APPLICATION NOTE

Optimisation of phosphorothioate oligonucleotide separation using BioPro IEX QF

Oligonucleotides are important in genetic testing, research and forensics. Phosphorothioate oligonucleotides (PS) are resistant to nucleases and prevent nucleolytic degradation. Therefore, PS are often used for in vivo and in vitro technologies as they are more robust than phosphodiester oligonucleotides (PO).

For this reason corresponding analytical methods had to be developed. Anion exchange chromatography (AEX) methods can provide one suitable approach. Usually PS show broader peaks than PO because of their higher acidity, so that a higher salt concentration is required (see fig. 2).

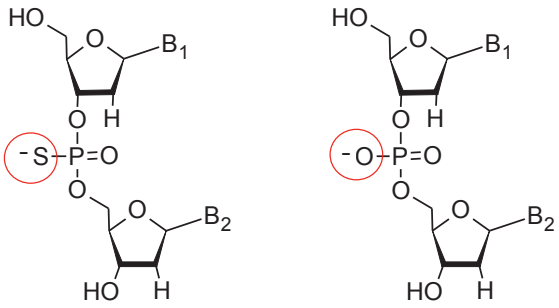

Figure 1: Structure of PS and PO linkage.

This application note shows how to achieve narrow peaks and high sensitivity by optimising the separation of phosphorothioate oligonucleotides in only 5 steps using the strong anion exchanger BioPro IEX QF.

Table 1: Final chromatographic conditions.

Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID
Part no.	QF00S05-1046WP
Eluent:	A) 10 mM NaOH/methanol (70/30)
	B) 10 mM NaOH containing 1.0 M $NaClO_4$/methanol (70/30)
Gradient:	40–100 % B in 6.3 min
Flow rate:	1.0 mL/min
Temperature:	60 °C
Detection:	UV at 260 nm
Injection:	2 µL (10 nmol/mL)

As a first step the PS RNA was analysed without using an organic modifier with a gradient of 32–80 % B in 24 min at 25 °C. Broad peaks and low sensitivity are obtained.

10	Single-stranded RNA	5'-U^C^A^U^C^A^C^A^C^U^G^A^A^U^A^C^C^A^A^U-3'	(All PS RNA 20mer)
11		5'-G^U^C^A^U^C^A^C^A^C^U^G^A^A^U^A^C^C^A^A^U-3'	(All PS RNA 21mer)

^=Phosphorothioated

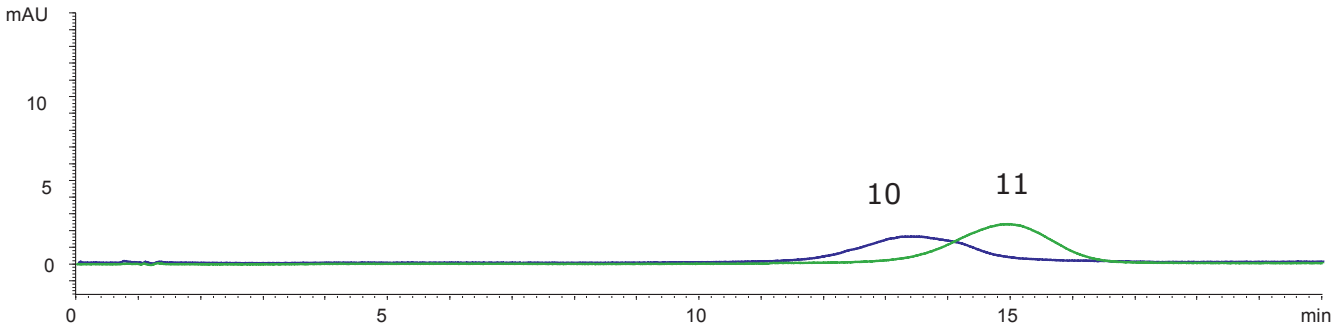

Figure 2: Separation of single-stranded PS RNA without modifier at 25 °C.

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Using a steeper gradient of 32–80 % B in 8 min the peaks became a bit narrower.

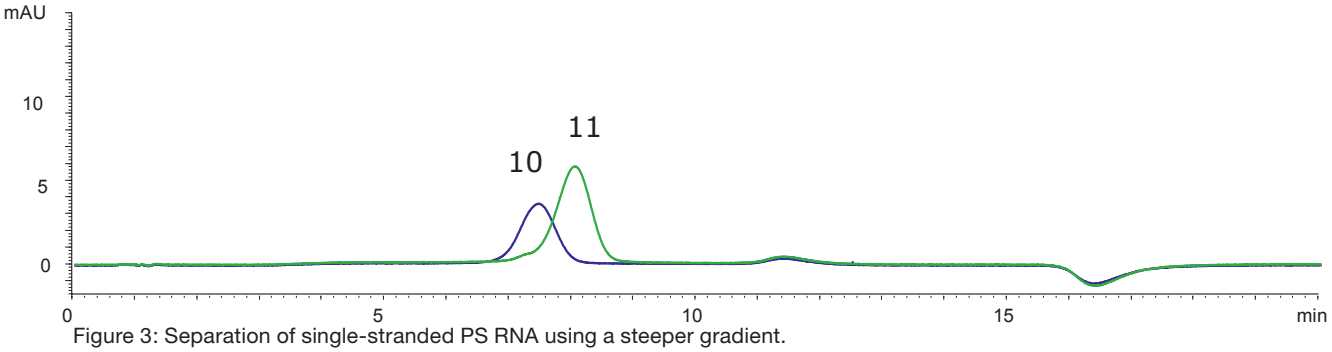

Figure 3: Separation of single-stranded PS RNA using a steeper gradient.

Increasing the column temperature to 60 °C lead to narrower peaks and improved resolution.

