Separation of RNA oligonucleotides using XTerra® MS C18, 2.5 µm column

The XTerra MS C18, 2.5 µm column is an excellent choice for separation of DNA and RNA oligonucleotides. The key to the successful separation is a combination of several factors:

- highly efficient columns packed with 2.5 µm sorbent
- elevated separation temperature
- shallow gradient used for separation

The efficient separation is based on an ion-pairing mechanism. The separation of oligonucleotides differing in the length by a single nucleotide is possible up to ~ 60 mer. Separation partially depends on the oligonucleotide sequence (hydrophobicity of the bases). For example, page 3 shows a more successful separation of the 29/30-mer than of the 20/21-mer pair. Alternative ion-pairing buffers were introduced in order to improve the separation. More detailed discussion of separation principles and sequence dependent retention using XTerra columns is discussed in literature (M.Gilar, Analytical Biochemistry, vol. 298 (2001) 196-206; and M.Gilar et al., Journal of Chromatography A, vol. 958 (2002) 167-182.

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Sequence for RNA oligos in test mixture

10-mer U.A.C . C.G.A . C.U.G . U

11-mer G.U.A . C.C.G . A.C.U . G.U

20-mer U.A.C . C.G.A . C.U.G . U.U.A . C.C.G . A.C.U . G.U

21-mer G.U.A . C.C.G . A.C.U . G.U.U . A.C.C . G.A.C . U.G.U

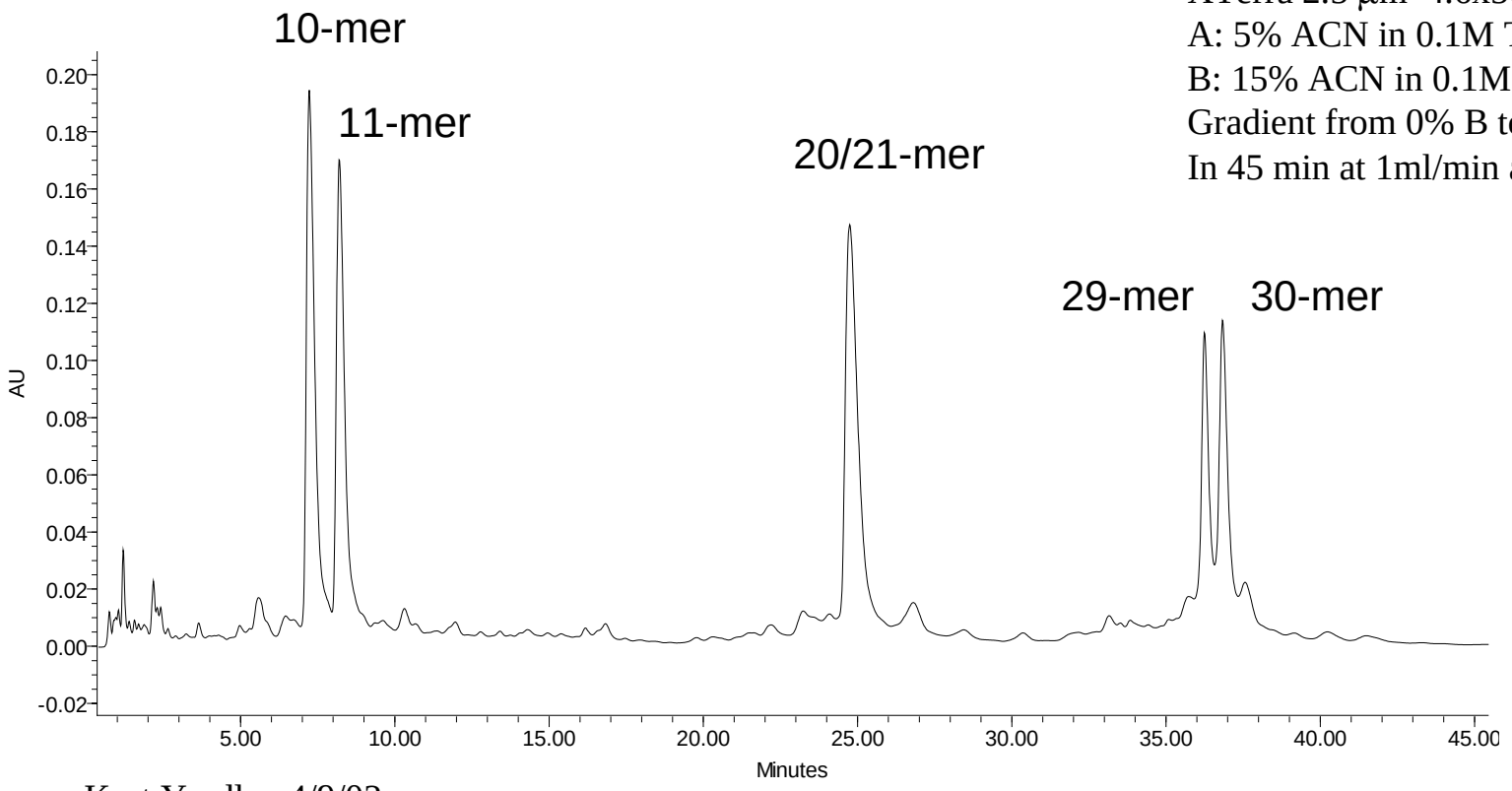
29-mer A.C.C . G.A.C . U.G.U . U.A.C . C.G.A . C.U.G . U.U.A . C.C.G . A.C.U . G.U

