Selectivity and Efficiency Effects of Pore Size of Hybrid Superficially Porous Particles on Nucleic Acids Separations

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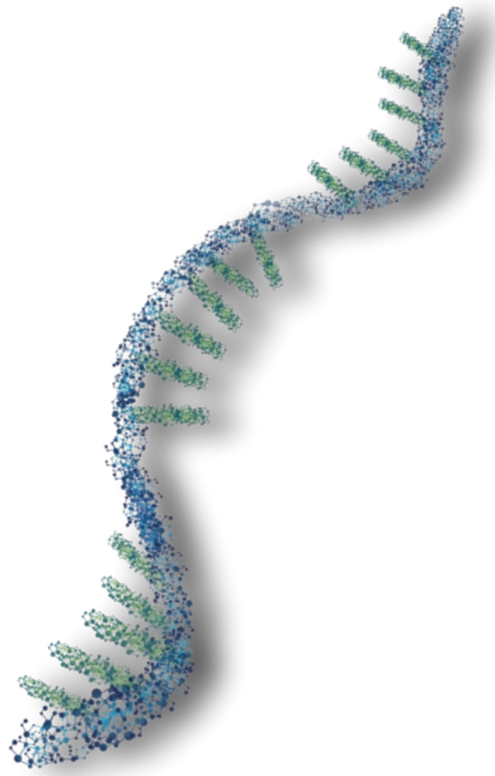
Introduction to Oligos

- **Role in Therapeutics and Research**
  Oligonucleotides are essential for therapies, diagnostics, and molecular biology research including antisense and siRNA drugs. Largely these are synthetic molecules, with variable byproducts of synthesis.
- **Unique Physicochemical Properties**
  These molecules have high polarity, multiple negative charges, and variable sizes (MW ≥ 4,000), with broad modifications, complicating their analysis.
- **Regulatory and Quality Challenges**
  Stringent regulations require purity, identity, and integrity to ensure safety and efficacy in clinical use.
- **Analytical Method Development**
  Specialized chromatographic and mass spectrometry techniques are crucial for accurate oligonucleotide characterization.



Complexity and Its Impact

- **Oligonucleotide Structural Forms**

  Oligonucleotides exist as single strands or duplexes with secondary structures affecting separation. Often adopt extended conformation.

- **Chemical Features and Modifications**

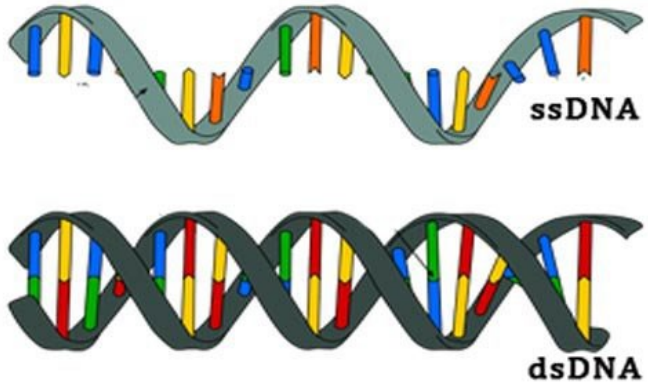
  Deoxy- and Ribo- polynucleotides, with phosphorothioate and 2’-O-methyl and fluorinated variants that alter oligonucleotide enzymatic, chemical and biological properties.

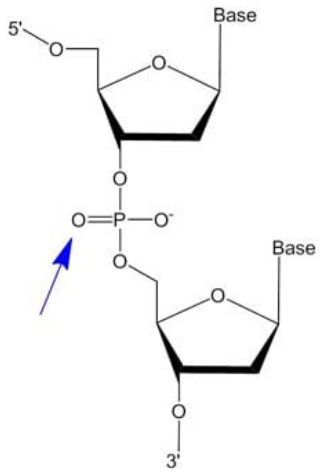
- **Conjugates in Oligonucleotides**

  Incorporating lipids, carbohydrates or peptides enhances delivery but adds complexity to chromatographic analysis.

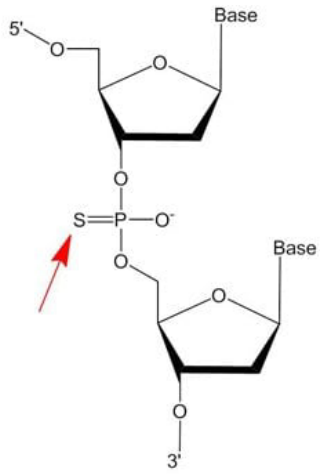
- **Chromatographic Challenges**

  Modifications affect retention, and can create positional isomers, requiring tailored chromatography strategies.

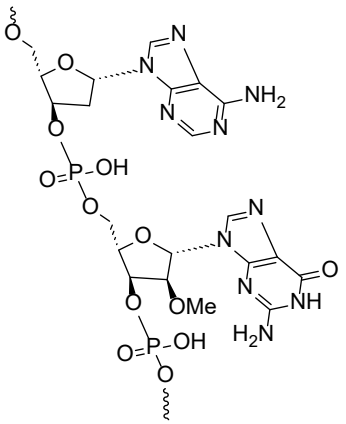




Phosphodiester Bond



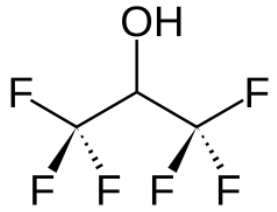
Phosphorothioate Bond



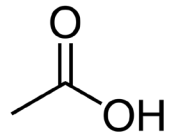
2’-O-modified RNA

Mobile Phase Design: IP-RP

- Key Properties of a Mobile-Phase Ion-Pairing Reagent
  - Volatile (MS-friendly).
  - High purity / chemically homogeneous to prevent secondary peaks.
  - Adequate solubility in HFIP/ACN systems (fluorinated solvents change amine solubility profile).
  - Designed to form stable, reversible ion pairs with polyanionic oligos.
- Why HFIP + Amine Systems?
  - HFIP (Hexafluoro-isopropanol) is a weak, volatile acid commonly paired with alkylamines in LC–MS mobile phases.
  - Lowers mobile-phase pH to ~8–9 (vs. pH 11–12 with amines alone).
  - At this pH, oligos remain partially protonated → better retention + lower metal adduct formation.
  - Weak acid → does not strongly compete for negative charge in ESI, improving sensitivity.
  - Reduces ion suppression associated with stronger acids (e.g., carboxylic acids).
- HFIP–Amine–Water–AcN/MeOH
  - System Considerations: HFIP and aliphatic amines form dynamic clusters → can form immiscible phase under high organic or cool autosampler conditions → "ghost peaks."
  - Increasing HFIP generally boosts ionization efficiency but may decrease hydrophobicity of the amine complex.

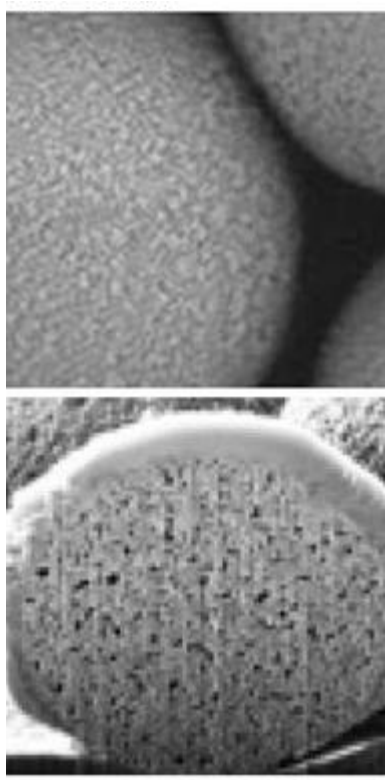


HFIP



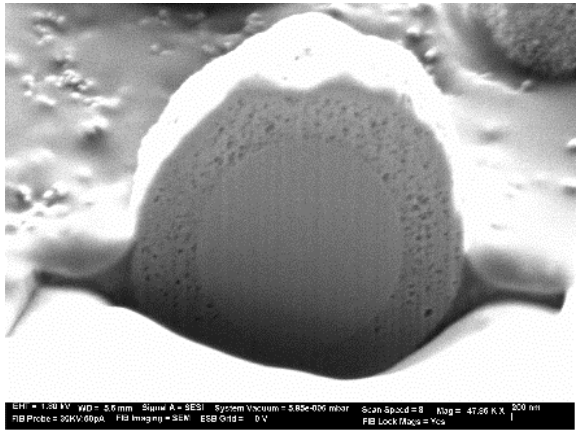
Acetic Acid

SPP Technology from AMT



Fully Porous Particle (FPP)

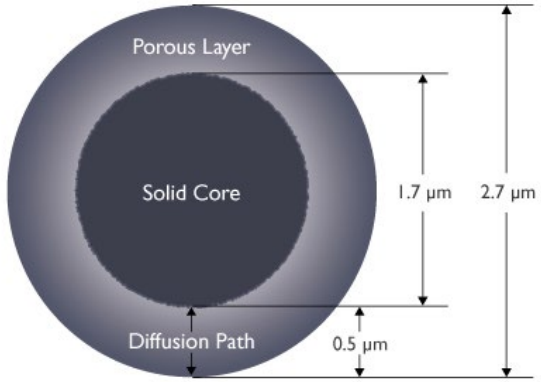
HALO 90 Å, 2.7 µm



Brief terminology lesson:

FPP – Fully Porous Particle

TPP- Totally Porous Particle

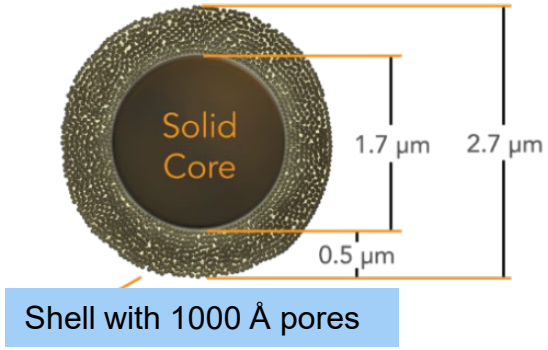


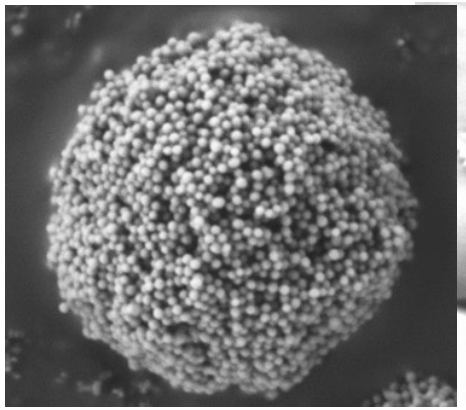
SPP – Superficially Porous Particle

Terms associated with SPP – Fused-Core®, solid-core, core shell

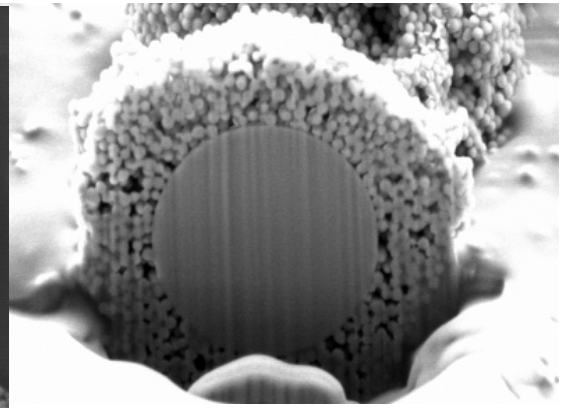
Superficially Porous Particle (SPP)

SPP Technology from AMT: 1000 Å Pore





SEM



Section analysis by FIB-SEM

- 2.7 µm particle with 0.5 µm thick shell and 1000 Å pores
- Surface area ~ 22 m^2/g
- Designed for larger molecules
- Hybrid silica particles
- Elevated pH stable

Wagner, Schuster, Boyes, Shields, Miles, Haynes, Kirkland, and Schure. Superficially porous particles with 1000 Å pores for large biomolecule high performance liquid chromatography and polymer size exclusion chromatography J. Chromatogr. A 1485 (2017) 75–85.

