Analysis of synthetic oligonucleotides using Mini Q 4.6/50 PE

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High quality separation of oligonucleotides requires a technology able to distinguish between closely related samples differing by as little as one or two bases. In order to achieve this level of resolution, high performance columns and media are needed. This application note shows that Mini Q 4.6/50 PE is a robust column that provides high resolution separation of DNA and RNA oligonucleotides at both elevated and ambient temperatures. The separation resolution of a mixture of 18-, 19-, and 20-mer DNA oligonucleotides in a single sample was compared after runs at 22°C and 40°C. The results demonstrate that Mini Q 4.6/50 PE, when operated at ambient temperature, delivers data of similar quality to runs performed at elevated temperatures. A high quality separation of DNA oligonucleotides of 13, 20, and 60 bases in length was also obtained at ambient temperature. Finally, the high reproducibility of Mini Q 4.6/50 PE is demonstrated by comparing the peak profile of a sample containing a 13-mer DNA oligonucleotide after 80 runs with that of the first run. Mini Q 4.6/50 PE delivers high resolution separation of synthetic oligonucleotides at micropreparative and analytical scale at both elevated and ambient temperatures.

Introduction

Ion exchange chromatography separates differently charged biomolecules based on reversible interactions between a charged biomolecule and an oppositely charged chromatographic medium. Biomolecules bind as they are loaded onto the medium. Conditions are then altered so that bound substances are eluted differentially. Elution is usually performed by applying a buffer gradient with gradually increasing salt concentration or by a change in pH. Target molecules are collected in a concentrated, purified form.

Mini Q 4.6/50 PE consists of a strong anion exchange chromatography medium prepacked in Tricorn™ columns. The chromatography medium is comprised of MiniBeads™ which are non-porous, monodisperse spherical beads with a small diameter (3 µm) enabling high resolution. The physically stable beads are packed in short (50 mm), compact, and high performance Tricorn columns that provide high resolution separation with brief running times.

Methods

Sample preparation

DNA oligonucleotides of 13, 18, 19, 20, and 60 bases, and an RNA oligonucleotide of 21 bases were synthesized using ÄKTA™ oligopilot™. The 13-, 18-, 19-, and 20-mer DNA oligonucleotides were synthesized using Primer Support™ 200. The 60-mer DNA was synthesized using Custom Primer Support 40s, and the 21-mer RNA oligonucleotide using Custom Primer Support 80s. Absorbance was measured at 260 nm, and each oligonucleotide was dissolved in water to produce a solution at a concentration between 10 and 30 OD/ml.

Resolution of oligonucleotides

The quality of peak resolution during elution using a salt gradient was tested by running 20- and 60-mer DNA oligonucleotides on Mini Q 4.6/50 PE. The effect of temperature on the quality of peak resolution was tested by running samples containing a mixture of 18-, 19-, and 20-mer DNA oligonucleotides at 22°C and 40°C.

Experimental conditions are listed in each figure with the resulting chromatograms. The elution gradients used with Mini Q 4.6/50 PE were set according to optimal, empirically derived levels as well as oligonucleotide length.

Reproducibility of oligonucleotide analysis

Eighty consecutive samples from a single stock solution of a synthetic 13-mer DNA oligonucleotide (5'-GGG CCC AAA TTTT-3') were run on a Mini Q 4.6/50 PE column.

Results

Analysis of oligonucleotide purity

Synthetic 20- and 60-mer DNA oligonucleotides were run on Mini Q 4.6/50 PE and peak profiles were analyzed (Fig 1A and 1B). A similar analysis was performed on a synthetic 21-mer RNA oligonucleotide (Fig 1C). Single, discrete, and compact peaks representing 20-mer DNA, 60-mer DNA, and 21-mer RNA oligonucleotides were detected after running unpurified samples on Mini Q 4.6/50 PE. These data demonstrate that the column can be confidently applied to analyze the purity of short and long synthetic DNA and RNA oligonucleotide samples.

High quality oligonucleotide separation

The quality of separation resolution of 18-, 19-, and 20-mer oligonucleotides was measured at 22°C and 40°C by comparing differences in retention time between the three oligomers at the tested temperatures. Figure 2 and Table 1 show that retention times were similar at both temperatures. Narrower peaks are obtained when the temperature is elevated, but the Mini Q 4.6/50 PE column gives sufficient resolution of oligonucleotides at ambient temperature. Thus, temperature control is not required to obtain high resolution with Mini Q 4.6/50 PE.

