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응용 자료

UPLC-MS Separation of Oligonucleotides in Less than Five Minutes: Method Development

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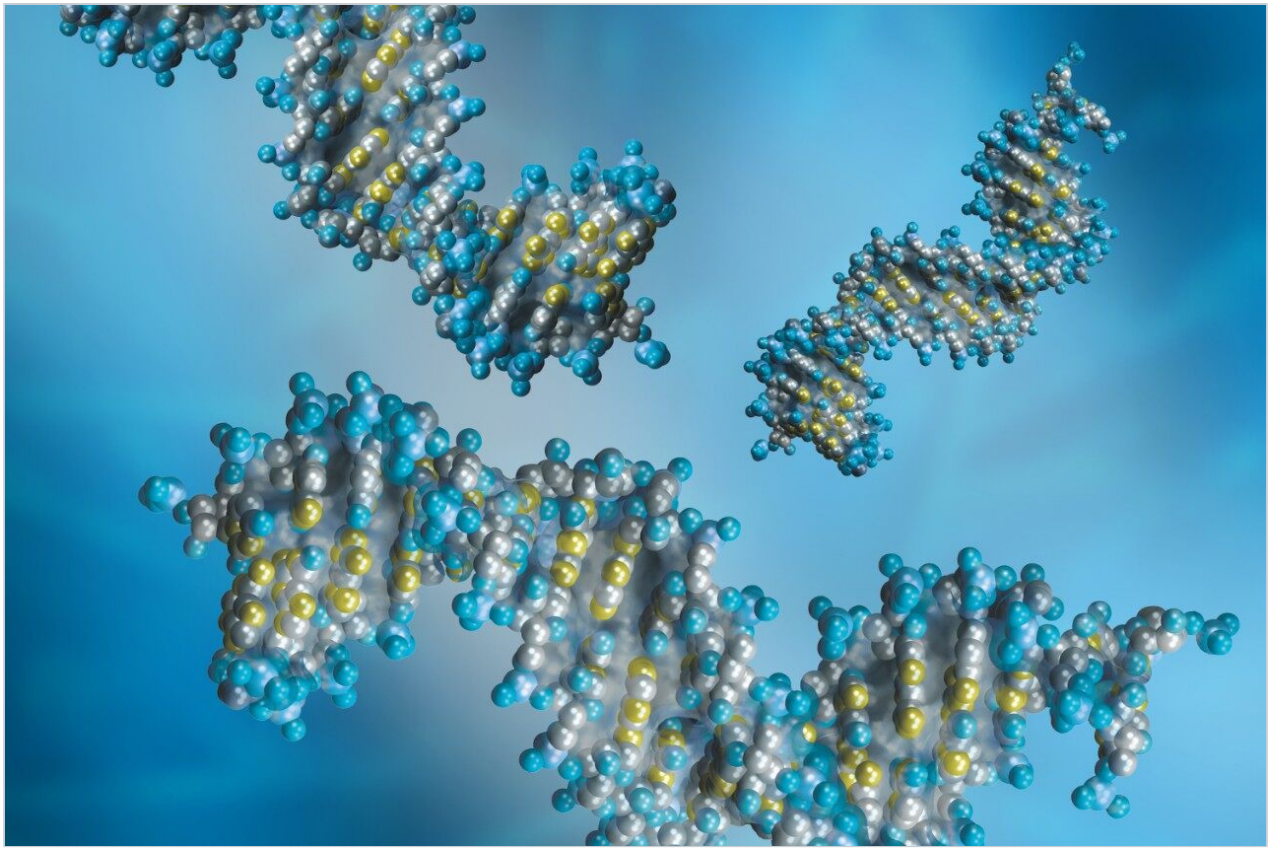

Abstract

In this application note details on the UPLC-MS separation of oligonucleotides in less than five method.

Introduction

The ACQUITY UltraPerformance LC (UPLC) System, when combined with Oligonucleotide Separation Technology (OST) Columns packed with 1.7 µm sorbent, offer superior analytical performance for oligonucleotide separations compared to HPLC. UPLC also enables users to significantly reduce analysis time.

In this work, we illustrate the UPLC analytical method development process and extend the topic discussed in a previous note, "UPLC Separation of Oligonucleotides: Method Development"[1] providing guidelines for producing high resolution, fast, LC-MS compatible oligonucleotide separations.

