OLIGONUCLEOTIDE ANALYSIS ON XBRIDGE™ AND XTERRA® COLUMNS

Waters

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INTRODUCTION

The analysis of oligonucleotides by reversed-phase HPLC poses challenges using traditional chromatographic sorbents. Traditional silica-based stationary phases have limited stability at elevated temperatures and high pH. In 1999, dramatic improvements in column stability were realized with the commercialization of the first generation hybrid silica-organic chromatographic XTerra® columns by Waters Corporation. XTerra® MS C_{18} columns have previously been demonstrated to provide enhancements in both performance and column lifetime over silica sorbents for this application, which employs elevated temperatures and high pH for optimal resolution. More recently, the second generation bridged-ethyl hybrid (BEH) technology has been commercialized with the introduction of XBridge™ columns, thus further improving column lifetime and performance. In this study, experiments were performed to compare column lifetime and selectivity of XBridge™ C_{18} and XTerra® MS C_{18} columns for the analysis of single stranded, synthetic DNA and RNA oligonucleotides.

INSTRUMENTATION

HPLC: Waters Alliance® 2695 with 2996 PDA UV detection
Column Heater: Systec
Column config.: Waters XBridge™ C_{18}, 4.6 x 50 mm, 2.5 µm (P/N: 186003090)
Waters XTerra® MS C_{18}, 4.6 x 50 mm, 2.5 µm (P/N: 186000602)

RESULTS

IA. Lifetime study — TEAA mobile phase *

*** TEAA:** aqueous triethylammonium acetate

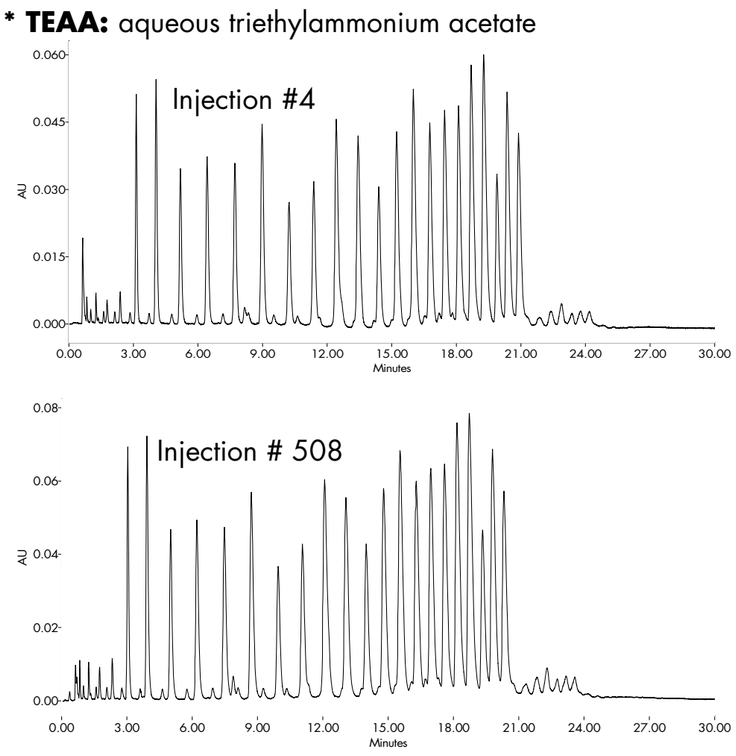

Figure 1. Lifetime study of an XBridge™ column in TEAA mobile phase using a mixture of polyT (5T-25T, ~200µM each). Conditions: 65°C, 1mL/min. Mobile Phase A: 100mM TEAA, pH 7. Mobile Phase B: 25% acetonitrile / 75% 100mM TEAA, pH 7. Gradient: 7% - 12% acetonitrile in 30 min. Detection: UV @ 260 nm. (Ref. 1)

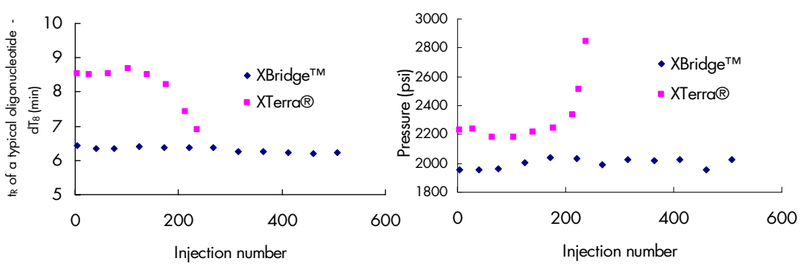

Figure 2. After over 500 injections, the retention time of oligonucleotides and the pressure of the system remained constant for an XBridge™ column. The retention time decreased and the pressure increased for an XTerra® column.

