[TECHNOLOGY BRIEF]

Separation of Small Intact Proteins on CORTECS UPLC C_8 1.6 µm Columns

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GOAL

To demonstrate narrower peak widths observed using of CORTECS® UPLC® C_8 1.6 µm Columns solid-core technology on the separation efficiency of small intact proteins.

BACKGROUND

The introduction of CORTECS UPLC C_8 1.6 µm Columns for UPLC separations provides significantly higher efficiency over fully porous particles. The optimized, solid-core particle structure allows for exceptional column efficiency to increase gradient peak capacity and sensitivity. CORTECS particles are made in both 1.6 µm and 2.7 µm diameters to provide seamless transfer between UPLC, UHPLC, and HPLC separations. The entire CORTECS family of columns are manufactured and quality tested with tight tolerances, making them ideal for many applications. Intact proteins can be difficult to analyze by LC, since achieving acceptable detection sensitivity is a challenge. For MS detection, many proteins are difficult to phase transition into gas, so sharper chromatographic peaks, as well as selective sample preparation can significantly improve their detection. Ribonuclease A (13.7 kDa), cytochrome C (12 kDa), and lysozyme (14.3 kDa) are three common, small proteins that are often separated by ion exchange. They can also be easily separated based on hydrophobicity using reverse phase chromatography.

Solid-core particles significantly improve chromatographic efficiency over fully porous particles of the same diameter. The advantage of solid-core particles becomes obvious in the separation of small intact proteins, which are commonly separated on short alkyl ligands, such as C_8.

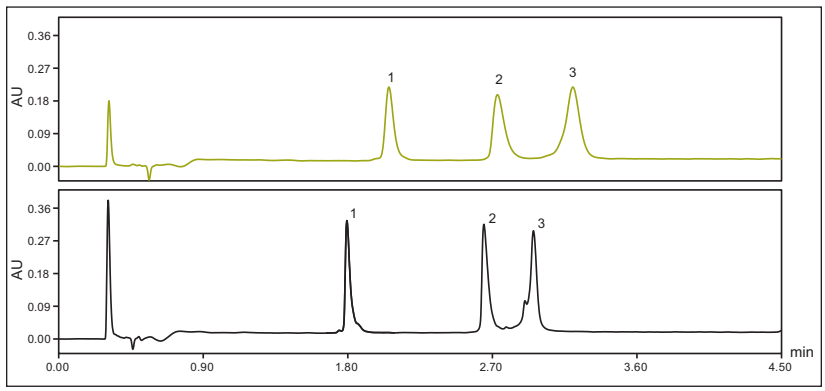

Figure 1. Separation of RNAse A, cytochrome C, and lysozyme on 2.1 mm x 50 mm, ACQUITY UPLC® BEH C_8 Column, 130Å, 1.7 µm, 2.1 mm x 50 mm (top) and CORTECS UPLC C_8 Column, 90Å 1.6 µm, 2.1 mm x 50 mm.

However, C_{18} ligands, in the absence of a controlled surface charge, often cause overly tailing peak shapes and excessive retention for proteins and large peptides.[1] Peptide separations are often achieved on C_{18} ligands, but improved peak shapes for proteins can be achieved on shorter ligands, like C_8, and solid-core particles.

THE SOLUTION

A CORTECS UPLC C_8 Column, 1.6 µm, 2.1 x 50 mm, (p/n 186008404) was compared to an ACQUITY UPLC BEH C_8 Column, 1.7 µm, 2.1 x 50 mm (p/n 186002877). The gradient used was 4 minutes, from 10 to 40% acetonitrile with 0.1% formic acid. The sample mixture used was the IEX Cation Test Standard (p/n 186006870), which is a lyophilized equal mass mixture of ribonuclease A, cytochrome C, and lysozyme. The IEX Cation Test Standard was dissolved in 1 mL of 95/5 H_2O/ACN with 0.1% formic acid, then diluted 10x with the same diluent for injection. The use of the CORTECS UPLC C_8 Column provided significantly improved peak capacity. The resultant peak widths from the CORTECS UPLC C_8 Column were, on average, about 20% narrower, measured at 4σ peak width, yielding a 22% increase in peak capacity, and 20% increase in peak height, compared to the ACQUITY UPLC BEH C_8 Column. The chromatograms in Figure 1 compare the peak heights and widths of the small proteins separated on the ACQUITY UPLC BEH C_8 Column, 1.7 µm, 2.1 x 50 mm (p/n 186002877) and CORTECS UPLC C_8 Column, 1.6 µm, 2.1 x 50 mm (p/n 186008404). CORTECS UPLC Columns have a slightly lower column phase ratio, due the solid particle cores, which reduces the observed retention time. The narrower peak widths and high peak heights reveal two minor impurities between the cytochrome C and lysozyme peaks, which were obscured on the ACQUITY UPLC BEH C_8 Column. The combination of high performance instrumentation and extremely high column efficiency afforded by CORTECS UPLC C_8 Columns allows for fast, sensitive separation of small intact proteins.

LC conditions	
LC system:	ACQUITY UPLC I-Class System with Binary Solvent Manager (BSM), Sample Manager (SM-FL), and Active Column Manager (CM-A)
Detection:	Tunable UV Detector (220 nm)
Columns:	CORTECS UPLC C_8 Column, 1.6 µm, 2.1 x 50 mm (p/n 186008404) and ACQUITY UPLC BEH C_8 Column, 1.7 µm, 2.1 x 50 mm (p/n 186002877)
Column temp.:	45 °C
Sample temp.:	15 °C
Injection volume:	1 µL
Flow rate:	0.25 mL/min
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in acetonitrile
Gradient:	10 to 40% B in 4 minutes, then 40 to 75% in 0.5 minutes and held for 1 minute
Vials:	TruView™ LCMS Certified Clear Glass 12 x 32 mm Screw Neck Max Recovery Vial, with Cap and Preslit PTFE/Silicone Septa, 1.5 mL volume (p/n 186005662CV)
Protein standard:	IEX Cation Test Standard (p/n 186006870)
Chromatography software:	Empower® 3 Chromatography Data Software
Keywords:	Large peptides, small proteins, UPLC, CORTECS, ribonuclease A, lysozyme, cytochrome C

SUMMARY

Narrow peak widths and detection sensitivity can be challenging to achieve in small protein separations. Compared to ACQUITY UPLC BEH C_8 1.7 µm Columns, markedly narrower peaks can be achieved on CORTECS UPLC C_8 1.6 µm Columns. The bonded ligand is exactly the same, so the improvement is due to the base particle morphology of the solid-core particles. The improvements of solid-core particles becomes more pronounced with larger molecules, which was the case for the intact proteins tested here.

References

1. "Increasing Peak Capacity in Reverseed-Phase Peptide Separations with Charged Surface Hybrid (CSH) C_{18} Columns" Mattew A. Lauber, Stephan M. Koza, and Kenneth J. Fountain, Waters Application Note 720004568en.

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