

Agilent PL-SAX Anion-Exchange Media for Nucleotide and Oligonucleotide Analysis

Technical Overview

Introduction

The physical stability of Agilent PL-SAX ensures rapid equilibration between separations, and permits the use of aggressive cleanup procedures employing high salt, sodium hydroxide, mineral and organic acids, and a wide range of organic solvents. The capability of PL-SAX is shown here in the analysis of different nucleotide and oligonucleotides.



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Analysis of nucleotides under acidic conditions

The physical stability of PL-SAX media allows the use of acidic conditions for the separation of nucleotides. For the analysis of the more strongly retained 5' di- and tri-phosphate nucleotides, a gradient is used to minimize the analysis time (Figure 1). The less strongly retained 2', 3' mono-phosphate nucleotides can be analyzed using an isocratic system, eluent A, in under 20 minutes (Figure 2).

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1802)
Eluent A: 0.01 M NaH₂PO₄ at pH 2.58
Eluent B: 0.5 M NaH₂PO₄ at pH 2.58
Gradient: Linear 0-100% B in 30 min
Flow Rate: 1.0 mL/min
Detection: UV, 280 nm

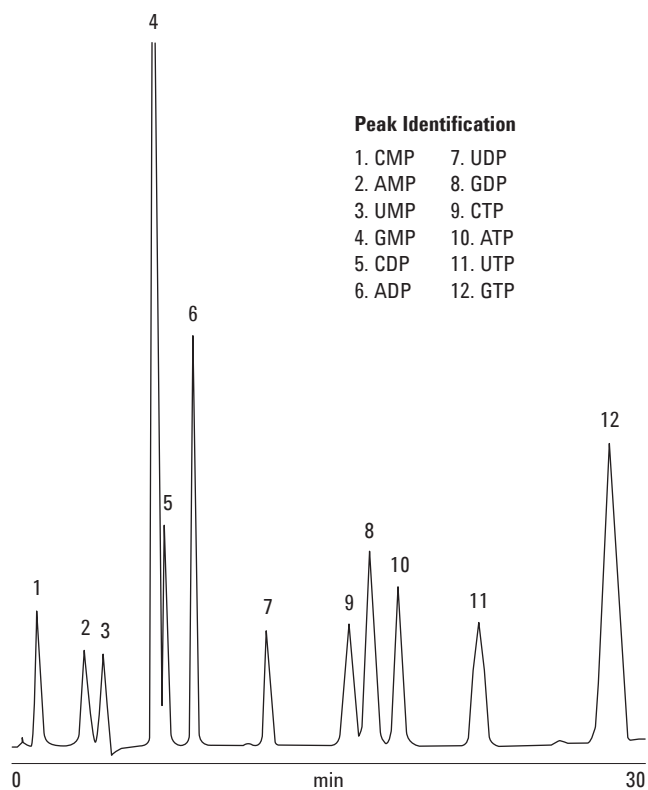


Figure 1. Gradient analysis of 5' di- and tri-phosphate nucleotides on Agilent PL-SAX.

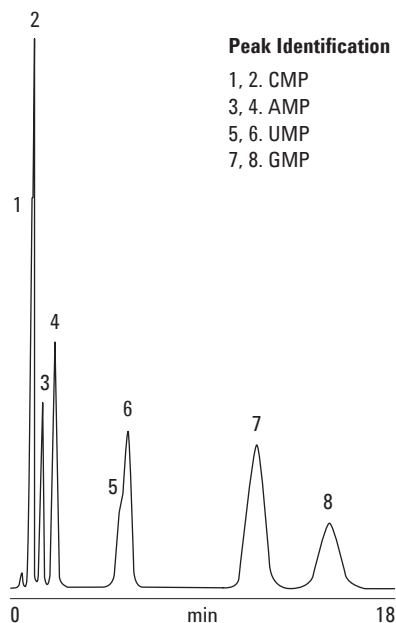


Figure 2. Isocratic analysis of 2', 3' mono-phosphate nucleotides on Agilent PL-SAX.

