, MO, USA). Solvents were purchased from EMD (San Diego, CA, USA). Fully porous 5 µm 300 Å C18 columns (100 x 4.6 mm), core-shell Aeris 3.6 µm WIDEPORE XB-C18 columns (100 x 4.6 mm), and BioSep-SEC-s3000 GFC columns (300 x 7.8 mm) were obtained from Phenomenex (Torrance, CA, USA). Competitor 3000-series GFC column (300 x 7.8 mm) was obtained from Tosoh Corporation (San Francisco, CA).

For reversed phase analysis, mouse immunoglobulin IgG samples were analyzed on an Agilent® 1200 HPLC system with autosampler, column oven, solvent degasser, and UV detector set at 214 or 280 nm. Data was collected using ChemStation software (Agilent, Santa Clara, CA, USA). Mobile phases used were 0.1 % TFA in water (A) and 0.1 % TFA in acetonitrile (B) and a gradient from 10 to 40 % B in 15 minutes was used at 1 mL/min. Column was maintained at 80 °C.

For gel filtration analysis, an isocratic method was used featuring 100 mM sodium phosphate at pH 6.8 with a flow rate of 1 mL/min. Column was maintained at ambient room temperature (22 °C).

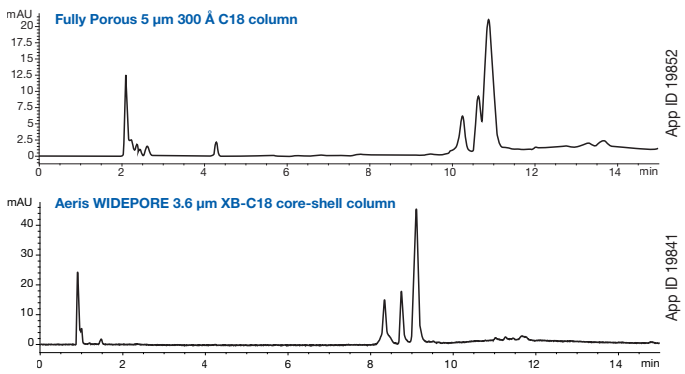
Results and Discussion

As is depicted in **Figure 1**, Aeris WIDEPORE 3.6 µm particle morphology is significantly different from small pore core-shell media (Kinetex® 2.6 µm, for example, uses a 0.35 µm shell on a 1.9 µm core). Aeris WIDEPORE core-shell particles are designed to maximize resolution of proteins greater than 10 kilodaltons molecular weight regardless of whether an HPLC or UHPLC is used. The thin porous shell minimizes protein peak band spreading due to diffusion in and out of the porous layer; while the larger particle size reduces column backpressure allowing for the use of longer columns for increased resolution. The result is a column with performance on par or better than sub-2 µm wide pore fully porous media at backpressures significantly lower than 3 µm fully porous columns.

APPLICATIONS

The performance advantage of Aeris WIDEPORE core-shell columns is demonstrated in **Figure 2** where an Aeris column is compared to a 5 µm fully porous wide pore column. IgG immunoglobulins are considered difficult proteins to separate by reversed phase due their large size (150 kDa) and hydrophobicity. Typically, elevated column temperatures and isopropanol mobile phase are required to improve recovery and resolution. In this example, mouse immunoglobulin IgG separation is compared on each column using an acetonitrile mobile phase at 80 °C. (Aeris columns are stable to 90 °C.) Note the significantly narrower peak width for the Aeris WIDEPORE core-shell column resulting in the resolution of the three main glycoforms of IgG versus the fully porous columns where only two components are baseline resolved. Of additional note is the greater recovery for the Aeris column; low hydrophobicity and good inertness results in greater recovery for hydrophobic proteins.

Figure 2.



Conditions for both columns:

Columns: Aeris WIDEORE 3.6 µm XB-C18
Fully Porous 5 µm 300 Å C18
Dimensions: 100 x 4.6 mm
Mobile Phase: A: Water with 0.1 % TFA
B: Acetonitrile with 0.1 % TFA

Gradient:

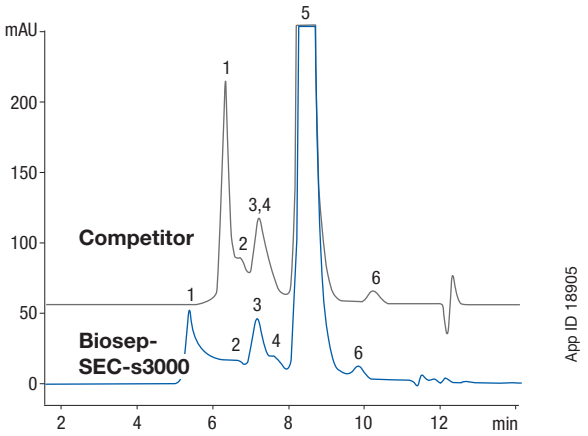
Time (min)	% B
0	10
1	20
15	40

Flow Rate: 1 mL/min
Temperature: 80 °C
Detection: UV @ 280 nm
Sample: Mouse monoclonal antibody

Comparison between a fully porous 5 µm 300 Å C18 column (top chromatogram) to an Aeris WIDEPORE 3.6 µm XB-C18 column (bottom chromatogram) for a mouse IgG sample. Note the significantly narrower peak width and greater protein recovery for the Aeris column. The thin shell minimizes protein diffusion distance resulting in narrower peak widths and greater resolution of closely related species.

