An Introduction to Ion-Paired Reverse-Phase Oligonucleotide Separations:

From Analysis to Purification

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Introduction to Ion-Paired Reverse-Phase (IP-RP) Separations

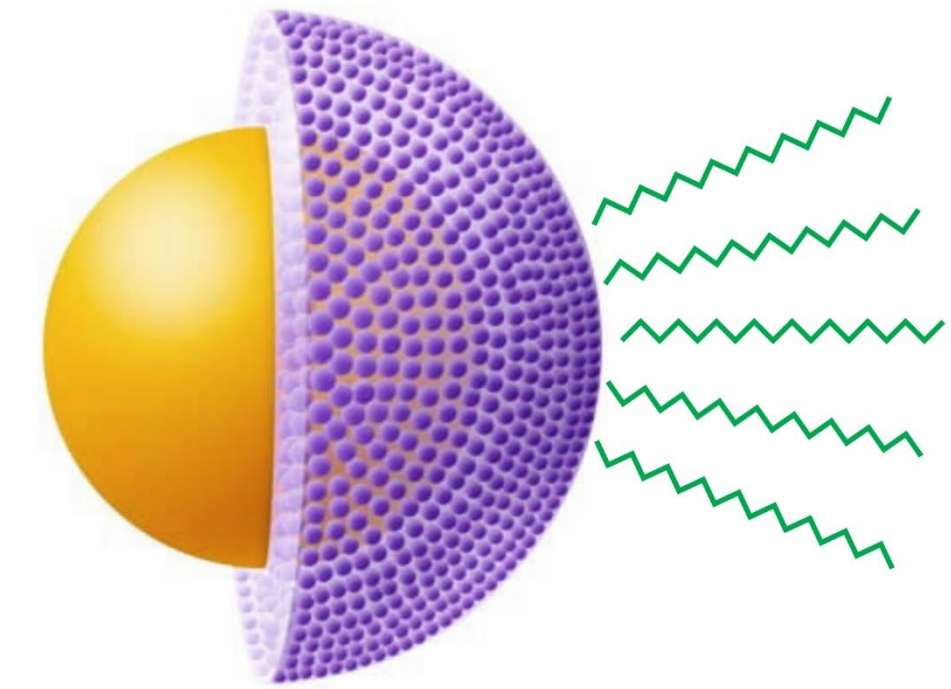
Agenda

- Introduction
- Method Objectives
- Column Selection
- Method Optimization
- Analytical Analysis
- Purification
- Resources

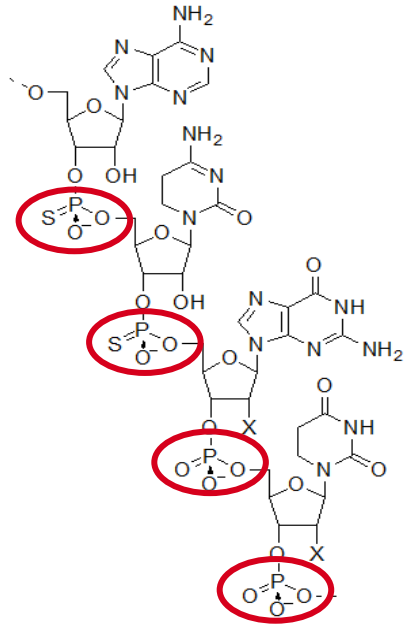
Introduction to IP-RP

Understanding IP-RP

Hydrophobic interface



Anionic backbone



The oligonucleotide's anionic phosphate backbone has no interaction with the hydrophobic reverse phase media on its own.

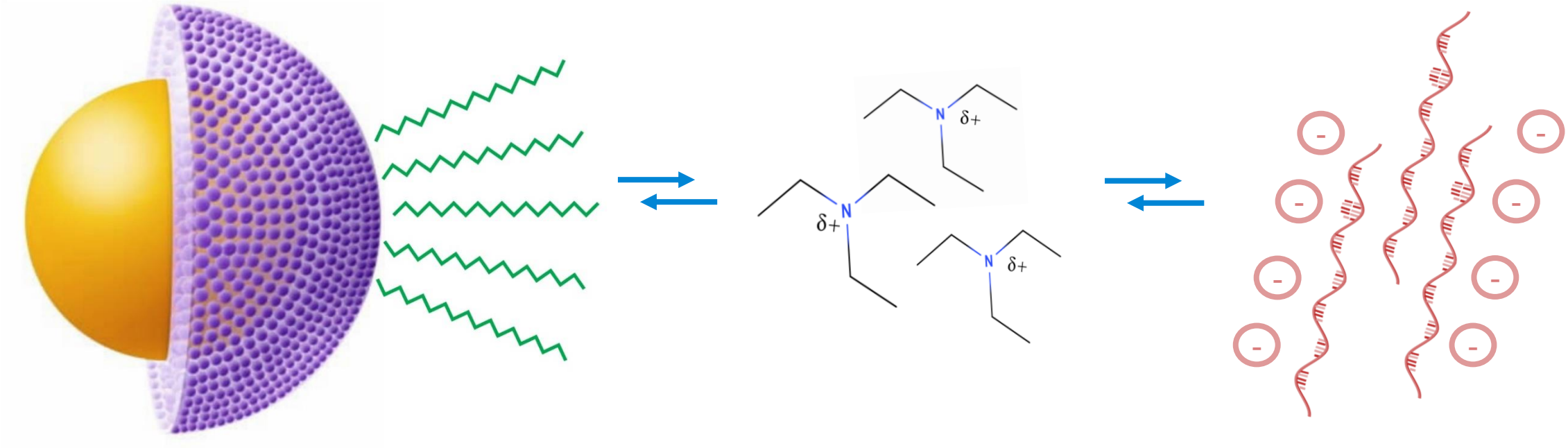
Introduction to IP-RP

Understanding IP-RP

Hydrophobic interface

Ion-pairing reagent

Anionic backbone

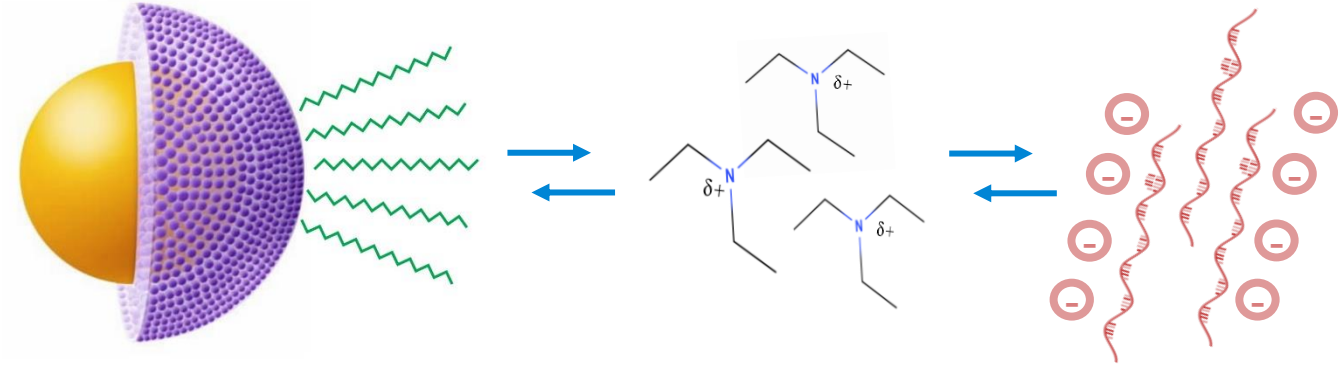


Introduction of an alkylamine ion-pairing reagent in the mobile phase facilitates the interaction and retention of oligonucleotides with the hydrophobic reverse-phase media.