Separating 5' mono-, di- and tri-phosphate nucleotides using a salt/pH gradient

By using the high performance, 8 μm particle size PL-SAX 1000 \AA adsorbent with a pH and salt gradient (Figure 3), it is possible to resolve 12 standard nucleotides in less than 20 minutes (Figure 4).

Conditions (Figure 3)

Column: PL-SAX 1000 \AA 8 μm , 50 x 4.6 mm (p/n PL1551-1802)
 Eluent A: 0.01 M KH_2PO_4
 Eluent B: 0.5 M KH_2PO_4
 Gradient: Linear 0-100% B in 20 min
 Flow Rate: 1.0 mL/min
 Detection: UV, 260 nm

Conditions (Figure 4)

Column: PL-SAX 1000 \AA 8 μm , 50 x 4.6 mm (p/n PL1551-1802)
 Eluent A: 0.01 M KH_2PO_4 at pH 2.6
 Eluent B: 0.5 M KH_2PO_4 at pH 3.5
 Gradient: Linear 0-100% B in 20 min
 Flow Rate: 1.0 mL/min
 Detection: UV, 260 nm

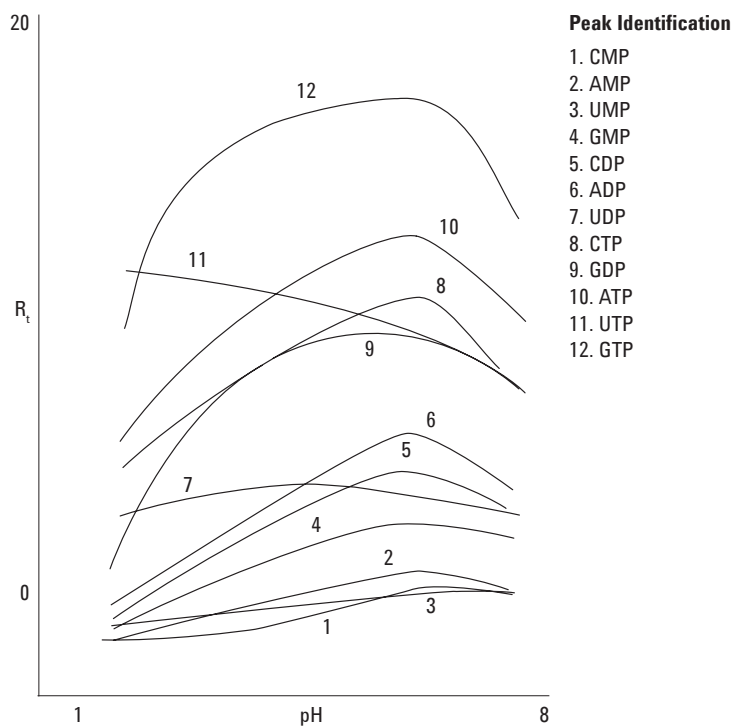


Figure 3. Resolution of nucleotides using a salt/pH gradient and Agilent PL-SAX.

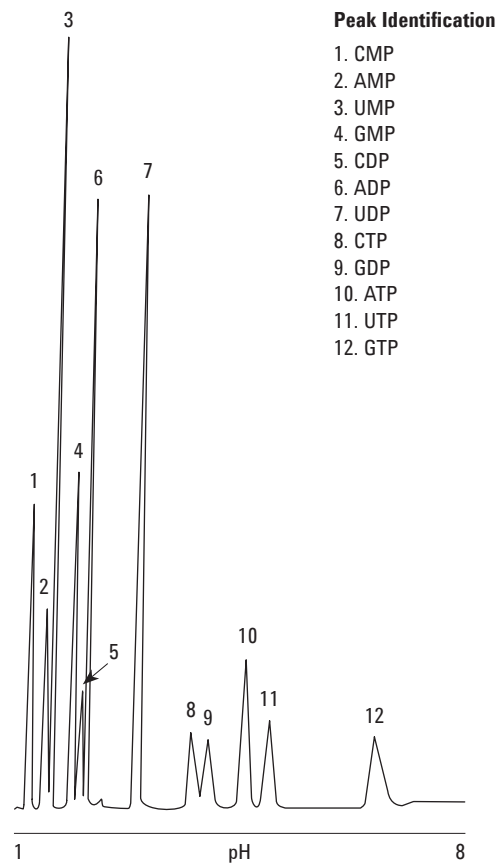


Figure 4. Resolution of phosphate nucleotides by Agilent PL-SAX in less than 20 min.

