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APPLICATIONS

Achieving Improved Intact IgG Separations Using Aeris™ WIDEPORE Core-Shell HPLC | UHPLC Columns

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Aeris WIDEPORE core-shell HPLC | UHPLC columns provide dramatic improvement in the separation of intact antibodies when compared to fully porous columns. The high permeability of the Aeris core-shell particle combined with low hydrophobicity and inert surface results in high recoveries and improved peak shape for even the most hydrophobic proteins.

Introduction

The use of core-shell technology has resulted in a new paradigm in ultra-high performance by decoupling increased column efficiency from high backpressure. The principal result for most small molecule applications has been reduced run times and increased throughput without the need for expensive new UHPLC instrumentation.

However, for intact protein separation, the principal focus is more on improving resolution of proteins from near-identical post-translationally modified impurities rather than reducing run times. A wide pore core-shell HPLC | UHPLC column (Aeris WIDEPORE) has been introduced that is specifically designed to improve protein separations. Rather than utilize a similar morphology of small molecule core-shell columns with larger pores, a completely different particle was developed that takes into account the slower diffusion of proteins into porous particles. A graphic representation of the Aeris particle is shown in **Figure 1**.

When one looks at various intact protein separations, the resolution of different glycoforms of therapeutic IgG antibodies stands out as one of the more difficult due to the large size and structure of IgG. In this technical note, improved separation of IgG glycoforms are shown using Aeris core-shell HPLC | UHPLC columns.

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 and core-shell Aeris 3.6 µm WIDEPORE XB-C18 columns (100x4.6 mm) were obtained from Phenomenex (Torrance, CA. USA).

Mouse Immunoglobulin IgG samples were analyzed on an Agilent® 1200 HPLC system with autosampler, column oven, solvent degasser, and UV detector set at 214 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 and a gradient from 10 to 40 % B in 15 minutes was used at 1 mL/min. Column was maintained at 80 °C.

Results and Discussion

As is shown in **Figure 1**, Aeris WIDEPORE 3.6 µm is a significantly different particle morphology compared to 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 were 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; 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.

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 HPLC 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-only 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.

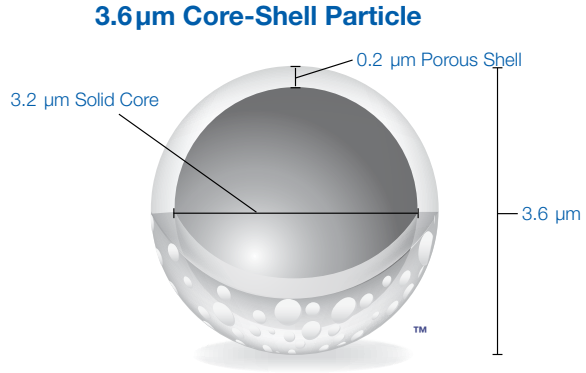

Figure 1.
Graphical representation of an Aeris WIDEPORE 3.6 µm particle. A 0.2 µm porous shell surrounds a 3.2 µm solid core. This particle geometry is specifically designed to narrow the peak width and improve resolution for proteins and other large molecules.

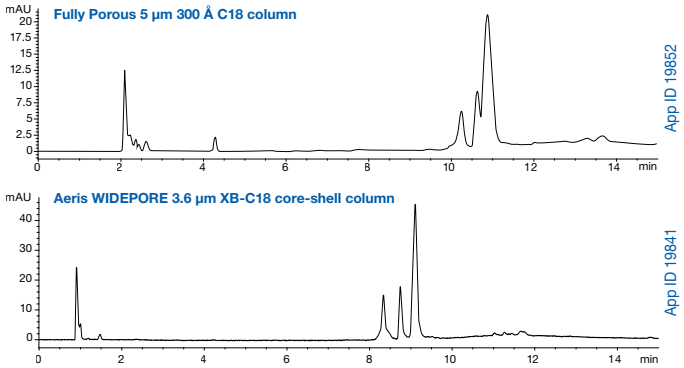

Figure 2.
Comparison between a fully porous 5 µm 300 Å C18 column (top chromatogram) to an Aeris WIDEPORE 3.6 µm XB-C18 column (bottom chromatogram) for a mouse IgG sample. Note the significantly narrower peak width and greater protein recovery for the Aeris column. The thin shell minimizes protein diffusion distance resulting in narrower peak widths and greater resolution of closely related species.

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Conclusion

The good resolution and recovery for this application demonstrate the utility of using core-shell Aeris™ WIDEPORE columns for immunoglobulin and large protein separations.

Ordering Information

Aeris WIDEPORE 3.6 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1
XB-C18	00B-4482-AN	00D-4482-AN	00F-4482-AN	00G-4482-AN
XB-C8	00B-4481-AN	00D-4481-AN	00F-4481-AN	00G-4481-AN
C4	00B-4486-AN	00D-4486-AN	00F-4486-AN	00G-4486-AN

Aeris WIDEPORE 3.6 µm Analytical Columns (mm)

	100 x 4.6	150 x 4.6	250 x 4.6
XB-C18	00D-4482-E0	00F-4482-E0	00G-4482-E0
XB-C8	00D-4481-E0	00F-4481-E0	00G-4481-E0
C4	00D-4486-E0	00F-4486-E0	00G-4486-E0

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

guarantee

If Aeris core-shell technology does not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

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