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Note d'application

UPLC Separation of Oligonucleotides: Effect of Increased Flow Rate and Faster Run Time

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Abstract

This application note highlights the utility of the ACQUITY UPLC system when used with an ACQUITY UPLC BEH column. The combined system provides accurate, reproducible, and rapid separation of oligonucleotides. It also illustrates that analysis times can be dramatically reduced by scaling both the gradient slope and flow rate proportionately, thus yielding constant gradient slope per volume of mobile phase.

Benefits

We highlight the utility of the ACQUITY UPLC System when used with a 1.7-µm column. The platform provides accurate, reproducible, and rapid separation of oligonucleotides. The method also illustrates that analysis times can be dramatically reduced by scaling both the gradient slope and flow rate proportionately, thus yielding constant gradient slope per volume of mobile phase.

Introduction

There is an increased desire to perform rapid and accurate analysis of oligonucleotides. The ACQUITY Ultra Performance LC (UPLC) System combined with Waters Oligonucleotide Separation Technology (OST) Columns packed with 1.7-µm sorbent as shown here allows for both rapid and accurate analysis that is not possible using conventional HPLC.

Conventional HPLC methods for the analysis of oligonucleotides generally suffer from low resolution of oligonucleotides, particularly when analysis times are reduced. The UPLC method presented here expands on the topic discussed previously in application note, "UPLC-MS Separation of Oligonucleotides in Less Than Five Minutes: Method Development,"[1] which provides guidelines for the rapid analysis of RNAi and DNA in less than 2.5 minutes in most cases.

Experimental

Samples

OST MassPREP Standard was dissolved in 100 mM TEAA. RNAi and DNA samples (IDT Technologies) were prepared by partial digestion of purified samples with phosphodiesterase II (Sigma).

LC conditions

LC System:	Waters ACQUITY UPLC System with ACQUITY UPLC PDA Detector
Column:	ACQUITY UPLC OST C_{18}, 2.1 x 50 mm, 1.7 µm
Column temp.:	60 °C
Flow rate:	0.2 to 0.8 mL/min
Mobile phase A:	100 mM hexylammonium acetate, pH 7.0
Mobile phase B:	Acetonitrile
Gradient:	28 to 39 %B
Detection:	PDA UV 260 nm

Results and Discussion

Ion-pairing reversed phase (IP-RP) liquid chromatography is a commonly accepted separation strategy for the analysis of oligonucleotides. Of the known ion-pairing systems, triethylamine hexafluoroisopropanol (TEA/HFIP) is commonly used due to its MS compatibility and impressive resolving power. Additionally, we utilized hexylammonium acetate (HAA), which also offers exceptional resolution.

We developed a method for the analysis of up to 35-mer oligonucleotides in under 2.5 minutes and oligonucleotides up to 25 mer in under 2 minutes with impressive resolution. This was accomplished by establishing appropriate UPLC conditions to successfully elute all species in under 10 minutes at a flow rate of 0.2 mL/min, which corresponds to a total flow of 2 mL. We then increased the flow rate incrementally

while maintaining a constant gradient slope. By scaling the overall analysis time so that the gradient was completed within the same pumped volume, we achieved a constant gradient slope per column volume. As shown in Figure 1, the ACQUITY UPLC coupled with an ACQUITY UPLC OST column offers exceptional resolution at all flow rates tested.

