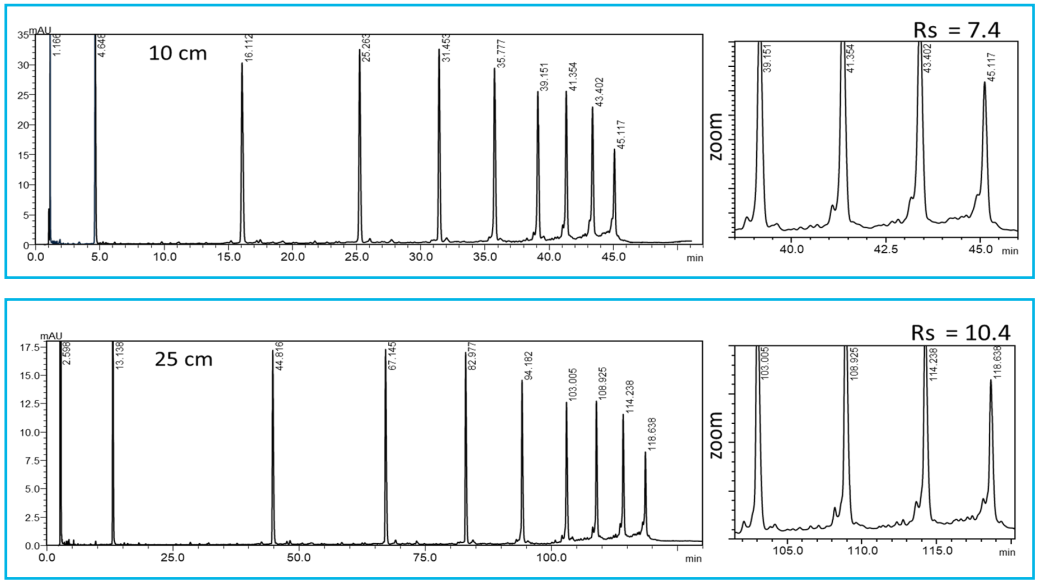
The Impact of Column Length on Oligonucleotide Resolution in HPLC



TEST CONDITIONS:

Column: HALO 1000 Å OLIGO C18, 2.7 µm, 2.1 x 100 mm
Part Number: P2762-602
Column: HALO 1000 Å OLIGO C18, 2.7 µm, 2.1 x 250 mm
Part Number: P2762-902
Mobile Phase A: (90)/5/5 (10mM DiBA/100mM HFIP)/ MeOH/ACN
Mobile Phase B: 80/20 Water/ACN

Gradient:	Time	%B
10cm	0.0	30
	60.0	80
25cm	0.0	30
	150.0	80

Flow Rate: 0.2 mL/min.
Back Pressure: 111 bar - 100mm
206 bar - 250mm
Temperature: 60 ºC
Injection: 1.0 µL of ssDNA, 20-100 ladder (10µg/mL)
Sample Solvent: 10mM Tris/1mM EDTA
Wavelength: PDA, 260 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2

The resolution of an oligonucleotide mixture was evaluated using a longer column (25 cm) under a shallow gradient, successfully resolving species up to 100 nucleotides in length. The resolution scaled approximately with the square root of column length (√L), consistent with chromatographic theory when the gradient rate was adjusted proportionally to column length—specifically, comparing 150-minute and 60-minute gradients for the 25 cm and 10 cm columns. Across the oligonucleotide size range, resolution increased by a factor of 1.34, which is close to the theoretical expectation of 1.58. This deviation may reflect practical limitations such as extra-column dispersion, gradient mixing effects, or non-idealities in oligonucleotide diffusion and interaction kinetics. Nonetheless, the data supports the expected trend that longer columns, when paired with appropriately scaled gradients, enhance resolution for complex oligonucleotide mixtures, particularly in the context of gradient elution with acetonitrile (AcN) under DiBA/HFIP conditions.