High temperature stability of BioPro IEX columns

Temperature is a common tool used in liquid chromatography to adjust e.g. selectivity. However, the effect of temperature on retention becomes more complex with large biomolecules. The molecules can unfold, which is dependent on the stability of their secondary structure, and interact with the stationary phase with varying strength. As the responses of proteins at higher temperatures are dependent on the conformation, it is difficult to predict the change in retention.

It was reported that temperature influences the peak capacity and resolution of proteins in ion exchange chromatography (IEX), while the effect on selectivity seems only to be limited. This behaviour is true for both pH- and salt-gradient separations. Therefore, it makes sense to also optimise the temperature in the process of IEX method development [1].

When high temperatures are useful

Even though most IEX analyses of proteins or antibodies are performed using relatively mild temperatures of 25–30°C, there is also a need for high temperatures in IEX as well. Oligonucleotides often require higher temperatures, up to 60°C in AEX mode, to achieve high resolutions and also to suppress unwanted intermolecular interactions, which would negatively influence the chromatography.

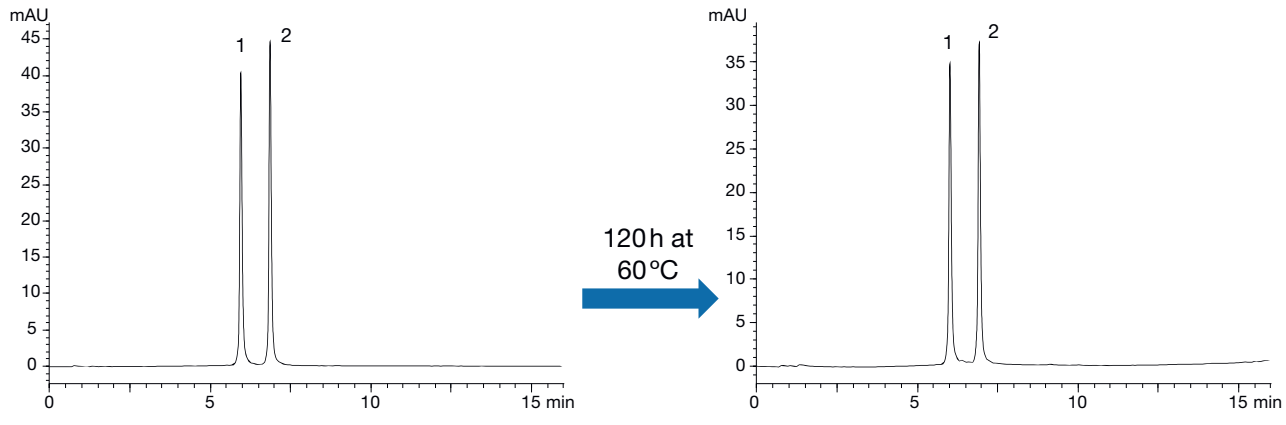
YMC's BioPro IEX columns can be used within the temperature range of 4–60°C. This allows for the application of relatively high temperatures which provides flexibility in method development.

Temperature stability of BioPro IEX columns

The temperature stability of the BioPro IEX columns is demonstrated using two examples: the analysis of 2 oligonucleotides and the separation of 2 proteins applying different chromatographic conditions. In both cases the non-porous anion exchanger BioPro IEX QF was used.

After obtaining the initial chromatogram using the conditions described below, the column was flushed at 60°C with either 10 mM NaOH for 120 h or 20 mM Tris-HCl for 33 h, respectively. Initial analyses were performed and again after 120 h in the first example and after 9 and 33 h in the second example.

Example 1 Analysis of 20 and 21 mer DNA



Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID	Temperature:	25 °C
Part No.:	QF00S05-1046WP	Detection:	UV at 260 nm
Eluent:	A) 10 mM NaOH	Injection:	4 µl (each 5 nmol/ml)
	B) 10 mM NaOH containing 1.0 M $NaClO_4$	Sample:	1) 5'-TCATCACACTGAATACCAAT-3'(DNA 20 mer)
Gradient:	25–55%B (0–15 min), 100%B (15–20 min)		2) 5'-GTCATCACACTGAATACCAAT-3'(DNA 21 mer)
Flow rate:	1.0 ml/min		

Figure 1: Initial chromatogram of 2 DNA oligonucleotides and after 120 h of flushing with NaOH at 60°C.