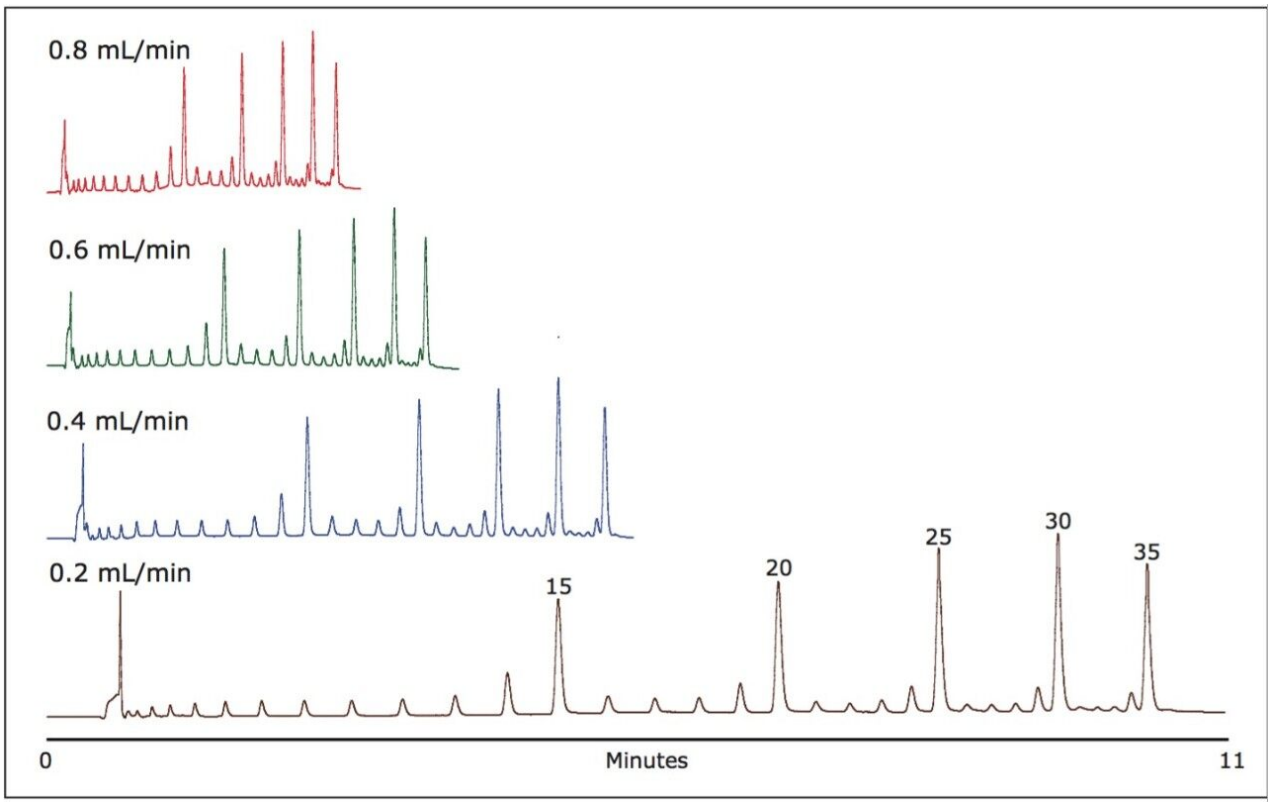

Figure 1. UPLC chromatograms for the separation of deoxythimidine sequences (OST MassPREP Standard) at various flow rates with constant gradient slope.

Additionally, system pressure increased significantly as the flow rate increased, ranging from 3200 psi at 0.2 mL/min to 12,000 at 0.8 mL/min. These pressures far exceed those possible with conventional HPLC but are accomplished routinely using the ACQUITY UPLC System.

To determine the reproducibility of retention time as a function of gradient slope, we determined the volume of eluent necessary to elute each oligonucleotide at the flow rates tested. We found exceptional reproducibility of delivered volume to oligonucleotide elution as shown in Figure 3. This highlights the selectivity of our system for oligonucleotides, regardless of flow rate.