Introduction to IP-RP

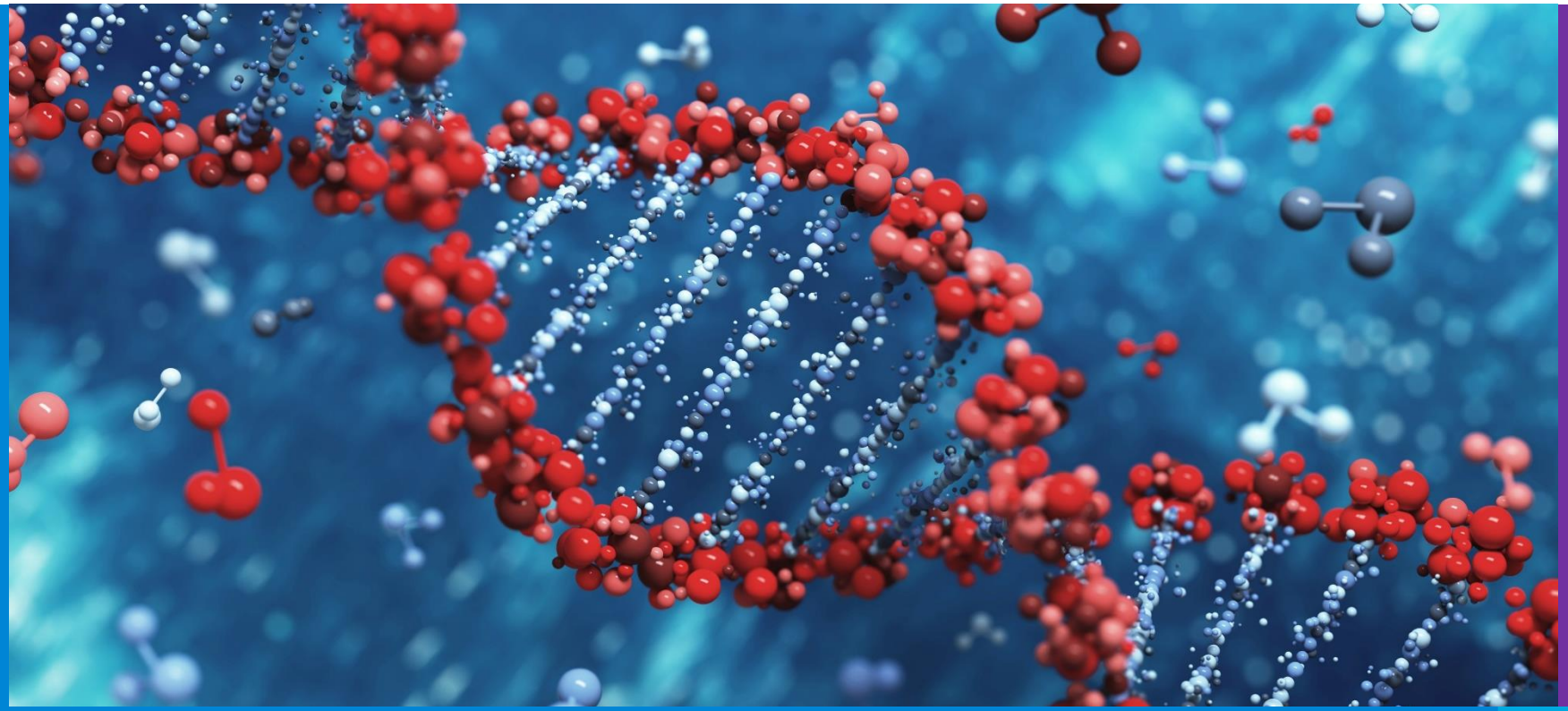
Advantages of IP-RP

- IP-RP can provide high resolution separation of fail sequences and other impurities (modifications, base loss, etc.)
- The method is compatible with UV or MS analysis
- IP-RP has denaturing conditions, elevated pH and temperature, which breaks up secondary interactions giving sharper peaks



An Introduction to IP-RP Oligonucleotide Separations

Establishing Method Objectives



Establishing Method Objectives

Analytical vs. Preparative

Analytical LC	Preparative LC
Identification	Purification

Pump

Injector

Column

Detector

Waste

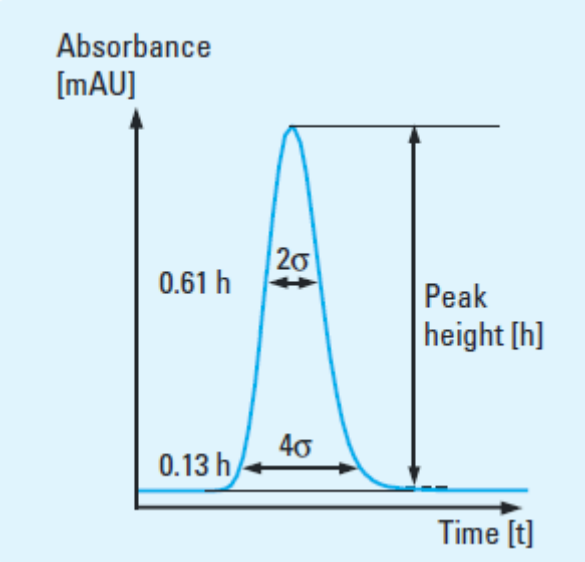
Fraction Collector

Establishing Method Objectives

Analytical Objectives

In analytical methods the overarching objective is **information**.

Gaussian peak shape



There is a linear relationship between sample load and peak height and area. The peak symmetry and the capacity factor remain unchanged.

Chromatographic parameters

- Accuracy
- Precision
  - Ruggedness
  - Robustness
- Selectivity/Specificity
- Linearity
- Range
- Quantitation Limit (LOQ, 10x S/N)
- Detection Limit (LOD, 3x S/N)

Establishing Method Objectives

Preparative Objectives

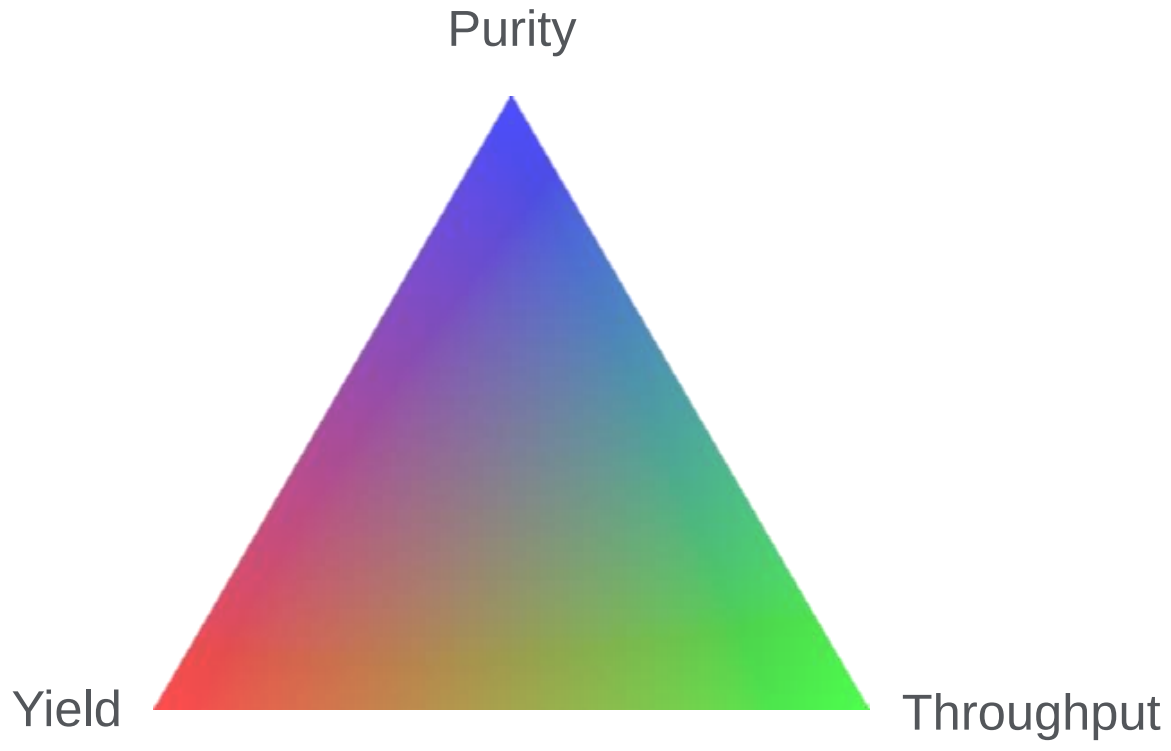
In preparative methods the main objective is purification.

Balance of purity, yield, and throughput

The three parameters used to judge the results of a preparative run are purity, yield, and throughput.

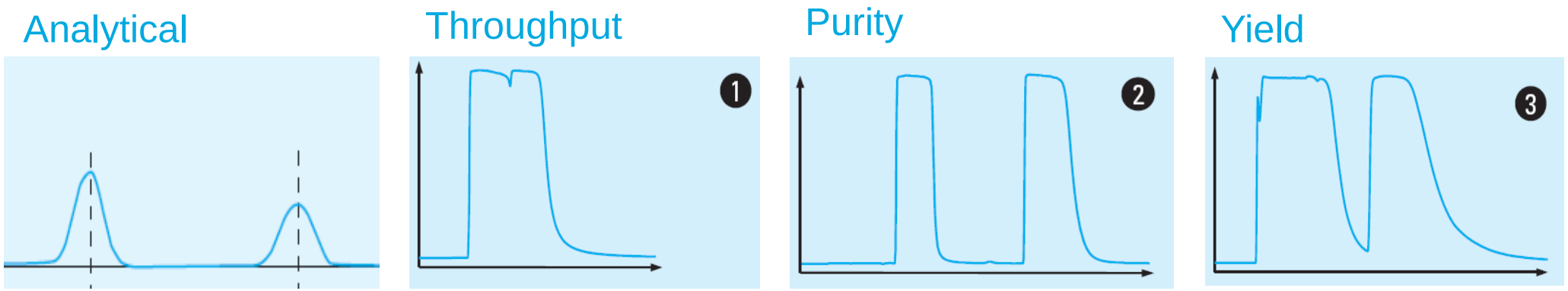
Since these parameters are interdependent, **a preparative method cannot be optimized with respect to all three parameters at once.**

The most important optimization parameter depends on the application.