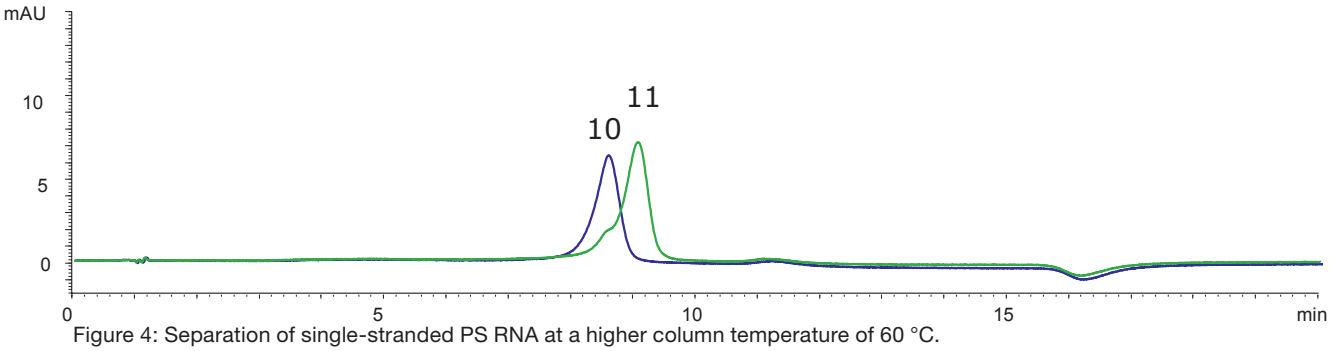

Figure 4: Separation of single-stranded PS RNA at a higher column temperature of 60 °C.

With the addition of methanol as organic modifier (Eluent A and B: 20 % MeOH) the resolution and sensitivity improved. To achieve the same salt concentration the gradient was adjusted to 40–100 % B in 8 min.

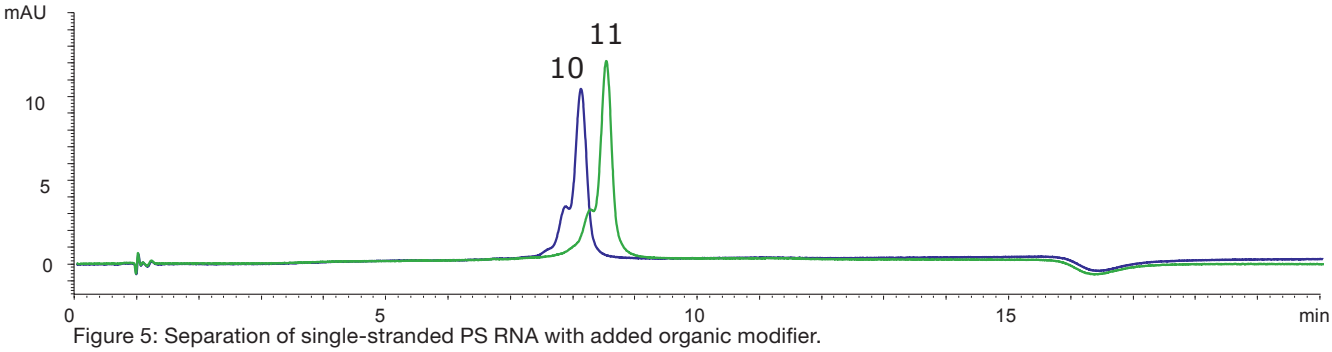

Figure 5: Separation of single-stranded PS RNA with added organic modifier.

By increasing the ratio of organic modifier (30 % MeOH in A and B) narrow peaks with partly resolved variants and high sensitivity were obtained. The gradient was adjusted to 40–100 % B in 6.3 min.

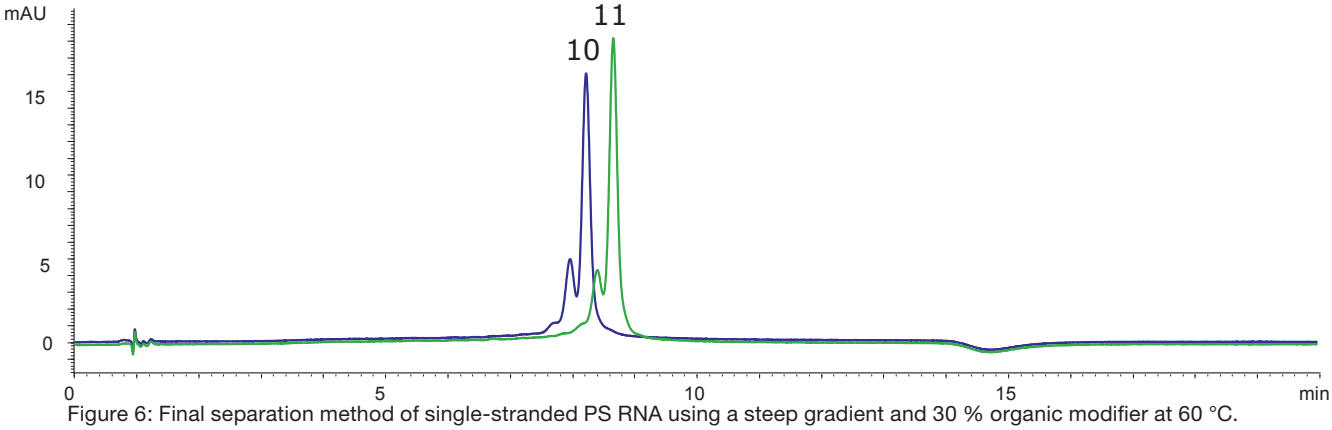

Figure 6: Final separation method of single-stranded PS RNA using a steep gradient and 30 % organic modifier at 60 °C.