# Using Longer Aeris™ PEPTIDE Core-Shell HPLC/UHPLC Columns for Improved Peptide Mapping

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*A new 3.6µm 100 Å HPLC/UHPLC column (Aeris PEPTIDE) has been introduced that is specifically designed to improve separations of peptide and peptide mapping applications. The Aeris PEPTIDE XB-C18 column was developed to complement Aeris WIDEPORE XB-C18 core-shell columns for protein characterization. When one looks at peptide mapping applications, performance requirements are significantly different versus intact protein separations, as increased retention and selectivity are required to separate the large number of peptides generated in peptide mapping applications. Because increased resolution is a higher priority versus speed, a larger particle (3.6µm) core-shell particle was developed allowing the use of longer columns at lower backpressures. In this application the increased resolution that longer Aeris PEPTIDE 3.6µm XB-C18 provide will be demonstrated.*

## Materials and Methods

All chemicals, standards and antibodies were obtained from Sigma Chemical (St. Louis, Missouri). Solvents were purchased from EMD (San Diego, California). Core-shell Aeris PEPTIDE 3.6µm XB-C18 columns (150 × 4.6mm and 250 × 4.6mm) were obtained from Phenomenex (Torrance, California). Bovine serum albumin was digested with trypsin and 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, California). Mobile phases used were 0.1 % Formic acid in water (A) and 0.1 % Formic acid in acetonitrile (B) with a gradient from 3 to 65 % B at a flow rate of 1.2 mL/min. Gradient times were adjusted based on column length (33 to 55 minutes respectively). Column was maintained at 40 °C.

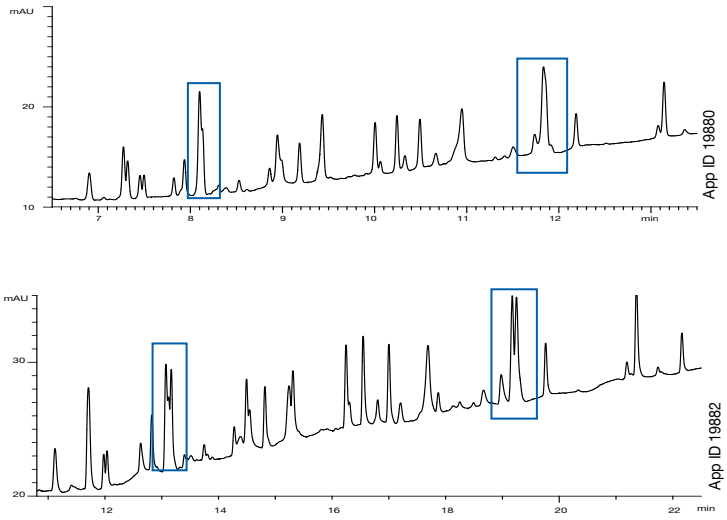
## Results and Discussion

Aeris PEPTIDE 3.6µm XB-C18 core-shell particles demonstrate similar or better performance than sub-2 µm fully-porous columns at a fraction of the backpressure, allowing the use of longer columns at backpressures compatible with existing HPLC systems. The 3.6µm core-shell media is of particular utility for peptide map applications where the increased resolution of longer columns is desired (for high-speed UHPLC applications the Aeris PEPTIDE 1.7µm XB-C18 can be used instead). An example of the utility is demonstrated in **Figure 1** where 150 × 4.6mm and 250 × 4.6mm Aeris PEPTIDE 3.6µm XB-C18 columns were compared for a peptide map of BSA. The 150 × 4.6mm column provides excellent separation of the peptide mixture at a low column backpressure (140 bar at 1.2 mL/min) such that a longer column could be used to achieve additional resolution if required. When the 250 × 4.6mm Aeris PEPTIDE 3.6µm XB-C18 column was used for the separation, additional peptides were resolved while still at a backpressure amenable to using standard HPLC systems (200 bar at 1.2 mL/min). These results demonstrate the performance advantage and utility of the Aeris PEPTIDE 3.6µm XB-C18 media for highly complex peptide mapping mixtures where one can utilize different column lengths to optimize resolution and separation time based on the needs of a specific application.

## Conclusion

Maximizing resolution between proteins and their modified impurities is critical in obtaining useful quantitation of post-translational modifications of biogeneric proteins. The different applications in this technical note show the utility of Aeris WIDEPORE columns for obtaining accurate data for intact protein applications. The optimized geometry of the core-shell Aeris WIDEPORE columns, as well as good selectivities of the three separate phases offered (XB-C18, XB-C8 and C4), deliver better resolution and recovery than existing fully porous 300 Å columns for intact protein analysis. Finally, the large particle size of the Aeris WIDEPORE column delivers a significantly lower backpressure than sub-2µm 300 Å columns which allows for more flexibility in instrument used (HPLC or UHPLC) as well as column length in developing biogeneric protein applications.

**Figure 1.**
BSA Tryptic map separated on different length Aeris PEPTIDE 3.6µm XB-C18 columns (150 × 4.6mm top, 250 × 4.6mm bottom). Note the good separation on the shorter Aeris PEPTIDE column and the increased resolution provided by the longer Aeris PEPTIDE (250 × 4.6mm) column. Because backpressure for the Aeris 3.6µm column is so low, one can optimize column lengths based on their separation time and resolution requirements.



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## Aeris PEPTIDE 1.7 µm Minibore Columns (mm)

| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | SecurityGuard* ULTRA Cartridges* 3/pk |
|---|---|---|---|---|
| XB-C18 | 00B-4506-AN | 00D-4506-AN | 00F-4506-AN | AJ0-8948 |

## Aeris PEPTIDE 3.6 µm Minibore Columns (mm)

| | 50 x 2.1 | 100 x 2.1 | 150 x 2.1 | 250 x 2.1 | SecurityGuard ULTRA Cartridges* 3/pk |
|---|---|---|---|---|---|
| XB-C18 | 00B-4507-AN | 00D-4507-AN | 00F-4507-AN | 00G-4507-AN | AJ0-8948 |

## Aeris PEPTIDE 3.6 µm Analytical Columns (mm)

| | 100 x 4.6 | 150 x 4.6 | 250 x 4.6 | SecurityGuard ULTRA Cartridges* 3/pk |
|---|---|---|---|---|
| XB-C18 | 00D-4507-E0 | 00F-4507-E0 | 00G-4507-E0 | AJ0-8946 |

* SecurityGuard ULTRA cartridges require holder part number, AJ0-9000

## SecurityGuard™ ULTRA Cartridge Holder (for 2.1 to 4.6 mm ID columns)

| SecurityGuard ULTRA Guard Cartridge Holder | ea |
|---|---|
| | AJ0-9000 |

guarantee

If Aeris core-schell columns do not provide at least an equivalent separation as compared to a competing column of the same phase, return the column with the comparative data within 45 days for a FULL REFUND.

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