Establishing Method Objectives

Preparative Objectives



Preparative HPLC-Gaussian to unsymmetrical peak shape

- Overloading the column will cause the peaks to shift from a Gaussian to a more triangular shape
- Common chromatographic characteristics with non-linear adsorption
  - Tailing
  - Fronting
  - asymmetrical peak shape

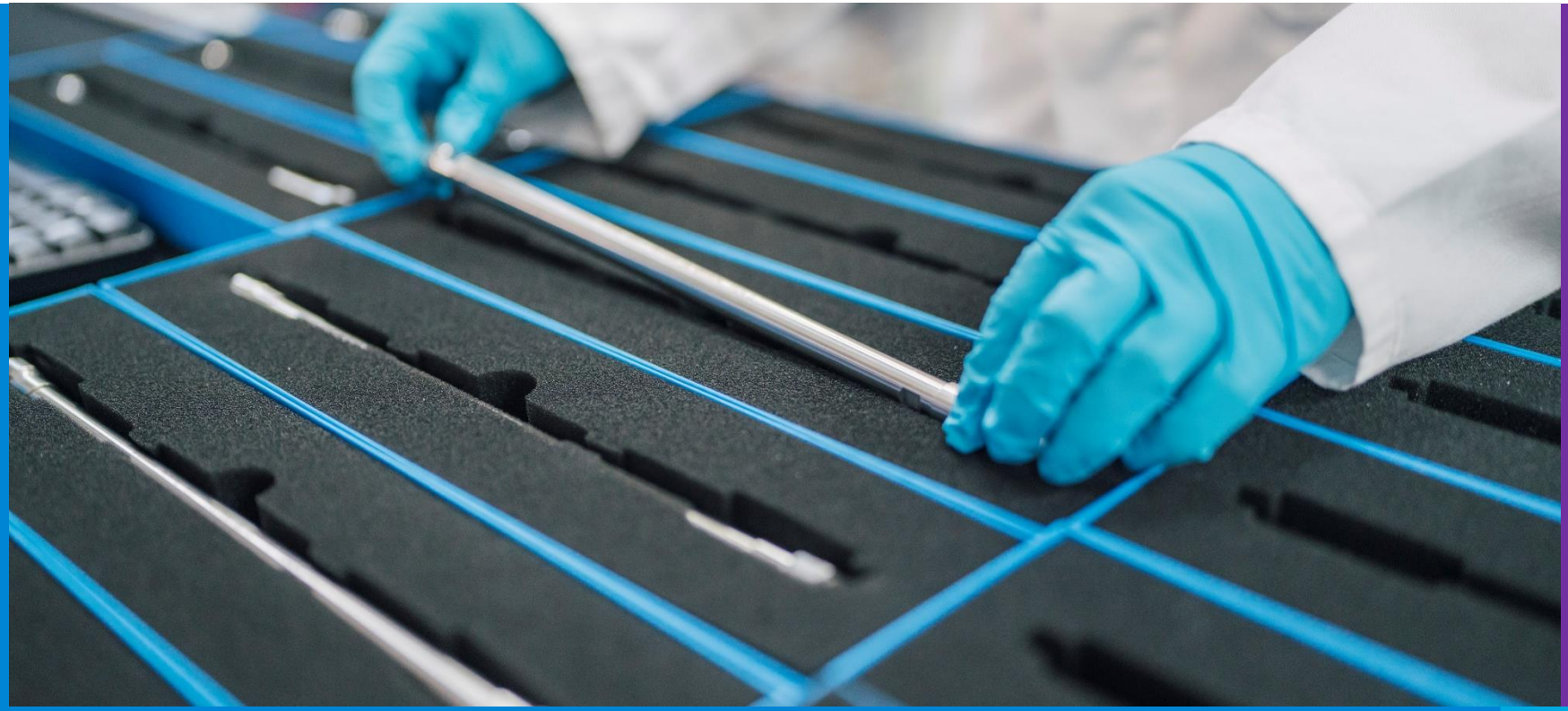
Establishing Method Objectives

Analytical vs. Preparative

	Analytical LC	Preparative LC
Objective	• Quantitation	• Purification
Peak Shape	• Gaussian	• Non-Gaussian
Priority	• Reproducibility	• Minimize sample loss

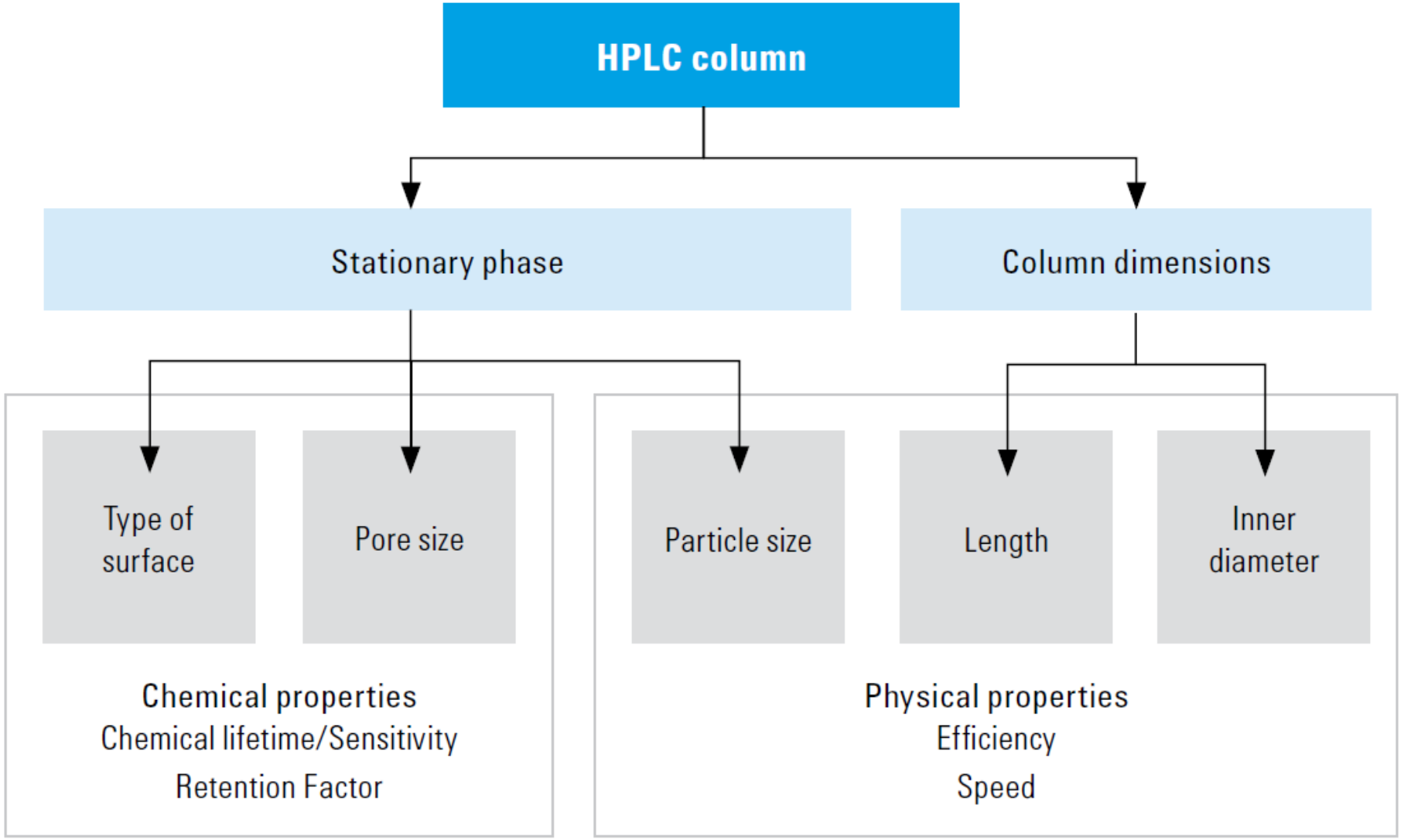
An Introduction to IP-RP Oligonucleotide Separations

Column Selection



Column Selection

Overview



LC-Handbook-Complete-2.pdf (agilent.com)