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
Mobile Phase A:	15 mM TEA, 400 mM HFIP, pH 7.9

Mobile Phase B:	50% A, 50% MeOH
Detection:	PDA TIC or UV 260 nm
Sample:	oligodeoxythymidines

Results and Discussion

Oligonucleotide separation in ion-pairing reversed-phase (IR-RP) liquid chromatography is typically performed with shallow gradients. Recently, a novel ion-pairing buffer compatible both with UV and electrospray MS detection has been described.[2,3] The buffer is comprised of triethylamine (TEA, an ion-pairing agent) and aqueous hexafluoroisopropanol (HFIP, a volatile weak acid used as buffering component to bring the pH to ~ 8).

While triethylammonium acetate (TEAA) is useful for oligonucleotide analysis, it is not compatible with MS detection. The LC retention behavior of oligonucleotides strongly depends on their nucleotide composition, requiring careful optimization of gradient elution conditions for each specific oligonucleotide.[4] TEA-HFIP ion-pairing buffer yields more consistent and predictable oligonucleotide retention behavior over a broad range of compositional differences as compared to TEAA.

Oligonucleotide retention also varies depending on its length (charge). Differences are also observed for different classes of oligonucleotides and chimeric oligonucleotides (DNA, RNA, LNA, phosphorothioates, morpholino backbone, 2′O-methylated species, and combination of all above).

Waters UPLC method development guidelines for oligonucleotide analysis can be summarized in three steps:

1. Identify a suitable initial gradient strength. If elution behavior of the oligonucleotide is unknown, start with a scouting gradient. Recommended flow rate for a 2.1 x 50 mm OST Column is 0.2 mL/min and separation temperature is 60 °C.
2. Adjust the gradient slope to achieve a desirable separation. In general, shallower gradients provide increased resolution.
3. Faster analyses can be achieved by increasing the flow rate while maintaining gradient column volumes.

If the gradient range and the gradient slope in column volumes remain constant, the separation selectivity is not negatively affected.

Figure 1 illustrates method development for a 30 to 60 nt oligonucleotide using TEA-HFIP. The high resolution separation in chromatogram Figure 1A has an initial mobile strength of 22.5% MeOH and slope 0.25% MeOH/min. The resolution can be further improved by using a shallower gradient (0.15% MeOH/min, Figure 1B), but at the expense of analysis time – which will negatively impact throughput.

The analysis time can be shortened by adjusting the initial gradient conditions (Figure 1C). Since the gradient slope was unchanged, the high resolution separation was preserved, with the possible exception of the early eluting peaks.

