Ultra-Fast RP UHPLC separation of DNA-Oligonucleotides using Evosphere MAX C18/AR without Ion-Pair reagents

Introduction

There is a growing need to separate and identify short nucleic acid fragments, such as oligonucleotides, especially therapeutic ones. This necessity arises from the expanding field of nucleic acid-based therapeutics and the critical need for ensuring the purity and quality of these compounds for safe and effective use in biomedical applications.

Experimental analysis:

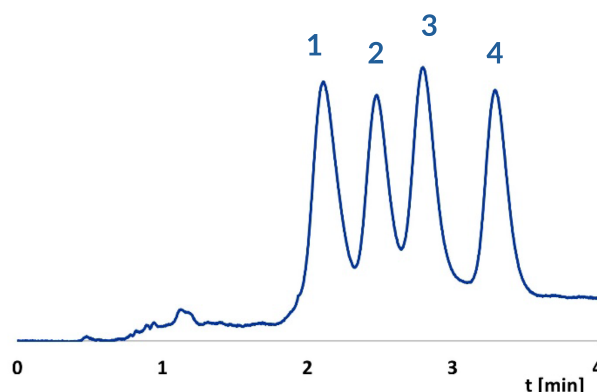
This research aims to develop a simple and efficient chromatographic method without ion-pair reagents for the separation of oligonucleotides with varying motif and sequence composition while maintaining the general size and length of the oligonucleotides.

This comprehensive approach allows for a thorough exploration of the chromatographic separation capabilities across various oligonucleotide sequences, ensuring the method's applicability to a wide array of therapeutic candidates. The alteration or substitution of one nucleotide in the sequence mimics synthetic impurities; consequently, oligonucleotides used in the study are an important class of possible impurities.

A higher resolution was observed for the mid-chain nucleotide exchange than for the difference on the 3' terminal nucleotide only. This is most likely due to the effect of the intrachain nucleotide on the shape of the molecule in solution.

Nevertheless, the resolution obtained in both cases is exceptional for a RP UHPLC system without the use of ion pair reagents.

1. **5' ATCGATCGATCGATCGATCG 3' (MW 6117 Da)**
2. **5' ATCGATCGATCGATCGATCT 3' (MW 6092 Da)**
3. **5' ATCGATCGATCGATCGATCC 3' (MW 6077 Da)**
4. **5' ATCGATCGATCGATCGATCA 3' (MW 6101 Da)**



In this technical note we have shown a robust LC-UV method for separation of oligonucleotides with varying motif and sequence composition using the Evosphere MAX C18/AR stationary phase.

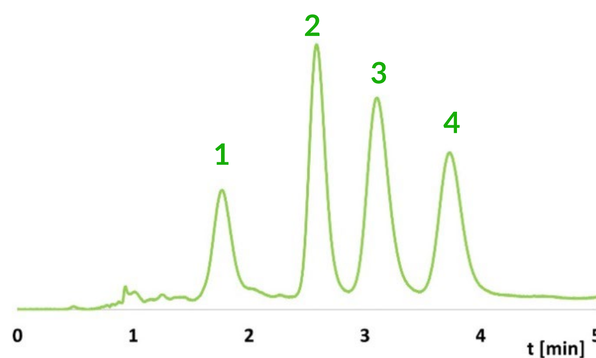
The separation of four-compound mixtures in 5 minutes by UHPLC in reversed-phase mode was achieved without the need of typical ion-pair reagent mixtures (i.e. TEA/HFIP, DIPEA/HFIP) usage.

The Evosphere MAX C18/AR column provided the separation of oligonucleotides differing in the position or type of one base in the sequence (with a minor difference in the molecular weight). It was demonstrated that the method is well suited for separating unmodified oligonucleotides, which is extremely important for purity determination or monitoring the efficiency of chemical synthesis for therapeutic candidates.

Conclusion

In summary, the study addresses the pressing need for advanced chromatographic methods capable of separating and identifying short nucleic acid fragments, particularly therapeutic oligonucleotides. Through systematic evaluation of oligonucleotides with diverse motifs and sequence compositions, as well as the incorporation of sequence-mimicking impurities, using the C18Ar column it is possible to enhance the analytical tools available for ensuring the quality and safety of nucleic acid-based therapeutics.

1. **5' ATCGATCGAACGATCGATCG 3' (MW 6126 Da)**
2. **5' ATCGATCGATAGATCGATCG 3' (MW 6141 Da)**
3. **5' ATCGATCGATCGATCGAACG 3' (MW 6126 Da)**
4. **5' ATCGATCGATCGATCGATCA 3' (MW 6101 Da)**



Experimental Conditions:

Column:	Evosphere MAX C18/AR, 100 Å, 1.7µm, 2.1 x 100 mm
PN#:	EVO18AR020501M
Mobile phase A:	25mM Ammonium Acetate (pH 6)
Mobile phase B:	MeOH
Gradient Program:	**Time [min]** **% B**
	0.0 12
	10.0 20
F **Flow rate**	0.3 mL/min
Temperature:	60 °C
UV-Vis Detection, λ:	260 nm
Injection Volume:	1 µl
LC system:	Thermo Scientific™ Vanquish™ Horizon UHPLC

Separation of DNA-Oligonucleotides Without Ion-Pair Reagents

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