Column Selection

Overview

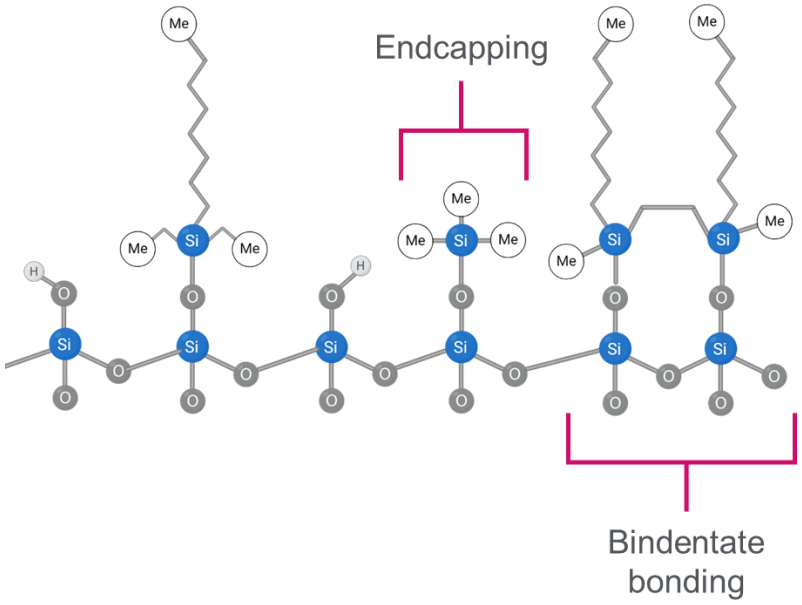
IP-RP Method Conditions

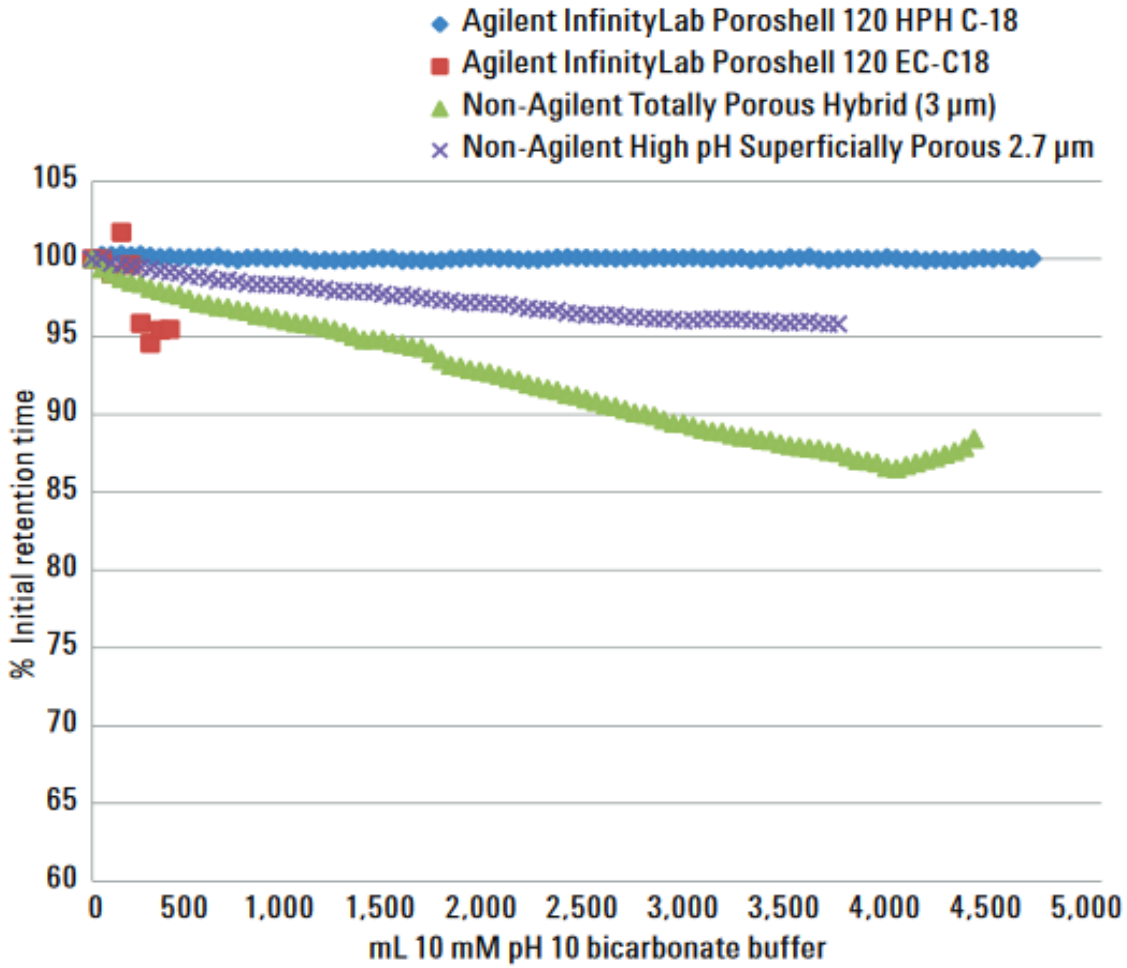
Mobile Phases (UV)	A: 100 mM TEAA in H_2O B: Acetonitrile
Mobile Phases (MS)	A: 15 mM TEA & 400 mM HFIP in H_2O B: Methanol
pH	≥7
Temperature	60 °C

Column Selection

Column lifetime

- High pH, temperature, and pressure can lead to the dissolution of the silica stationary phase
- Double end-capping and/or bidentate bonding improves peak shape of basic compounds and high pH stability





Column Selection

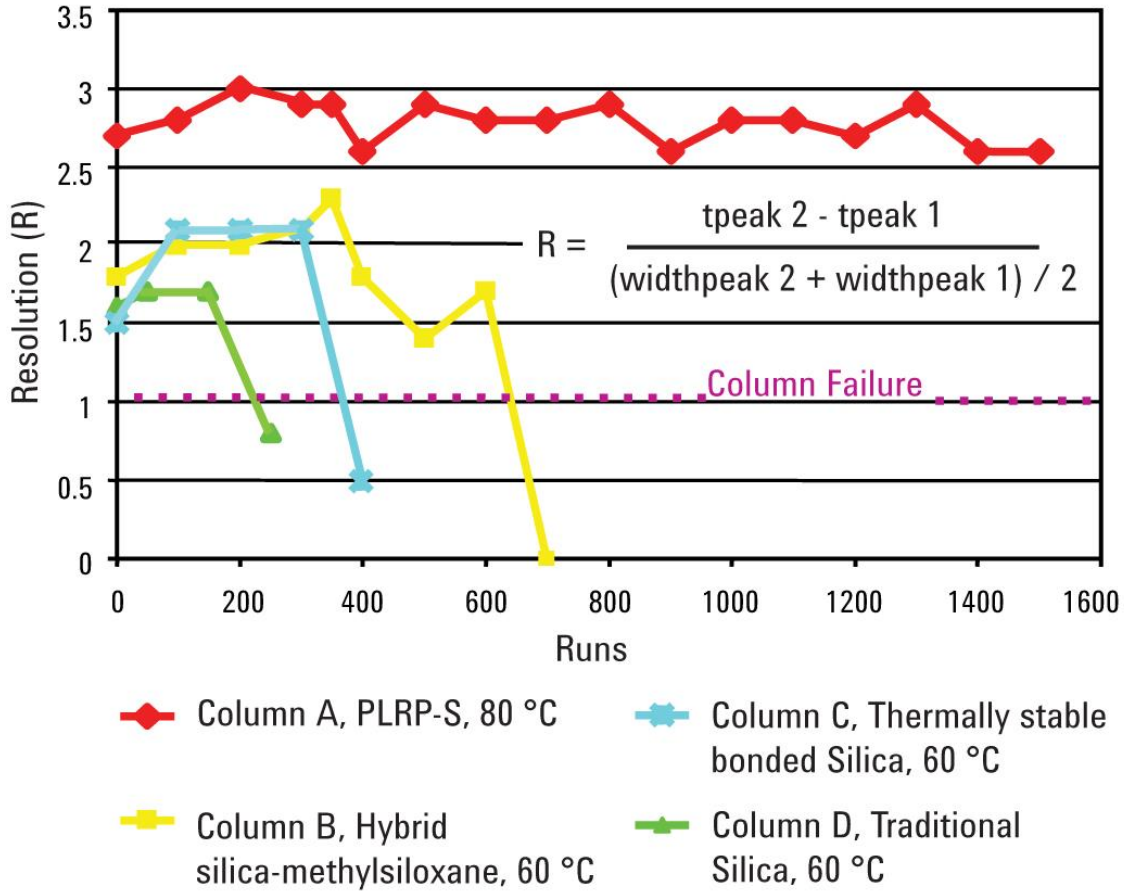
Overview

	AdvanceBio Oligonucleotide	PLRP-S
Description	Silica-based C18	Polymeric
pH Range	3-11	1-14
Max Temperature	65 °C	200 °C
Pore Sizes	100 Å	100, 300, 1000, 4000 Å
Particle Sizes	2.7, 4 µm	3, 5, 8, 10, 10-15, 15-20, 30 µm
Inner Diameters	2.1, 4.6, 10, 21.2 mm	2.1, 4.6, 7.5, 25, 50, 100 mm
Lengths	50, 100, 150 mm	50, 150, 250, 300 mm
Bulk Media	No	Yes

Column Selection

Stability of PLRP-S Columns

The thermal stability and lifetime of the columns was evaluated. Columns were subjected to 25-minute-long gradient cycles at either 80 °C (PLRP-S) or 60 °C (all other columns). The resolution (R values) of poly(dT)19-24 and 29/30 mer samples were calculated after every 100 gradient cycles. Columns were considered dead when R values dropped by more than 25%.