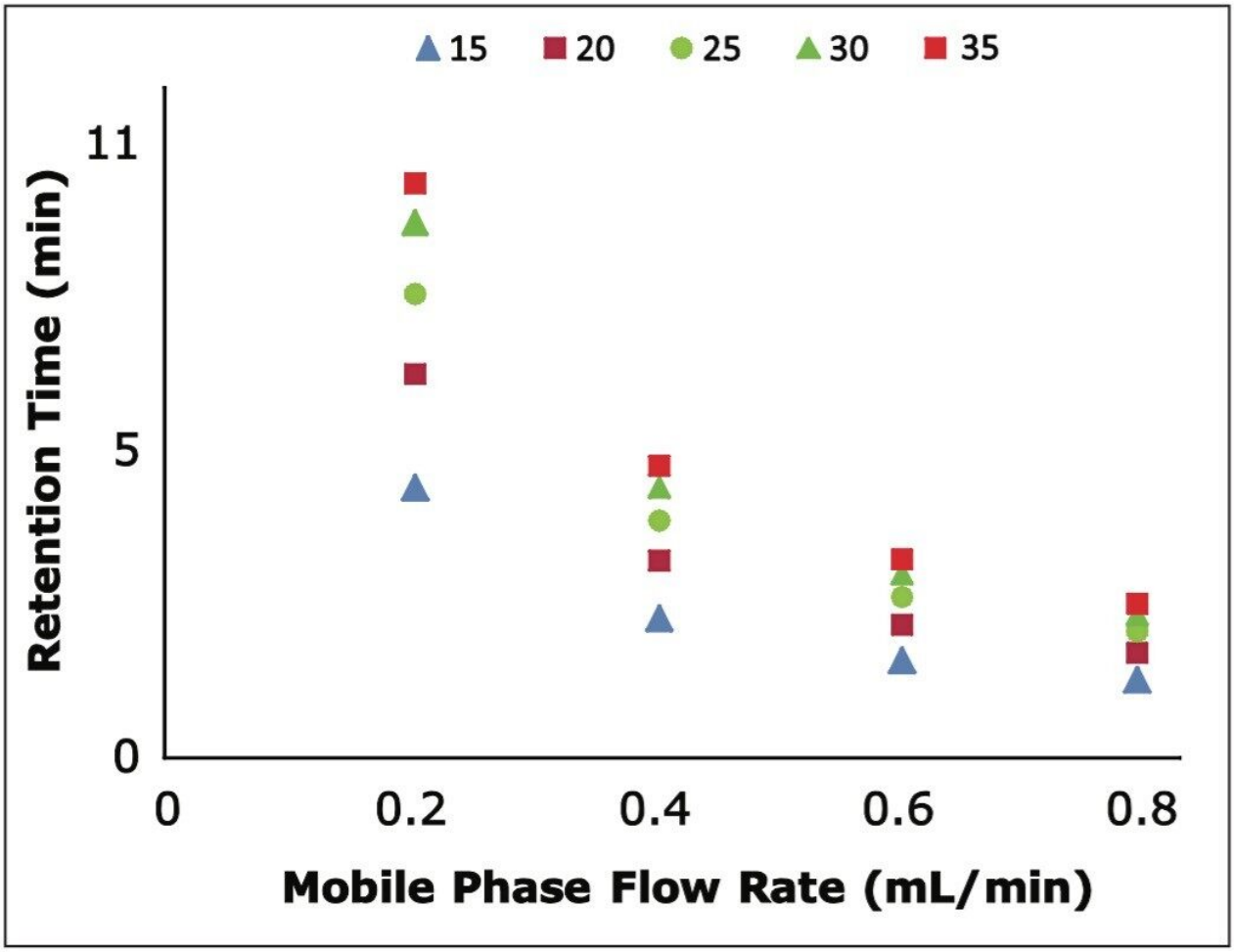

Figure 2. Comparison of mobile phase flow rate and oligo retention time at various flow rates.