P. Dechadilok, W.M. Deen, Hindrance factors for diffusion and convection in pores, Ind. Eng. Chem. Res. 45 (2006) 6953–6959.

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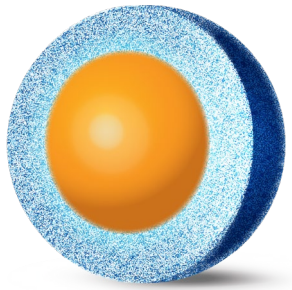
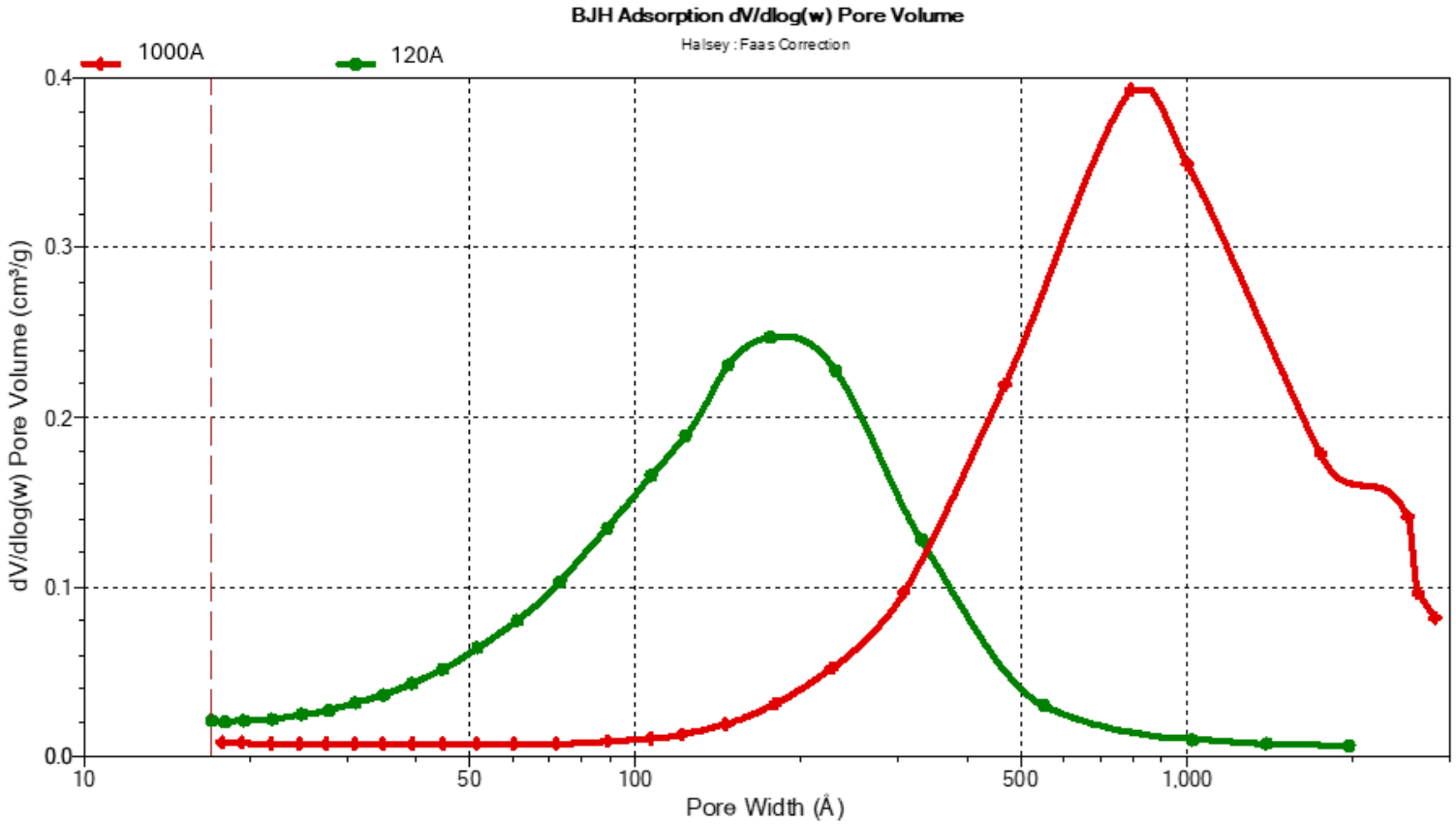
M. R. Schure, R. S. Maier, T. J. Shields, C. M. Wunder, B. M. Wagner, Intraparticle and interstitial flow in wide-pore superficially porous and fully porous particles, Chem. Eng. Sci. 174 445–458 (2017).

Larger Pore Hybrid Superficially Porous Particles

- A novel organo-silane surface modification has been applied to larger pore 2.7 µm diameter silica with a 0.5 µm porous shell, shown below.
- Surface area was maintained at ~24 m^2/g, with no pore restriction apparent. Mode Pore Size = 830 Å



120 Å



1000 Å

Importance of High pH Stability

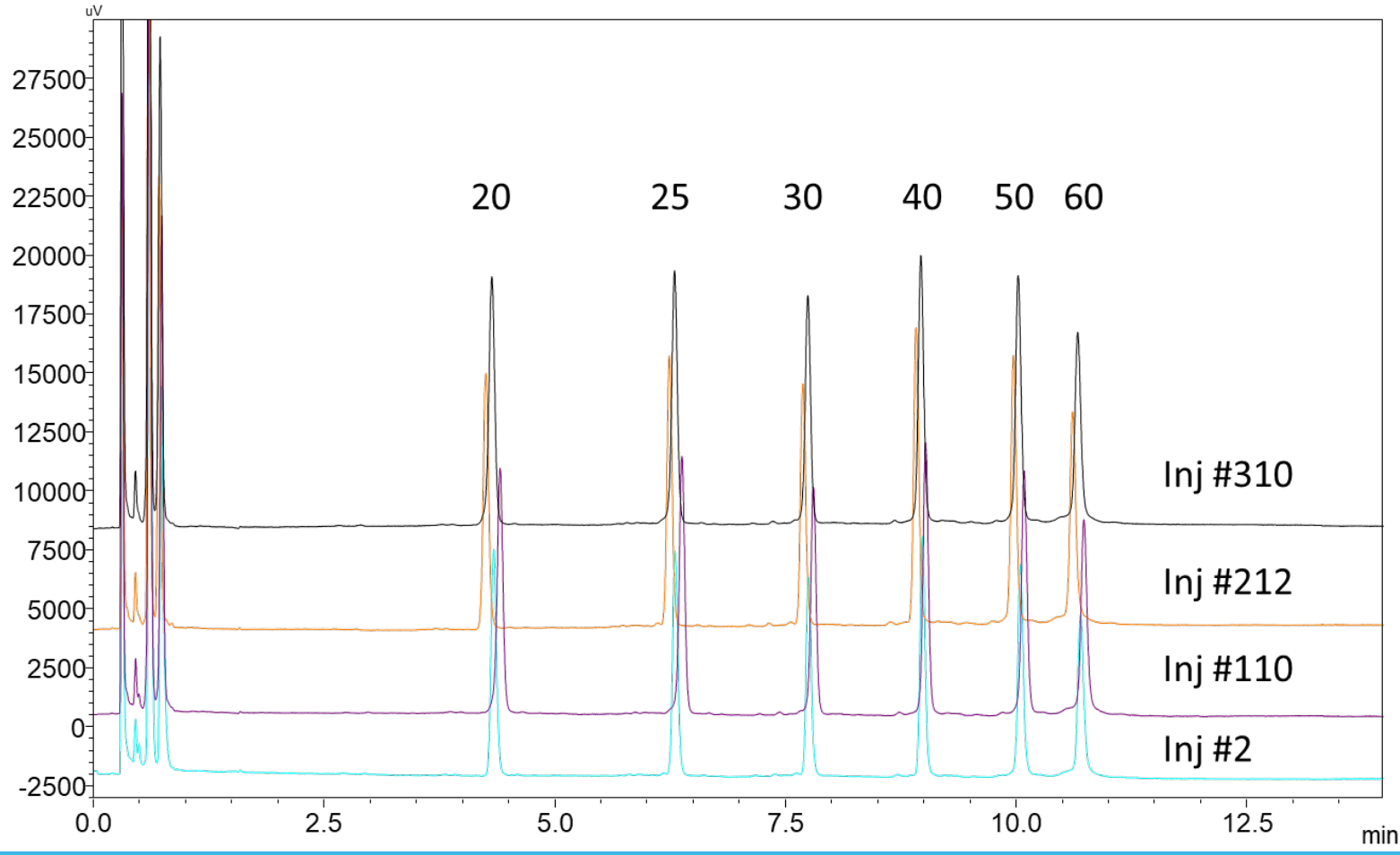
Elevated pH and Temperature Testing of Halo Oligo C18

Columns: 2.1 x 50 mm, Oligo C18
Flow Rate: 0.5 mL/min
Temp: 60°C
A- 15 mM TEA/50 mM HFIP, pH 8.9
B- MeOH
Detection: 260 nm, 10 nm
Sample: 1 µL, 10/60 IDT Standard @ 10ng

Gradient:

Time	%B
0.0	5.0
14	12
14.1	30
14.4	30
14.5	5
20.0	Stop

- A stability example on the HALO® 120Å oligo C18 bonded phase hybrid SPP silica
- Over 300 injections, retention and PW of oligos shifts very little
- With mass spec friendly ion pairing conditions (TEA/HFIP)
- Average GC content oligonucleotides