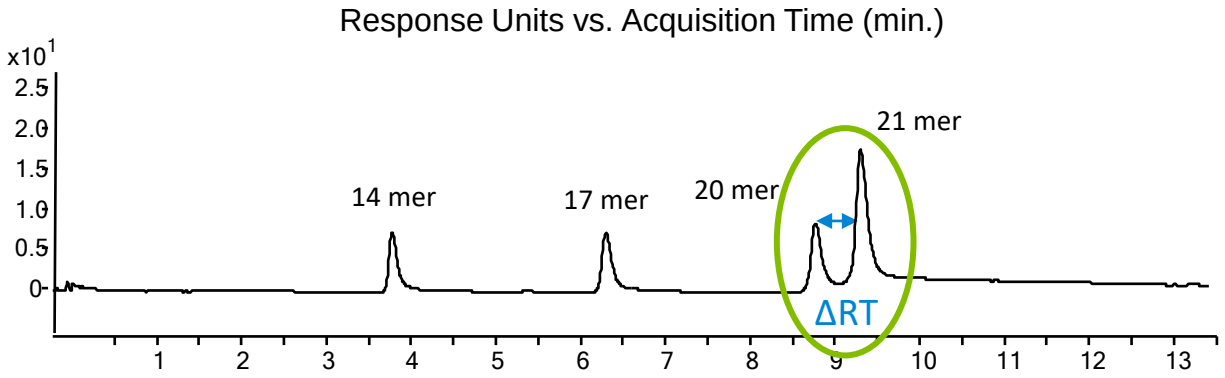
Mobile phase A:	100 mM TEAA in water
Mobile phase B:	100 mM TEAA in 25:75 acetonitrile
Gradient:	6 to 8%B in 10 min
Flow rate:	1.0 mL/min
Sample:	Poly(dT)19-24 and 29/30 mer oligonucleotide
Injection:	10 µL
Detection:	UV at 254 nm

Improved Column Lifetime with Thermally Stable Polymer Columns for Oligonucleotide IP-RP HPLC

Column Selection

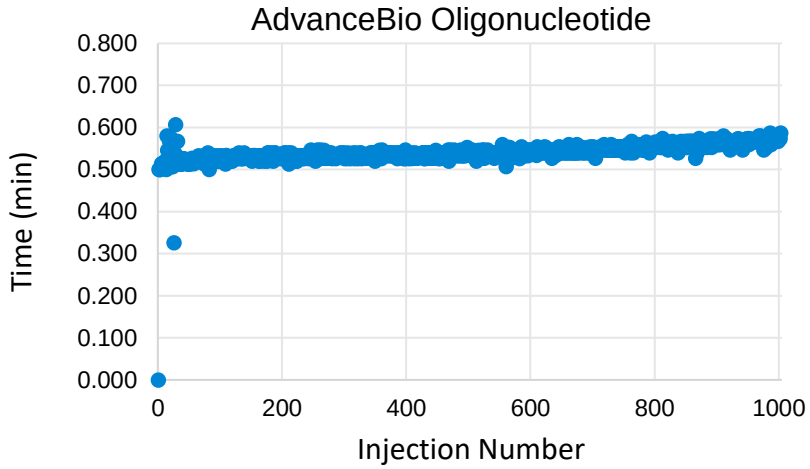
Stability of AdvanceBio Oligonucleotide Column

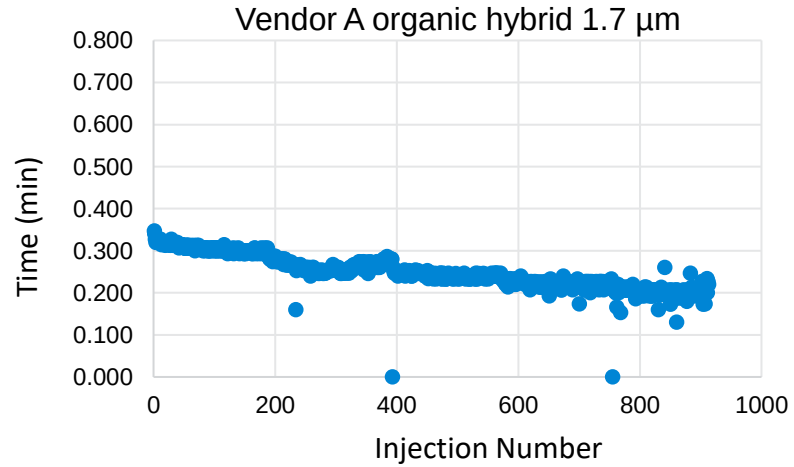
AdvanceBio Oligonucleotide



Column:	AdvanceBio Oligonucleotide, 2.1 x 50 mm
Mobile phase A:	100 mM TEAA in water
Mobile phase B:	100 mM TEAA in acetonitrile
Gradient:	6 to 8%B in 10 min
Stop time:	11 min
Post run:	5 min
Flow rate:	0.6 mL/min
Sample:	Agilent Oligonucleotide Resolution Standard
Temperature:	65 °C
Injection:	5 µL
Detection:	UV at 260 nm

ΔRT Between Peak 20 and 21mer





Column Selection

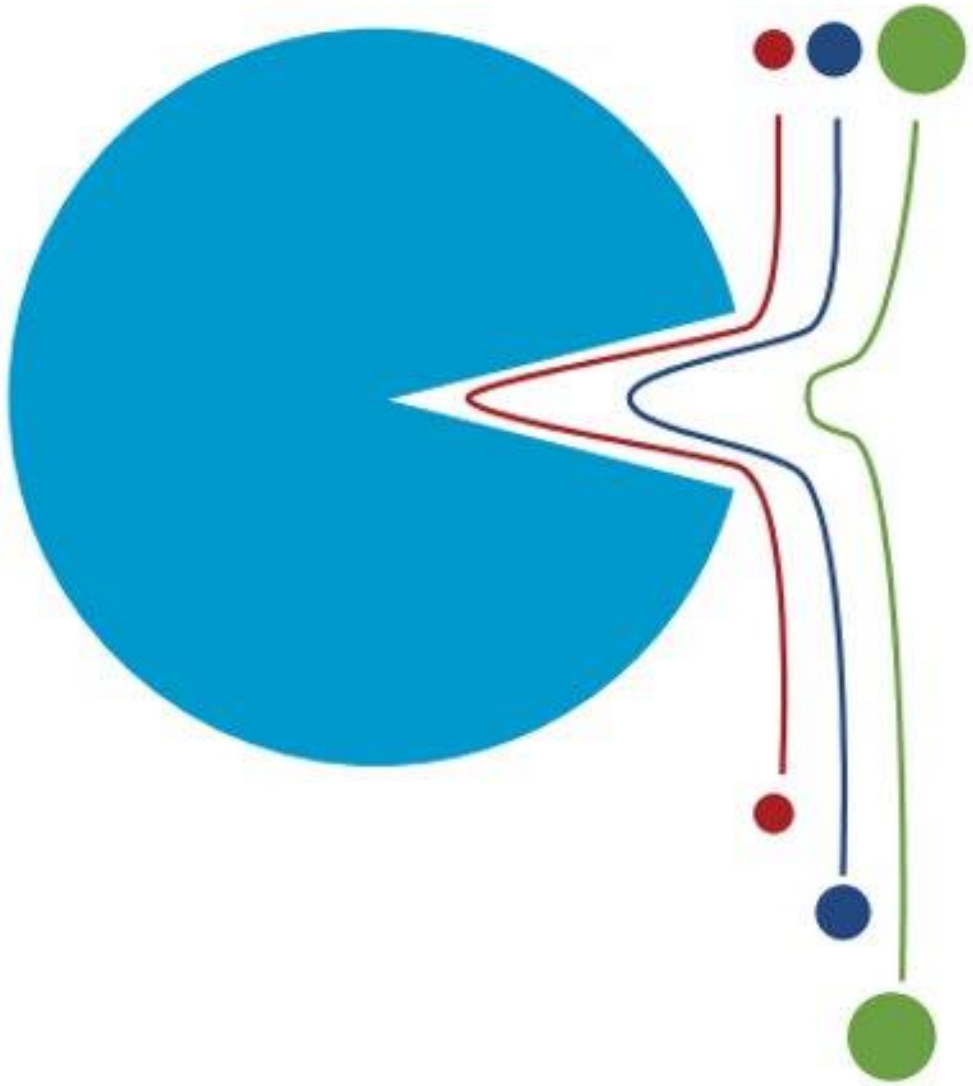
Pore Size

	AdvanceBio Oligonucleotide	PLRP-S
Description	Silica-based C18	Polymeric
pH Range	3-11	1-14
Max Temperature	65 °C	200 °C
Pore Sizes	100 Å	100, 300, 1000, 4000 Å
Particle Sizes	2.7, 4 µm	3, 5, 8, 10, 10-15, 15-20, 30 µm
Inner Diameters	2.1, 4.6, 10, 21.2 mm	2.1, 4.6, 7.5, 25, 50, 100 mm
Lengths	50, 100, 150 mm	50, 150, 250, 300 mm
Bulk Media	No	Yes

