TOSOH

Application Note

Optimization parameters for anion exchange analysis of oligonucleotides

The development of oligonucleotide therapeutics is on the rise since the first approval in 1998. The 15-25 nucleotide long single or double-strands are designed to interact with a specific RNA sequence and thus modulate mRNA splicing or stability[1]. In order to do so, the sequence and length of oligonucleotide therapeutics are essential. However, due to errors in the oligonucleotide synthesis process, nucleotides may either be missing (N-x) or are attached in excess (N+x). The addition or lack of a nucleotide goes along with an additional or missing phosphate/phosphorothioate changing the overall negative charge of the molecule, which makes anion-exchange a method to analyze N+/-x impurities.

This application note demonstrates how to optimize a (U)HPLC method for oligonucleotide analysis by modifying the buffer composition and temperature. This helps adjusting the method to the need of individual oligonucleotides and results in a universal method serving as a starting point for in-depth method optimization. A single-stranded oligonucleotide was employed as an exemplary sample and optimization was performed on a TSKgel® DNA-NPR anion exchange column. With its small, non-porous particles with a weak anion exchange ligand, it is well suited for fast nucleic acid analysis.

Material and Methods

Column:	TSKgel DNA-NPR (4.6 mm ID x 7.5 cm L)
UHPLC:	Thermofisher Dionex Ultimate 3000 UHPLC system
Mobile phase:	A: 10 mM NaOH pH 12 or 20 mM Tris-HCl pH 8 as indicated B: 10 mM NaOH pH 12, 2 M NaCl or 0.8 M NaBr as indicated
Gradient:	0 min (0% B) 3 min (0% B) 16 min (100% B)
Flow rate:	0.5 mL/min
Detection:	UV @ 260nm
Injection vol.:	5 µL
Temperature:	25 ° C if not stated differently
Sample:	ssDNA full phosphorothioate

Results

The separation of an oligonucleotide sample was tested at pH 8 and 12 (*Figure 1*). At the more basic pH of 12, the major product as well as shorter impurities (eluting earlier) and longer ones (eluting later) were separated. In contrast, these sample components were hardly separated at lower pH. This is explained by reduced ionization of the oligonucleotide at the given pH of 8. Further experiments were, hence, conducted at pH 12.

Figure 2 shows the analysis of an oligonucleotide at room temperature (25 °C) and 60 °C. For both conditions, the major product, shortmers, and longmers were separated. No difference in retention was observed for the analyzed oligonucleotide.

Figure 1. Influence of pH on the analysis of oligonucleotides by AEX.

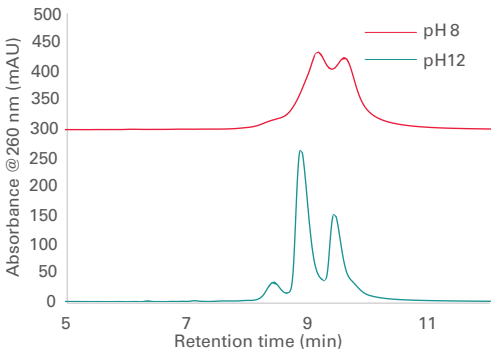

Figure 2. Influence of the temperature on the characterization of oligonucleotides by AEX.

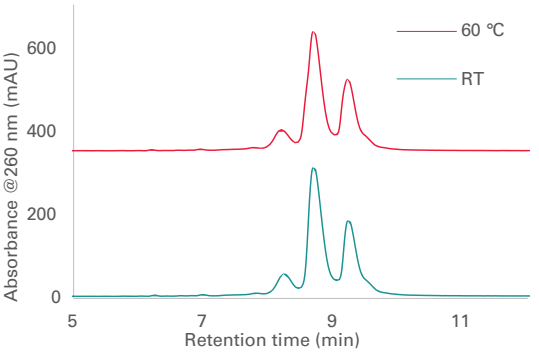

Figure 3. Influence of the type of salt on analysis of oligonucleotides by AEX.