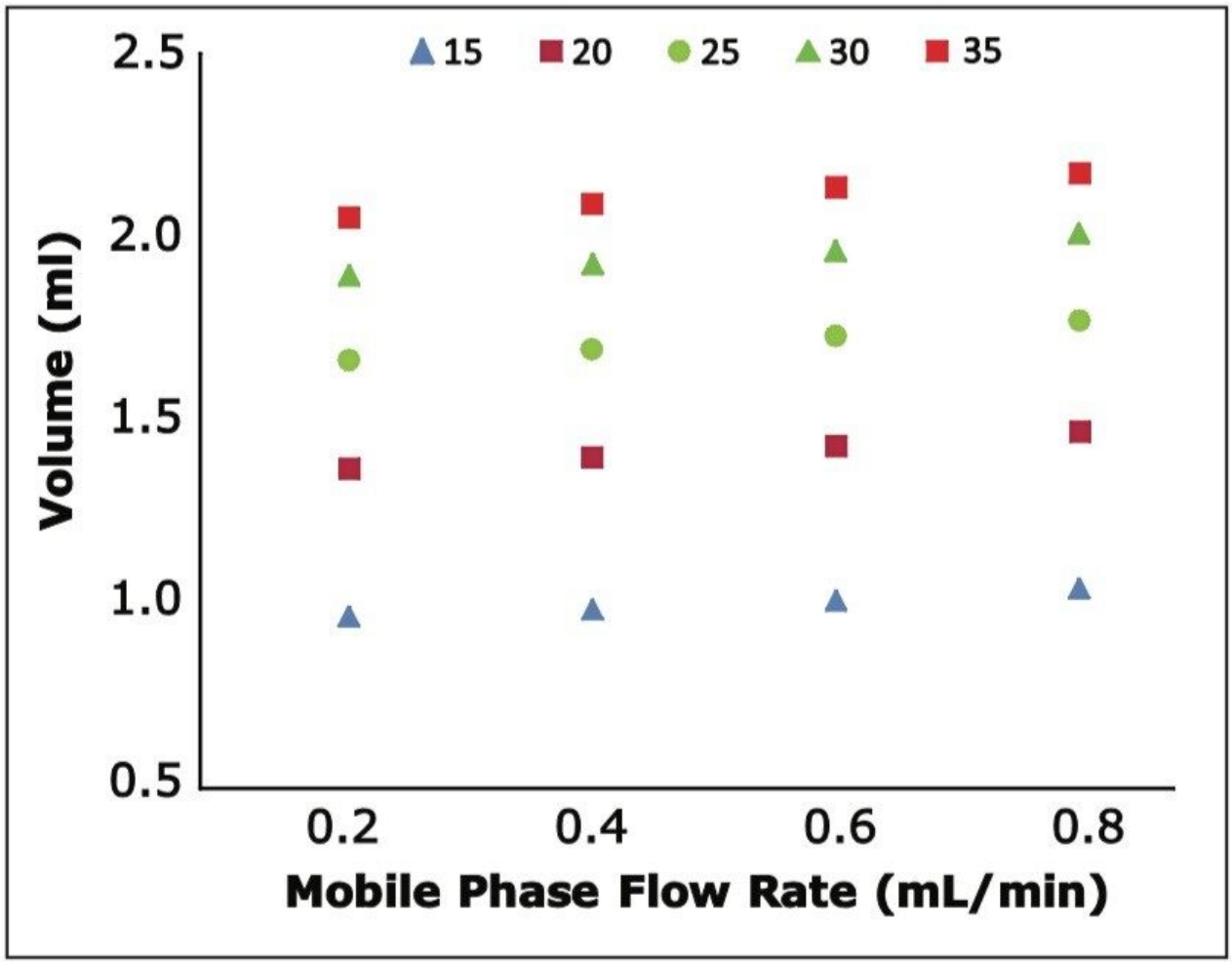

Figure 3. Comparison of mobile phase volume pumped at various flow rates for the retention of various oligonucleotide sequences.

We tested our method with a more challenging sample, a heteromolecular DNA sequence, Figure 4. Our rapid separation method provided regular retention based on oligo length with impressive resolution from failure sequences.