Column Selection

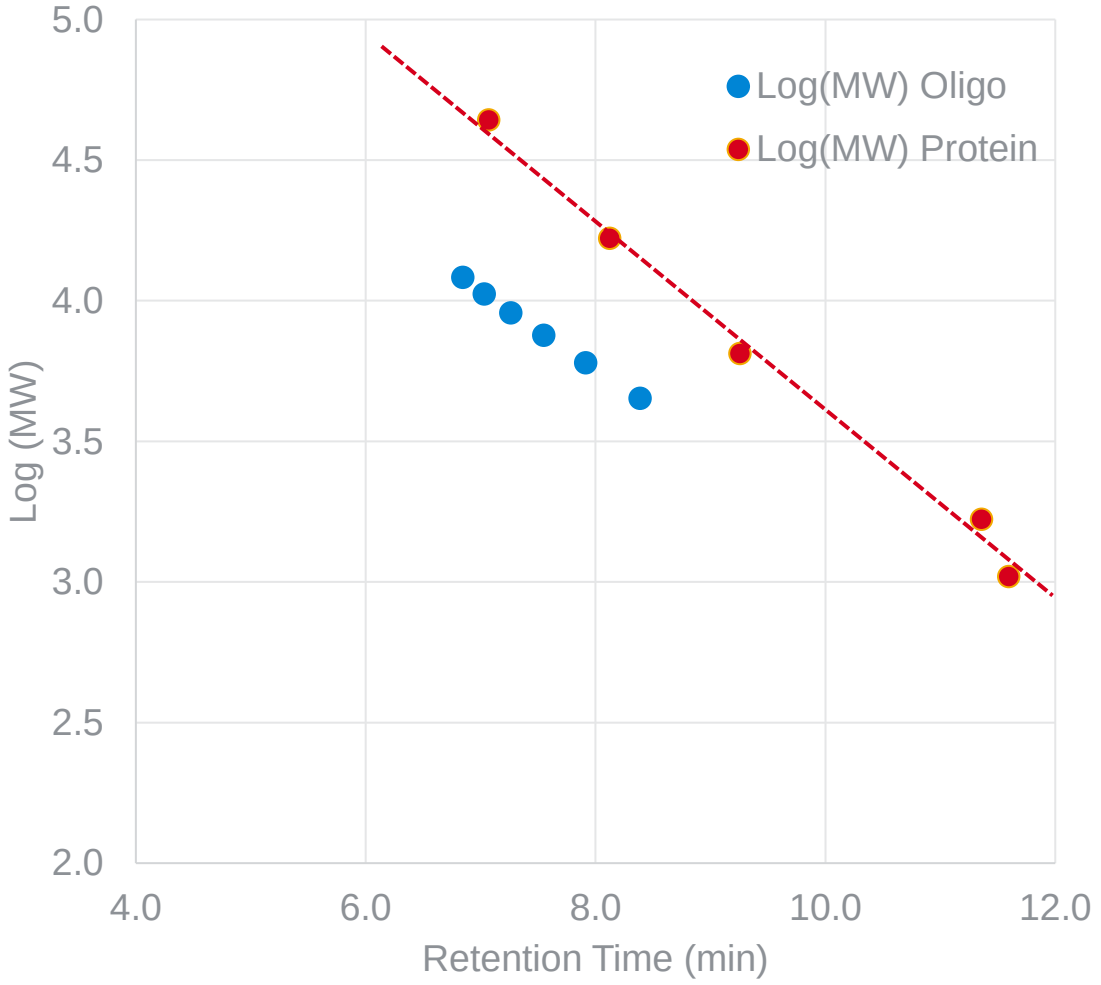
Pore Size

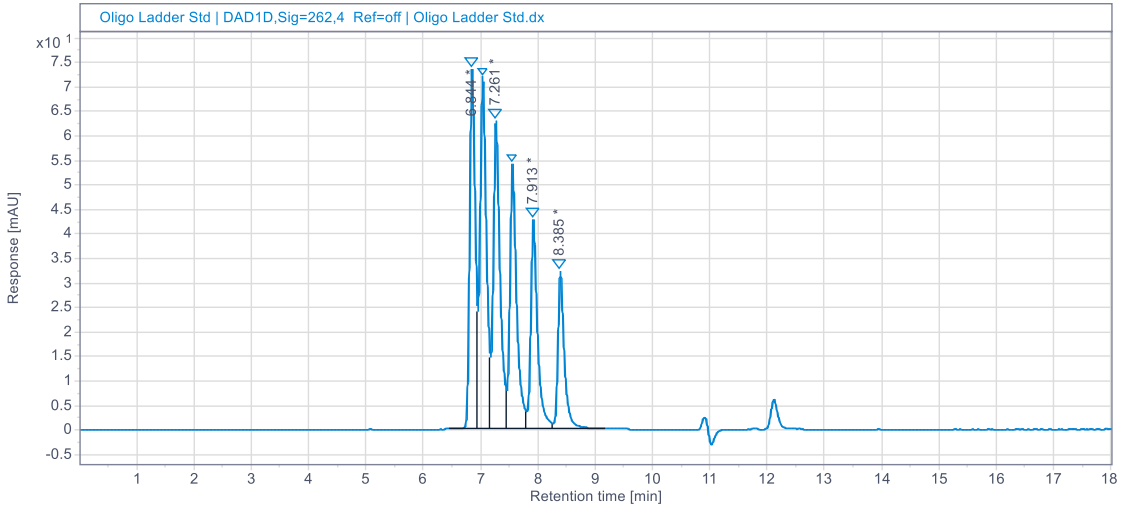
- As a general rule, the pore size should be 3X the hydrodynamic radius of your analyte
- Larger pore sizes can increase stationary phase mass transfer and improve resolution
- Columns with smaller pores have higher surface area and potentially higher binding capacities



Column Selection

Pore Size





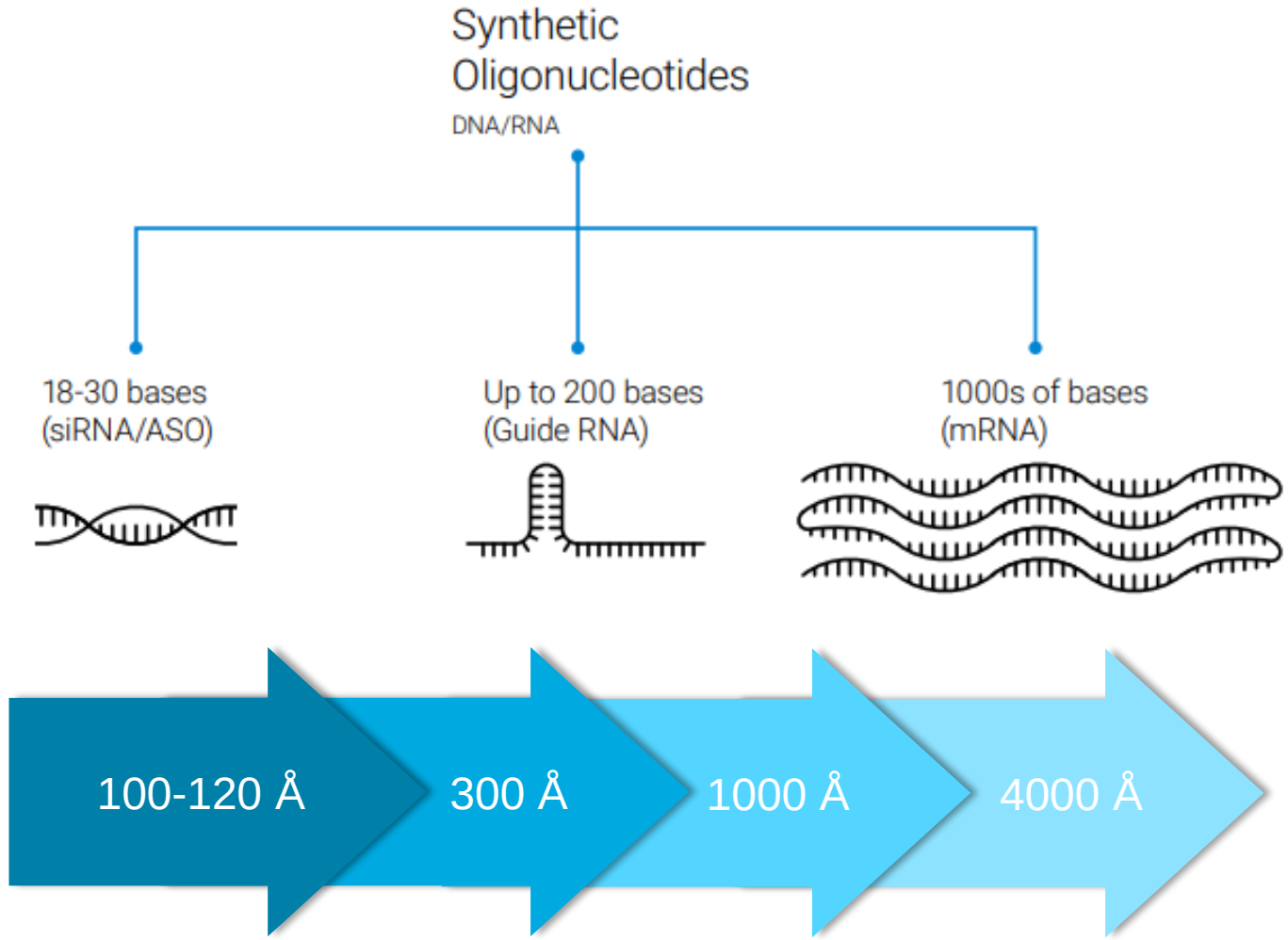
Size exclusion chromatogram of oligonucleotide ladder standard containing dT_{15}, dT_{20}, dT_{25}, dT_{30}, dT_{35}, dT_{40}.

dT_{40} has a MW of around 12.1 kDa but elutes at a time that corresponds to a globular protein of around 50 – 60 kDa.