Oligomer Ladder 20/100: TEAA/Acetonitrile

HALO®

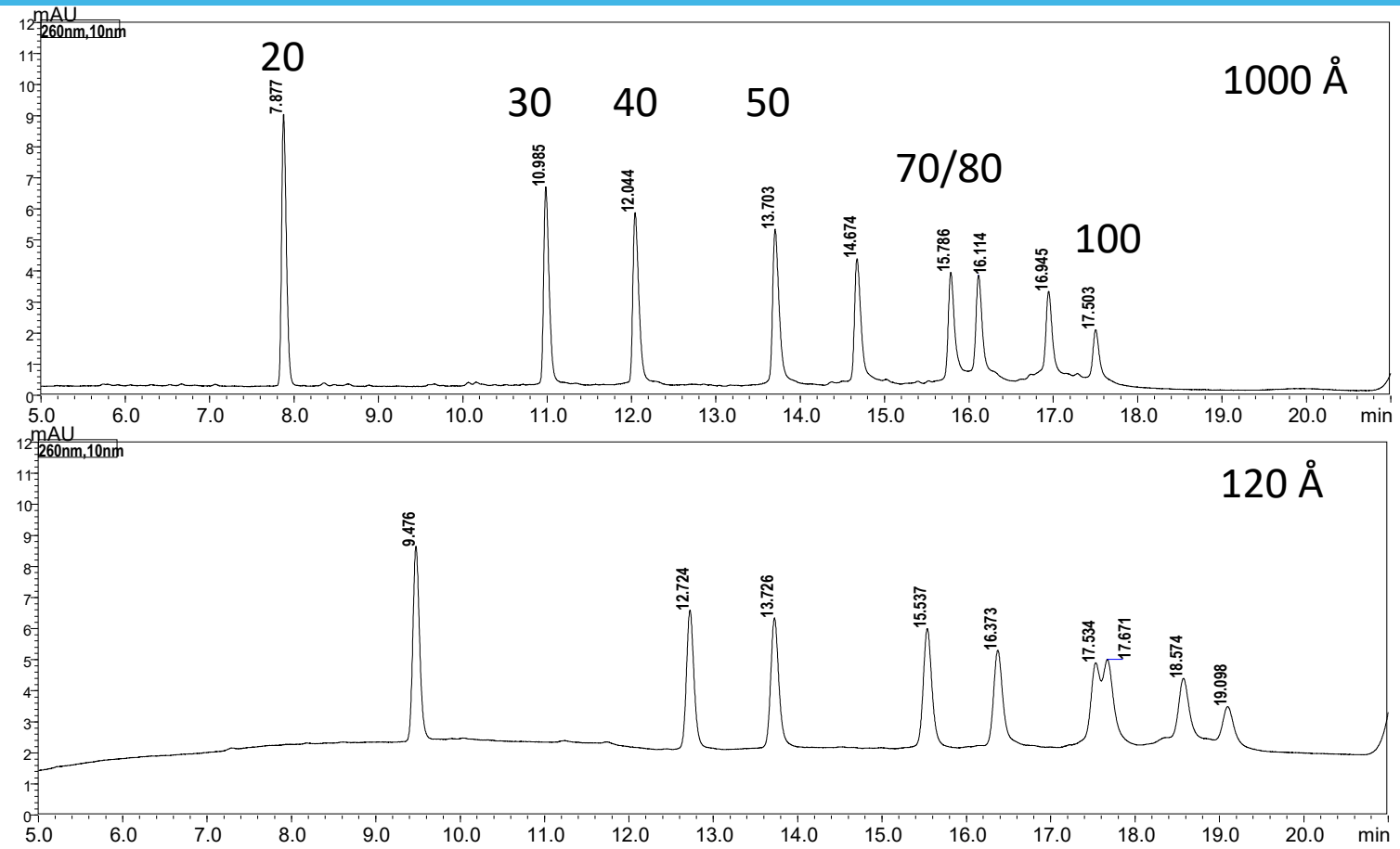
TEST CONDITIONS:

Columns: 2.7 µm, 2.1 x 100 mm, 120/1000 Å pore
Mobile Phase A: 100 mM TEAA (pH 7.0)
Mobile Phase B: Acetonitrile

Gradient:	Time	%
	0.0	6
	20	11
	21	30
	22	30
	22.5	6
	30	Stop

Detection: 260 nm, 10 nm
Sample: 1 µL, 20/100 IDT @ 10ng
Flow Rate: 0.5 mL/min
Temperature: 60 °C
Detection: UV/PDA, 260 nm
Injection Volume: 1.0 µL

ssDNA: 20 nts to 100 nts; avg G/C content



- Larger pore - decreased retention, lower solvent strength, similar gradient range, narrowed peak widths
- Modest selectivity differences

Greater Peak Capacity for Larger Pore SPP: TEA/HFIP Acetonitrile

(0.25% AcN/min)

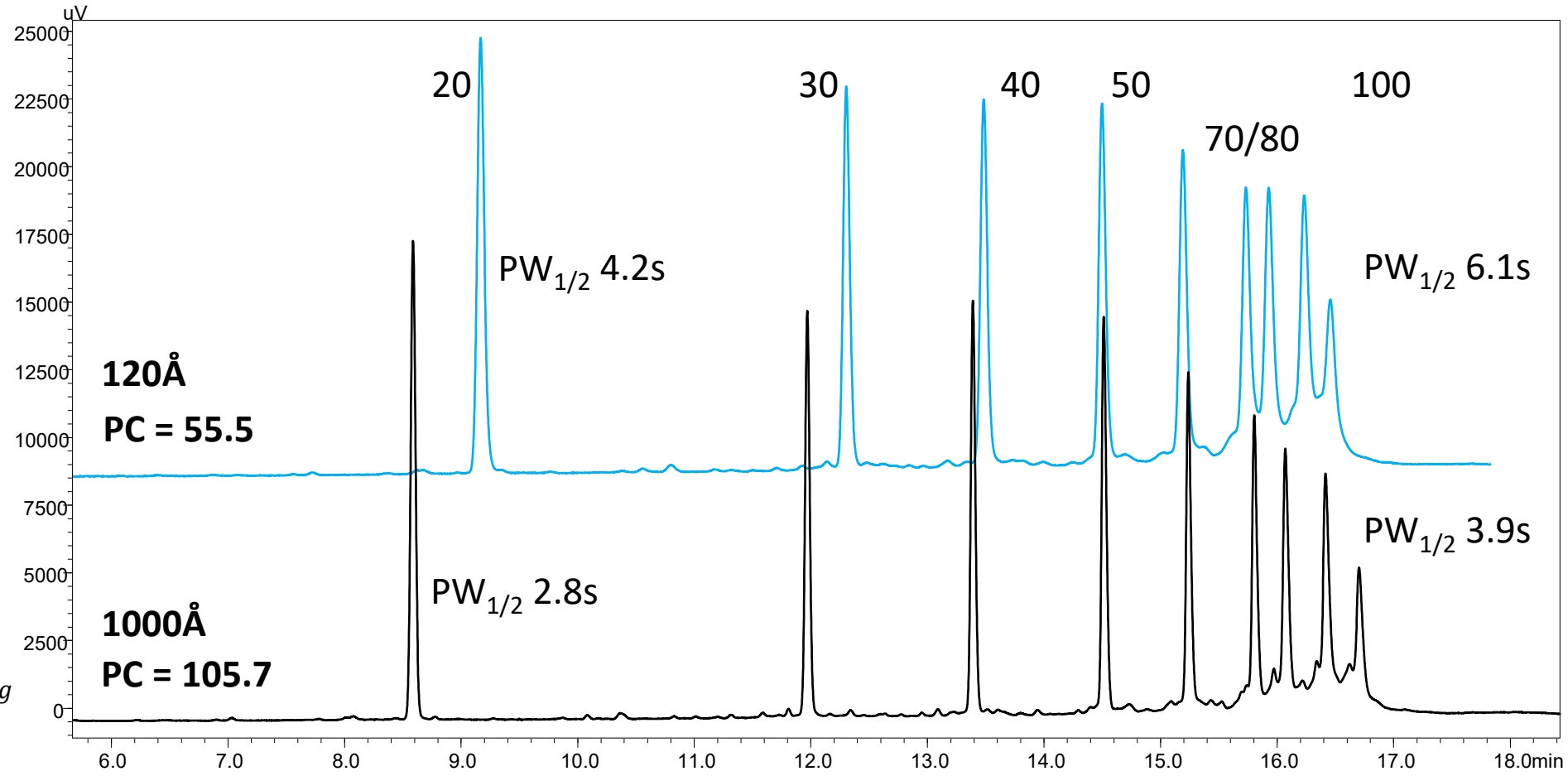
Conditions:

Columns: 2.1 x 100 mm, Inert HW
Flow Rate: 0.5 mL/min
Temp: 60°C
A- 15 mM TEA/50 mM HFIP, pH 8.9
B- Acetonitrile

Gradient:	Time	%
	0.0	1.5
	20	6.5
	21	15
	22	15
	22.5	1.5
	30	Stop

Detection: 260 nm, 10 nm
Sample: 1 µL, 20/100 IDT @ 10ng

$$PC = \Delta(Rt_{100} - Rt_{20})/4\sigma_{avg}$$



Resolution of Longer Oligonucleotides

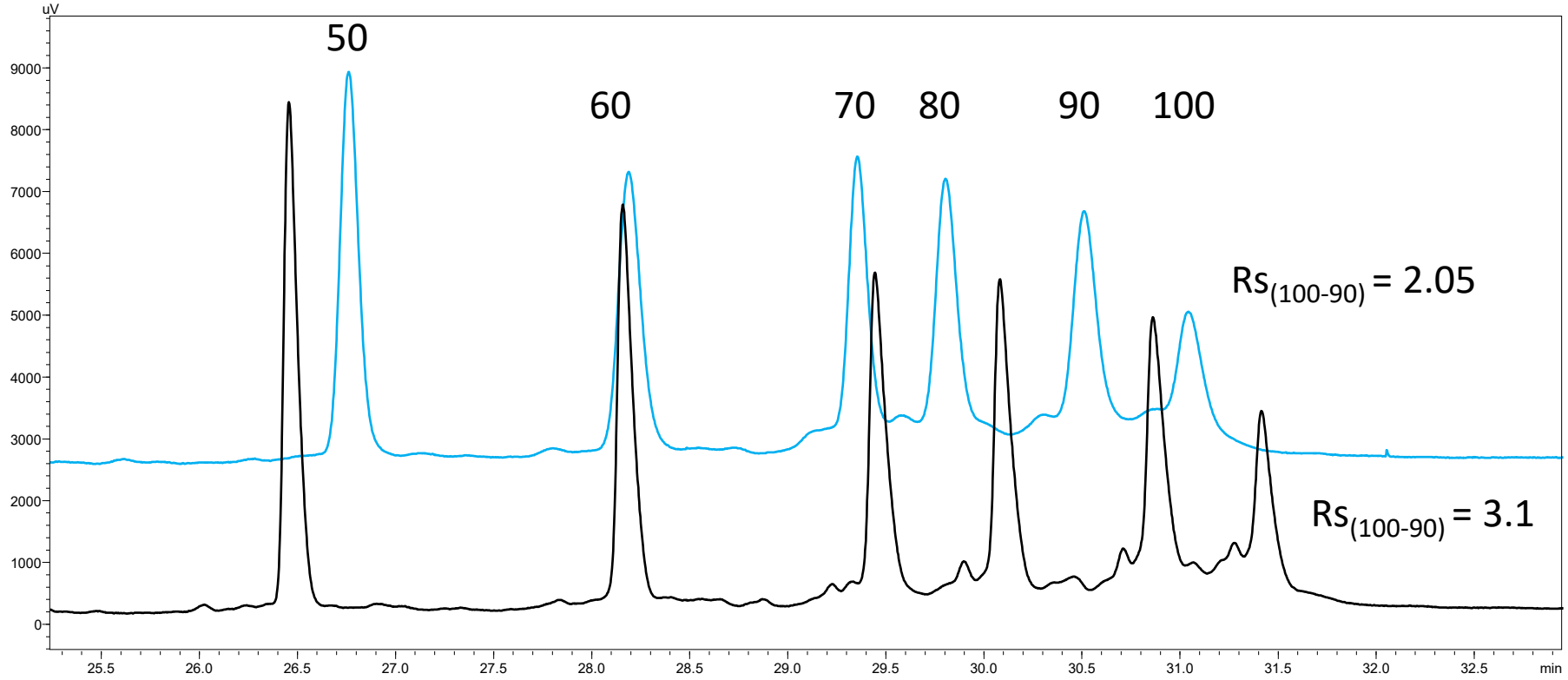
Conditions:

Columns: 2.1 x 100 mm, Inert HW
Flow Rate: 0.5 mL/min
Temp: 60°C
A- 15 mM TEA/50 mM HFIP, pH 8.9
B- Acetonitrile

Gradient:	Time	%
	0.0	1.5
	40	6.5
	41	15
	42	15
	43	1.5
	50	Stop

Detection: 260 nm, 10 nm
Sample: 1 µL, 20/100 IDT @ 10ng

(0.125% AcN/min)



- Adjusting gradient for closer matching of Rt across this sample yields little effect on Rs or PC

Flow Rate Effect on 1000A Prototype C18: TEAA/Acetonitrile Gradient Elution Parameters

Series of oligonucleotides separated by using Halo® 120 Å Oligo or Prototype 1000 Å – 2.1 mm x 100 mm.

Acetonitrile gradients with 100 mM TEAA (pH 7.0), 60°C, Abs detection 260 nm.

Varied flow rate at constant gradient volume (V0/VG: t_0/t_G) with time varied. Use relevant k* and solvent range ($\Delta\phi$).

