Separation of RNA-Oligonucleotides Without Ion-Pair Reagents

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

Introduction

Ribonucleotide synthesis, like any chemical process, can introduce impurities due to various factors inherent in the synthesis method and the nature of the reagents involved. To mitigate the presence of impurities in synthesized ribonucleotides, rigorous purification methods have to be applied, such as Ultra High-Performance Liquid Chromatography (UHPLC), gel filtration, or solid-phase extraction, to isolate and obtain high-purity products suitable for downstream applications. Separating oligonucleotide impurities is crucial in analytical and preparative chromatography to obtain pure nucleic acid samples.

Ion-pair reagents and inorganic salts are commonly used in RP-UHPLC to enhance the separation of nucleic acids. However, their presence complicates downstream applications, increase instrument maintenance requirements, requires dedicated instrumentation, and reduces the MS sensitivity due to ion suppression. Hence, MAC-MOD in conjunction with Nicolaus Copernicus University – Team of Biomolecules Chromatography group has developed an application that does not need ion-pair reagents to analyze and purify RNA based oligonucleotides and there process impurities and intermediates.

Experimental analysis:

This research aimed to develop a simple and efficient ion-pair reagent-free chromatographic method for the separation of ribooligonucleotide impurities.

Experimental Conditions:

Column:	Evosphere MAX C18/AR, 100 Å, 1.7 µm, 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	2
	30.0	8
Flow rate	0.3 mL/min	
Temperature:	60 °C	
UV-Vis detection, λ:	260 nm	
Injection volume:	6 µl	
LC system:	Thermo Scientific™ Vanquish™ Horizon UHPLC	

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In this technical note we have shown a robust LC-UV method for the separation of ribooligonucleotide impurities using the Evosphere MAX C18/AR UHPLC Column. This stationary phase exhibits exceptional potential for the separation of oligonucleotides impurities in RP UHPLC applications

Successful separation of a significant number of impurities was completed in 25 minutes. The novel C18/AR phase chemistry is bonded to the highly efficient monodisperse fully porous particles in an inert-coated column hardware delivers excellent efficiency and selectivity to deliver similar resolution as the industry standard ion-pair approach.

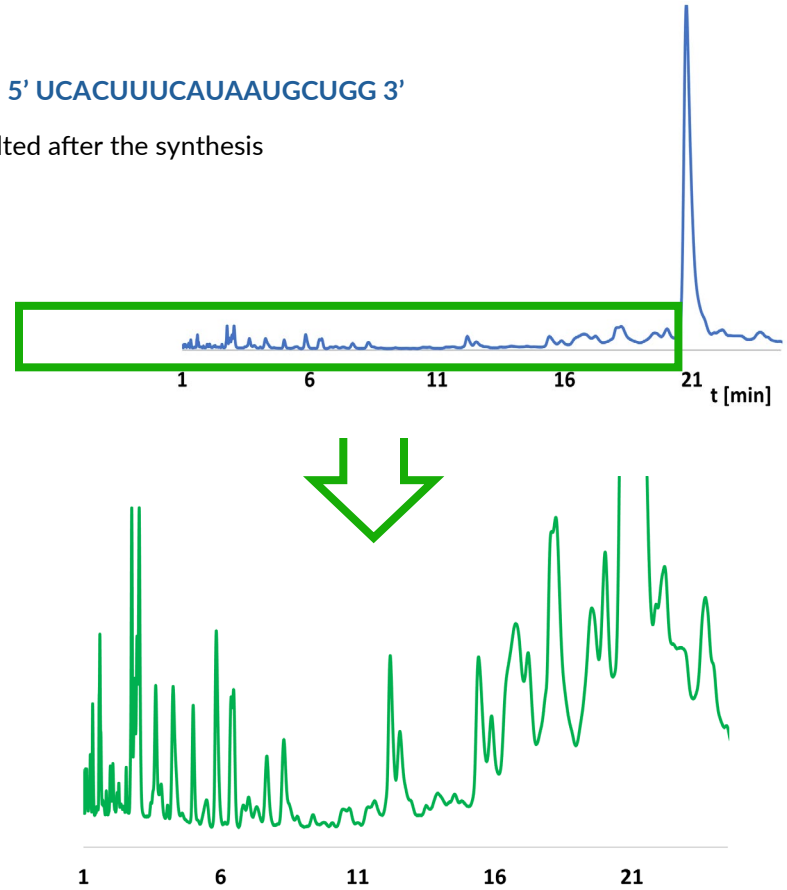
The key advantage of this method is the use of ion-pair-free and inorganic salts-free mobile phases. This developed method eliminates these additives, simplifying the chromatographic system and making the analysis more compatible with downstream applications such as mass spectrometry or biological assays. This approach also reduces the risk of ion suppression effects, which can occur when ion-pair reagents interfere with ionization in mass spectrometry.

Conclusion

In summary, the information provided highlights the effectiveness of using RP UHPLC with the C18/AR stationary phase bonded to the Evosphere monodisperse fully porous particle technology for separating oligonucleotide impurities. The method offers advantages such as compatibility with downstream applications, simplicity in mobile phase composition, and rapid separation within a shortened timeframe.

A raw product was **5' UCACUUUCAUAAUGCUGG 3'**

was analyzed desalted after the synthesis



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Technical Note

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