IB. Lifetime study—HFIP mobile phase **

**** HFIP:** 1,1,1,3,3,3-hexafluoroisopropanol F_3CCHCF_3 (OH)
HFIP is a weak acid used to adjust pH of triethylamine. This buffer is volatile and MS – compatible. The separation for oligonucelotides using this buffer is comparable or better than with conventional TEAA. (Ref. 2,3)

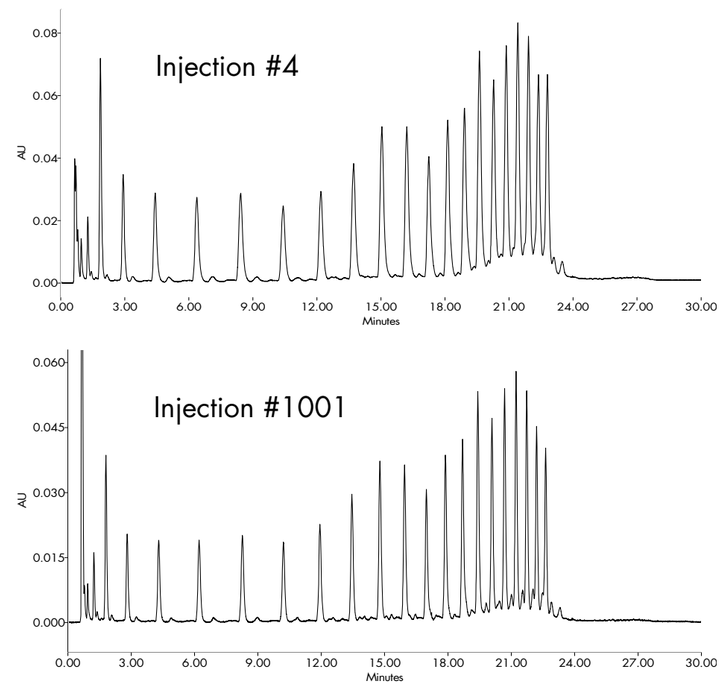

Figure 3. Lifetime study of an XBridge™ column in HFIP mobile phase using a mixture of polyT (5T-25T, ~200µM each). Conditions: 60°C, 1mL/min. Mobile phase A: 10% methanol / 90% (385mM HFIP + 14.3 mM TEA), Mobile Phase B: 25% methanol / 75% (385 mM HFIP + 14.3 mM TEA). Gradient: 100% A — 100% B in 30 min. Detection: UV @ 260 nm.

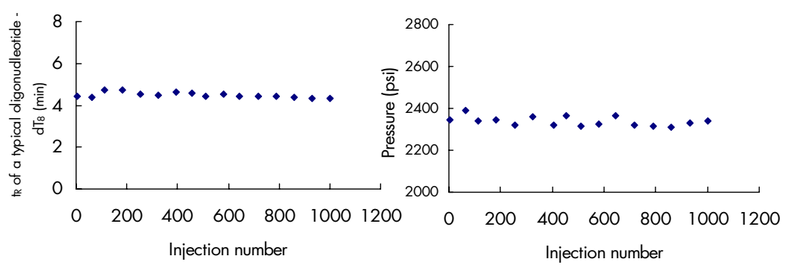

Figure 4. After over 1000 injections, the retention time of oligonucleotides and the pressure of the system remained constant for an XBridge™ column.

XBridge™ C_{18} columns exhibited no apparent loss in retention or efficiency for over 1000 injections using gradient separation conditions.

IIA. Application — dye-labeled oligonucleotides

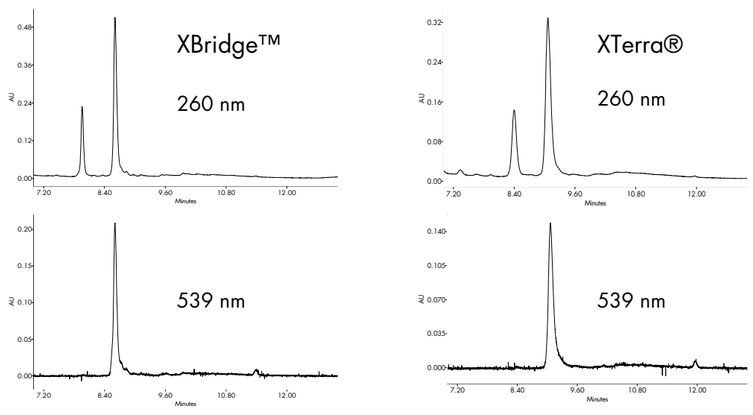

Figure 5. HEX-labeled 25mer DNA detected at 260 nm and 539 nm on XTerra® and XBridge™ columns. The unlabeled failure sequence was detected and separated from the labeled sequence at 260 nm. 60 °C, 1mL/min. Mobile phase A: 100mM TEAA, pH7. Mobile phase B: 100% acetonitrile. Gradient: 5-30% acetonitrile in 15 min. Sample: (HEX) - TTT GAC TTA GAC TTA GAC TTA GTT T. (Ref.4)

REFERENCES

1. M. Gilar, et al., *J. Chromatogr. A*, **958**, 167–182, 2002.
2. K. J. Fountain, et al., *Rapid Comm. Mass Spectrom.*, **17**, 646–653, 2003.
3. M. Gilar, et al., *Oligonucleotides*, **13**, 229–243, 2003.
4. K. J. Fountain, et al., *J. Chromatogr. B*, **783**, 61–72, 2003.