Assess recovery by area counts, dispersion by PW1/2

Flow rates varied from 0.125 mL/min to 1.0 mL/min; %AcN 6.5-11.5

Values determined at 0.4 mL/min flow rate.

Oligo	S-value		Ln k_0		k*	
	1000	*120*	*1000*	*120*	*1000*	*120*
30	106.5	93.0	9.50	8.86	7.6	8.7
60	122.9	112.5	12.0	11.5	6.6	7.2
90	130.2	122.6	13.4	13.2	6.3	6.6

$$\ln(k^*) = -S\Delta\phi + \ln(k_w)$$

wherein

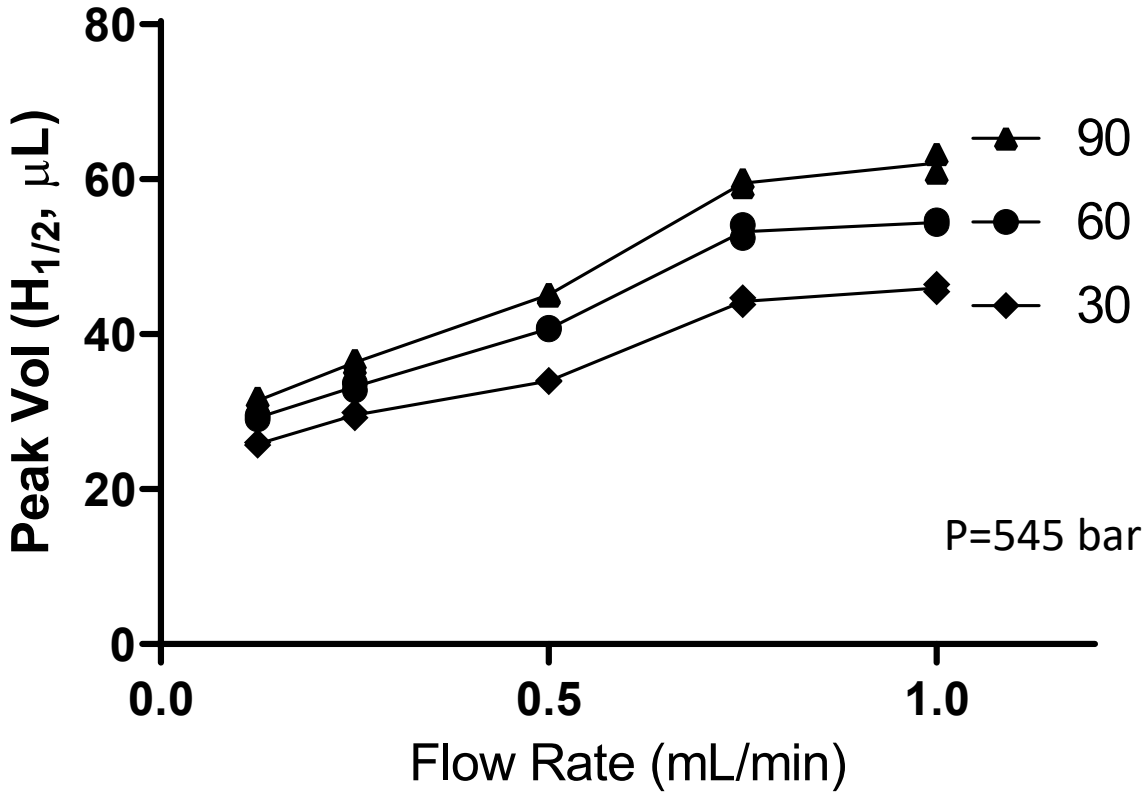
$$\ln(k_w) = \ln(k_0) + S\Delta\phi_w$$

Flow Rate Effect on 1000A Prototype C18

Conditions:

Columns: 2.1 x 100 mm, inert HW
1000 Å C18 Prototype Hybrid SPP
Temp: 60°C
A- 100 mM TEAA, pH 7.0
B- Acetonitrile
Constant Gradient Volume - 6.5-11.5% AcN
Detection: 260 nm, 10 nm
Sample: 1 µL, 20/100 IDT @ 10ng

Fixed Volume Gradient Conditions (10.0 mL); Peak Volume = $PW_{1/2}$ x Flow Rate

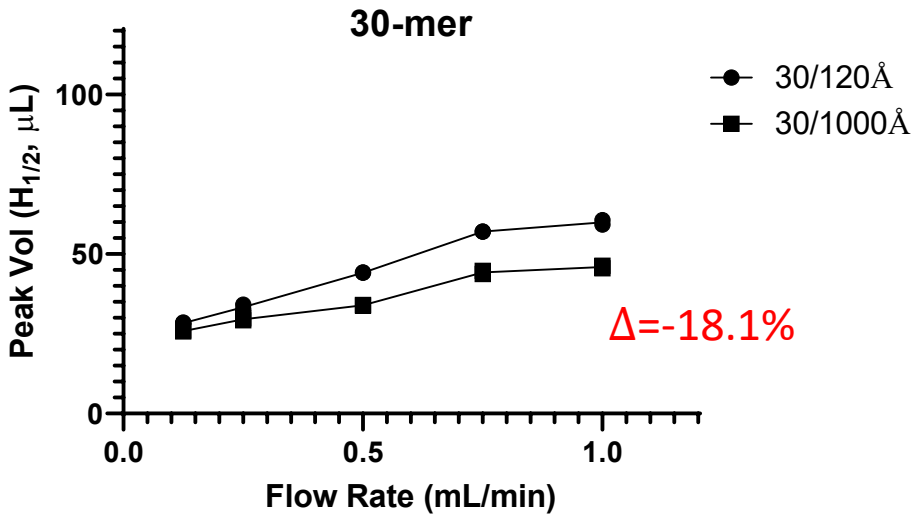


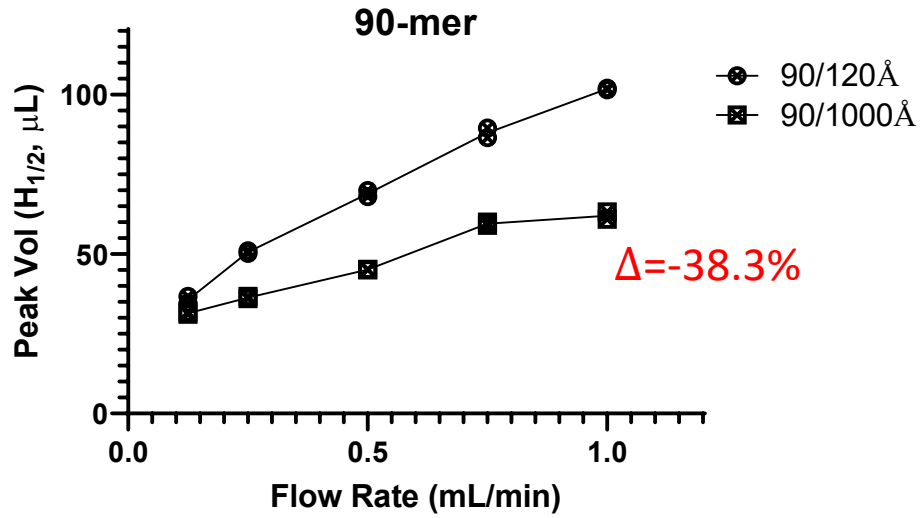
- Longer oligonucleotides have greater PW, at all flow rates

Flow Rate Effect on Band Widths: Effect of Pore Size

Conditions:

Columns: 2.1 x 100 mm, inert HW
Halo Oligo C18 Hybrid SPP
Temp: 60°C
A- 100 mM TEAA, pH 7.0
B- Acetonitrile
Detection: 260 nm, 10 nm
Sample: 1 µL, 20/100 IDT @ 10ng





- Increased oligonucleotide length increases band width
- Larger pore size reduces band width at all flow rates
- At very low flow, very narrow peaks
- Longer oligonucleotides show band width improvement at higher flow rate (example shown for 1 mL/min)
- Modest effect with smaller oligonucleotides at lower flow rate

Isocratic Retention is Pressure Dependent

Retention changes under isocratic conditions in response to pressure changes, caused by flow rate change or column length change (amongst other conditions). The conditions for column efficiency analysis as a function of linear velocity are not met (van Deemter or Knox analysis). This has been described in by Stoll, Maloney and coworkers for both RP and HILIC with smaller oligonucleotides (23-mer).

TEST CONDITIONS:

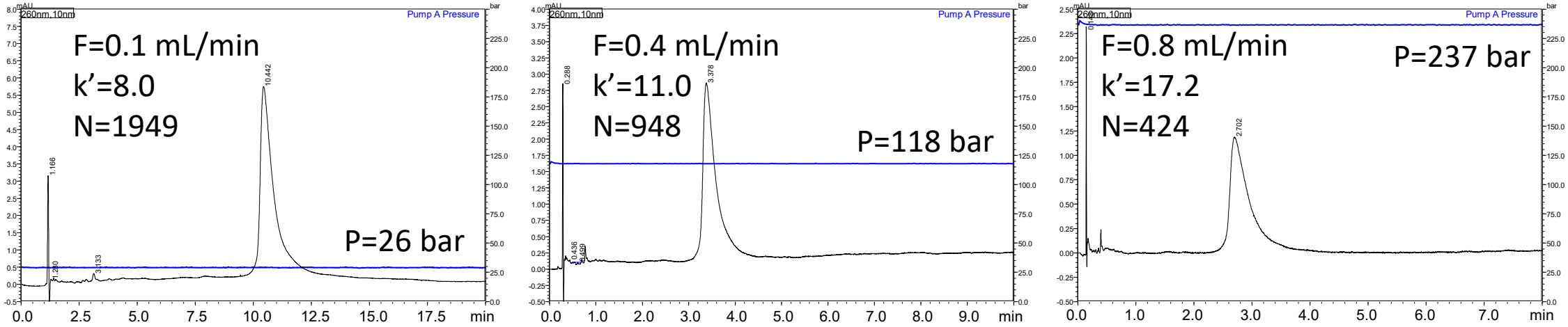
Columns: 2.7 µm, 2.1 x 50 mm, Halo 1000 Oligo

Mobile Phase A: 100 mM TEAA (pH 7.0), Mobile Phase B: Acetonitrile – Isocratic 11.3% B (9.0% AcN)

Sample: 1 µL, 90-mer ssDNA, 10ng

Temperature: 60 °C

Detection: UV/PDA, 260 nm



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TEST CONDITIONS:

Columns: 2.7 µm, 2.1 x 50 mm, Halo 1000 Oligo C18

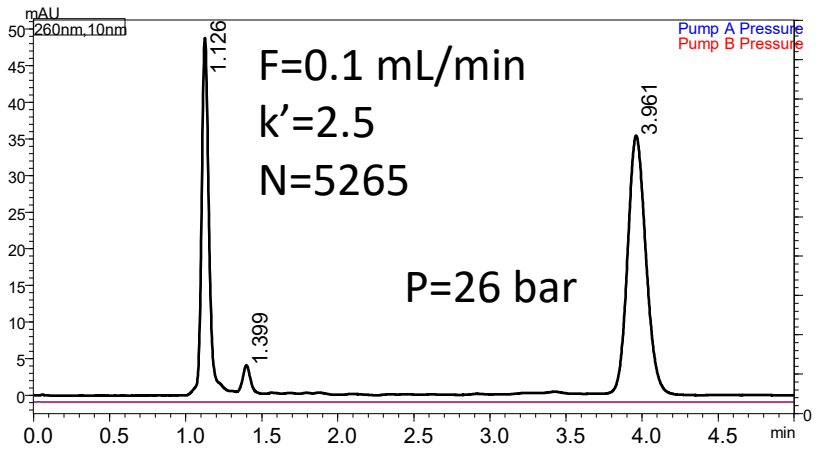
Mobile Phase A: 5.0 mM DiPEA/50 mM HFIP/5% MeOH , Mobile Phase B: 50%Acetonitrile