Column:	Mini Q 4.6/50 PE
Sample:	20-mer DNA
Sample load:	2 µl of 10 OD/ml sample
Buffer A:	1 mM Tris, 10 mM $NaClO_4$, pH 9.3
Buffer B:	1 mM Tris, 300 mM $NaClO_4$, pH 9.3
Flow rate:	1 ml/min
Gradient:	1 to 70% buffer B, 30 min
Detection:	UV 260 nm

Column:	Mini Q 4.6/50 PE
Sample:	60-mer DNA
Sample load:	2 µl of 10 OD/ml sample
Buffer A:	1 mM Tris, 10 mM $NaClO_4$, pH 9.3
Buffer B:	1 mM Tris, 300 mM $NaClO_4$, pH 9.3
Flow rate:	1 ml/min
Gradient:	1 to 99% buffer B, 30 min
Detection:	UV 260 nm

Column:	Mini Q 4.6/50 PE
Sample:	21-mer RNA
Sample load:	2 µl of 10 OD/ml sample
Buffer A:	1 mM Tris, 10 mM $NaClO_4$, pH 9.3
Buffer B:	1 mM Tris, 300 mM $NaClO_4$, pH 9.3
Flow rate:	1 ml/min
Gradient:	1 to 70%
Detection:	UV 260 nm

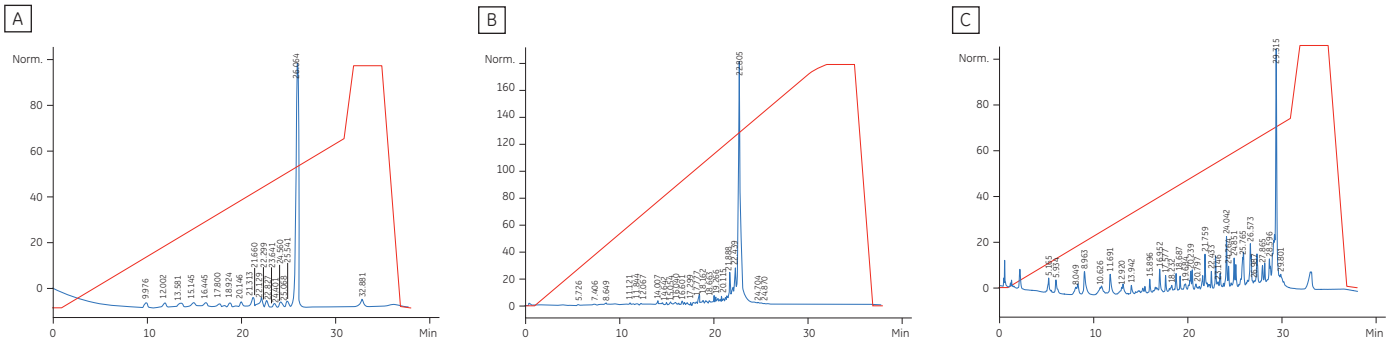

Fig 1. Analysis of synthetic DNA and RNA oligonucleotides: A) 20-mer DNA, B) 60-mer DNA, and C) 21-mer RNA.

Column:	Mini Q 4.6/50 PE, room temperature (22°C)
Sample:	18-, 19-, and 20-mer DNA
Sample load:	2 µl of 10 OD/ml sample
Buffer A:	1 mM Tris, 10 mM $NaClO_4$, pH 9.3
Buffer B:	1 mM Tris, 300 mM $NaClO_4$, pH 9.3
Flow rate:	1 ml/min
Gradient:	1 to 50%
Detection:	UV 260 nm

Column:	Mini Q 4.6/50 PE, 40°C
Sample:	18-, 19-, and 20-mer DNA
Sample load:	2 µl of 10 OD/ml sample
Buffer A:	1 mM Tris, 10 mM $NaClO_4$, pH 9.3
Buffer B:	1 mM Tris, 300 mM $NaClO_4$, pH 9.3
Flow rate:	1 ml/min
Gradient:	1 to 55%
Detection:	UV 260 nm