IIB. Application — RNAi

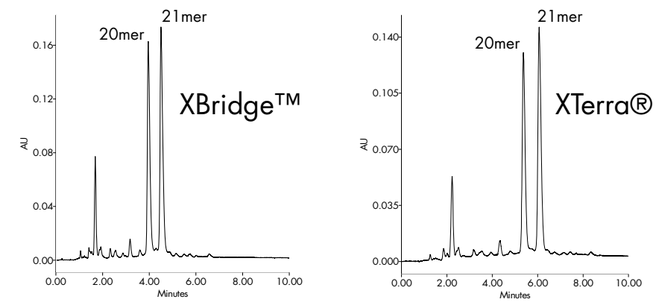

Figure 6. 20mer and 21mer RNAi separated on XTerra® and XBridge™ columns. 60 °C, 1mL/min. Mobile phase A: 100mM TEAA, pH7. Mobile phase B: 100% acetonitrile. Gradient: 7-11% acetonitrile in 15 min. Sample: 21mer 5′-AUU GUG UAC CUU UAG CUU dTdT-3′ and 20mer 5′-UUG UGU ACC UUU AGC UU dTdT-3′

The chromatographic selectivity is similar on XBridge™ C_{18} and XTerra® MS C_{18} columns.

III. Effect of Temperature

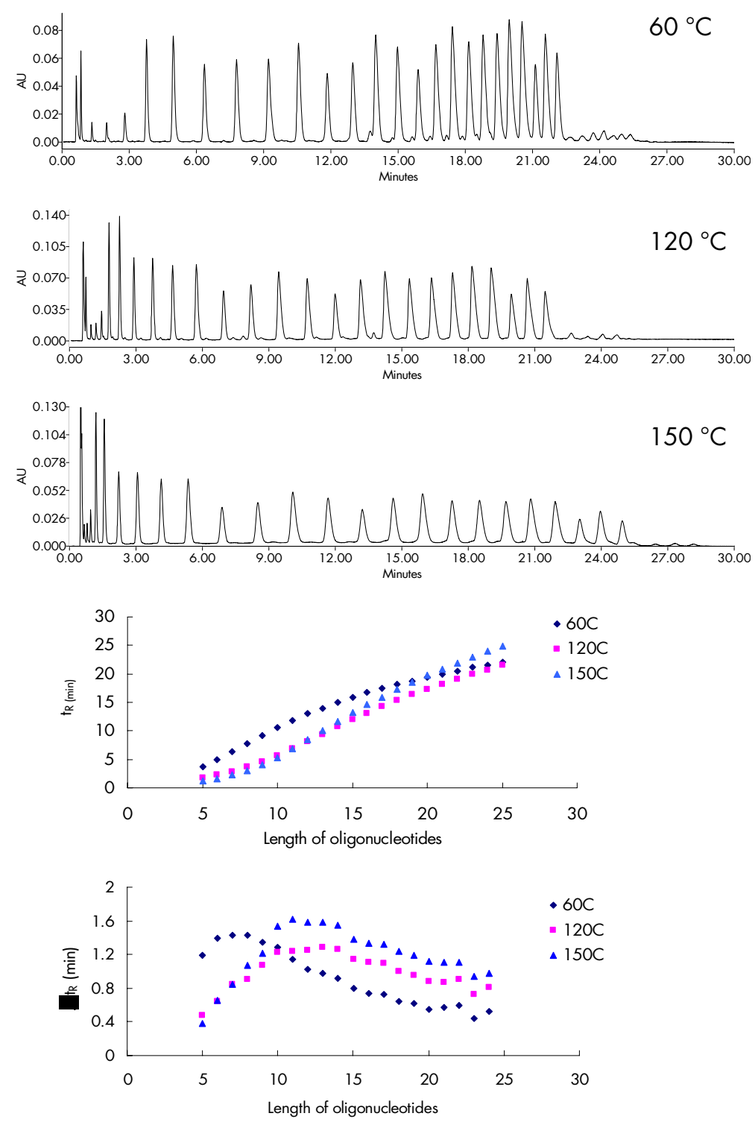

Figure 7. Separation of polyT DNAs carried out at elevated temperatures on an XBridge™ column. 1mL/min. Mobile phase A: 100mM TEAA, pH7. Mobile phase B: 100% acetonitrile. Gradient: 7-12% acetonitrile in 30 min at 60 °C; 4-9% acetonitrile in 30 min at 120 °C; 0-5% acetonitrile in 30 min at 150 °C.

At higher temperatures, the chromatographic separation improves for higher molecular weight oligonucleotides.

CONCLUSIONS

1. XBridge™ columns have much longer life time than XTerra® columns at 60—65°C. XBridge™ columns can operate for extended periods (>1000 injections) using either TEAA or HFIP mobile phases at temperatures above 60°C.
2. Comparable chromatographic selectivity is observed for both XTerra® and XBridge™ coumns, with XBridge™ columns being slightly less retentive and more efficient.
3. Improved resolution for longer length oligonucleotides is observed at elevated temperatures.

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