FlexFire
HPLC & UHPLC Columns

Selection of UHPLC columns for nucleic acid oligomer analysis

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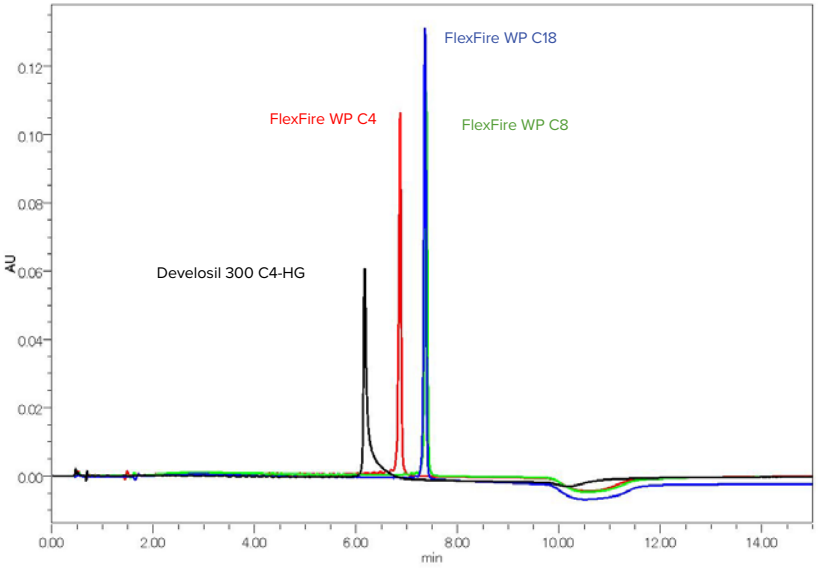
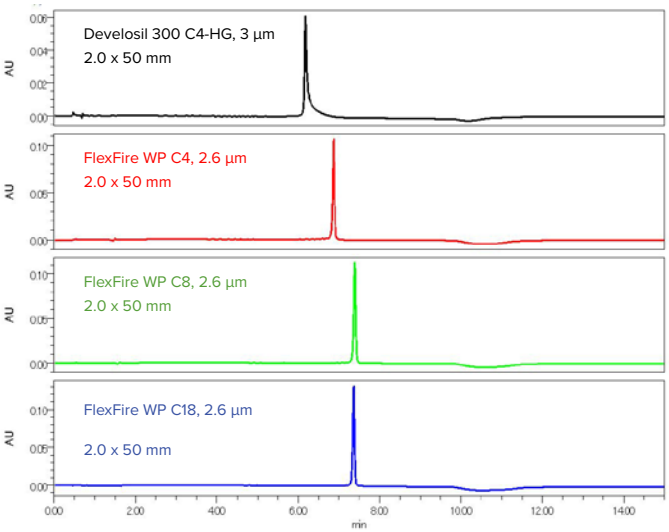
■ Introduction

Nucleic acid-based drugs are located between small molecule drugs and biopharmaceuticals, and synthetic oligonucleotides up to about 30-mer are the mainstream, and they are attracting a lot of attention as they expand in scale in the future.

We examined FlexFire columns and its analytical conditions using the synthesized miRNA samples. For this study, we applied inert UHPLC columns to prevent sample loss.