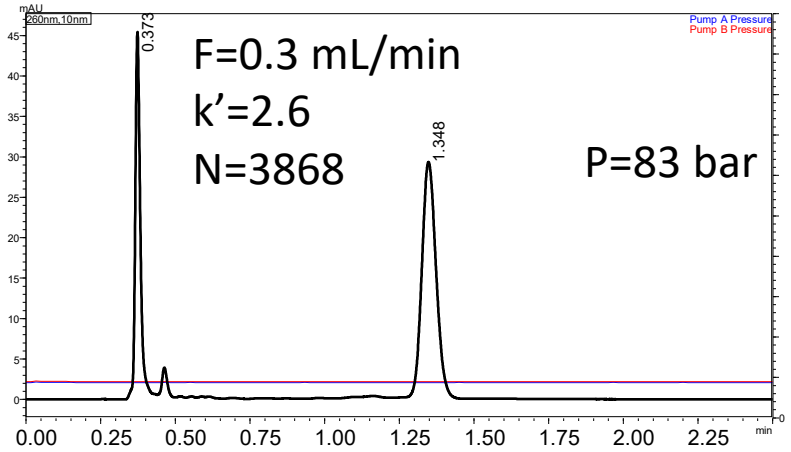
Isocratic 8.0% AcN

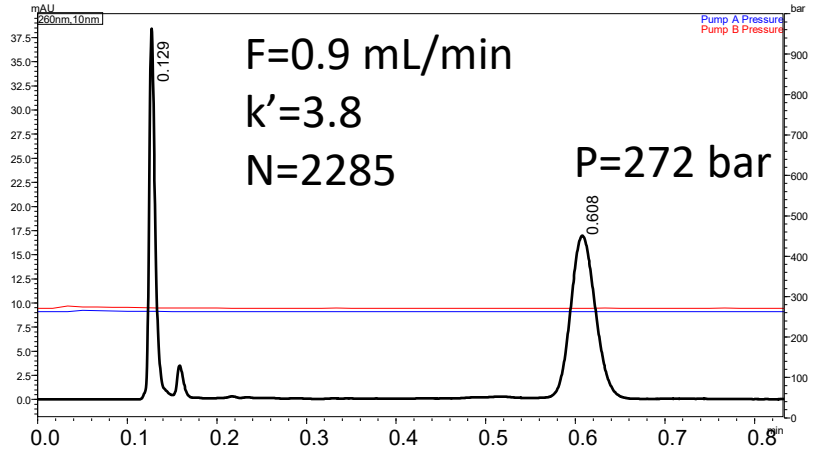
Sample: 1 µL, 30-mer ssDNA, 10ng

Temperature: 60 °C

Detection: UV/PDA, 260 nm







Retention is Pressure Dependent

Journal of Chromatography A 1744 (2025) 465687

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Does column length still matter? A case study of the effect of column length on resolution of therapeutic oligonucleotides

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ARTICLE INFO

Keywords:
Ion-pairing reversed-phase
Oligonucleotide
Phosphorothioate
Column length
Resolution

ABSTRACT

As the importance of therapeutic oligonucleotides (ONs) continues to grow in the pharmaceutical industry, the importance of high performing analytical methods needed to characterize them also grows. The characteristics of these molecules (e.g., highly charged phosphate backbone, and small but important modifications such as methylation and fluorination) make them difficult to analyze thoroughly using conventional liquid chromatography (LC) conditions. Recently, other research groups have been emphasizing the utility of ultra-short (<< 50 mm) columns for proteins and other large biomolecules, and have remarked that long columns only add unnecessary peak dispersion without providing additional resolution over short columns. These statements naturally call into question the long-established theory for small molecule LC separations that asserts that separation performance is maximized by working at the highest available operating pressure, and then choosing the longest column possible while working at the van Deemter optimum flow rate. This apparent contradiction in turn raises the question – for which types of large biomolecule does the established chromatographic theory no longer apply? In this study we have carried out experiments and calculations aimed at answering this question for ion-pairing reversed-phase separations of therapeutic ONs with masses on the order of 6 kDa. This included measuring isocratic plate heights for these molecules after establishing an empirical relationship between retention, mobile phase composition, and flow rate, because retention of the ONs is extremely sensitive to pressure (20 % increase in k per bar pressure drop), and thus retention varies with flow rate at a constant mobile phase composition. After taking these factors into account, we find that resolution of the oligonucleotides does increase with the square root of column length, as predicted by the well-established theory for small molecules. However, we also find that this relationship is only found when the gradient slope is held constant while varying the column length, and that if this is not done it is actually possible to observe that resolution decreases with increasing column length. Thus, the design of experiments used to evaluate the role of column length in separation performance is critical. In addition to the importance of these findings to development of LC methods for ON separations in general, they will be especially impactful in two-dimensional (2D) separations of ONs where there is more or less freedom to choose parameters from a wide range of possibilities depending on the mode of 2D separation that is used.

1. Introduction

Oligonucleotides are a class of therapeutics that is gaining mo- [3].

In recent years there has been renewed interest in the use of very short (<< 50 mm) LC columns for rapid analysis of large molecules such

Journal of Chromatography A 1742 (2025) 465643

Contents lists available at ScienceDirect

Journal of Chromatography A

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Effect of flow rate on plate height and resolution for antisense oligonucleotides under hydrophilic interaction liquid chromatography conditions

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ARTICLE INFO

Keywords:
Method development
2D-LC
HILIC
Retention modelling
Oligonucleotides

ABSTRACT

Determination of quality attributes of antisense oligonucleotides (ASOs) such as purity, potency, and sequence is challenging due to their relatively large size, polyanionic nature, and large number of synthetic modifications. Chromatography technologies are evolving rapidly to meet these challenges, and one area of particularly rapid change at this time is the use of hydrophilic interaction liquid chromatography (HILIC) for oligonucleotide (ON) separations. Relatively little has been published on the factors that dictate the kinetics of these separations. This knowledge gap consequently makes it difficult to know what gains might be made during method development by changing flow rate or particle size, for example. In this work we have taken initial steps to address this gap by examining the dependence of plate height and resolution on flow rate for separations of 23-mer ASOs under HILIC conditions. Such work is complicated by the fact that the retention of these molecules decreases dramatically with increasing pressure. After adjusting mobile phase composition to hold retention factor nominally constant for each flow rate used, we find that plate height increases strongly with increasing flow rate such that the plate height increases about ten-fold over the range of flow rate of 0.1 to 4.0 mL/min. when using a 4.6 mm i.d. column. However, the minimum reduced plate height observed at the lowest flow rate is quite impressive at around 2. Finally, we find that this dependence of plate height on flow rate translates, as expected, to an improvement in resolution as flow rate is decreased, both in conventional one-dimensional separations, and in the second dimension of a two-dimensional separation. We expect to use this work as a foundation to build on as we deepen our understanding of the kinetics of ON separations.

1. Introduction

Oligonucleotides (ONs) constitute a rapidly growing pharmaceutical class with tremendous clinical potential. Antisense oligonucleotides (ASOs) are a subset of ON therapeutics composed of single stranded RNA, typically 20–30 nucleotides in length. Determination of quality attributes of ASOs such as purity, potency, and sequence is challenging due to their relatively large size, polyanionic nature, and large number of synthetic modifications [1]. Although formal regulatory guidelines are currently not defined for impurity reporting thresholds for synthetic ONs, many investigators follow the levels (0.1–0.3 %) proposed by Capaldi, et al., or the recent European Medicines Agency (EMA) chromatography coupled with mass spectrometry (HPLC-MS) is a powerful technique capable of determining many impurities with the required sensitivity [4,5]. However, growing concerns over the environmental impact of per- and poly-fluoroalkyl substances (PFAS), led to the recommendation of the European chemicals agency (ECHA) to restrict the use of PFAS in Europe [6]. The absence of an alternative to the use of PFAS as mobile phase modifiers (such as the hexafluoroisopropanol used in this work) will hamper the performance of methods based on IPRP separations.

Other separation types including anion-exchange [7,8] and hydrophilic interaction liquid chromatography (HILIC) [9–11] may be beneficial for certain types of RNA separations. Moreover, the

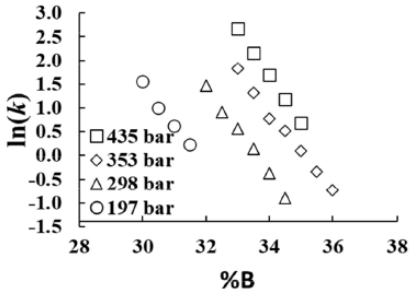


Fig. 2. Dependence of ln(k) on mobile phase composition at different pressures. The reported pressures are estimates for pressure at the midpoint of the column, which were adjusted using different lengths of post-column restriction capillaries. Chromatographic conditions: Column, 50 mm x 2.1 mm i.d. BEH C18 (1.7 µm); Temperature, 65 °C; Flow rate, 0.4 mL/min.; Solvent A, 10mM triethylammonium phosphate in water, pH 7.8; Solvent B, 30/70 (ACN/water). Sample – 0.2 µL injection of LR10 (1 mg/mL in water).

Pressure and composition as independent variables affecting retention

- verified for RP-IP in HOAC and HFIP
  - Also observed in IP-HILIC

Column length matters, especially at lower gradient steepness.

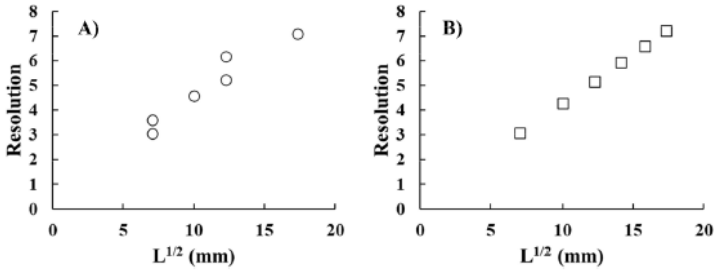
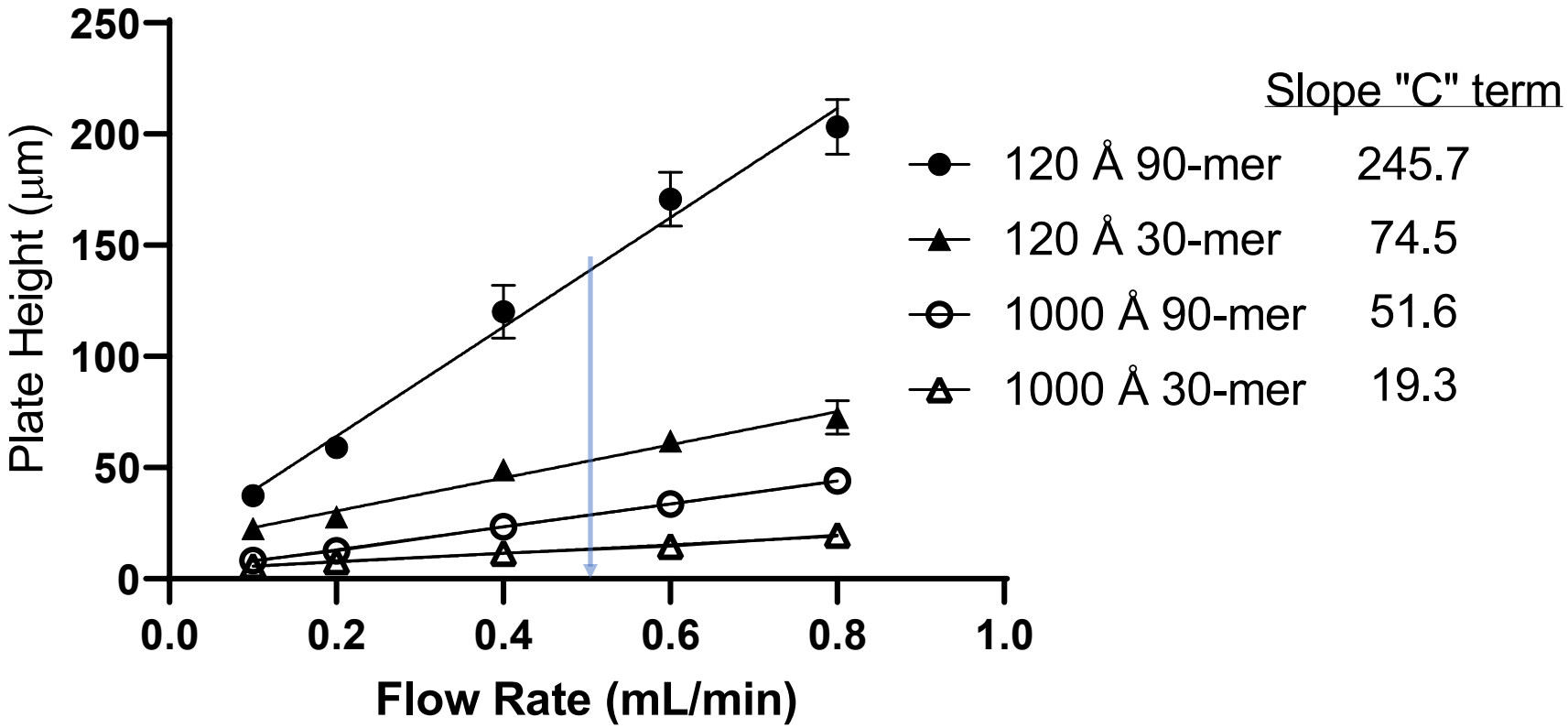


