

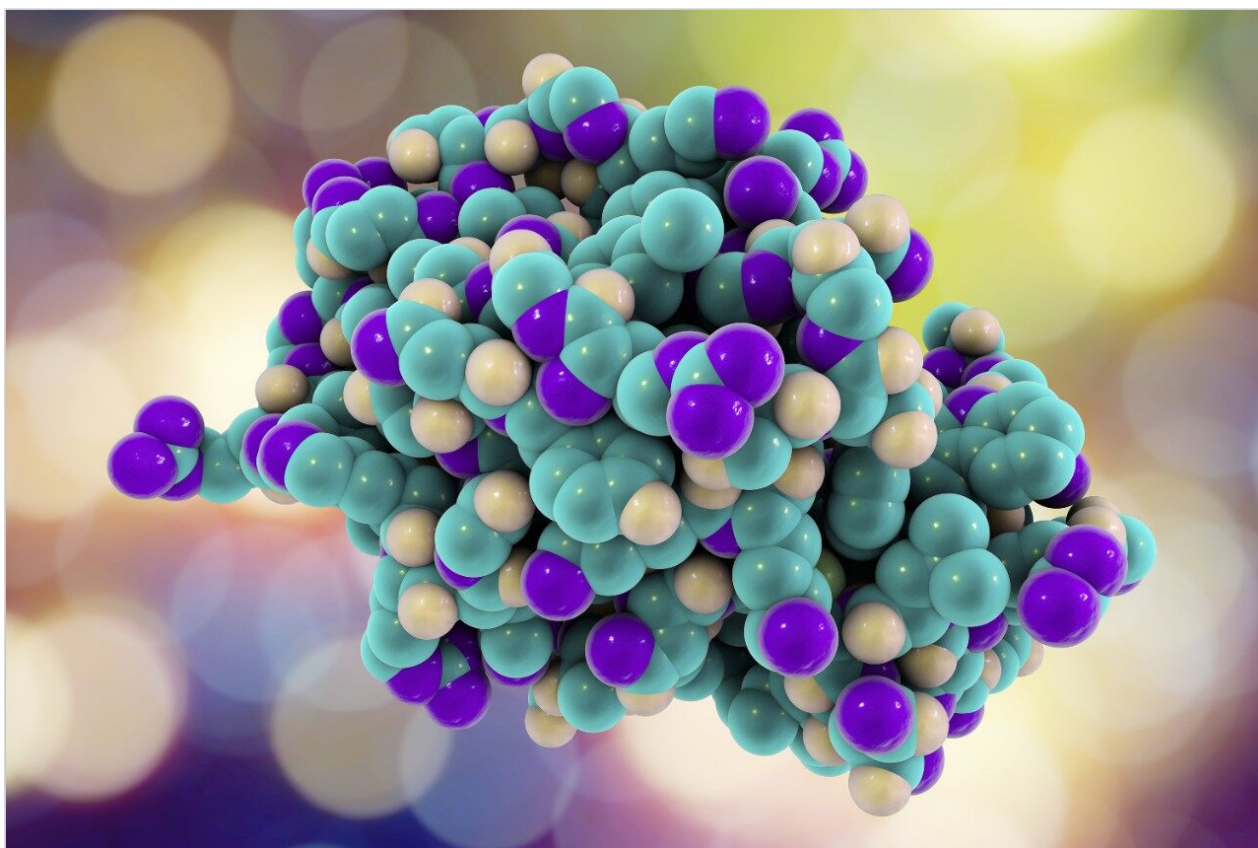
## Application Note

# Separation of Small Intact Proteins on CORTECS UPLC C<sub>8</sub> 1.6 μm Columns

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Jacob N. Fairchild, Erin E. Chambers

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

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## Abstract

This Application brief demonstrates narrower peak widths observed using of CORTECS UPLC C<sub>8</sub> 1.6 µm Columns solid-core technology on the separation efficiency of small intact proteins.

### Benefits

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<sub>8</sub>.

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## Introduction

The introduction of CORTECS UPLC C<sub>8</sub> 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.

However, C<sub>18</sub> ligands, in the absence of a controlled surface charge, often cause overly tailing peak shapes and excessive retention for proteins and large peptides.<sup>1</sup> Peptide separations are often achieved on C<sub>18</sub> ligands, but improved peak shapes for proteins can be achieved on shorter ligands, like C<sub>8</sub>, and solid-core particles.

