Chromatography Solutions

Technical note #020

Ion-Pair Reversed-Phase Analysis of an Oligonucleotide Ladder Standard

INTRODUCTION

Oligonucleotides are short lengths of DNA/RNA which are increasingly being used as therapeutic agents for the treatment of genetic disorders and cancers.

With oligonucleotide treatments, it is possible to synthesise a single-strand of DNA/RNA which has a complementary sequence to a disease causing gene. Once this therapeutic DNA sequence has bound to the target gene in the body, the gene is deactivated. This method of treatment is known as antisense therapy.

Currently, over 100 oligonucleotide-based therapies are in the clinical pipeline with many more in the pre-clinical development stage.

Several impurities can be produced during oligonucleotide synthesis (e.g. failure sequences), due to a less than 100% efficient process, and these need to be removed from the desired product. This purification process can be performed using ion-pair reversed-phase HPLC (RPLC).

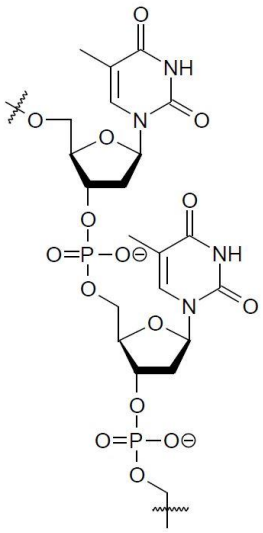

Figure 1: Structure of thymidine oligonucleotide

Avantor® ACE®

The use of a hydrophobic, positively-charged ion-pair reagent allows the highly negatively-charged oligonucleotides to interact with the hydrophobic stationary phase employed in RPLC. The target oligonucleotides can then be separated from chemically modified by-products by hydrophobicity.

Use of high temperatures are common for these types of analyses in order to temporarily destroy the secondary structure of the oligonucleotides which improves peak shape of longer length oligomers and improves resolution.[1]

Figure 2 shows a method for the separation of an oligonucleotide ladder standard comprising of six thymidine monophosphate polymers of varying length (15 – 40 nucleotides, see general structure in figure 1).

This separation has been carried out on the novel ACE Oligo phase, with gradient elution using TEAA as an ion-pair reagent.

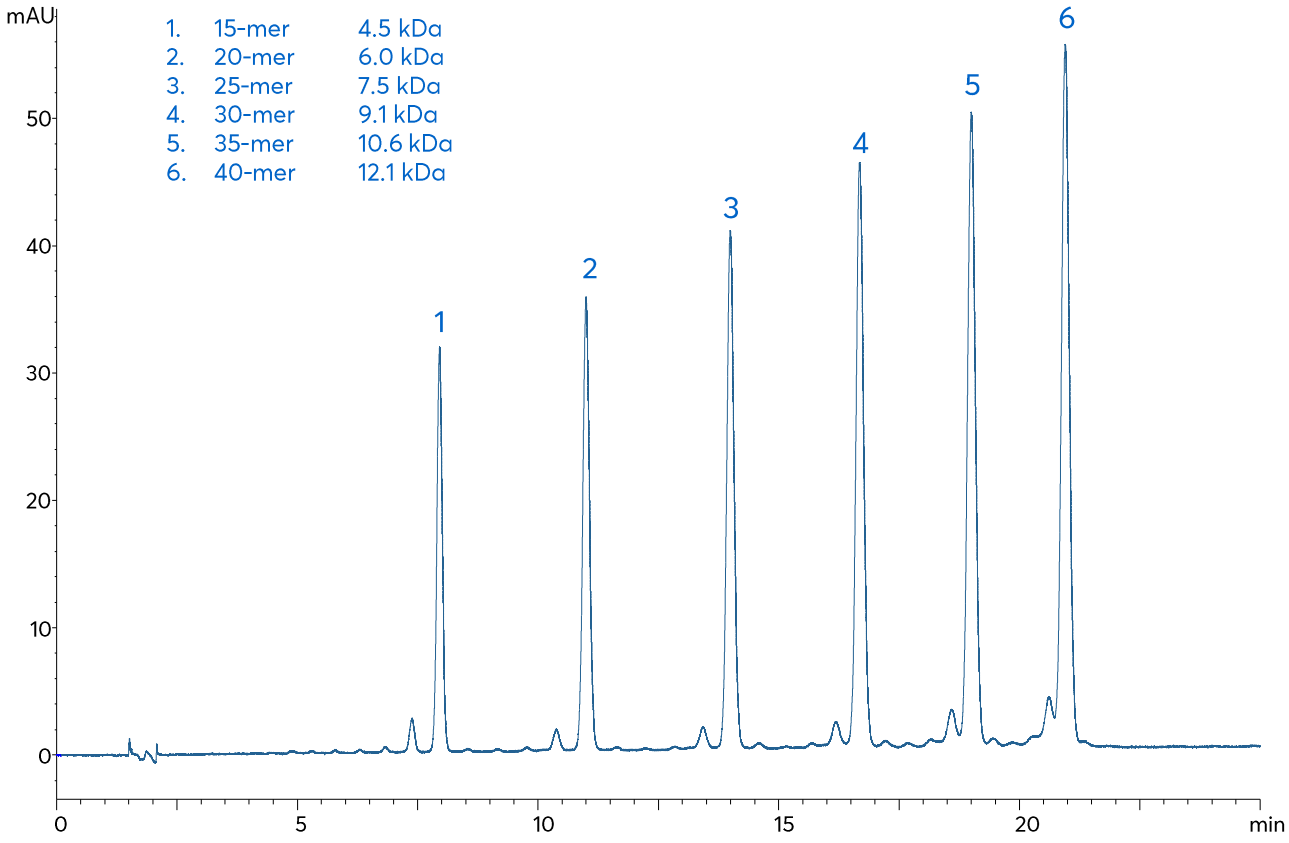

Figure 2: Separation of thymidine oligomers

[1] Brown T, Brown T (Jnr), Nucleic Acids Book, ATDBio Ltd,

Method Details

CONDITIONS

Column: ACE Excel 3 Oligo

Dimensions: 150 x 4.6 mm

Mobile Phases: A: 80 mM triethylammonium acetate in H_2O (pH 7.0)

B: 80 mM triethylammonium acetate in MeCN

Gradient:

Time (mins)	%B
0	12
30	15
36	15
36.5	12
66.5	12

System Dwell Volume: 550 µL

Flow Rate: 1.0 mL/min

Injection: 20 µL

Temperature: 60 °C

Detection: UV, 260 nm

ORDERING TABLE

Product	Details	Size	Part number
ACE Excel 3 Oligo	UHPLC column	150 x 4.6 mm	EXL-1115-1546
Acetonitrile	VWR CHROMANORM® gradient grade	2.5 L	83639.320
Water	VWR CHROMANORM® gradient grade	2.5 L	83650.320
Triethylamine	VWR CHROMANORM® for HPLC	100 mL	28757.184
Acetic acid	VWR CHROMANORM® for HPLC	1 L	87023.290
UHPLC guard cartridge	For 4.6 mm i.d. columns	3 pk	EXL-1P15-GD4U
UHPLC guard holder	Required for use of UHPLC guard cartridges	1	H0011