30-mer U.A.C . C.G.A . C.U.G . U.U.A . C.C.G . A.C.U . G.U.U . A.C.C . G.A.C . U.G.U

RNA Test Mixture; generic gradient

Gradient 0.067% ACN/ minute

Gradient conditions
XTerra 2.5 µm 4.6x50mm column
A: 5% ACN in 0.1M TEAA
B: 15% ACN in 0.1M TEAA
Gradient from 0% B to 30% B
In 45 min at 1ml/min and 60° C



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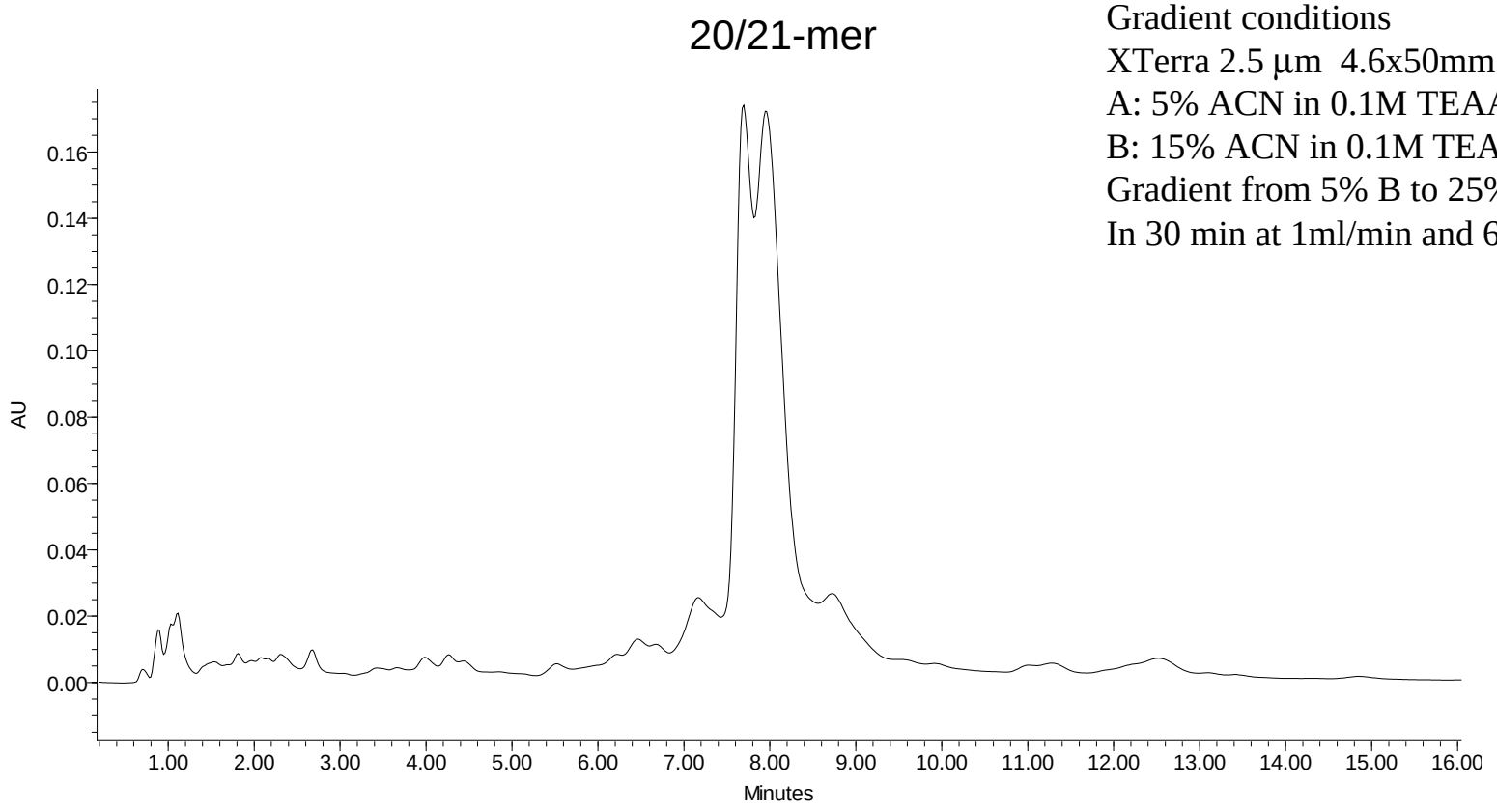
RNA oligos 20 and 21 mer

