

Development of a UHPLC column for proteins specifically antibody analysis.

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■ Introduction

We launched the FlexFire series in 2019 with the objective to achieve higher strength and purity in its silica. This product line provides greater versatility for UHPLC applications with a wider range of functional groups such as C18 and C30.

We recently developed a new type of FlexFire with wider pore (WP, 300 Å) C18, C8, C4, and C1 columns than previously developed, to analyze large-size proteins such as antibodies. They were designed to analyze the characterization of the first-dimensional structure of peptides in reverse phase condition. Using 1.6 μm or 2.6 μm particle sizes provides clearer data than conventional methods.

■ FlexFire Wide Pore vs. Conventional Wide Pore

We compared FlexFire Wide Pore and conventional wide pore with C4 columns (Fig. 1). Both showed relatively good peak shapes for the lower molecular weight but Bovine Serum Albumin gave poorly resolved peaks in the conventional column. This impaired peak could be caused by the impurity of the silica or unmasked silanol. FlexFire showed much better results.

It appeared that the higher molecular weight showed a more impaired peak (Fig. 2). A different brand's wide pore column presented a broadened peak for ovalbumin but FlexFire Wide Pore C4 and C1 showed a clear peak.

■ Application for Monoclonal Antibody

FlexFire Wide Pore can be applied to antibody analyses. Higher than 60°C produced peaks and 70°C presented sharp, clean peaks. This may indicate that the analyte is absorbed in the column but it did not show any difference inside a metal-free housing. Thus this may not be related to metal ion of housing but the packing material. The FlexFire is durable and usable up to 80°C, proving that a higher temperature has no negative effects on the column.

■ Conclusion

FlexFire Wide Pore presented better peak shapes for large proteins including antibodies in comparison to conventional columns. The column may be useful for the characterization of digested monoclonal antibodies as well. We will continue to report these applications.

