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アプリケーションノート

Successful Analysis of siRNA Using the ACQUITY UPLC H-Class Bio System

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

The goal of this application brief is to separate a heterogeneous mixture of a siRNA oligonucleotide and its failed sequences using the ACQUITY UPLC H-Class Bio System with Auto · Blend Plus Technology.

Benefits

The ACQUITY UPLC H-Class Bio System is used to efficiently separate the targeted 21 nt siRNA from multiple by-products of the synthesis.

Introduction

Synthetic oligonucleotides are now widely applied in molecular biology, clinical diagnosis, and the development of new therapeutic agents. RNAi therapeutics have attracted significant attention since the discovery of the RNA interference mechanism, prompting the development of analytical methods for short RNA oligonucleotides, typically 21 nucleotides (nt) in length.

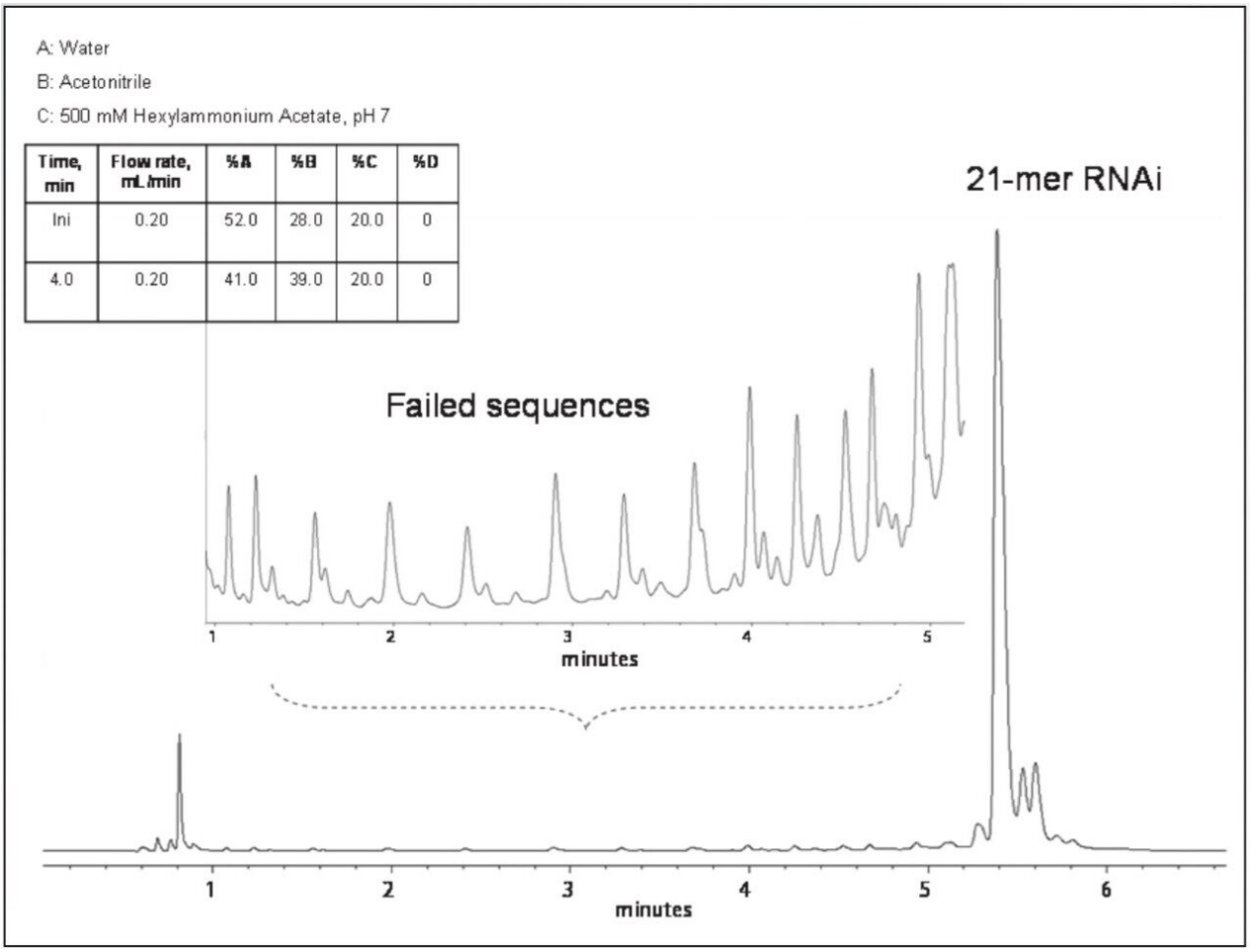
RNA oligonucleotides are produced by a step-wise solid-phase synthesis. The product released from the resin contains the desired sequence contaminated with truncated sequences and synthesis-related impurities. These contaminants need to be quantitated and characterized to ensure drug safety and efficacy.

Ion-pair reversed-phase UPLC is an efficient method for characterizing the full range of impurities in synthetic oligonucleotides. Several ion-pair reagents are available for the separation of siRNA, including some that are suitable for LC-MS applications and for non-denaturing conditions that preserve siRNA duplexes. In addition, modified oligonucleotides may require special mobile phase compositions. Optimization of the ion-pairing system for a particular sample can require the preparation of multiple formulations of mobile phases for testing. This tedious, iterative process can be simplified by using an instrument that accurately and precisely blends mobile phase mixtures from pure solvents and concentrated stocks as required by the experiments. The four-solvent blending feature of the ACQUITY UPLC H-Class Bio System was developed for this purpose.

Results and Discussion

Binary gradients are commonly used for the separations of synthetic oligonucleotides. With the Auto · Blend function of the ACQUITY UPLC H-Class Bio System, a ternary gradient was used for the analysis of a 21 nt siRNA sample. Solvent C was chosen to contain the ion-pairing reagent, hexylammonium acetate,

pH 7, at 500 mM concentration. Solvent A and solvent B are 100% water and 100% acetonitrile, respectively. In this way, mobile phase preparation is straight forward with reduced risk of mixing errors. The concentration of the ion-pairing modifier was kept constant at 20% throughout the separation while applying 28% to 39% gradient of the organic component. The siRNA sample was separated on a UPLC Oligonucleotide Separation Technology (OST) C_{18} column followed by UV detection on a tunable UV detector (TUV).



UPLC separation of 21 nt siRNA targeted product from its failed sequences serves as control of oligonucleotides synthetic quality. The Auto•Blend function of the ACQUITY UPLC H-Class Bio System allows an automatic mixing of three solvents resulting in fast and accurate analysis procedure.

With UPLC, the undesirable impurities are successfully resolved from the targeted 21 nt siRNA sequence. The main peak is a full-length product, while the small peaks are failed sequences. These multiple contaminants are efficiently resolved within 6 minutes of separation. Several of the oligonucleotides detected eluting past the target peak have longer sequences than the full-length RNA product. These are longer oligonucleotides with nucleotides added during a step-wise synthesis, or the full-length product

with incompletely removed protection groups.

Conclusion

We have demonstrated the ability of an ACQUITY UPLC H-Class Bio System to separate the targeted 21 nt siRNA from multiple by-products of the synthesis in 6 minutes. Auto · Blend Plus Technology provides flexibility for the method development because the manipulation of solvents composition is simplified. This highly efficient and cost-effective separation satisfies regulatory requirements and is expected to provide more robust and reliable characterization of the oligonucleotides.

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Auto · Blend Plus <https://www.waters.com/134623262>

ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>

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