Gradient 0.067% ACN/ minute



Gradient conditions
XTerra 2.5 µm 4.6x50mm column
A: 5% ACN in 0.1M TEAA
B: 15% ACN in 0.1M TEAA
Gradient from 5% B to 25% B
In 30 min at 1ml/min and 60° C

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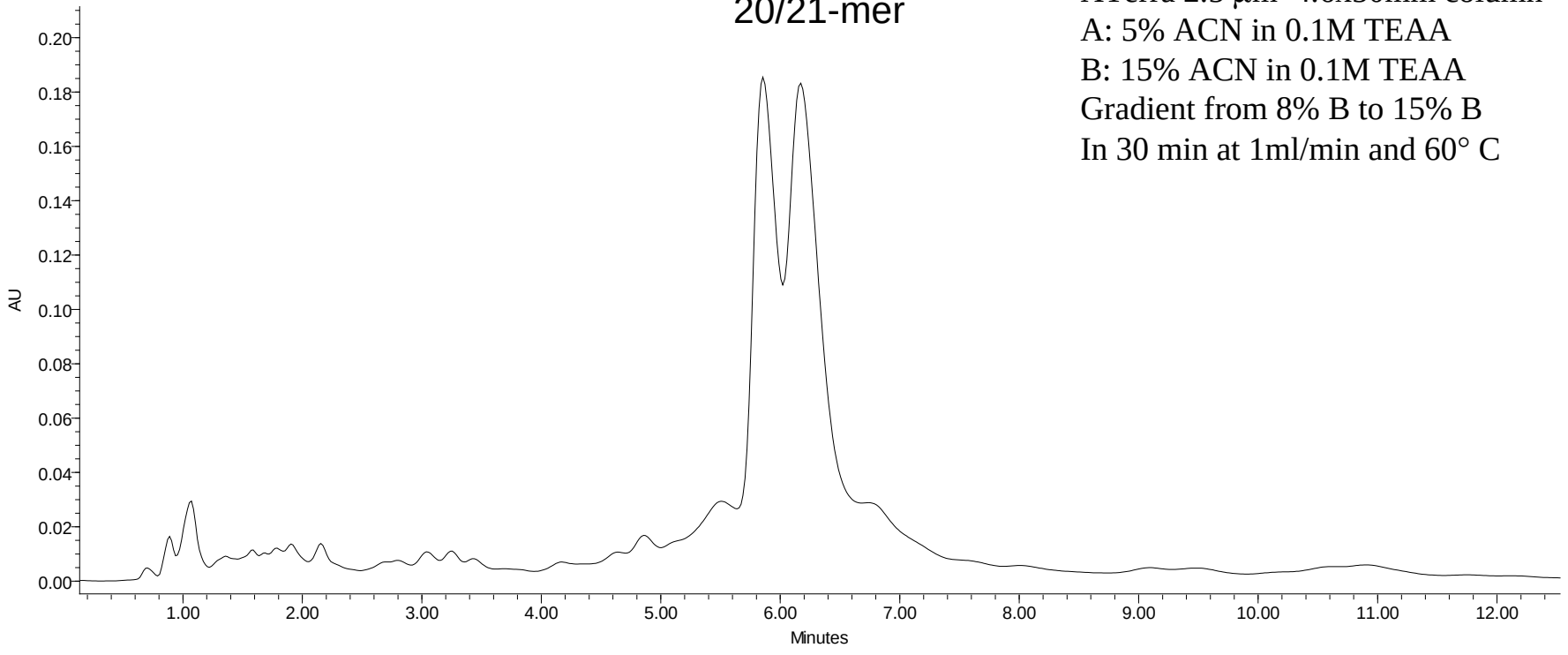
RNA oligos 20 and 21 mer; optimized gradient

Gradient 0.023% ACN/ minute



Gradient conditions
XTerra 2.5 µm 4.6x50mm column
A: 5% ACN in 0.1M TEAA
B: 15% ACN in 0.1M TEAA
Gradient from 8% B to 15% B
In 30 min at 1ml/min and 60° C

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