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UPLC Separation of Oligonucleotides: Method Development

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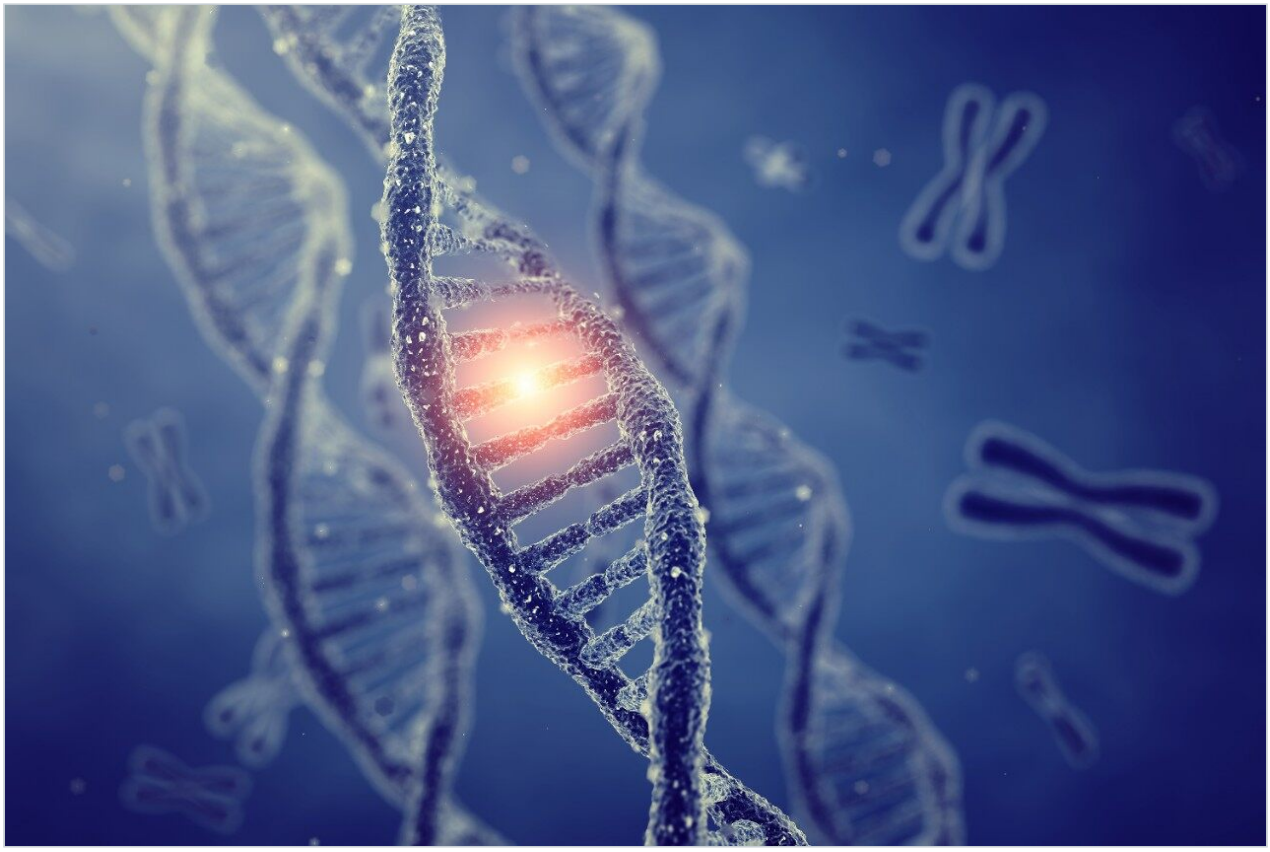

Abstract

This application note illustrates the impact of chromatographic parameters on UPLC oligonucleotide separations and general guidelines for developing high resolution, fast analytical methods.

Introduction

The Waters ACQUITY UltraPerformance (UPLC) System, combined with Oligonucleotide Separation Technology (OST) Columns packed with 1.7 µm sorbent, offer superior analytical performance for oligonucleotide separations compared to HPLC and fast LC separations.

