Develosil®

Develosil UHPLC C18 and C30 for Oligonucleotide Analysis

Reverse phase HPLC is common for analysis of small to midsize molecules, but careful experimental design and selection of columns are both required for successful separation.

Here we applied our newly developed UHPLC C18 and C30 columns to oligonucleotide analysis. This new type of column is designed and optimized for the rapid separation of small to midsize molecules, utilizing 1.6 µm diameter particles with 11 nm diameter pores to achieve higher resolution. Theoretically, this pore size should allow separation of molecules of up to 25,000 kDa with this column.

■ Experimental Condition

Table 1 shows the analytical conditions. Table 2 shows the specifications for Develosil 1.6 µm UHPLC C18 and C30 columns. For this series of columns, C18 has a higher density of alkyl chains on the silica gel surface than C30. This results in the C18 columns having higher retention of hydrophobic compounds. The lower density of alkyl chains in the C30 column allows the mobile phase more access to the endcapped silica, which can influence the separation of hydrophilic compounds. Because of this, modulation of the pH and organic solvent content of the mobile phase can result in larger changes in retention of hydrophilic species such as oligonucleotides on C30 when compared to C18

Table 1. Analytical Conditions

Column	Develosil UHPLC C18, 1.6 µm (2.0 x 50 mm)
Mobile Phase	A) 15 mM TEA + 400 mM HFIP, pH ≈ 8.0 B) Mobile phase A / Methanol = 50/50
Gradient	min / mL/min / %B / Curve 0.0 / 0.2 / 40 / linear 10.00 / 0.2 / 50 / linear 15.0 / 0.2 / 50 / linear 15.1 / 0.2 / 40 / linear
Temperature	50°C
Detection	UV 260 nm
Sample	Oligonucleotide Ladder Standard (15, 20, 25, 30, 35 and 40-mer)
Injection Volume	1.0 µL
System	Thermo Fisher SCIENTIFIC Vanquish_H
Mixer Volume	10 µL

Table 2. Develosil UHPLC Series Specifications

Column	**UHPLC C30**	**UHPLC C18**
Functional Group	Triacontyl	Octadecyl
Particle Size	1.6 µm	
Surface Area	340 m2/g	
Pore Volume	1.0 mL/g	
Pore Diameter	11 nm	
Carbon Load	13%	17%
Endcapping	Yes	
pH Range	pH 1-9	
Temperature Range	Max 60°C	
Maximum Pressure	80 MPa (11,600 psi)	

■ Results and Discussion

Figure 1 shows an oligodeoxythymidine ladder standard mixture (DNA ladder standard, oligos at 15, 20, 25, 30, 35 and 40-mer) separated using Develosil UHPLC C18 1.6 µm. The sample has higher concentrations at each 5-mer increment from 15-mer and up. This chromatogram shows each additional individual nucleotide length is a clearly separated peak.

We then applied Develosil UHPLC C30 1.6 µm to this analysis (Fig. 2). Compared to C18, the C30 column retains nucleotides more strongly, leading to longer retention times under the same conditions. Increasing the final concentration of mobile phase B by 0.5% results in similar retention times to that of C18 column. During the process of optimizing the mobile phase, we also found that small differences in mobile phase composition will affect retention time on C30 much more so than on C18.

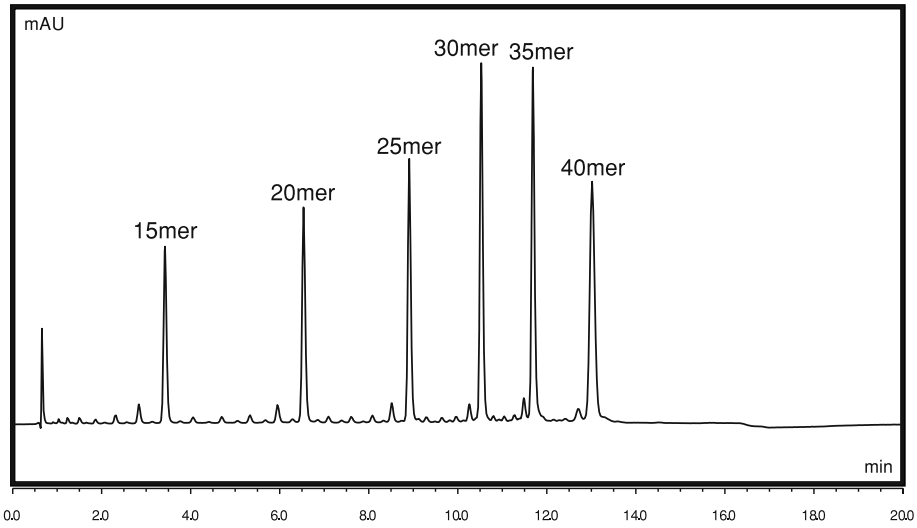

Figure 1. UHPLC C18 column separation of oligodeoxythymidine mixture.

