Application NOTE

Waters

RP-HPLC Method For Quality Control Of Synthetic Oligonucleotides Using XTerra® MS C_{18} Columns

Oligonucleotide synthesis yield

Although state-of-the-ort oligonucleotide synthesis is a fast and reliable process, the yield of the target product is limited. The full-length oligonucleotide (N) is typically contaminated with shorter failure products called N-1, N-2, N-3… Oligonucleotide purity decreases with the complexity of synthesis. The typical purity of crude 25mer product is ~ 75 % while the purity of a 60mer product is ~ 20 %. Occasionally the synthesis or post synthesis deprotection result in a product of inferior purity, which may compromise quality of the polymerase chain reaction (PCR) or other molecular biology assays.

XTerra® columns for efficient oligonucleotide separations

XTerra® MS C_{18} columns are packed with porous 2.5 µm, hybrid particles. The sorbent has extended stability at temperatures and pH's typically used for oligonucleotide separations (50-60° C; pH 7-9). The column yields high resolution efficiency required for successful analysis of oligonucleotides ***(Figure 2***). Volatile ion-pairing mobile phases are utilized to separate oligonucleotides according to their length. The resolution performance rivals that seen with capillary gel electrophoresis ***(Figure 1).*** With XTerra® MS C_{18} columns, baseline resolution of N from N-1 oligonucleotides is typically achieved for <30mer. Good resolution can be achieved for larger oligonucleotides (e.g. 60mer or larger: see ***(Figures 2 & 3)).***

HPLC system:	Alliance® 2690 Separations Module
Column:	XTerra® MS C_{18}, 2.5 µm, 4.6 x 50 mm
Part Number:	18600602
Flow rate:	0.5 ml/min
Solvent A:	10 % MeOH in 16.3 mM TEA, 400 mM HFIP buffer, pH 7.9
Solvent B:	40 % MeOH in 16.3 mM TEA, 400 mM HFIP buffer, pH 7.9
Gradient:	0-10 min from16.7 to 30 % B, 10-30 min from 30 % to 47.2 % B
Injection volume:	5 µl
Column temp.:	60 °C
Detection:	UV @ 260 nm

CGE system:	Waters Quanta® 4000 CE
Column:	BioRad BioCAP™ 75 µm, 34.5 cm
Separation:	15 kV
Sieving matrix:	PEG 35 kDa, 2 g/10 ml 0.1mM Tris-Boric acid, pH 8.3
Injection:	4 s @ 5kV
Column temp.:	30 °C
Detection:	UV @ 254 nm

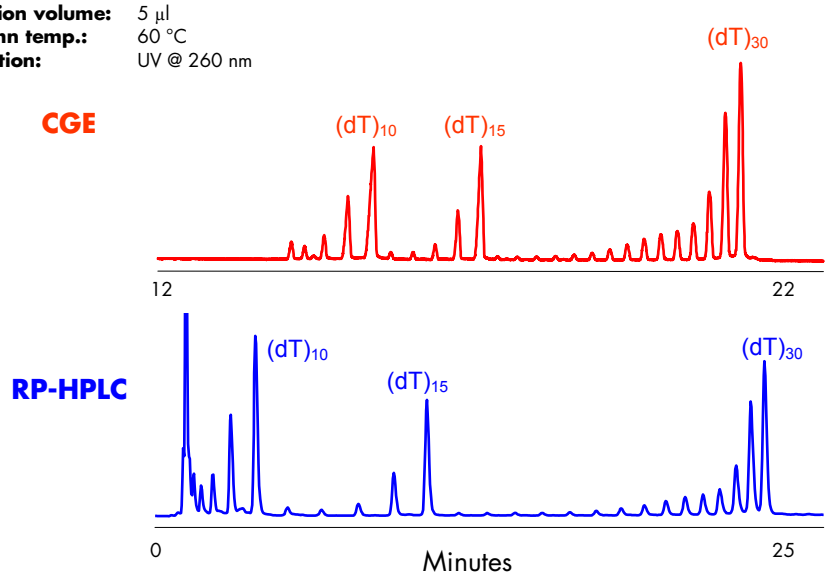


Figure 1. Separation of 6-30mer oligodeoxythymidines by CGE and RP-HPLC using XTerra® MS C_{18}