Investigating the decay of oligoribonucleotides-oligo (rA)_n

The PL-SAX 1000Å material has the optimum pore size for maximum loading with low band broadening for the analysis of oligoribonucleotides. A sample of oligo (rA)_n was prepared by alkaline hydrolysis of the homopolymer poly (rA), 7.2 mg/mL at 60 °C. After termination of the reaction by neutralizing the pH, the oligonucleotides were precipitated using ethanol:acetone (Figure 5).

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1802)
Eluent A: 0.2 M K₂HPO₄ + 5 M urea, pH 5.5
Eluent B: A + 1 M NaCl
Gradient: Linear, see Figure captions
Flow Rate: 1.0 mL/min
Detection: UV, 260 nm

Peak Identification

1. 2', 3' cAMP
2. 5' AMP
3. 2' AMP
4. 3' AMP
5. 2' (rA)₂
6. 3' (rA)₂
7. 2' (rA)₃
8. 3' (rA)₃
9. 2', 3' (rA)₄

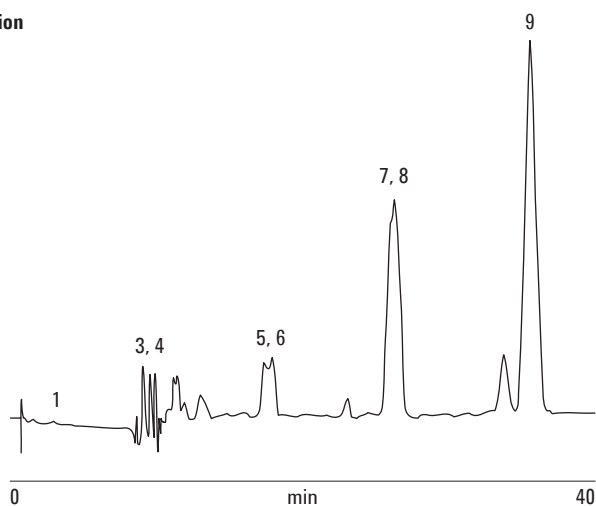


Figure 5. Oligo (rA) on Agilent PL-SAX (gradient 0-100% B in 20 min).

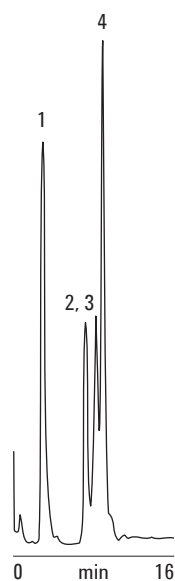


Figure 6. Oligonucleotide standards on Agilent PL-SAX (gradient 0-100% B in 20 min).

A freshly prepared sample of oligo (rA) has a secondary series of peaks that are unstable with time. This is probably the 2', 3' cyclic phosphate, which is intermediate in the conversion of the 3' phosphate to the 2' phosphate that has been identified using reference materials (Figure 7).

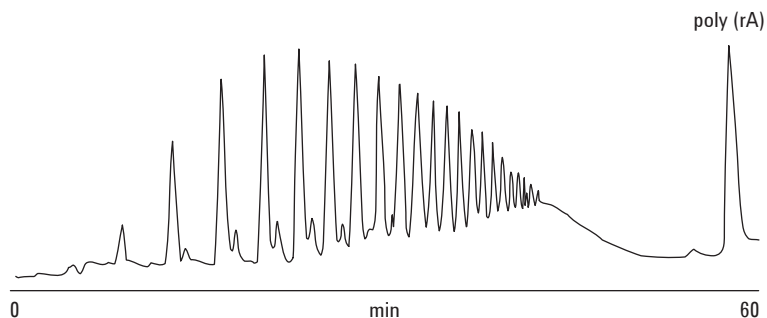


Figure 7. The decay of a fresh sample of oligonucleotide (rA) on Agilent PL-SAX (gradient 0-100% B in 60 min).