Column Selection

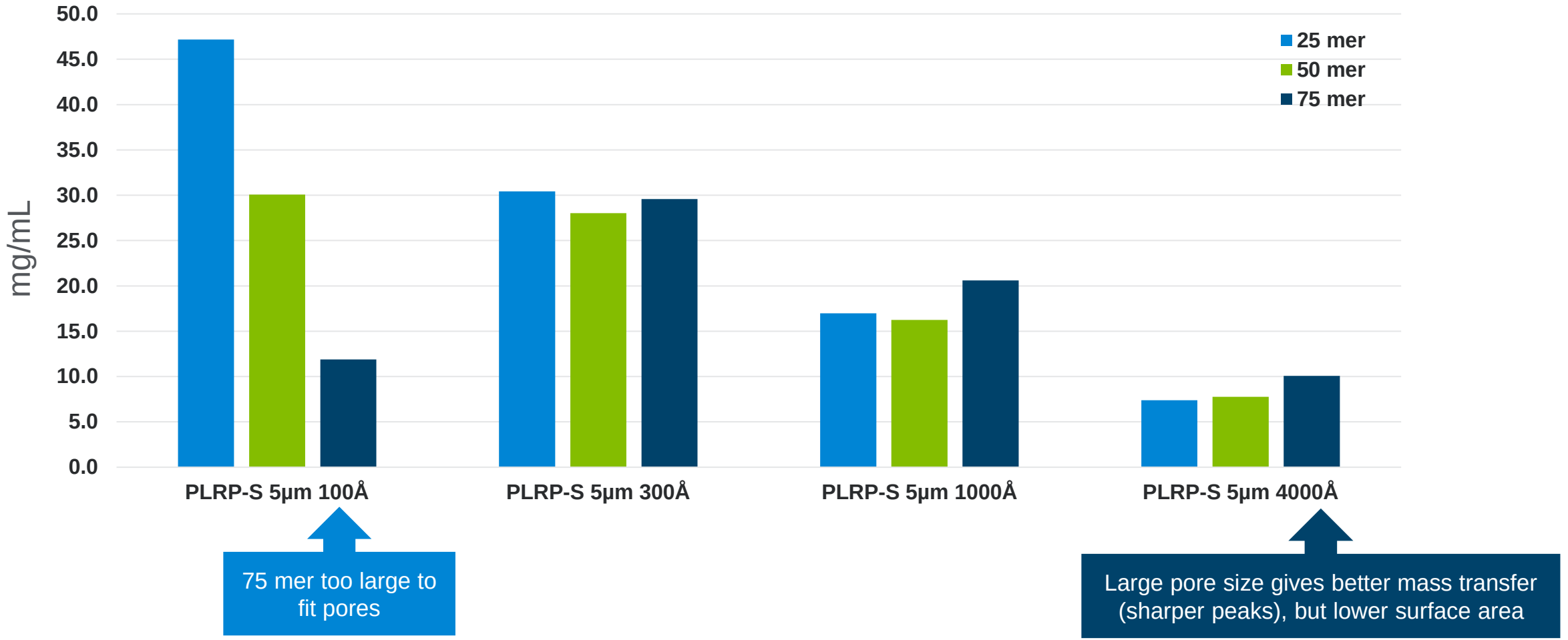
Pore Size

- Purification requires determination of the optimal pore size to ensure effective mass transfer of an oligonucleotide in solution.
- Determining the balance between pore size and binding capacity is important for ensuring the highest resolution and yield.



Column Selection

Pore Size



Dynamic Binding Capacity of Oligonucleotides on PLRP-S Columns and Stationary Phases

Column Selection

Particle Size

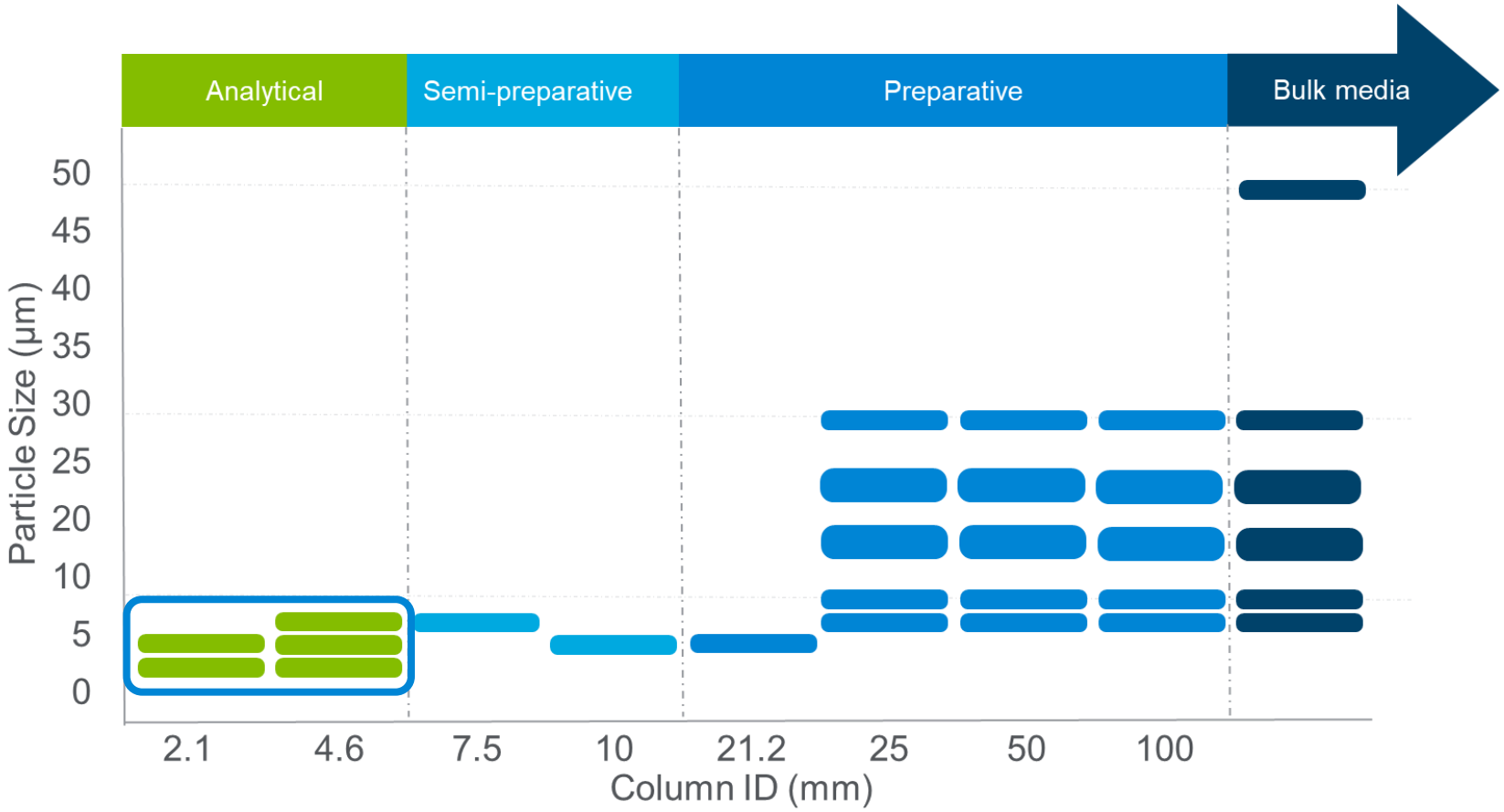
	AdvanceBio Oligonucleotide	PLRP-S
Description	Silica-based C18	Polymeric
pH Range	3-11	1-14
Max Temperature	65 °C	200 °C
Pore Sizes	100 Å	100, 300, 1000, 4000 Å
Particle Sizes	2.7, 4 µm	3, 5, 8, 10, 10-15, 15-20, 30 µm
Inner Diameters	2.1, 4.6, 10, 21.2 mm	2.1, 4.6, 7.5, 25, 50, 100 mm
Lengths	50, 100, 150 mm	50, 150, 250, 300 mm
Bulk Media	No	Yes

Column Selection

Particle Size

In analytical HPLC, a smaller particle size is better.

- Higher throughput, more sensitivity, less solvent
- Smaller particles increase backpressure