HPLC system:	Alliance® 2690 Separations Module	**Gradient:**	From 9.3 % B to 44 % B in 20.8 min
Column:	XTerra®MS C_{18}, 2.5 µm, 4.6 x 50 mm	**Injection volume:**	5 µl (1 nmole of oligonucleotide)
Part Number	186000602	**Column temp.:**	60 °C
Flow rate:	1 ml/min	**Detection:**	UV @ 260 nm
Solvent A:	5 % ACN in 0.1 M TEAA, pH 7		
Solvent B:	20 % ACN in 0.1 M TEAA, pH 7		

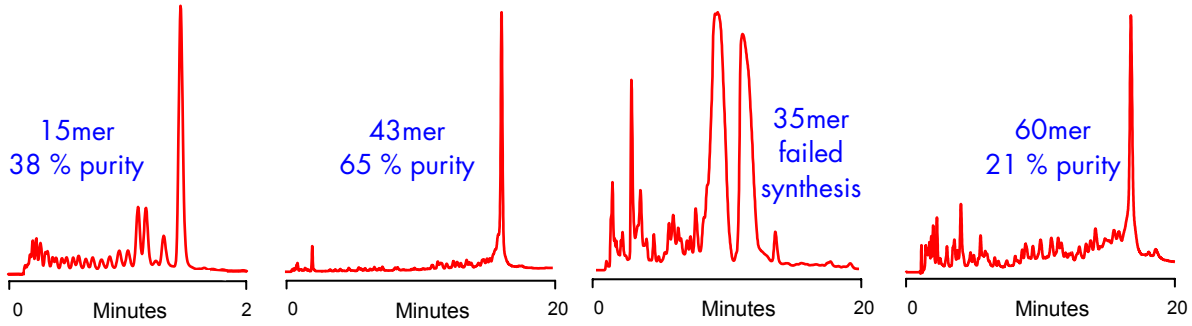
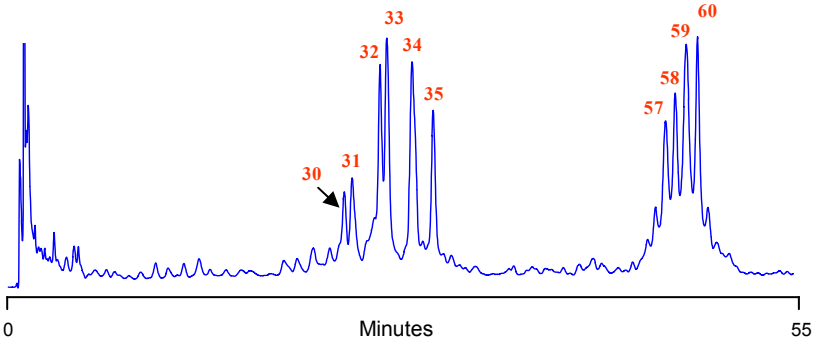


Figure 2. RP-HPLC quality control of synthetic oligonucleotides. Purity was measured as the % of peak area at $UV_{260\ nm}$.

Triethylammonium acetate (TEAA) is an inexpensive volatile ion-pairing agent used for routine oligonucleotide analysis ***(Figure 2)*** and purification. Alternatively, triethylammonium-hexafluoroisopropanol buffer (TEA-HFIP) can be used for more efficient separation of long oligonucleotides (>45mer, ***Figure 3***), and for applications using mass spectrometry detection.

Preparation of 0.1 M TEAA buffer: Dissolve 5.6 mm glacial acetic acid in ~950 ml of water. While mixing add 13.86 ml of TEA. Adjust pH with diluted acetic acid to ~ 7 and adjust volume to 1L with water.
Preparation of 16.3 mM TEA - 400 mM HFIP buffer: Dissolve 42.1 ml of HFIP in ~950 ml of water. While mixing vigorously add 2.28 ml of TEA. Adjust volume to 1L with water**.** The pH of solution should be close to 7.9.



HPLC system:	Waters CapLC® System
Column:	XTerra®MS C_{18}, 2.5 µm, 1.0 x 50 mm
Part Number	186000979
Flow rate:	23.6 µl/min
Solvent A:	16.3 mM TEA, 400 mM HFIP aqueous buffer, pH 7.9
Solvent B:	MeOH
Gradient:	0-55 minutes, liner gradient 21– 26.5 % B
Injection volume:	0.5 µl
Column temp.:	50 °C
Detection:	UV @ 260 nm

Figure 3. RP-HPLC separation of 30-35mer and 57-60mer heterooligonucleotides.

WATERS CORPORATION
34 Maple St.
Milford, MA 01757 U.S.A.
T: 508 478 2000
F: 508 872 1990
www.waters.com