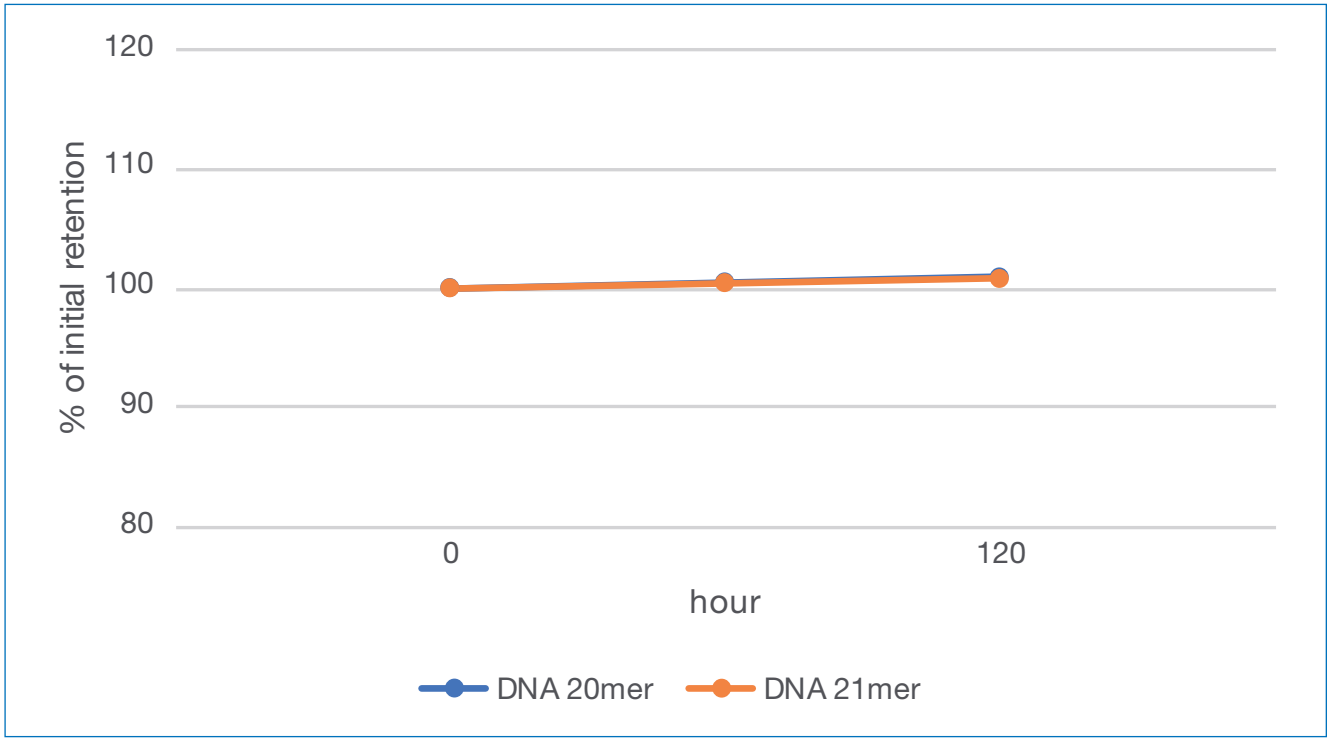


Figure 2: No change in retention time for both oligonucleotides after 120 h.