Fig. 6. Dependence of resolution on the square root of column length. A) Experimental results; B) Simulation results. Chromatographic conditions for A: Columns, 50 mm x 2.1 mm i.d. HPH C18 (2.7 µm), 100 mm x 2.1 mm i.d. HPH C18 (2.7 µm), 150 mm x 2.1 mm i.d. HPH C18 (2.7 µm), and 300 mm x 2.1 mm i.d. HPH C18 (2.7 µm); Flow rate, 0.1 mL/min.; Gradient elution from 33.6 to 38.6 %B, 33.7 to 38.7 %B, 36.5 to 41.5 %B in gradient times of 20, 30, or 60 min; Solvent A, 10 mm triethylammonium acetate in water, pH 8.0; Solvent B, 10mM triethylammonium acetate in 30/70 (ACN/water), pH 8.0. Sample – 0.2 µL injection of a mixture of LR8 and LR10 (0.5 mg/ml each in water). Calculation parameters are described in Section 4.3 of the text.

Pore Size Effects in SPP

- Analysis of flow rate effect on efficiency at constant k’ requires mobile phase adjustment.
- 90-mer greater peak width than 30-mer at all flow rates.
- Pore size strongly impacts peak width at constant k’.
- Restricted diffusion in smaller pores increases mass-transfer resistance, even with SPP morphology.
- At low flow, high performance is achieved.

Flow Rate Effect on Column Efficiency



Conditions:

2.1 x 100 mm columns of 2.7 µm particles 60˚C; Mobile Phases are 10 mM DiBA/100 mM HFIP (8.4)/5% MeOH
Acetonitrile volume fraction adjusted using 10% in Pump A and 20% in Pump B to yield ***k’=10.0 (+/- 6%)***

Pore Size Effects in SPP

- At low flow, high performance is achieved (near 2 dp).
- Even with large pore SPP, patience is required for very high efficiency.

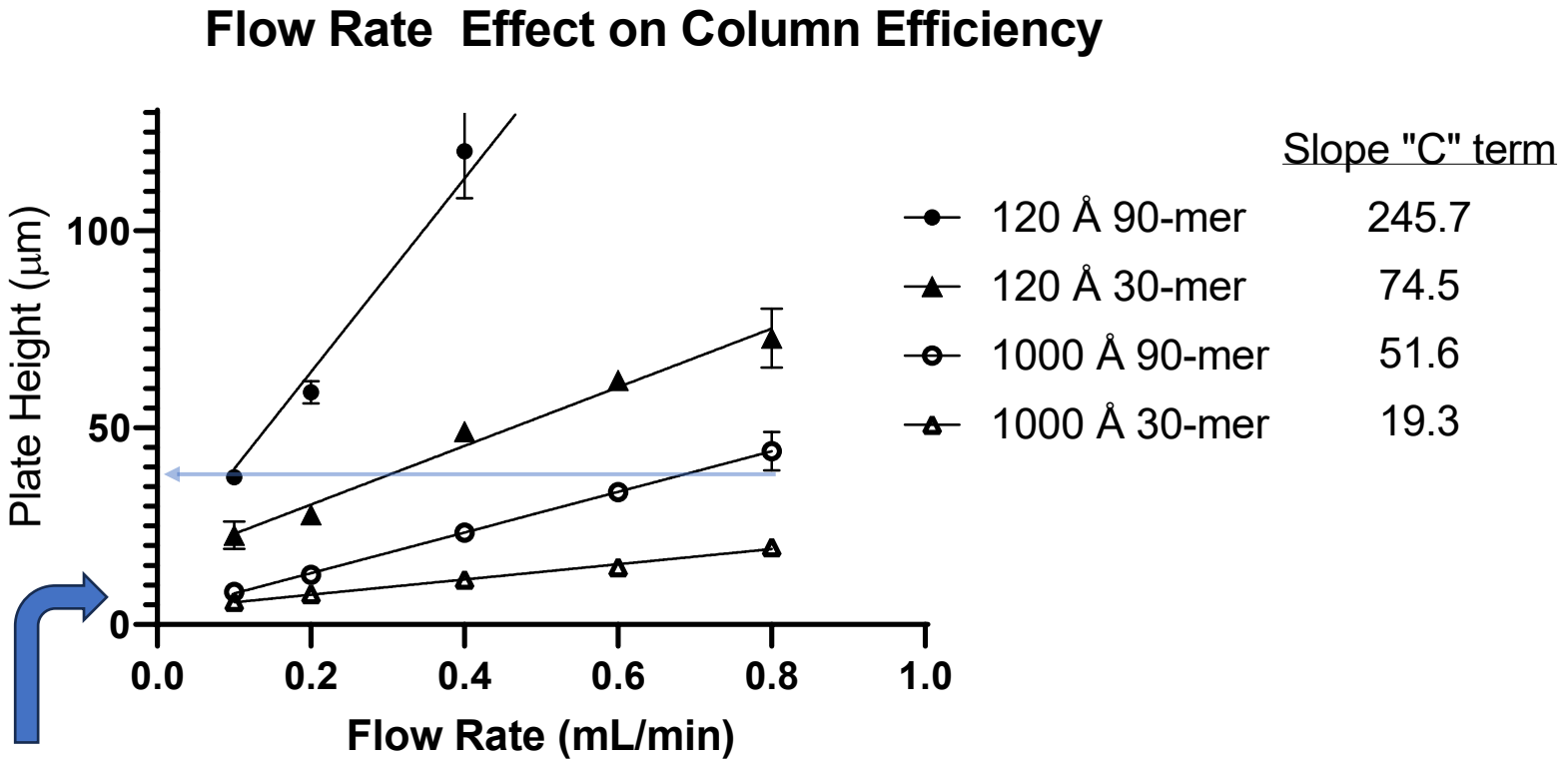


Plate height @0.1 mL/min close to 2.0 dp (5.5 µm for 30-mer; 2.04 dp)

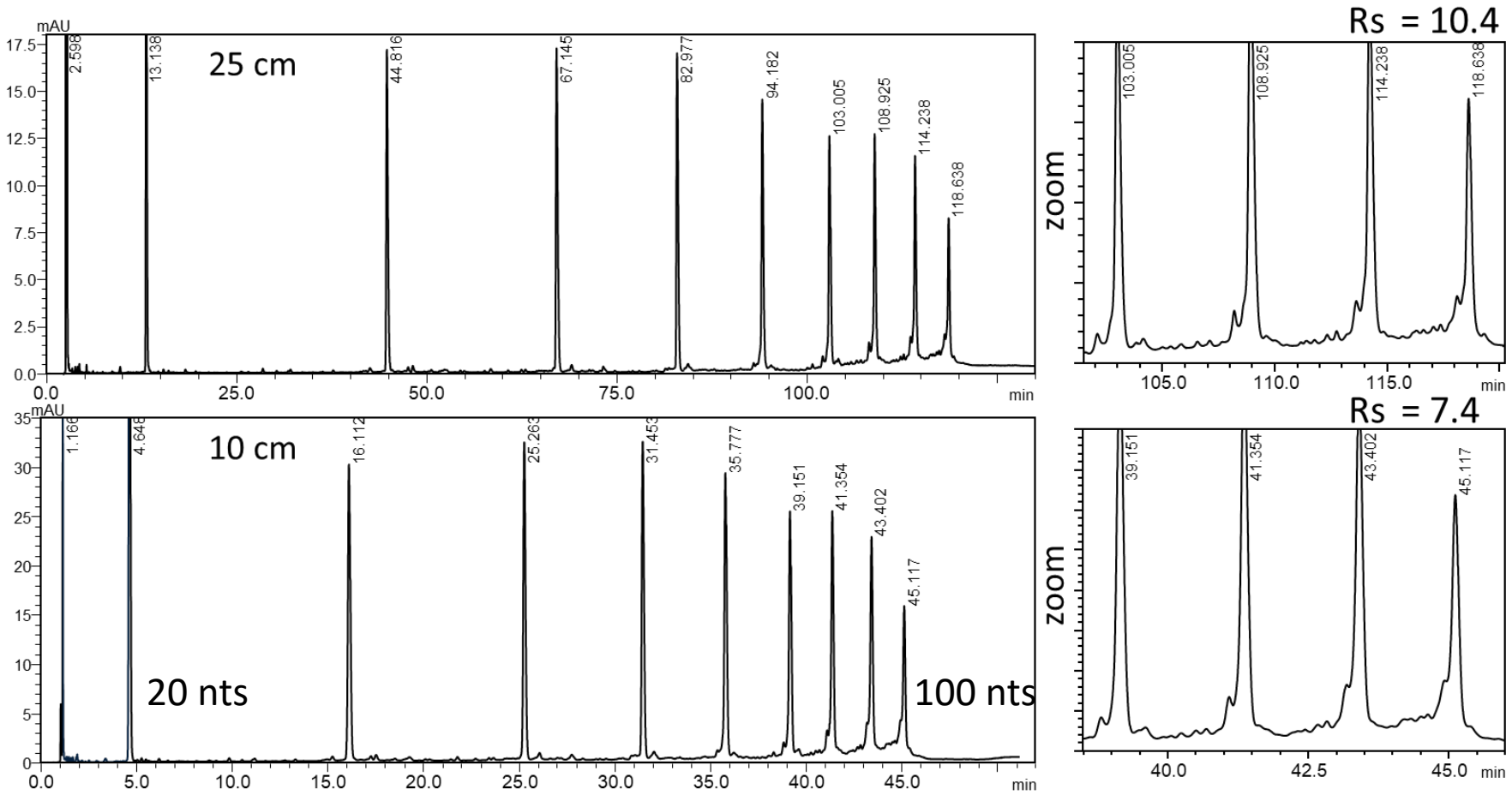
Conditions:

2.1 x 100 mm columns of 2.7 µm particles 60˚C; Mobile Phases are 10 mM DiBA/100 mM HFIP (8.4)/5% MeOH Acetonitrile volume fraction adjusted using 10% in Pump A and 20% in Pump B to yield ***k'=10.0 (+/- 6%)***

Column Length to Increase Resolution of Longer Chain Nucleic Acids

Conditions: 2.1 mm ID columns; 60˚C; 0.2 mL/min; 2 µL, 20/100 IDT @ 20ng
Mobile Phase: A – 10 mM DiBA/100 mM HFIP/5% MeOH/5% AcN; B – A at 20% AcN; 15-75%B in 150 min (top) or 60 min (lower);

- Resolution of the oligonucleotide mixture on a longer column (25 cm) using a shallow gradient, resolving out to 100 nts.
- Resolution of 90/100 nt pair close to expected for √L dependence. The gradient rate is scaled to L (150 min to 60 min) for comparing the 25 and 10 cm columns (across the range increase by 1.34; expected 1.58).
- Longer columns, greater resolution at the cost of time.