Figure 3 compares performance of a BioSep™-SEC-s3000 gel filtration to a competitor GFC column in separating human IgG2 aggregates. The sample was incubated for two weeks at 37 °C to show aggregates. The flow rate is 1 mL per minute at an ambient temperature. Note the improved resolution on the BioSep-SEC-s3000 column over the popular competitor 3000-series GFC column. The IgG dimer 1 and IgG2 dimer 2 (peaks 3 and 4) co-elute on the competitor column; on the BioSep-SEC-s3000 column, the two peaks are partially resolved.

Figure 3.



Conditions for both columns:

Column: BioSep-SEC-s3000
Competitor 3000-series GFC Column
Dimensions: 300 x 7.8 mm
Mobile Phase: 100mM Sodium Phosphate pH 6.8
Flow Rate: 1 mL/min
Temperature: Ambient
Detection: UV @ 214 nm
Sample: 1. HMW aggregates
2. IgG trimer
3. IgG dimer 1
4. IgG2 dimer 2
5. Hu IgG2 monomer
6. Low MW impurity

Comparison between a competitor 3000-series GFC column and a BioSep-SEC-s3000 GFC column for Human IgG2k aggregates. Note the resolution between peaks 3 and 4 on the BioSep-SEC-s3000 column, and how the same peaks co-elute on the competitor GFC column.

BioSep-SEC-s3000 offers a high optimal molecular weight selectivity window for a wide range of proteins. It can accommodate samples ranging between 1,000 to 700,000 daltons, depending on whether the sample is run in its native state, under 0.5 % SDS, or under 6 M GnHCl.

Conclusion

Improved resolution and recovery using Aeris WIDEPORE and BioSep offer improved options for analyzing large recombinant proteins. These columns feature new particle technology that improves the accuracy and reliability of intact protein analysis methods.

APPLICATIONS

Ordering Information

Aeris™ Columns

WIDEPORE 3.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges*
	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1	3/pk
XB-C18	00B-4482-AN	00D-4482-AN	00F-4482-AN	00G-4482-AN	AJ0-8783
XB-C8	00B-4481-AN	00D-4481-AN	00F-4481-AN	00G-4481-AN	AJ0-8785
C4	00B-4486-AN	00D-4486-AN	00F-4486-AN	00G-4486-AN	AJ0-8899

Aeris Columns

WIDEPORE 3.6 µm Analytical Columns (mm)				SecurityGuard ULTRA Cartridges*
	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
XB-C18	00D-4482-E0	00F-4482-E0	00G-4482-E0	AJ0-8769
XB-C8	00D-4481-E0	00F-4481-E0	00G-4481-E0	AJ0-8771
C4	00D-4486-E0	00F-4486-E0	00G-4486-E0	AJ0-8901

* SecurityGuard ULTRA cartridges require holder, Part No.: AJ0-9000

For more information on Aeris Core-Shell HPLC / UHPLC columns visit www.phenomenex.com/Aeris

BioSep™ Columns

Stainless Steel Columns (mm):	Narrow Bore	Analytical		Preparative	SecurityGuard Cartridges (mm)	
Phases	300 x 4.6	300 x 7.8	600 x 7.8	300 x 21.2	4 x 3.0**	15 x 21.2***
					/10pk	ea
BioSep-SEC-s2000	00H-2145-E0	00H-2145-K0	00K-2145-K0	00H-2145-P0	AJ0-4487	AJ0-8588
BioSep-SEC-s3000	00H-2146-E0	00H-2146-K0	00K-2146-K0	00H-2146-P0	AJ0-4488	AJ0-8589
BioSep-SEC-s4000	00H-2147-E0	00H-2147-K0	00K-2147-K0	00H-2147-P0	AJ0-4489	AJ0-8590
					for ID: 4.6-7.8 mm	for ID: 21.2 mm

** SecurityGuard Analytical cartridges require holder, Part No.: KJ0-4282
*** PREP SecurityGuard Cartridges require holder, Part No.: AJ0-8223

For more information on BioSep GFC columns, visit www.phenomenex.com/BioSep

guarantee

If columns in this technical note do not provide at least an equivalent separation as compared to columns of similar phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

APPLICATIONS

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