APPLICATION NOTE

Benefits of Daicel Columns in the Separation of Oligonucleotides

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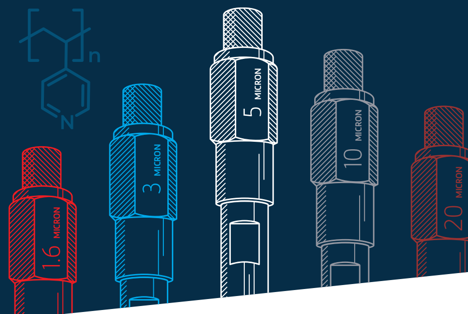
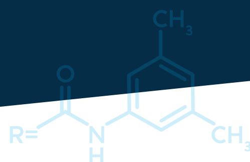


DAICEL Chiral Technologies



INTRODUCTION

In recent years, Daicel has continuously innovated in the life sciences with new developments in achiral columns, genomics, labelled standards, method validation, intracellular injections, and excipients, among many other areas.

An area of particular challenge and interest is that of the separation of oligonucleotides. The very close structure of the impurities to the end-product often makes the complete resolution difficult. Daicel has several chiral and achiral columns that have been established to work well in this area. This Application Note demonstrates the resolution of a 6-component oligonucleotide mixture containing typical “n+1”, “n+2”, and end/mid modified impurities.

EXPERIMENTAL

All oligonucleotides used for this study (Table 1) were purchased from Sigma Aldrich in their desalted form. They were dissolved in pure water to 1 mM, and used with a 1 µL injection volume. Purified water was generated from an Elix® Advantage 15 system from Millipore, Acetonitrile (ACN) was HPLC-grade and was purchased from Carlo Erba, and Glacial acetic acid (purity >99.5%), and Triethylamine were purchased from Sigma Aldrich.

Screening and optimization were performed using an Agilent 1100 chromatographic system equipped with binary pump, autosampler, thermostatic oven and Diode Array Detector. A gradient elution mode was used with a time program of: 0-5min at 100%A, 5-20min to 100%B, 20-25min at 100%B, re-equilibration to 100%A for 5min, where Mobile Phase A was Buffer/ACN = 95/5 (v/v) and Mobile Phase B was Buffer/ ACN = 5/95 (v/v). The buffer used was triethylammonium acetate (TEAA) with varying salt concentration and pH-values (described below in the specific examples).

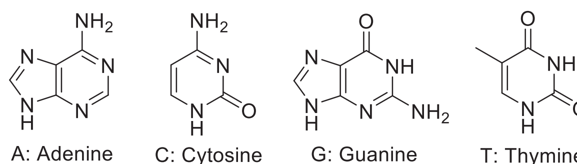


Figure 1: The Four Nucleotide Bases

All columns used for the investigation are listed in Table 2. All columns were 150 mm length x 4.6 mm internal diameter packed with 5 µm particle size, with the exception of the CHIRALPAK® ZWIX columns which were 4.0 mm internal diameter packed with 3 µm particle size.

Compound	Sequence
Basic 6-mer	CGTACT
“n+1” 7-mer	CGTACT**G**
“n+2” 8-mer	CGTACT**GA**
5’ end modified 6-mer	**GC**TACT
3’ end modified 6-mer	CGTA**TC**
Mid modified 6-mer	CG**AT**CT

Table 1: Oligonucleotides Used for Separation Study

Column	Column Characteristics
DCpak® PTZ DCpak® P4VP	Achiral Polymeric
CHIRALPAK® IB-N CHIRALPAK® IC CHIRALPAK® IG	Immobilized Polysaccharide Chiral
CHIRALPAK® QN-AX CHIRALPAK® QD-AX	Quinine- and Quinidine-based Anionic exchangers Chiral
CHIRALPAK® ZWIX(+) CHIRALPAK® ZWIX (-)	Quinine- and Quinidine-based Zwitterionic exchangers Chiral

Table 2: Selected Columns for Evaluation

DISCUSSION

Investigations were focused on one buffer type, namely triethylammonium acetate (TEAA), which is a commonly used ion-pairing agent in the oligonucleotides field. It offers the following advantages:

- Volatility and Compatibility with MS-detection
- Cost-effectiveness
- Limited toxicity
- Sufficient solubility of the oligonucleotides when mixed with acetonitrile (ACN).

The different column types showed good ability to elute and differentiate these short chain oligonucleotides with small sequence modifications. The use of TEAA as an ion-pair agent together with ACN in gradient mode resulted in sharp peaks eluting within a short run time, as well as in unique recognition mechanisms. Trends regarding pH-value, buffer concentration and temperature were established for the different types of columns, and shared in a more detailed white paper available from Daicel Chiral Technologies.