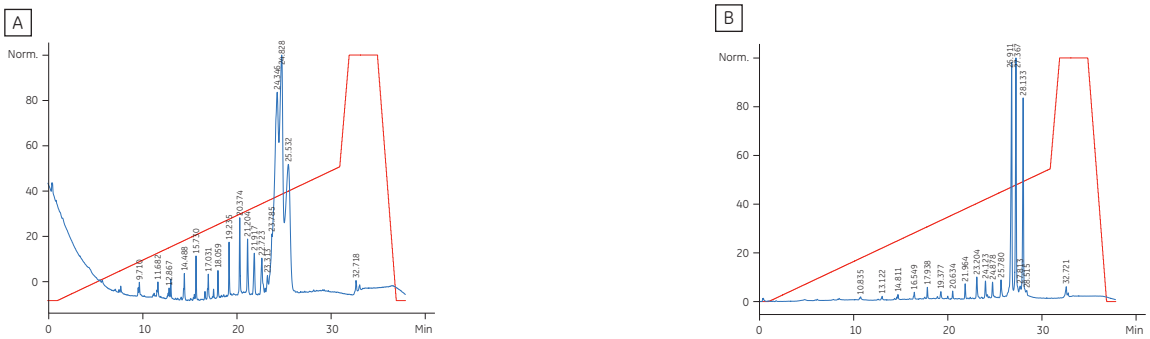

Fig 2. Separation of 18-, 19-, and 20-mer DNA oligonucleotides at (A) room temperature and (B) 40°C.

Table 1. Effect of temperature on oligonucleotide retention time

Temperature	Retention time of 18-mer oligonucleotide (min)	Additional retention time of 19-mer oligonucleotide[1] (min)	Additional retention time of 20-mer oligonucleotide[1] (min)
RT (22°C)	24.346	0.482	1.186
40°C	26.911	0.456	1.222

[1] Compared to retention time of 18-mer oligonucleotide

Reproducibility of oligonucleotide analysis

The consistency of data over repeated analyses was tested by running 80 consecutive samples of a 13-mer DNA oligonucleotide on Mini Q 4.6/50 PE. Figure 3A and 3B show peak profiles after the 1st and 80th runs, respectively. No significant difference in peak profile was observed after 80 consecutive runs compared with the first run, demonstrating the reliability and consistency of Mini Q 4.6/50 PE.

Column: Mini Q 4.6/50 PE
Sample: 13-mer DNA
Sample load: 2 µl of 10 OD/ml sample
Buffer A: 1 mM Tris, 10 mM $NaClO_4$, pH 9.3
Buffer B: 1 mM Tris, 300 mM $NaClO_4$, pH 9.3
Flow rate: 1 ml/min
Gradient: 1 to 70%
Detection: UV 260 nm

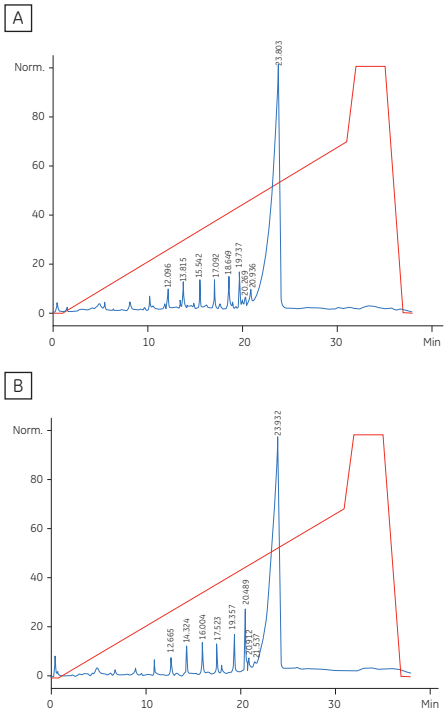

Fig 3. Reproducible analyses of 13-mer DNA: (A) 1st run and (B) 80th run.

Conclusion

Mini Q 4.6/50 PE is a robust chromatography column that provides high resolution separation of DNA and RNA oligonucleotides at both elevated and ambient temperatures. No significant difference in the separation resolution of a mixture of DNA oligonucleotides, differing by only one or two bases, was observed when samples were run at 22°C compared to 40°C. The reliability and robustness of the column was illustrated by the consistency of the peak profile over multiple runs. No significant differences were recorded in the peak profile after 80 consecutive runs compared with the first run. These data show that Mini Q 4.6/50 PE can be used to separate DNA and RNA oligonucleotides with high resolution and reproducibility.

Ordering information

Product	Quantity	Code no.
MiniQ 4.6/50 PE*	1	17-5177-01

* Tricorn columns have Valco fittings for ÄKTA™ design systems and are supplied with M6 connectors for FPLC™ System.

Related products

Related literature	Code no.
Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	11-0004-21
Tricorn MiniBeads - High Performance Columns, Data file	18-1165-93

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