■ Comparison with conventional products

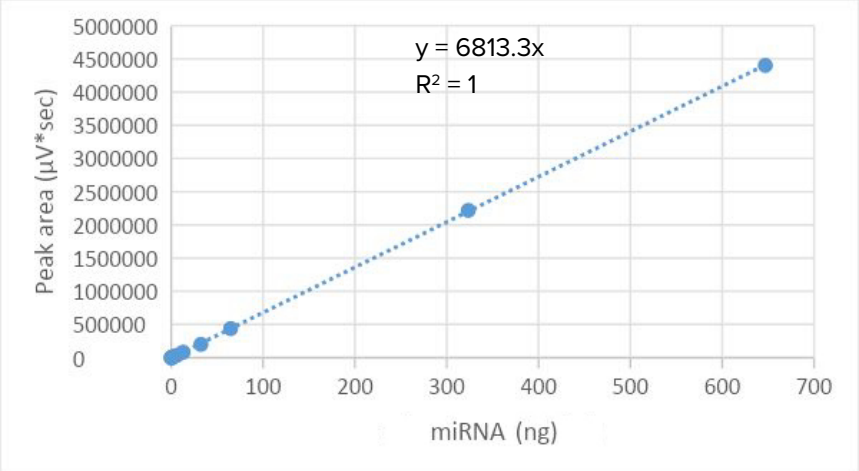


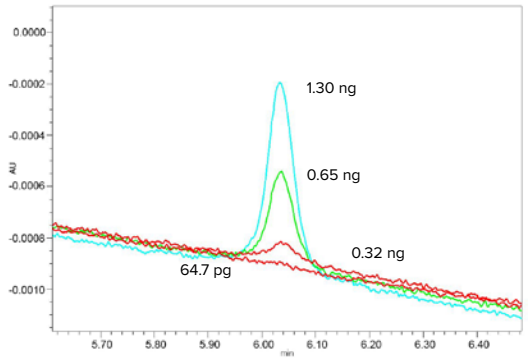


Analytical Conditions					
Column	Develosil 300 C4-HG, 3 µm (2.0 x 50 mm) FlexFire WP C4, 2.6 µm (2.0 x 50 mm) FlexFire WP C8, 2.6 µm (2.0 x 50 mm) FlexFire WP C18, 2.6 µm (2.0 x 50 mm)				
Mobile Phase	A) 100 mM HFIP + 10 mM TEA B) Methanol				
Gradient	min	mL/min	%A	%B	Curve
	0.00	0.3	95	5	
	8.60	0.3	80	20	6
	8.61	0.3	95	5	6
Detection	UV 260 nm				
Sample	40-mer miRNA ACACACACACACACACACACACACACACACACACACAC				
Injection Volume	1.0 µL				
System	Waters ACQUITY UPLC H-Class PLUS				
Mixer Volume	100 µL				

Synthesized 40-mer miRNA has been analyzed using both a conventional column and the FlexFire series with the new silica gel. The conventional Develosil 300 C4-HG showed peak tailing and less peak height. The FlexFire series has improved peak shapes and resulted in better sensitivity. High-quality packing materials with few impurities are essential for nucleic acid analysis.

■ Calibration curve of 40 mer miRNA

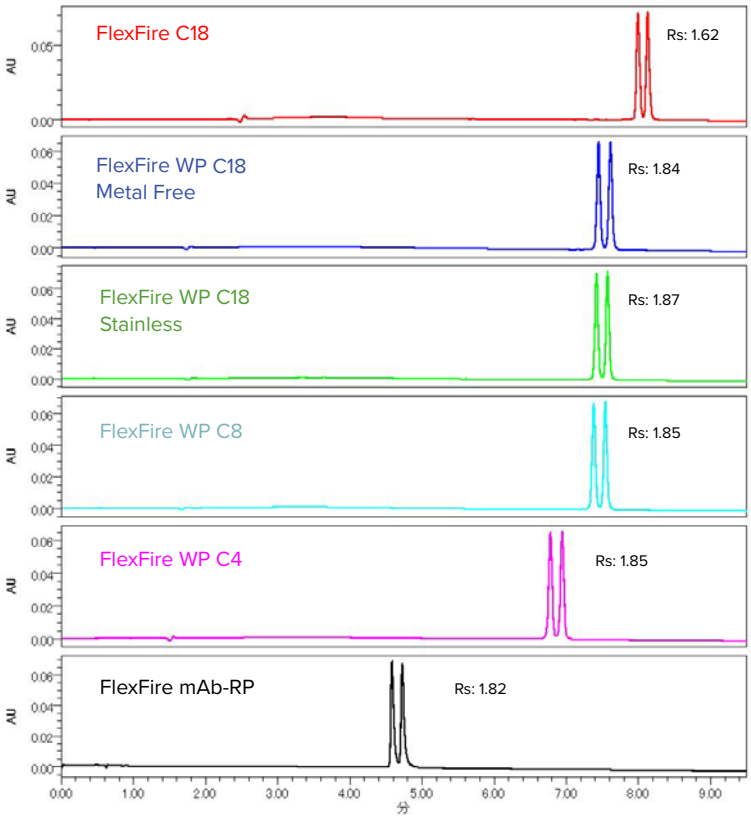




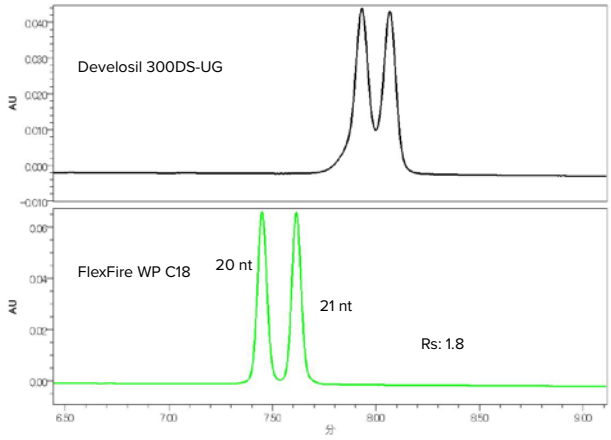
40-mer miRNA calibration on FlexFire WP C18

The calibration curve obtained by the UV detector presented a clean straight line in the range of 0.32-650 ng of miRNA. LC/MS should yield similar results at even lower concentrations.

