Oligonucleotides—analysis of antisense therapeutics

Application Note

Nucleic Acid Analysis

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Abstract

Advances in biopolymer therapeutics have resulted in the development of antisense oligonucleotides to act as anti-viral and anti-cancer drugs. These molecules attach to RNA in the cell cytoplasm preventing either translation of cellular mRNA into protein or the reverse transcription of viral RNA to DNA. Such oligonucleotides must therefore be determined for purity testing of drug products and therapeutic drug monitoring. Oligonucleotides cannot be successfully separated according to size by conventional CZE since they have similar mobilities independent of their chain length. However, using a sieving polymer filled capillary such analytes can be separated according to their chain length.

Antisense oligonucleotides can be separated using the Agilent Capillary Electrophoresis system. The sieving polymer solutions A and B have been optimized for separating oligonucleotides with a chain length of 5 to 80 nucleotides. Phosphodiester antisense oligonucleotides have an optimal activity with chain lengths greater than 17–mer.

Chromatographic conditions

Buffer:	200 mM 2,2 Bis-(hydroxyethyl)-(iminotris)-(hydroxymethyl) methane (BisTris), pH 7.2 200 mM Boric Acid
Sieving Solution:	Polymer
Capillary:	PVA coated 24.5 cm eff x 100 µm id (G1600-60419)
Flush Regimen:	High pressure flush from outlet -7.5 bar for 3 min
Injection:	7 sec at -10 kV
Run:	-25 kV, 30 °C
Detection:	260 nm/8 nm DNA filter (G1600-62700)
Sample:	Antisense pd(T) 6-025

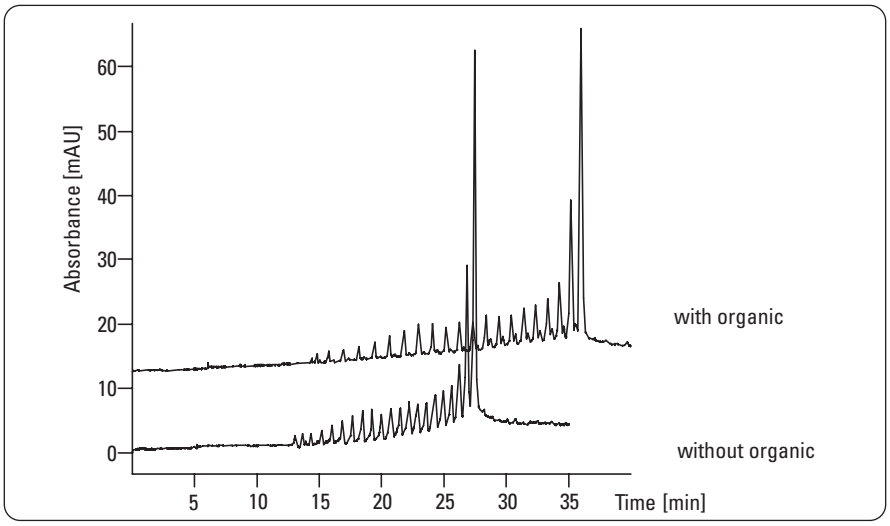

Figure 1
Improved resolution of antisense DNA by including organic modifier in sieving polymer.

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Experimental

All experiments were performed using the Agilent Capillary Electrophoresis system equipped with diode array detection and Agilent ChemStation software.

Figure 1 shows the separation of an antisense oligonucleotide standard pd(A) 6–25 indicating the enhanced resolution obtained using polymer solution B with organic additive when separating these phosphodiester backboned polymers. A reproducibility of migration time at 0.17 % RSD and of the reported area (1.52 % RSD) using hydrodynamic injection was obtained.

Equipment

- Agilent Capillary Electrophoresis system
- Agilent high-sensitivity detection cell
- Agilent ChemStation

Conclusion

The polymer solution B with added organic modifier is suitable for the analysis of phosphodiester antisense oligonucleotides. Migration time reproducibility can be achieved at less than 1 % RSD and even with hydrodynamic injection acceptable reproducibility of reported peak area can be achieved.