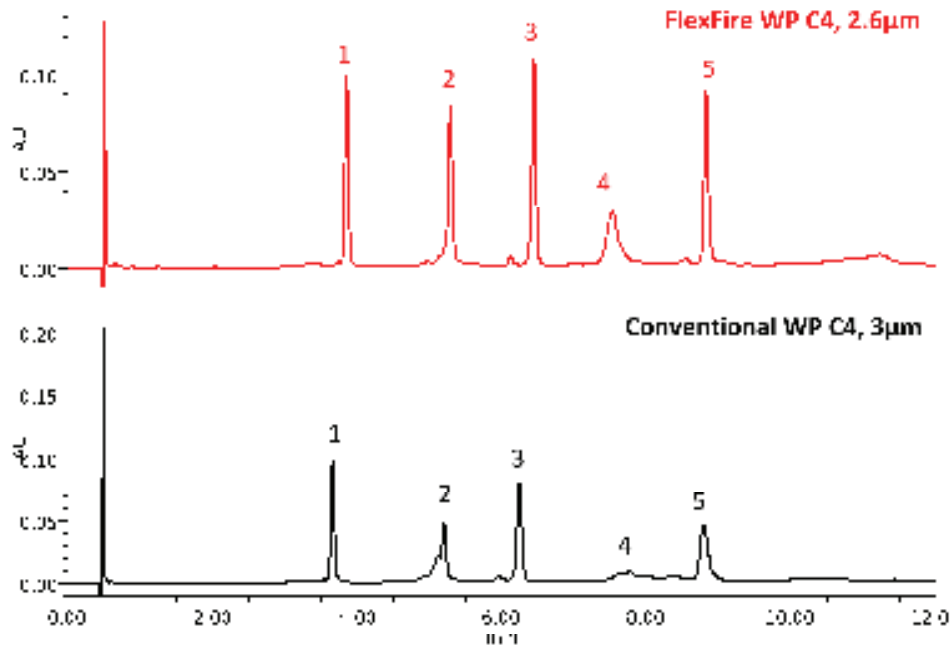


Fig. 1 Chromatograms of Large Molecule Analysis

Table 1. Analytical Conditions					
Column	Develosil FlexFire WP C4, 2.6 µm (2.0 x 50 mm) Conventional WP C4, 3 µm (2.0 x 50 mm)				
Mobile Phase	A) Water + 0.1%TFA B) Acetonitrile + 0.1%TFA				
Gradient	Time	mL/min	%A	%B	Curve
	0.00	0.3	80	20	
	12.60	0.3	40	60	6
	12.63	0.3	80	20	6
Temperature	40°C				
Detection	UV 220 nm				
Sample	1. Ribonuclease A (0.46 mg/mL) 2. Cytochrome C (0.59 mg/mL) 3. Lysozyme (0.30 mg/mL) 4. BSA (0.60 mg/mL) 5. Myoglobin (0.59 mg/mL)				
Injection Volume	1.0 µL				
System	Waters ACQUITY UPLC H-Class Plus_2				
Mixer Volume	100 µL				

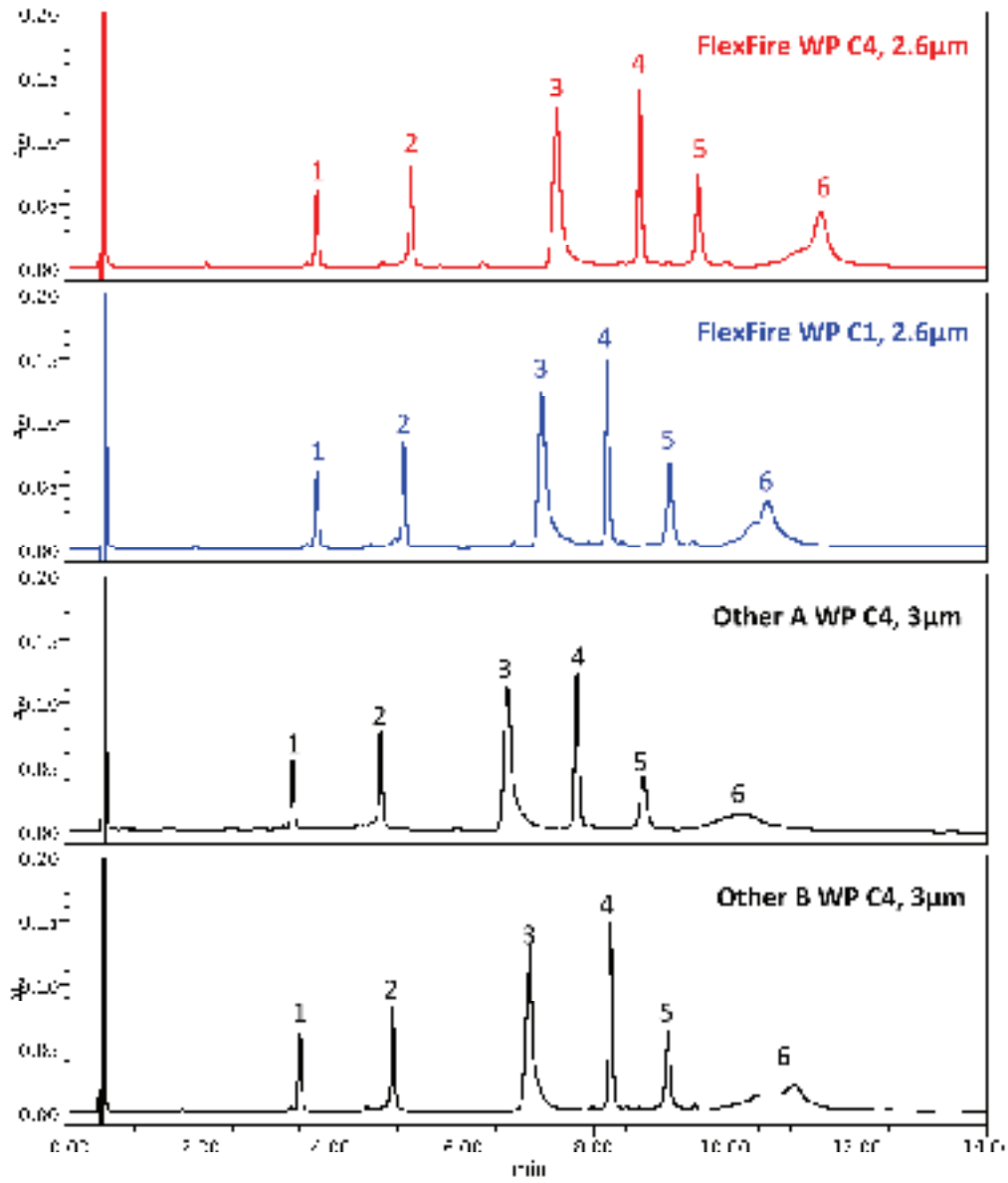


Fig. 2 In addition to Table 1, #6 peak is Ovalbumin (1.84 mg/mL)