Analysis of synthetic oligonucleotides

Oligonucleotides were produced using a commercial synthesizer by CED phosphoramidite chemistry. After deprotection, their purity was assessed using PL-SAX. Further purification from the shorter chain length failure sequences can also be done. Urea was added to the mobile phase to prevent self hybridization and the production of secondary structure of palindromic single stranded oligonucleotides (Figure 8).

Figure 9 shows a mixture of four synthetic oligonucleotides of different chain lengths. Note the reversal in expected elution order for peaks 3 and 4, the 30-mer and 29-mer.

Conditions

Column: PL-SAX 1000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1802)
Eluent A: 0.2 M K_2HPO_4 + 5 M urea, pH 5.5
Eluent B: A + 1 M NaCl
Gradient: Linear 0-100% B in 20 min
Flow Rate: 1.0 mL/min
Detection: UV, 260 nm

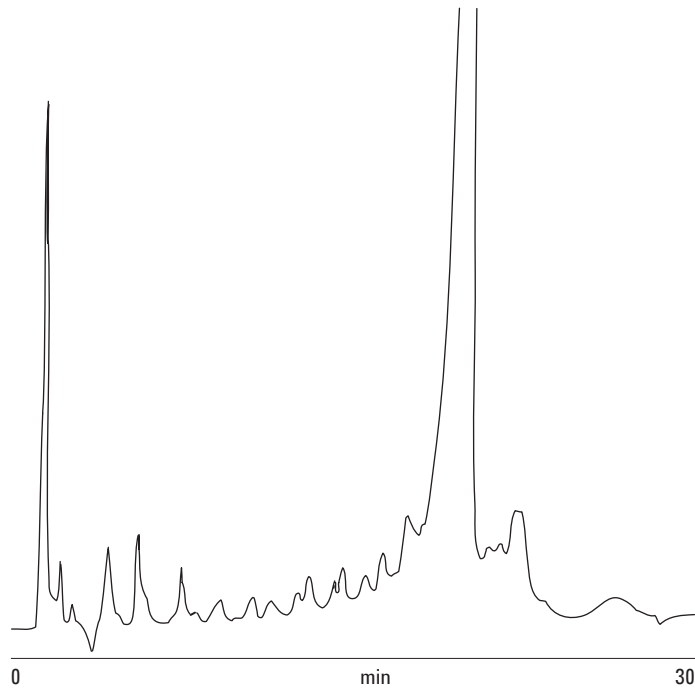


Figure 8. 24-mer chain of synthetic oligonucleotide ATTATGCTGAGTGATATCCCTAGG on Agilent PL-SAX.

Peak Identification

- 1. 6 mer: GGATCC
- 2. 24 mer: TAATAGGACTCACTATAGGGATCC
- 3. 30 mer: GCGTCCCACGGTTTCGACAGAACAGCCGAG
- 4. 29 mer: CATCCATTTGAGGTAGGTCAAATTTACCT