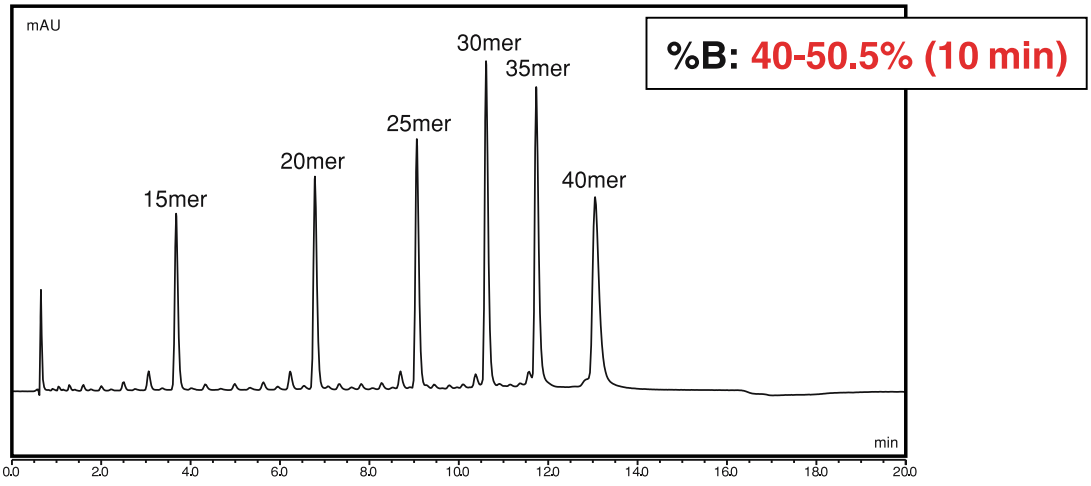

Figure 2. UHPLC C30 column separation of oligodeoxythymidine mixture.

■ Conclusion

Using the 1.6 µm particle of Develosil UHPLC C18 and C30 showed clear and sharp peaks of oligonucleotides up to 40-mers. The resolution was higher on C30. This indicates that choosing different column lengths or mobile phase conditions can enable analysis of 40nt or even longer oligonucleotides.

■ Order Information

Develosil UHPLC 1.6 µm Series

Size	C30	C18	C8	C1	HILIC
2.0 x 35 mm	201-I20035W	202-I20035W	203-I20035W	204-I20035W	205-I20035W
2.0 x 50 mm	201-I20050W	202-I20050W	203-I20050W	204-I20050W	205-I20050W
2.0 x 75 mm	201-I20075W	202-I20075W	203-I20075W	204-I20075W	205-I20075W
2.0 x 100 mm	201-I20100W	202-I20100W	203-I20100W	204-I20100W	205-I20100W
2.0 x 150 mm	201-I20150W	202-I20150W	203-I20150W	204-I20150W	205-I20150W

Develosil UHPLC 1.6 µm Metal-free Series

Size	C30	C18	C8	C1	HILIC
2.0 x 35 mm	201-I20035MFW	202-I20035MFW	203-I20035MFW	204-I20035MFW	205-I20035MFW
2.0 x 50 mm	201-I20050MFW	202-I20050MFW	203-I20050MFW	204-I20050MFW	205-I20050MFW
2.0 x 75 mm	201-I20075MFW	202-I20075MFW	203-I20075MFW	204-I20075MFW	205-I20075MFW
2.0 x 100 mm	201-I20100MFW	202-I20100MFW	203-I20100MFW	204-I20100MFW	205-I20100MFW
2.0 x 150 mm	201-I20150MFW	202-I20150MFW	203-I20150MFW	204-I20150MFW	205-I20150MFW

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