The Waters UPLC-based OST solution for the high-resolution, highthroughput analysis of synthetic oligonucleotides was developed following a series of comprehensive investigations that helped Waters scientists and engineers better understand limitations of existing analytical techniques for compounds. This research led to innovations designed to assist manufacturers deliver quality products that can help researchers make impactful discoveries that can lead to novel diagnostics or drug therapies. Failure to achieve these goals can seriously impede the ability of an organization to achieve desired results, such as obtaining the necessary FDA approval for product commercialization.

This application note illustrates the impact of chromatographic parameters on UPLC oligonucleotide separations and general guidelines for developing high resolution, fast analytical methods. For further method development guidelines for separation of oligonucleotides, please refer to other available application notes.

Experimental

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 mL/min, unless indicated otherwise in figures
Mobile phase A:	100 mM TEAA, pH 7
Mobile phase B:	80% A, 20% acetonitrile
Gradient figure 1:	40 to 62.5% B, for gradient time see figure
Gradient figure 2:	45 to 64.5% B, for gradient time see figure
Gradient figure 3:	Gradient started at 50, 45, 40, and 35% B, respectively. 0.75% B/min (0.15% acetonitrile/min)
Detection:	UV 260 nm
Sample:	15 to 60 nt oligodeoxythymidines

Results and Discussion

Oligonucleotide analysis in ion-pairing reversed-phase liquid chromatography (IP-RP LC) is typically performed with shallow gradients. The impact of gradient slope on oligonucleotide resolution is illustrated in Figure 1.