■ Detection of 1-base difference in miRNA

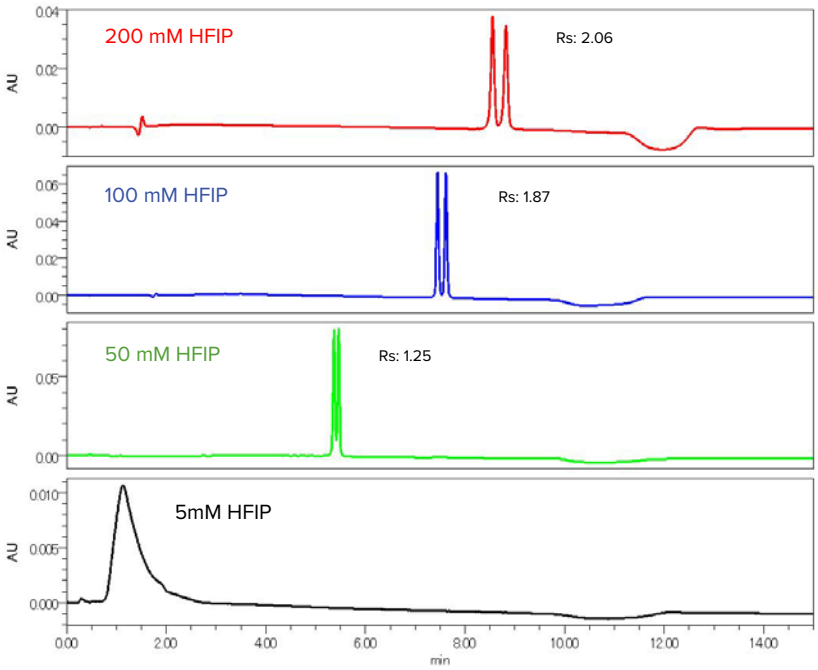


Resolution has been improved in the FlexFire wide pore columns, and the FlexFire Wide Pore (WP) C8 has the same retention as the FlexFire WP C18. The performance of these columns is dramatically improved compared to the conventional product.



Analytical Conditions	
Column	FlexFire C18, 2.6 µm (2.0 x 50 mm) FlexFire WP C18, 2.6 µm (2.0 x 50 mm) - Metal free FlexFire WP C18, 2.6 µm (2.0 x 50 mm) - Stainless FlexFire WP C8, 2.6 µm (2.0 x 50 mm) FlexFire WP C4, 2.6 µm (2.0 x 50 mm) FlexFire mAb-RP, 2.6 µm (2.0 x 50 mm)
Mobile Phase	A) 100 mM HFIP + 10 mM TEA B) Mobile Phase A/Methanol=50/50
Gradient	min 0.00 / 8.60 / 8.61 mL/min 0.3 / 0.3 / 0.3 %A 93 / 73 / 93 %B 7 / 27 / 7 Curve 6 / 6
Detection	UV 260 nm
Sample	20 nt (61.4 µg/mL) 21 nt (63.9 µg/mL)
Injection Volume	2.0 µL
System	Waters ACQUITY UPLC H-Class PLUS
Mixer Volume	100 µL

■ Effect of HFIP concentration



Analytical Conditions					
Column	FlexFire WP C18, 2.6 µm (2.0 x 50 mm)				
Mobile Phase	A) 200 mM HFIP + 20 mM TEA A) 100 mM HFIP + 10 mM TEA A) 50 mM HFIP + 5 mM TEA A) 5 mM HFIP + 0.5 mM TEA B) Mobile Phase A/MeOH+50/50				
Gradient	min	mL/min	%A	%B	Curve
	0.00	0.3	93	7	
	8.60	0.3	73	27	6
	8.61	0.3	93	7	6
Temperature	60°C				
Sample	1. 5'-GUACGCGGAAUACUUCGAdTdT-3' (20 nt) 2. 5'-CGUACGCGGAAUACUUCGAdTdT-3" (21 nt)				
Injection Volume	1.0 µL				
System	Waters ACQUITY UPLC H-Class PLUS				
Mixer Volume	100 µL				

HFIP concentration and retention.

HFIP concentration was altered while the ratio of [HFIP] : [Ion Pair (base)] was kept at 10:1. At 5 mM HFIP, the peak appeared in the front peak. At 50 mM HFIP, a sharp peak was detected. However, the peaks of 20 nt and 21 nt tended to overlap slightly.

Increasing HFIP concentration to 100 mM showed good separation. At 200 mM, the separation power was better but the effect of HFIP seemed to have plateaued. We recommend using 100 mM HFIP as it shows good separation performance while minimizing damage to the column.

■ Conclusion

Important points when selecting a column for the analysis of nucleic acid oligomers are:

- Using a wide pore column
- Use a silica gel substrate with very low impurities

In addition to HFIP, TEAA (triethylammonium acetate) and ion pair concentrations affect the separation. When using LC/MS, the mobile phase constituents can affect both the sensitivity and pollution of the detector.

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