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## Experimental

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 <sub>8</sub> Column, 1.6 µm, 2.1 x 50 mm (p/n <a href="#">186008404</a> ) and ACQUITY UPLC BEH C <sub>8</sub> Column, 1.7 µm, 2.1 x 50 mm (p/n <a href="#">186002877</a> )
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 <a href="#">186005662CV</a> )
Protein standard:	IEX Cation Test Standard (p/n <a href="#">186006870</a> )
Chromatography software:	Empower® 3 Chromatography Data Software
Keywords:	Large peptides, small proteins, UPLC, CORTECS, ribonuclease A, lysozyme, cytochrome C

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## Results and Discussion

A CORTECS UPLC C<sub>8</sub> Column, 1.6 µm, 2.1 x 50 mm, (p/n 186008404) was compared to an ACQUITY UPLC BEH C<sub>8</sub> 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<sub>2</sub>O/ACN with 0.1% formic acid, then diluted 10x with the same diluent for injection. The use of the CORTECS UPLC C<sub>8</sub> Column provided significantly improved peak capacity. The resultant peak widths from the CORTECS UPLC C<sub>8</sub> Column were, on average,

about 20% narrower, measured at  $4\sigma$  peak width, yielding a 22% increase in peak capacity, and 20% increase in peak height, compared to the ACQUITY UPLC BEH C<sub>8</sub> Column. The chromatograms in Figure 1 compare the peak heights and widths of the small proteins separated on the ACQUITY UPLC BEH C<sub>8</sub> Column, 1.7  $\mu\text{m}$ , 2.1 x 50 mm (p/n 186002877) and CORTECS UPLC C<sub>8</sub> Column, 1.6  $\mu\text{m}$ , 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<sub>8</sub> Column. The combination of high performance instrumentation and extremely high column efficiency afforded by CORTECS UPLC C<sub>8</sub> Columns allows for fast, sensitive separation of small intact proteins.

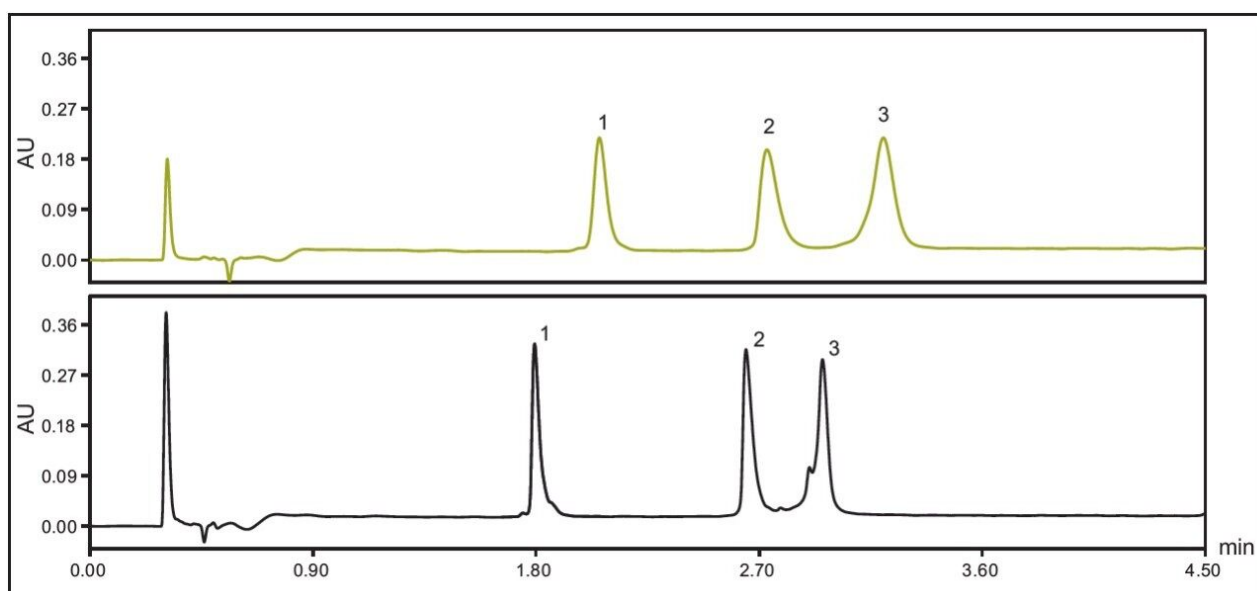


Figure 1. Separation of RNase A, cytochrome C, and lysozyme on 2.1 mm x 50 mm, ACQUITY UPLC BEH C<sub>8</sub> Column, 130 $\text{\AA}$ , 1.7  $\mu\text{m}$ , 2.1 mm x 50 mm (top) and CORTECS UPLC C<sub>8</sub> Column, 90 $\text{\AA}$  1.6  $\mu\text{m}$ , 2.1 mm x 50 mm.

## Conclusion

Narrow peak widths and detection sensitivity can be challenging to achieve in small protein separations. Compared to ACQUITY UPLC BEH C<sub>8</sub> 1.7  $\mu\text{m}$  Columns, markedly narrower peaks can be achieved on CORTECS UPLC C<sub>8</sub> 1.6  $\mu\text{m}$  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.

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## References

1. "Increasing Peak Capacity in Reversed-Phase Peptide Separations with Charged Surface Hybrid (CSH) C<sub>18</sub> Columns" Matthew A. Lauber, Stephan M. Koza, and Kenneth J. Fountain, Waters Application Note 720004568en.

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## Featured Products

[ACQUITY UPLC I-Class PLUS System <https://www.waters.com/134613317>](https://www.waters.com/134613317)

[ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>](https://www.waters.com/514228)

[Empower 3 Chromatography Data Software <https://www.waters.com/10190669>](https://www.waters.com/10190669)

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