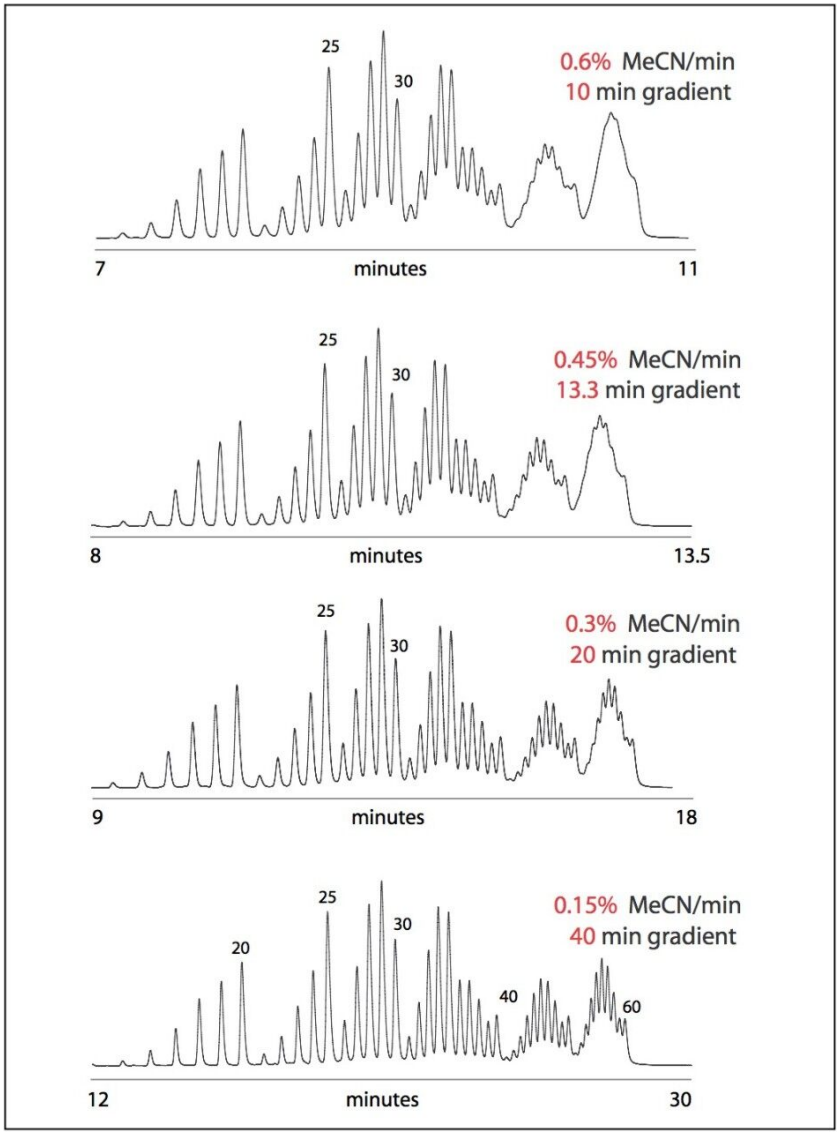

Figure 1. Impact of the gradient slope on separation of 15 to 60 nt oligodeoxythymidines and analysis time.

As expected, decreasing gradient slope increases resolution, but negatively impacts analysis throughput by increasing the run time. Another approach to maintaining resolution while decreasing analysis time is to increase mobile phase flow rate while proportionally reducing the gradient time (Figure 2). In such a scenario, the number of column volumes remains constant. Therefore, the separation selectivity remains unchanged with only the potential for some loss of resolution (Figure 2a). The constant gradient volume method is preferable as it enables faster analysis times with minimal deterioration in resolution. The increased operational pressures generated by 1.7 µm sorbent and higher flow rates require the capabilities of the ACQUITY UPLC System.