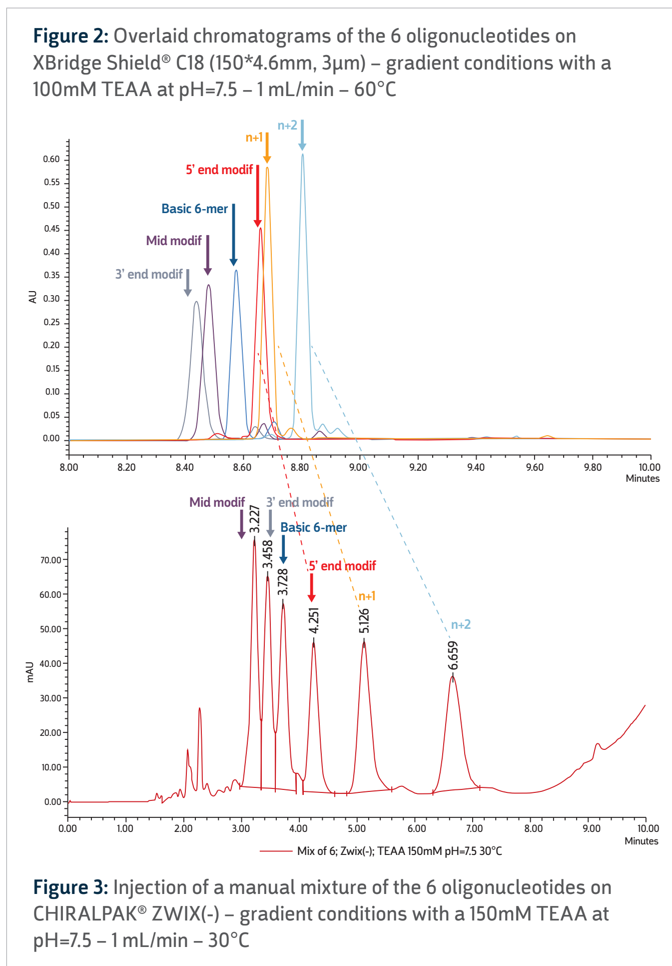


Figure 2: Overlaid chromatograms of the 6 oligonucleotides on XBridge Shield® C18 (150*4.6mm, 3µm) – gradient conditions with a 100mM TEAA at pH=7.5 – 1 mL/min – 60°C

Figure 3: Injection of a manual mixture of the 6 oligonucleotides on CHIRALPAK® ZWIX(-) – gradient conditions with a 150mM TEAA at pH=7.5 – 1 mL/min – 30°C

CHIRALPAK® ZWIX(-), offered the highest selectivity. When compared to a well established C18 column, the selectivity was significantly increased (Figures 2 and 3).

With their versatility and orthogonality, other Daicel columns also brought encouraging recognition behaviour as well as complementary elution patterns (Figures 4-6).

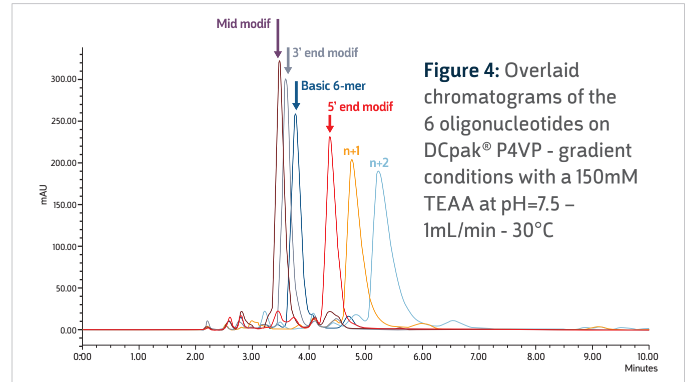


Figure 4: Overlaid chromatograms of the 6 oligonucleotides on DCpak® P4VP - gradient conditions with a 150mM TEAA at pH=7.5 – 1mL/min - 30°C

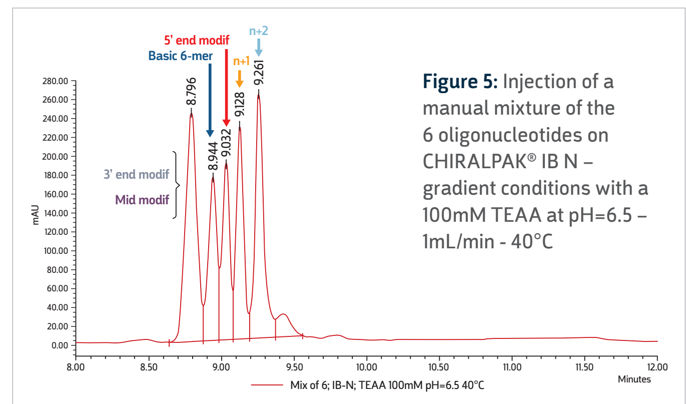


Figure 5: Injection of a manual mixture of the 6 oligonucleotides on CHIRALPAK® IB N – gradient conditions with a 100mM TEAA at pH=6.5 – 1mL/min - 40°C

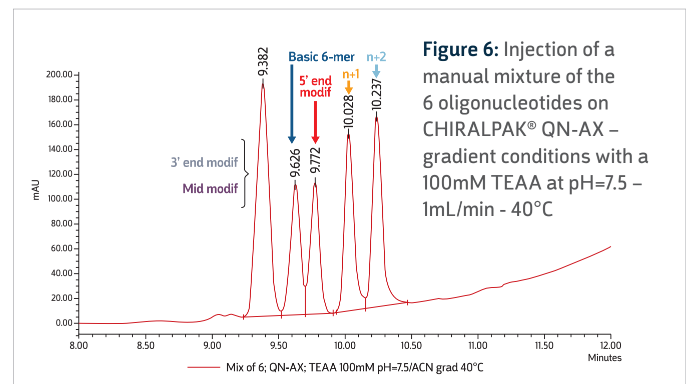
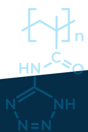


Figure 6: Injection of a manual mixture of the 6 oligonucleotides on CHIRALPAK® QN-AX – gradient conditions with a 100mM TEAA at pH=7.5 – 1mL/min - 40°C

These results open very promising investigation paths to apply and further improve any existing separation conditions. This could include use of smaller particle sizes, hyphenation with MS-detection and combinations with other columns in a 2D-configuration. It also offers potential for preparative separations.



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