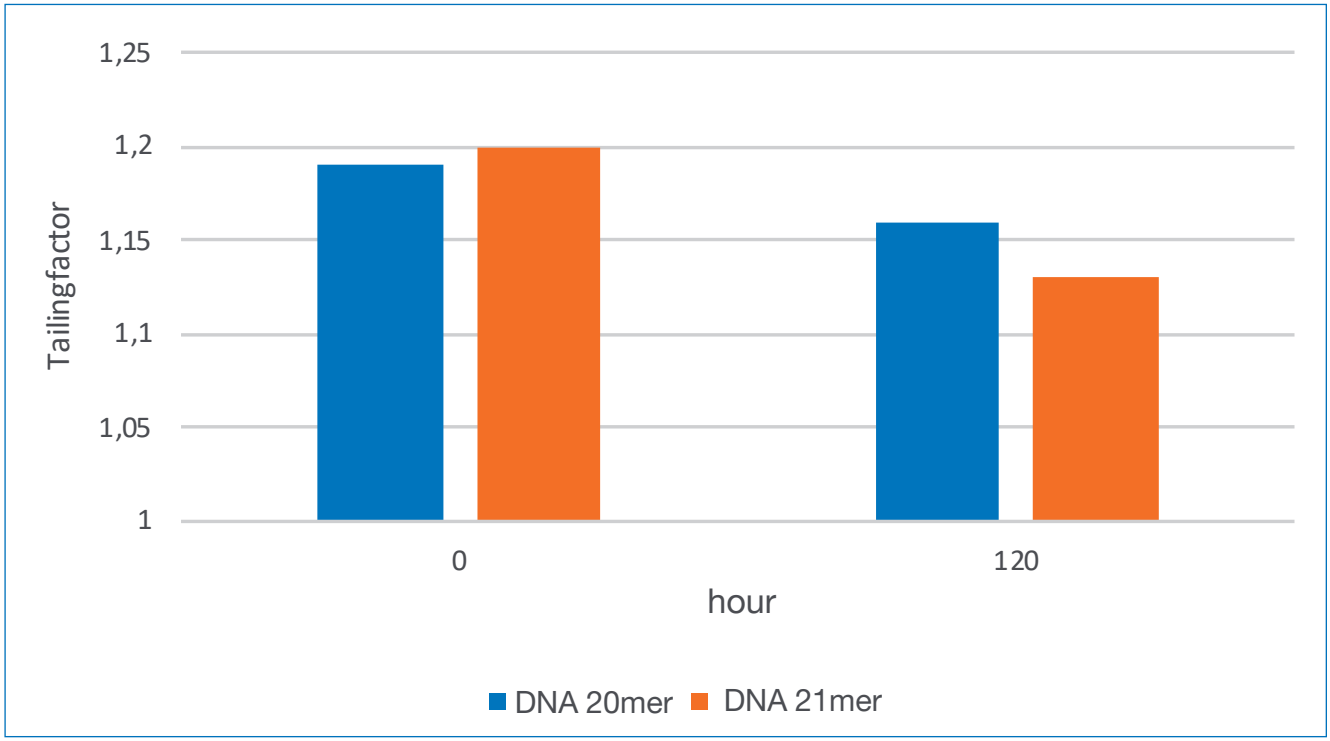
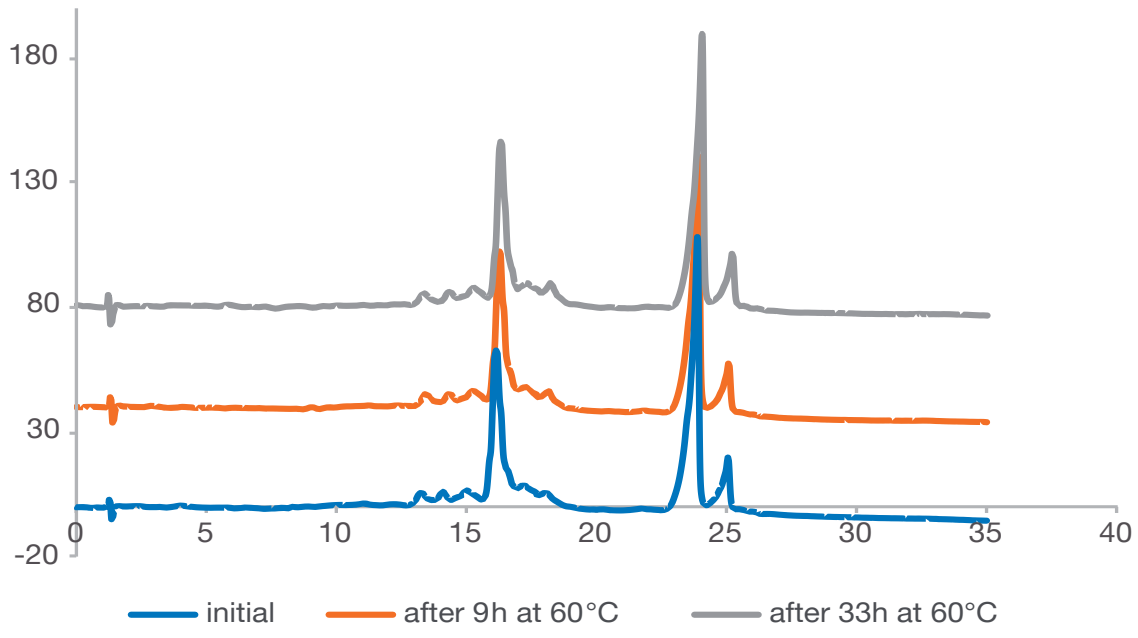


Figure 3: Tailing factors of both oligonucleotides obtained in the initial analysis and after 120 h of flushing.