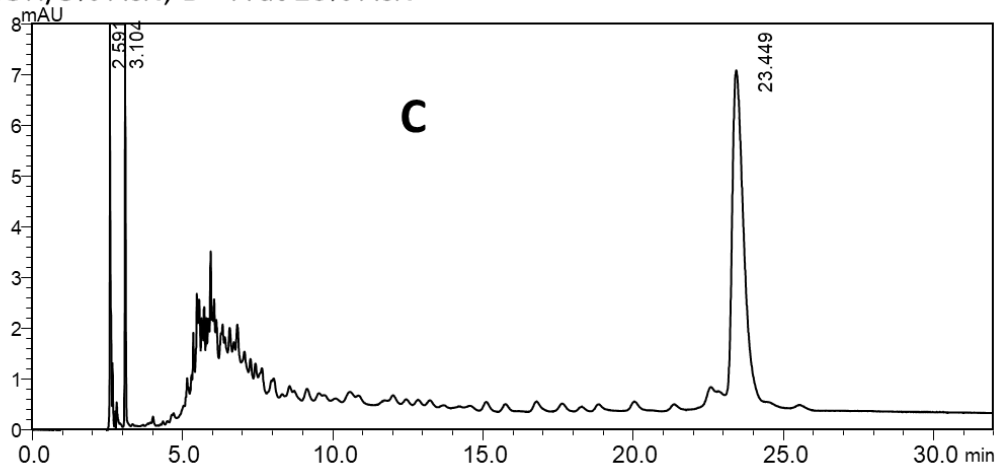


Utility for Resolution of Longer Chain Nucleic Acids – Impurity Analysis

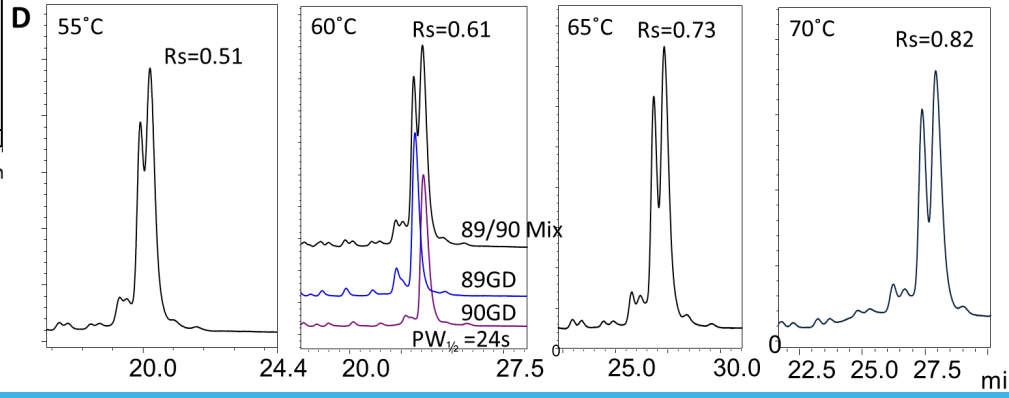
Resolution the GD EX 90-mer and synthesis impurities on a 25 cm column of the 1000Å SPP material (Panel C), with a shallow gradient (0.015% AcN/min). A synthetic 89-mer (deleted G at 3'-end of GD EX) was obtained. Separation of the 89/90-mer pair is difficult, even with a shallow gradient (0.015% AcN/min). Resolution and retention is dependent on temperature, as shown below in Panel D.

Conditions: 2.1 x 250 mm,1000Å; A - 0.2 mL/min; 60°C; Mobile Phase:
A- 10 mM DiBA/100 mM HFIP/5% MeOH/5% AcN; B – A at 20% AcN

Gradient:	Time	%B
	0.0	40.0
	1.0	40
	2.0	64
	30.0	67
	31.0	100
	33.0	100
	34.0	40.0
	40.0	STOP

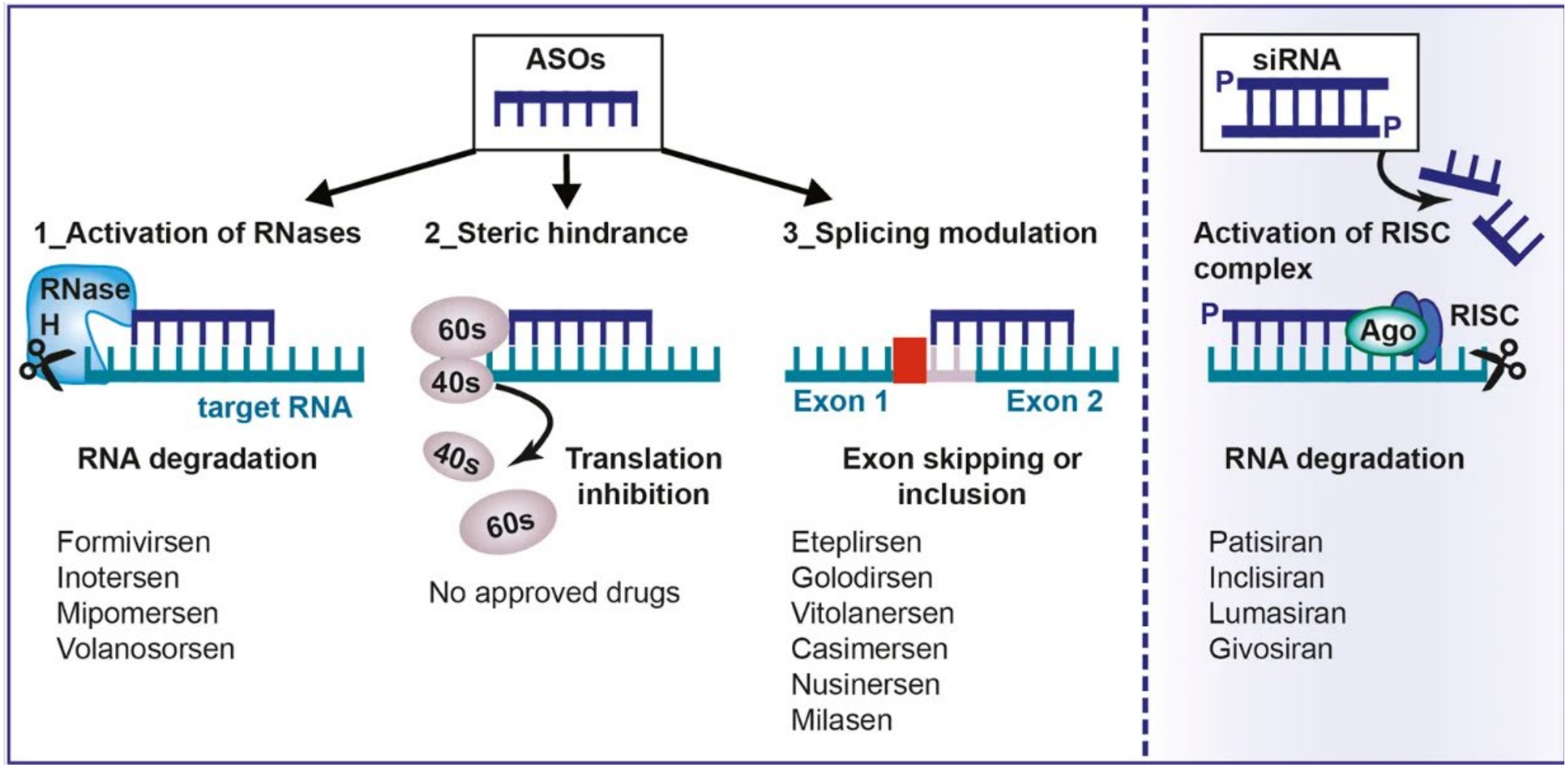


Spiked mixtures of 89/90 ssDNA



Oligonucleotide Therapeutics

"Short" Oligonucleotides (< 25 bases): modifications are common



Collotta D, Bertocchi I, Chiapello E and Collino M (2023) Antisense oligonucleotides: a novel Frontier in pharmacological strategy. Front. Pharmacol. 14:1304342. doi: 10.3389/fphar.2023.1304342

Method Development Challenges

- **Duplex Stability Impact**

  Duplex structure causes variable retention, requiring precise temperature and mobile phase control for consistent separation.

- **Ion-Pairing Agent Selection**

  Choosing ion-pairing agents compatible with both siRNA strands and mass spectrometry is essential for separation.

- **Gradient Optimization**

  Optimizing gradients resolves closely related species, including heavily modified siRNAs

- **Chemical Modifications Effects**

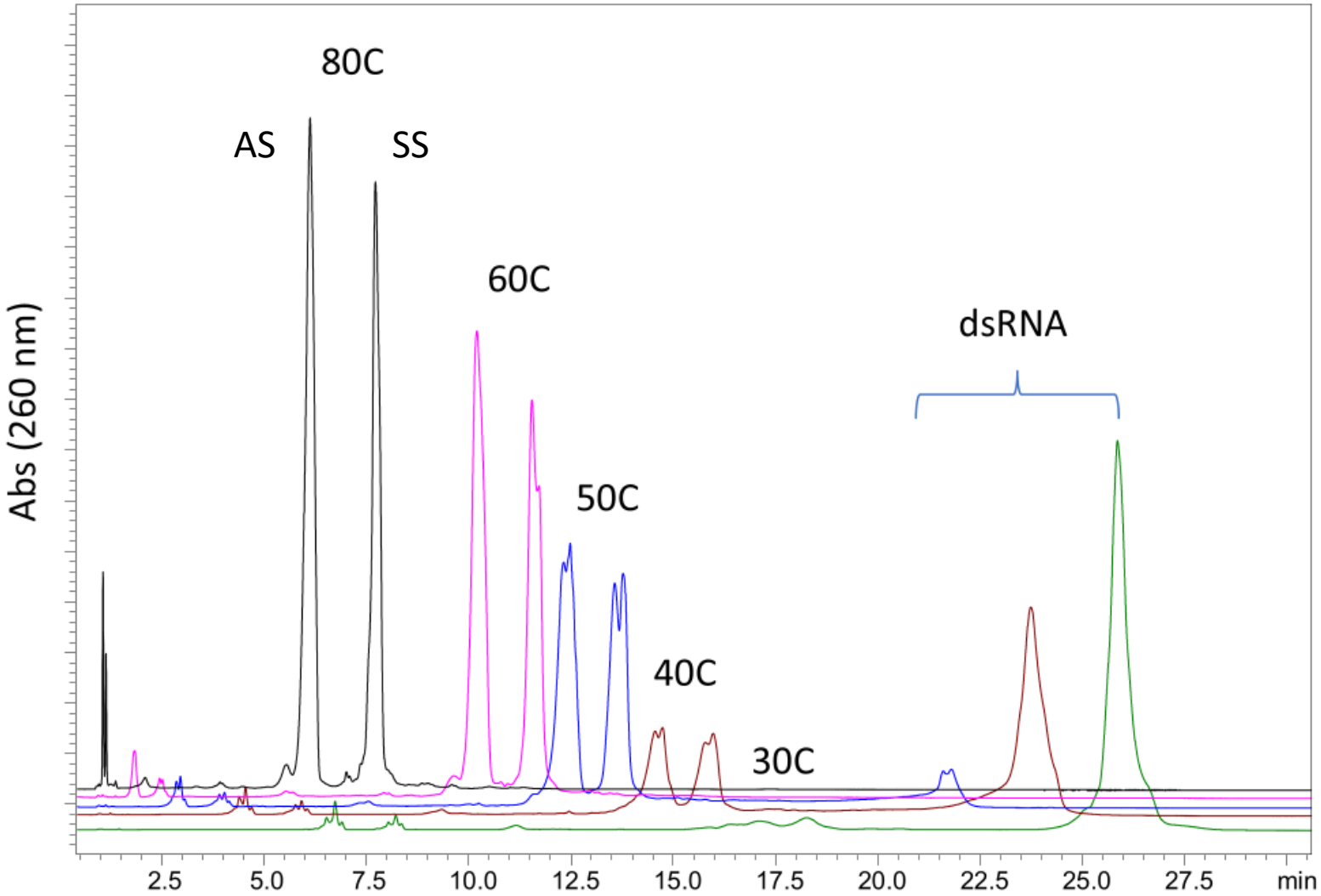
  siRNA modifications like phosphorothioates alter hydrophobicity and ionization, complicating method development.