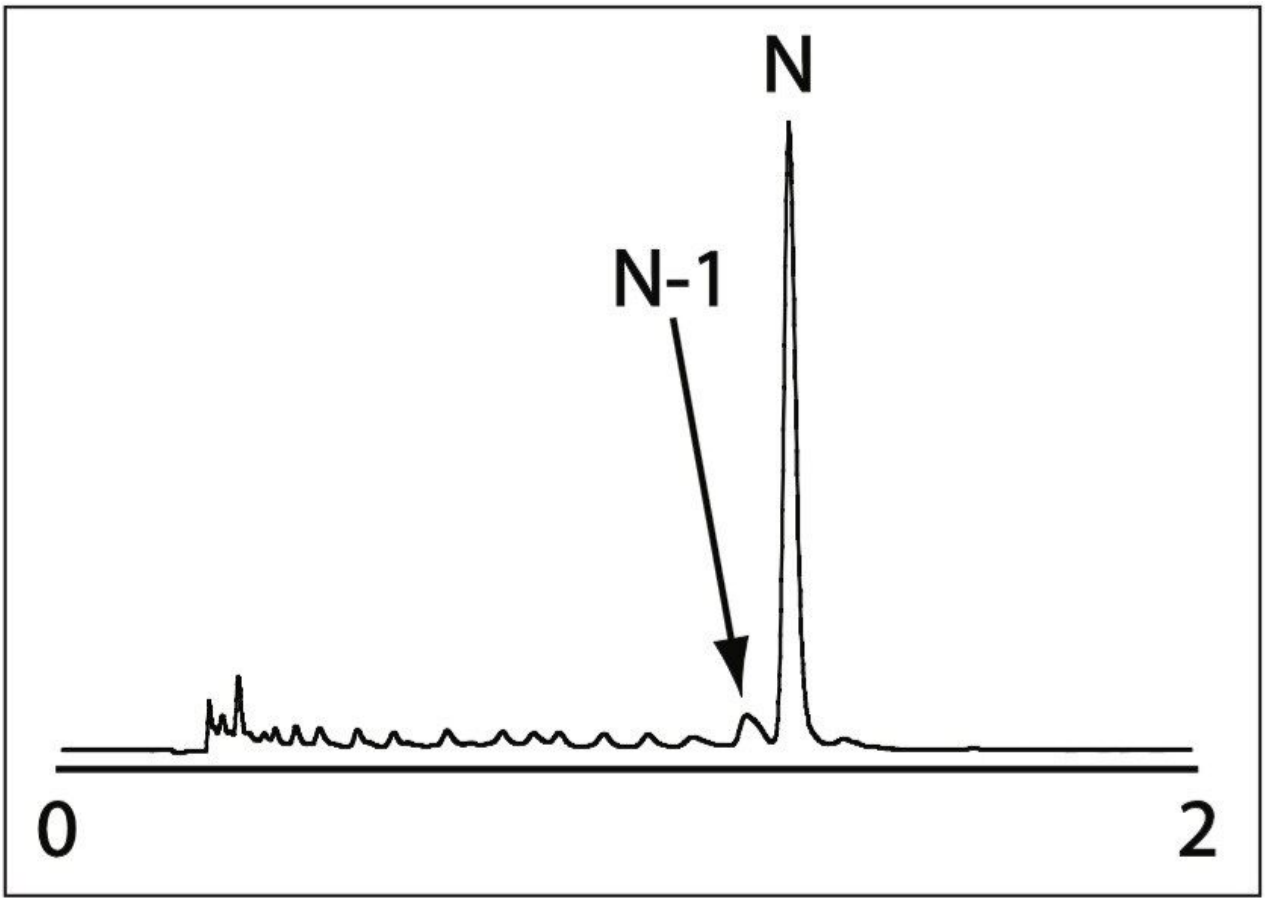

Figure 4. UPLC separation of RNA (top trace) and DNA (bottom trace) in under 2 minutes. N-x sequences are 5′ truncated.

Conclusion

The method presented in highlights the utility of the ACQUITY UPLC system when used with an ACQUITY UPLC BEH column. The combined system provides accurate, reproducible, and rapid separation of oligonucleotides. The method also illustrates that analysis times can be dramatically reduced by scaling both the gradient slope and flow rate proportionately, thus yielding constant gradient slope per volume of mobile phase. This is accomplished by using sub-2-micron particles and the high pressure capabilities of the ACQUITY UPLC. The method provides accurate and predictable retention of both homo- and hetero-molecular oligonucleotides.

References

1. UPLC/MS Separation of Oligonucleotides in Less than Five Minutes: Method Development. Waters Application Note. 2007; 720002387en.

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ACQUITY UPLC System <https://www.waters.com/514207>

ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

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