Example 2 Separation of proteins



Column:	BioPro IEX QF (5 µm) 100 x 4.6 mm ID	Temperature:	25 °C
Part No.:	QF00S05-1046WP	Detection:	UV at 220 nm
Eluent:	A) 20 mM Tris-HCl (pH 8.3)	Injection:	20 µl
	B) 20 mM Tris-HCl (pH 8.3) containing 0.5 M NaCl	Sample:	1) Ovalbumin
Gradient:	10–100%B (0–54 min)		2) Trypsin inhibitor
Flow rate:	0.5 ml/min		

Figure 4: Chromatograms of 2 proteins before flushing and after 9h and 33h of flushing with Tris-HCl at 60 °C.

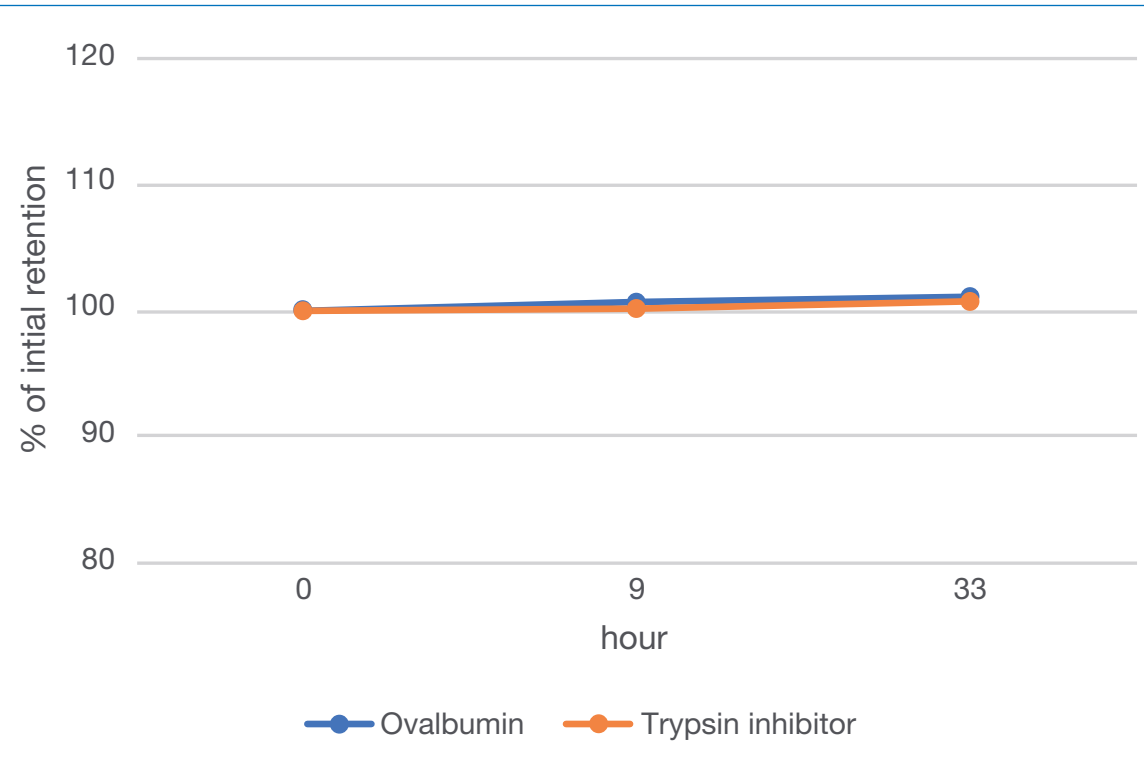


Figure 5: No change in retention time for both proteins after 9 and 33 h of flushing.

Temperature stability of BioPro IEX columns

These 2 examples nicely demonstrate that BioPro IEX show reproducible performances, even when they are used at their maximum temperature with different mobile phases for a long time period. The retention times as well as peak shapes remain stable.

This feature provides more flexibility in the method development and long-term use of BioPro IEX columns making them applicable to a broad range of analytes, such as antibodies and proteins, in addition to oligonucleotides, which in particular often require the use of high temperatures.

[1] S Fekete et al, Ion-exchange chromatography for the characterization of Biopharmaceuticals, J Pharm Biomed Anal (2015) 113:43-55.