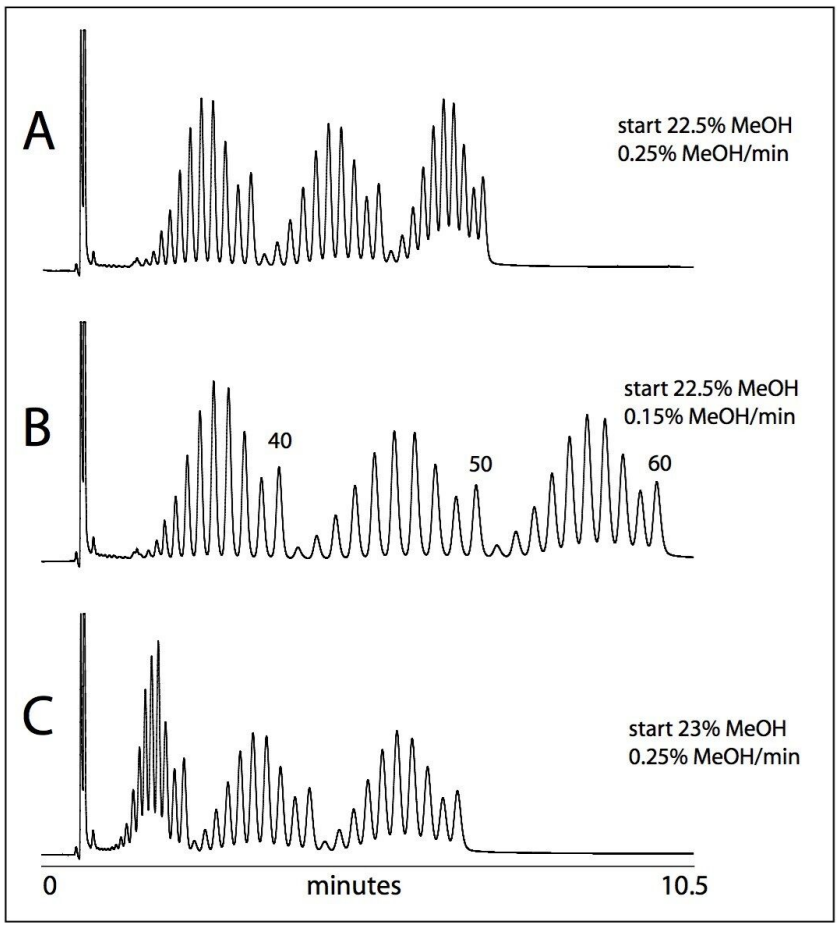

Figure 1. Separation of 30 to 60 nt oligodeoxythymidines using 2.1 x 50 mm, 1.7 µm ACQUITY UPLC OST C_{18} Column.

Figure 2 highlights a strategy for reducing analysis time without compromising high resolution by using

faster flow rates. The chromatogram in Figure 2B shows baseline resolution of 15 to 35 nt peaks in less than 10 minutes. To optimize the separation for throughput while maintaining high resolution, the flow rate was doubled and the initial gradient conditions were adjusted while holding constant the gradient slope in column volumes. The resulting chromatogram in Figure 2A shows a final separation accomplished in less than four minutes.

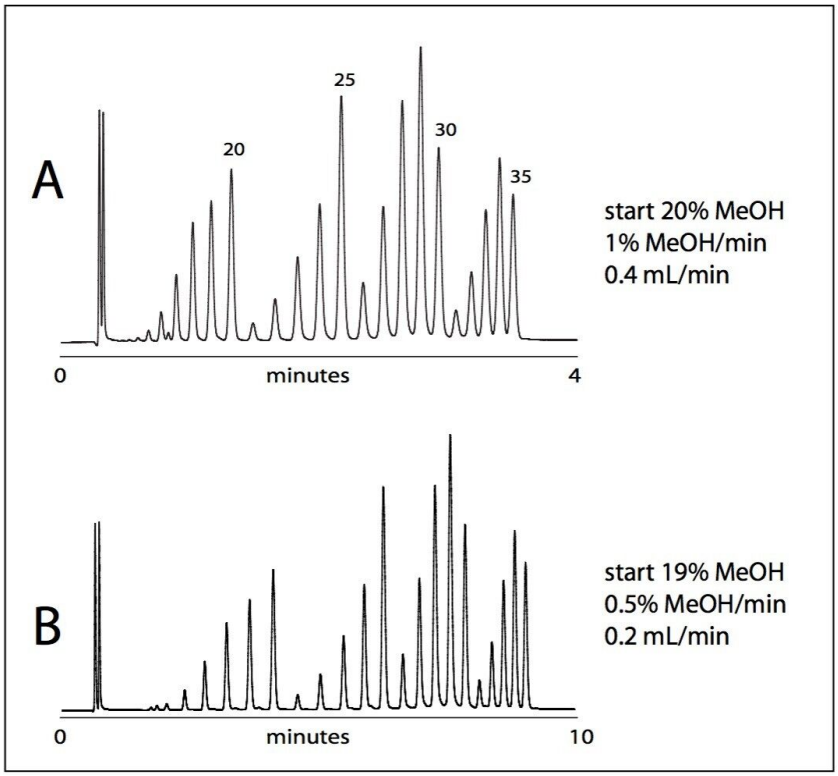

Figure 2. Separation of 15 to 35 nt oligodeoxythymidines.

Conclusion

UPLC has significant advantages for the LC and LC-MS analysis of different classes 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, LC-MS compatible methods offer easier quantitative analysis with increased

throughput generating better data in shorter time. ACQUITY UPLC Systems and Columns thus will increase the productivity of any laboratory developing LC and LC-MS methods and analyzing oligonucleotides.

References

1. UPLC Separation of Oligonucleotides: Method Development, Part 1. Waters Technical Note. 2007: 720002383EN <https://www.waters.com/webassets/cms/library/docs/720002383en.pdf> .
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