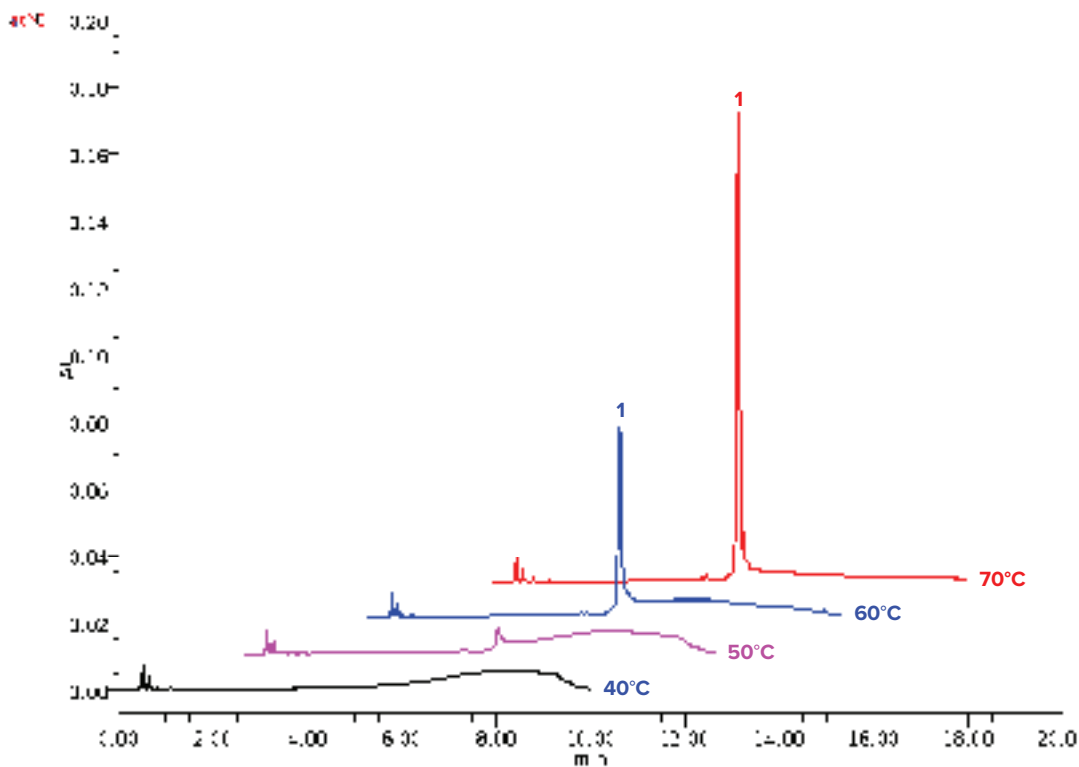


Fig.3

Table 2. Analytical Conditions

Column	Develosil FlexFire WP C4, 2.6 μ m (2.0 x 50 mm)				
Mobile Phase	A) Water + 0.1% TFA B) Acetonitrile + 0.1% TFA				
Gradient	Time	mL/min	%A	%B	Curve
	0.00	0.3	80	20	
	8.40	0.3	40	60	6
	8.42	0.3	80	20	6
Temperature	40°C, 50°C, 60°C, 70°C				
Detection	UV 280 nm				
Sample	Sample: 1. Intact Mouse IgG1 (5.0 mg/mL)				
Injection Volume	1.0 μ L				
System	Waters ACQUITY UPLC H-Class Plus_2				
Mixer Volume	100 μ L				

■ Contact Us



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