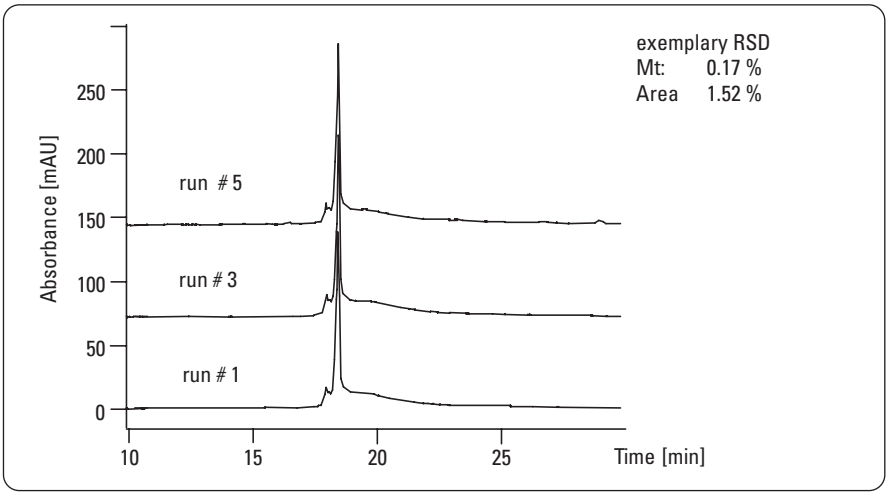

Figure 2
Quantitative reproducibility—hydrodynamic injection.

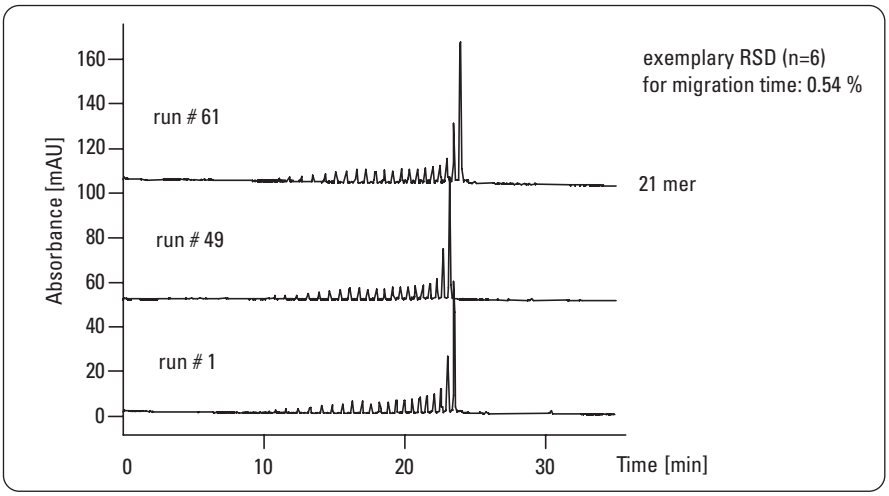

Figure 3
Reproducibility of migration time—polymer solution including organic additive.

Chromatographic conditions (figures 2, 3)

Buffer:	200 mM 2,2 Bis-(hydroxyethyl)-(iminotris)-(hydroxymethyl) methane (BisTris), pH 7.2 200 mM Boric Acid
Sieving Solution:	Polymeric solution B: 27 (w/v)% Polyethylenglykol 35000 (PEG) in 200 mM BisTris buffer solution, 20 (w/v)% acetonitrile
Capillary:	PVA coated 24.5 cm eff x; 100 µm id
Flush Regimen:	-7.5 bar for 3 min
Injection:	6 sec at 1 bar
Run:	-25 kV, 30 °C
Detection:	260 nm/8 nm DNA filter
Sample:	Antisense oligonucleotide 21 mer

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