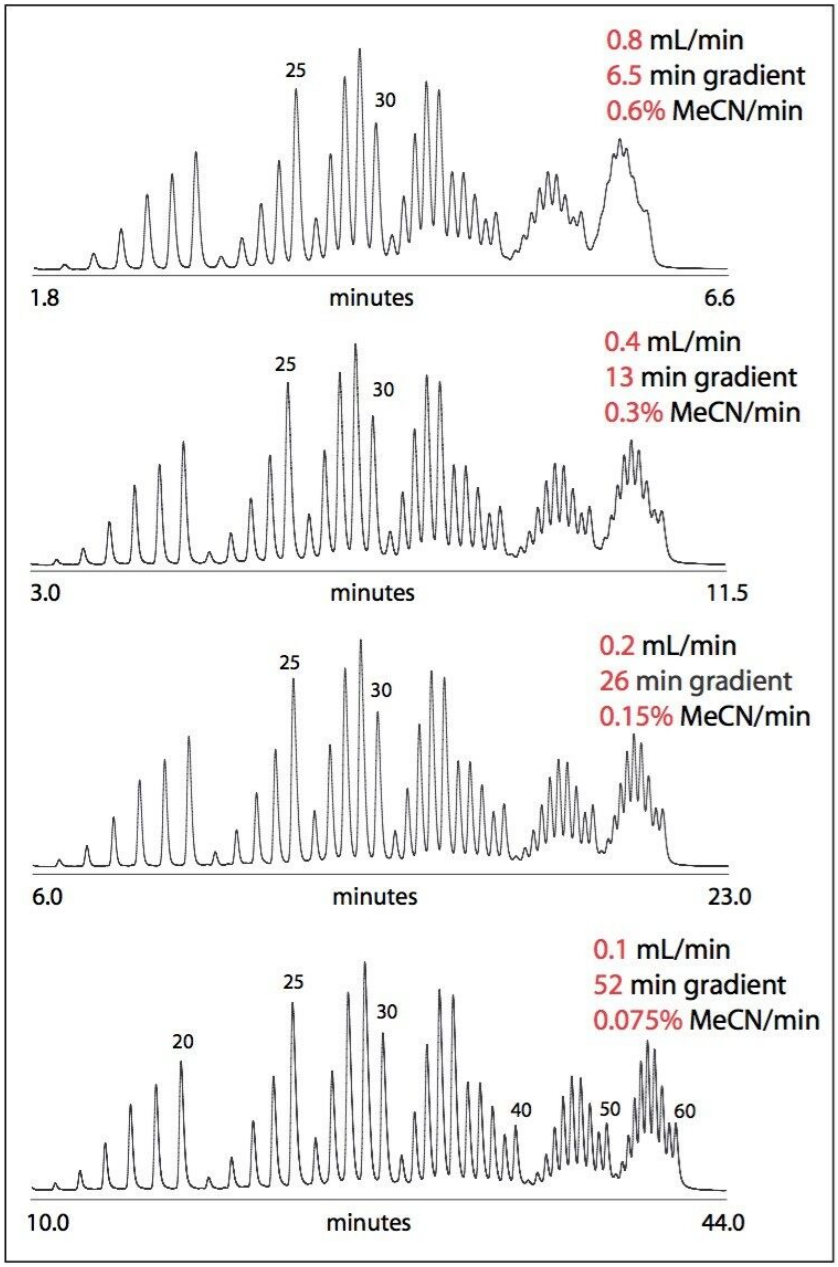

Figure 2. Separation of 15 to 60 nt oligodeoxythymidines at constant gradient volume in various mobile phase flow rates.

Oligonucleotides tend to elute in very narrow gradient ranges (mobile phase elution strength). If initial and final gradient conditions are not optimized properly, the resulting analysis time can be considerably longer than necessary, as the majority of the separation space in the typical HPLC chromatogram is unused for the separation. The preferable UPLC approach is to select a gradient slope providing high resolution and adjust the gradient initial conditions while keeping the gradient slope constant. In this way it is possible to

significantly reduce analysis time without sacrificing resolution, as shown in Figure 3.

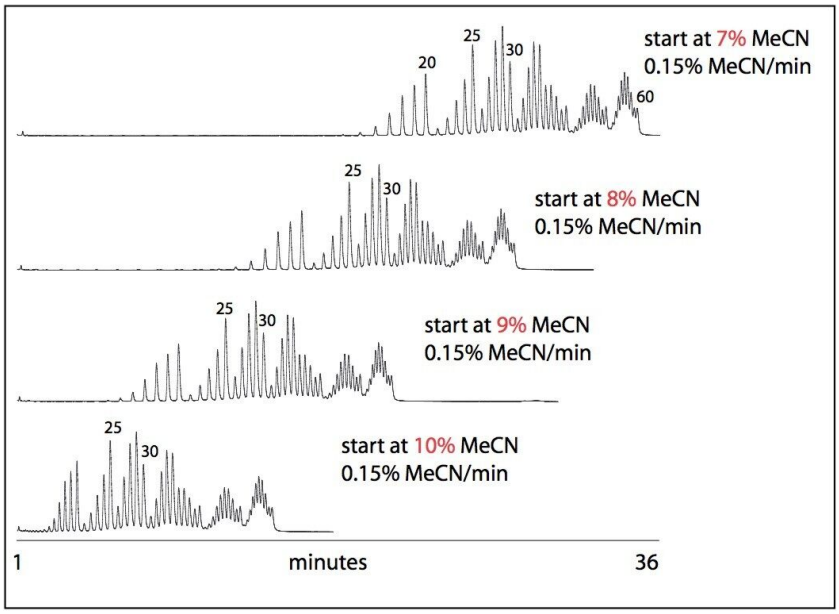

Figure 3. Reducing the analysis time by adjustment of initial gradient strength. Gradient slope remains constant.

Conclusion

The Waters ACQUITY UPLC System with OST Columns solution offers significant advantages to manufacturers or researchers who require improved technology for the analysis of oligonucleotides. The impact of optimized gradient slope, flow rate, and initial gradient strength on the separation of oligonucleotides has been demonstrated. UPLC enables improved resolution, resulting in improved separations with very fast run times.

High-resolution, high-throughput methods offer easier quantitative analysis with increased throughput, generating better data in shorter time with cost savings. The ACQUITY UPLC System will increase the productivity of any laboratory developing methods and analyzing oligonucleotides.

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