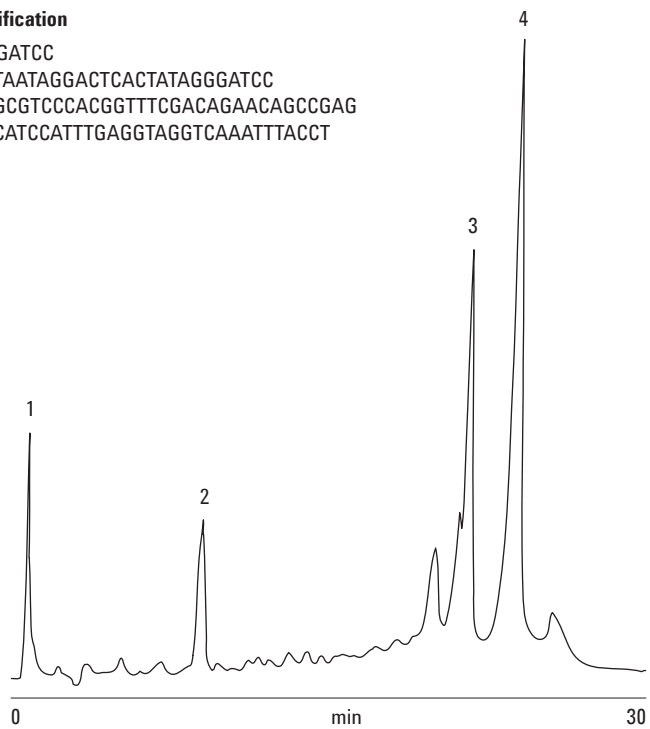


Figure 9. A mixture of four synthetic oligonucleotides separated by Agilent PL-SAX.

Analysis of heptadecanucleotides

By using a pH and salt gradient, PL-SAX can be used for the analysis of a heptadecanucleotide (Figure 10). The sample was a crude deprotection mixture from the phosphotriester chemical condensation synthesis of the heptadecanucleotide d(GCCATTCCATTACCA)rC.

Conditions

Column: PL-SAX 4000Å 8 µm, 50 x 4.6 mm (p/n PL1551-1803)

Eluent A: Water, pH 8.95

Eluent B: 1 M (NH₄)₂SO₄ at pH 2.58

Gradient: Linear 1-75% B in 60 min, then 75-100% in 9 min

Flow Rate: 1.0 mL/min

Detection: UV, 254 nm

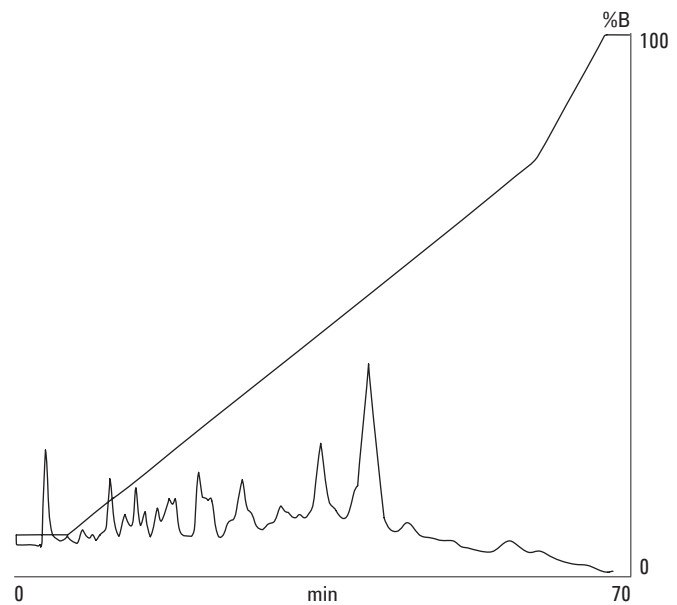


Figure 10. Analysis of heptadecanucleotide by Agilent PL-SAX (separation courtesy of D Carlson, Purdue University, USA).

Agilent PL-SAX Strong Anion-Exchange Columns

PL-SAX is designed for anion-exchange HPLC separations of proteins and deprotected synthetic oligonucleotides under denaturing conditions. The strong anion-exchange functionality, covalently linked to a chemically stable polymer, extends the operating pH range. What's more, anion-exchange capacity is independent of pH. For synthetic oligonucleotides, separations using denaturing conditions of temperature, organic solvent, and high pH are all possible. PL-SAX delivers improved chromatography for self-complementary or G-rich sequences that may associate to form aggregates or hairpin structures. The 5 μm material provides high efficiency separations of n and n-1 sequences.

These data represent typical results. For further information, contact your local Agilent Sales Office.

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Published in UK, August 10, 2011

5990-8779EN



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