Duplex Stability at Varying Temperatures

Conditions:

A- 3 mM DiPEA/150 mM HFIP, 5% MeOH
B- 40% H_2O, 15% IPA, 45% MeOH
2.1 x 150 mm Halo 1000 Oligo C18
Flow Rate: 0.35 mL/min
Temp: See Graph
Sample: Inclisiran, 1 µL, 1mg/mL in H_2O

- Duplex stability decreases progressively from 30 → 80 °C.
- Higher temperatures result in less intact duplex and increased strand dissociation.
- Peak shapes become narrower as temperature increases.
- Hybrid stability varies with sequence composition (GC content, length, and modification pattern).

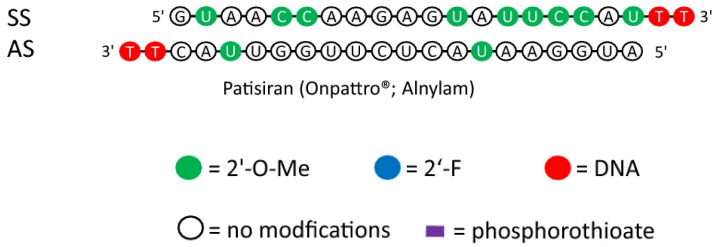


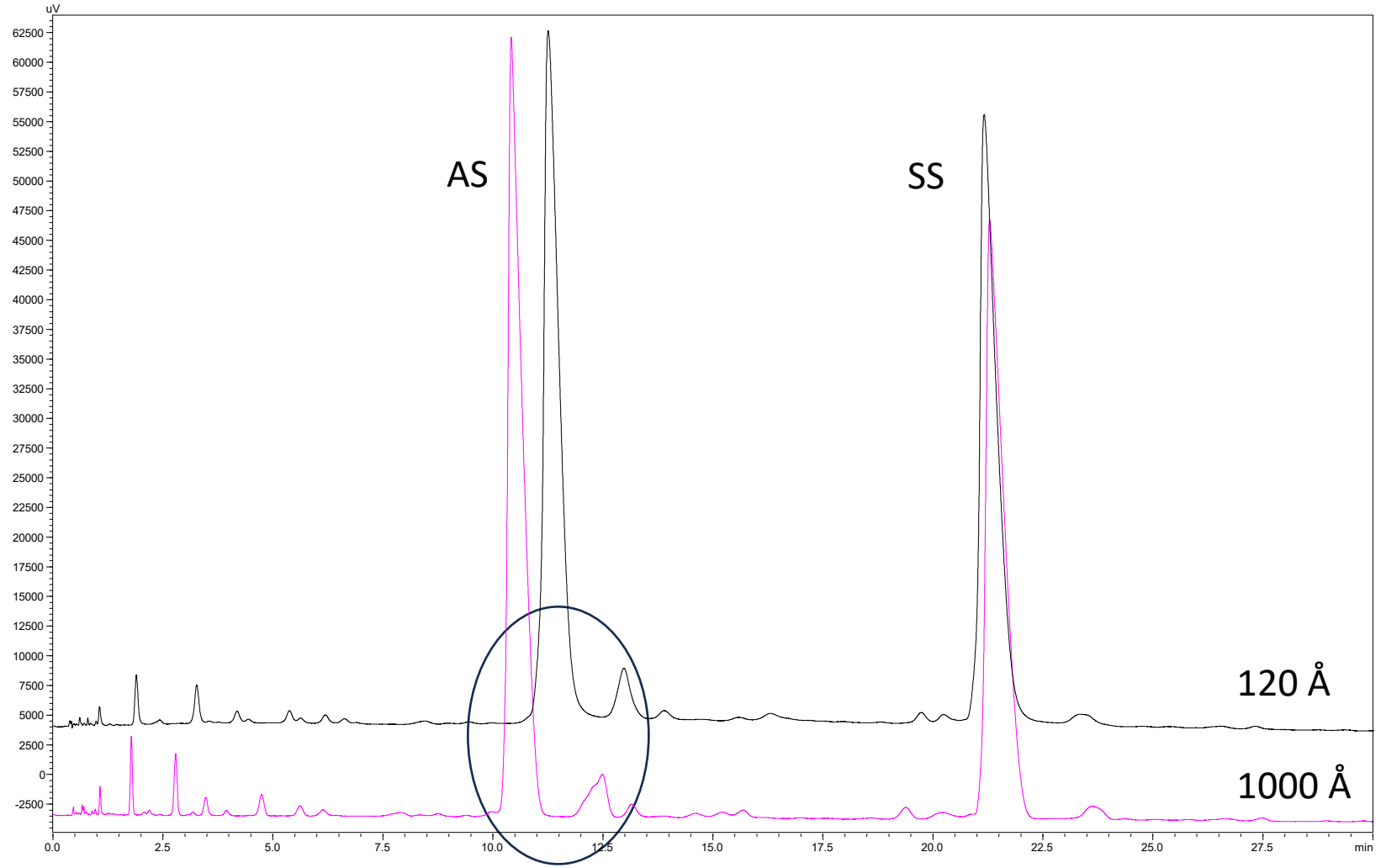
siRNA Impurities 120Å Compared to 1000Å SPP Oligo

HALO®

Conditions:

A- 10 mM DiBA/100 mM HFIP, 5% MeOH
B- 50%AcN
30 min gradient, 2.1 x 50 mm SPP C18
Flow Rate: 0.25 mL/min
Temp: 70˚C
Sample: Patisiran, 0.5 µL, 1mg/mL in H_2O
(21 nts each strand)





7.5-10.0%AcN

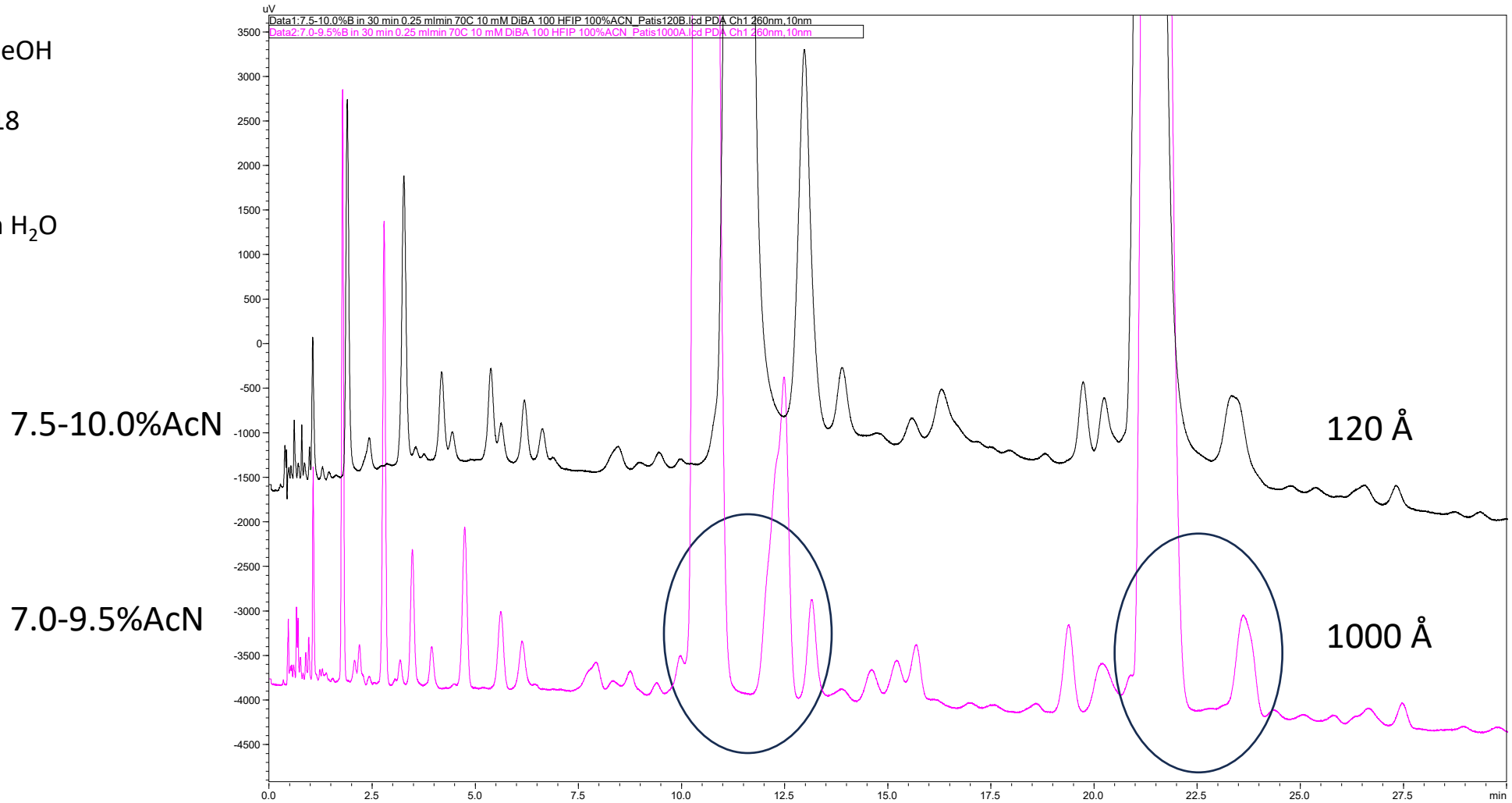
7.0-9.5%AcN

siRNA Impurities 120Å Compared to 1000Å SPP Oligo

HALO®

Conditions:

A- 10 mM DiBA/100 mM HFIP, 5% MeOH
B- AcN
30 min gradient, 2.1 x 50 mm SPP C18
Flow Rate: 0.25 mL/min
Temp: 70˚C
Sample: Patisiran, 0.5 µL, 1mg/mL in H_2O



Summary

- **Materials Design for Purpose**
  Hybrid silica SPP operation at elevated pH permits a wide range of mobile phase conditions (amine ion pairing reagents) with high mechanical, thermal and chemical stability.

- **Pore Size Importance**
  Selecting larger pore size reduces mass transfer limitations and improves separation for long or modified oligonucleotides. Separation selectivity can be affected by pore size.

- **High Resolution Requires Time and Length**
  Larger pores and lower flow rates benefit high resolution, particularly for longer chain lengths.

- **Sample Complexity**
  siRNAs' and longer synthetic oligonucleotides (sgRNAs) structure and chemical modifications require customized analytical methods for accurate characterization. Higher resolution HPLC and MS approaches will be welcomed.

Thank you for your Attention

Thanks to co-workers at AMT for their efforts and contributions –

Jason Lawhorn
Bryant McKinght
Mark Haynes
Abigail Antosz