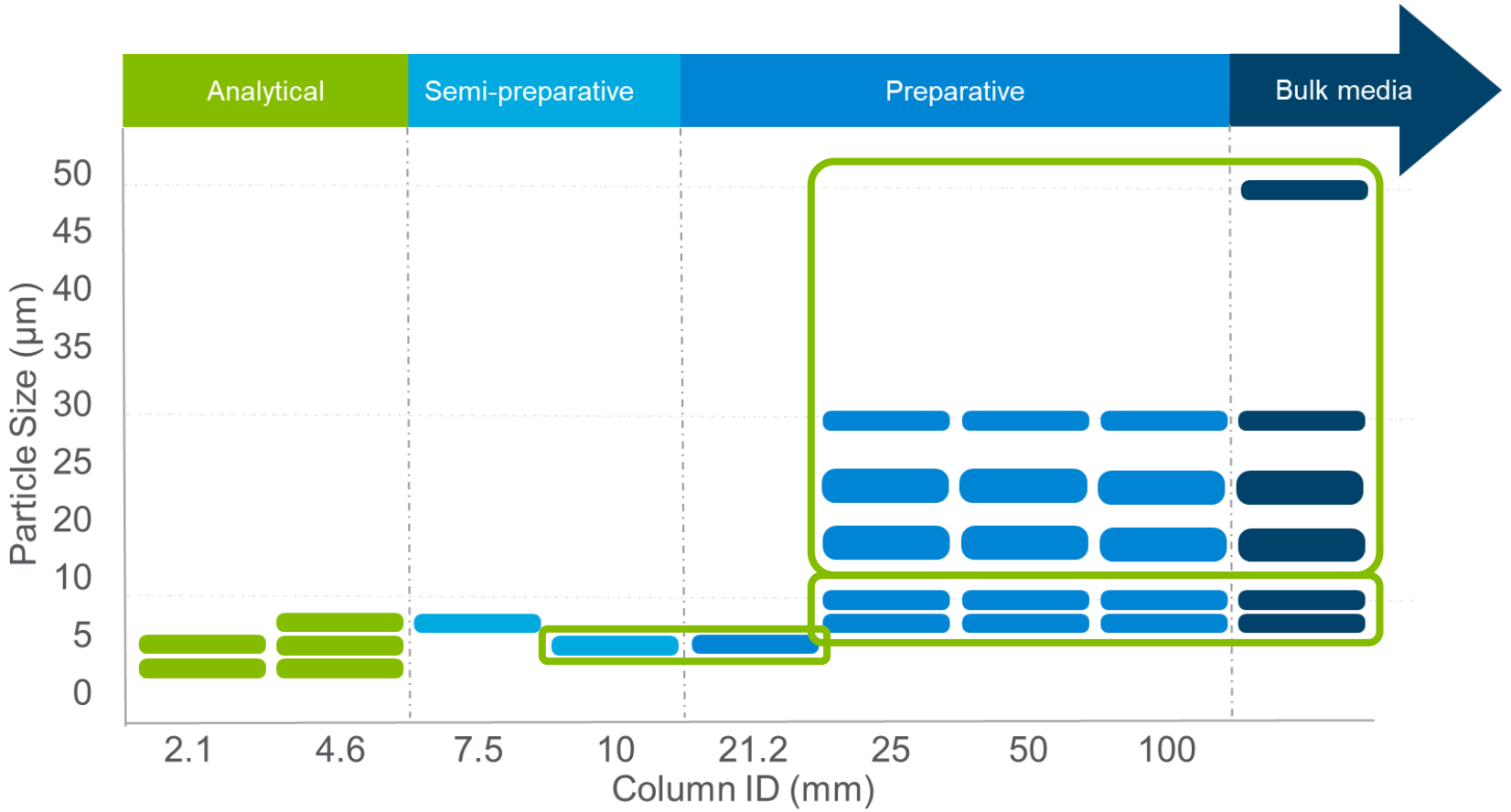


Column Selection

Particle Size

In preparative HPLC, the optimal particle size depends on the method objective.

- For complex solutions with closely eluting peaks, 4-5 µm particles will increase resolution
- For well defined separations, 7-10 µm particles can be used
- For high throughput, ≥10 µm particles can decrease column backpressure and allow fast flowrates, shorter methods



Column Selection

Column Dimensions

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Max Temperature	65 °C	200 °C
Pore Sizes	100 Å	100, 300, 1000, 4000 Å
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Inner Diameters	2.1, 4.6, 10, 21.2 mm	2.1, 4.6, 7.5, 25, 50, 100 mm
Lengths	50, 100, 150 mm	50, 150, 250, 300 mm
Bulk Media	No	Yes

Column Selection

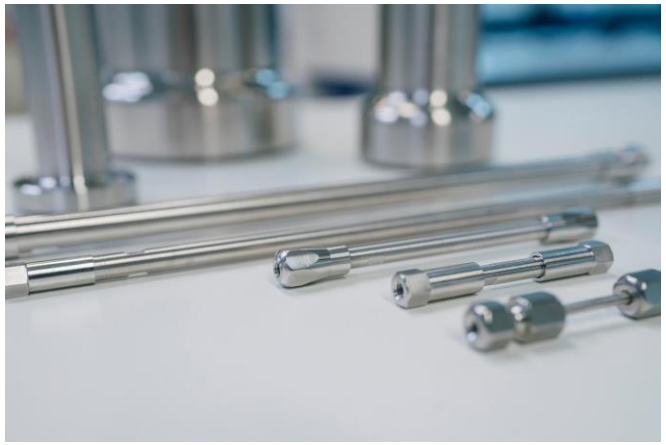
Column Dimensions: Analytical

Inner diameter (ID)

ID	Optimum Flow Rate	Recommended Use
4.6 mm	1.00-1.25 mL/min	Legacy methods
3.0 mm	0.8-1.0 mL/min	Lower solvent use
2.1 mm	0.4-0.5 mL/min	MS applications, lowest solvent use

Column length

Column Length	Recommended Use
50 mm	High throughput
100 mm	High resolution
≥150 mm	Ultra-high resolution



Column Selection

Column Dimensions: Purification

Preparatory methods are developed on an analytical column, then scaled to a preparatory column that meets purification objectives.

- Analytical columns with the same stationary phase, particle size and length will provide the most consistent results

An Introduction to IP-RP Oligonucleotide Separations

Method Optimization

Method Optimization

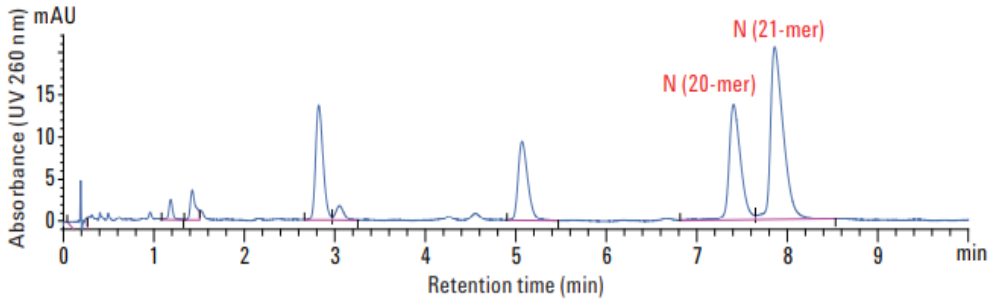
Method Optimization

Ion-Pairing Reagent	• Amines with longer alkyl chains can increase oligo hydrophobicity and increase retention • Increasing the concentration of ion-pairing buffer can increase retention
Gradient Steepness	• A steeper gradient can shorten method • A shallower gradient improves resolution
Temperature	• Increase temperature reduces secondary oligo structures • Reduces mobile phase viscosity and secondary column interactions → improved peak shape

Method Optimization

UV vs. MS Compatibility

LC/UV

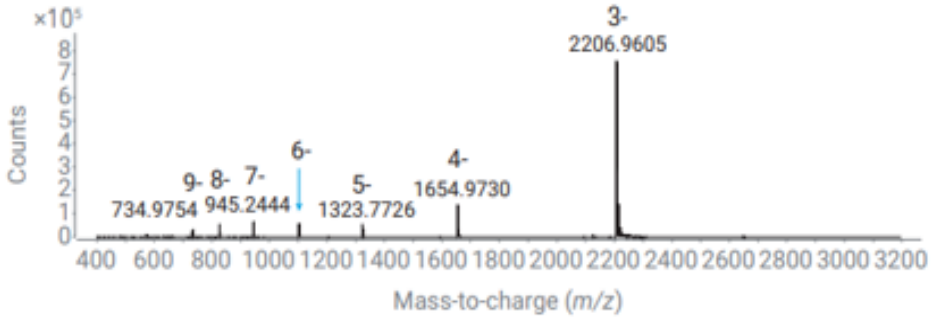


Ion pair: TEA, DBA, HA

Acidic counter ion: Acetate

Organic solvent: Acetonitrile

LC/MS



Ion pair: TEA, DBA, HA

Acidic counter ion: Hexafluoroisopropanol (HFIP)

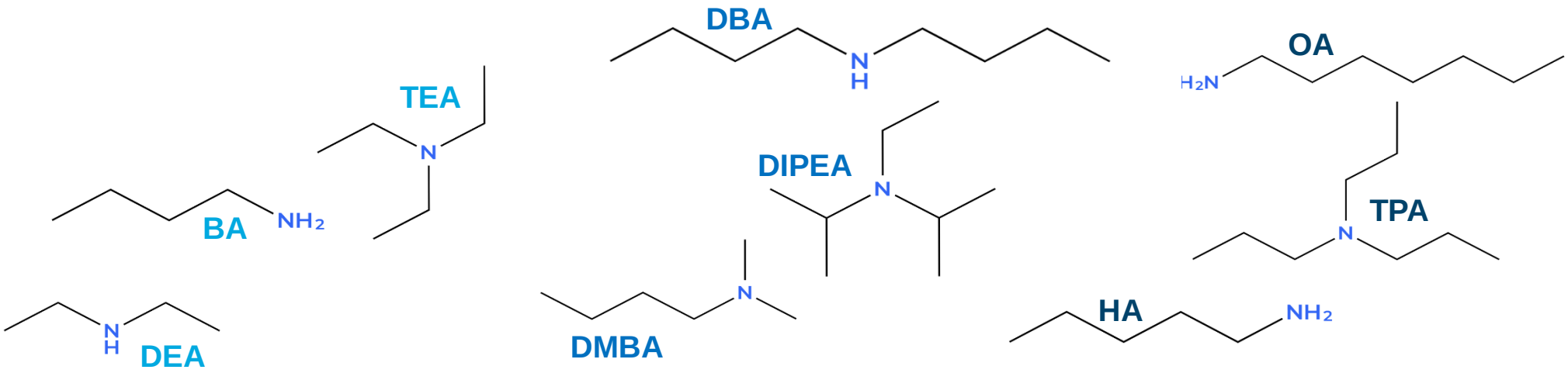
Organic solvent: Methanol

HFIP becomes immiscible in acetonitrile when mixed with certain alkylamines

Method Optimization

Ion-Pairing Reagents

The ion-pairing strength of the alkylamine depends on both its hydrophobicity and how easily it ionizes



Weak | Moderate | Strong

Diethylamine (DEA), butylamine (BA), triethylamine (TEA), dimethylbutylamine (DMBA, diisopropylethylamine (DIPEA), dibutylamine (DBA), hexylamine (HA), tripropylamine (TPA), octylamine (OA)

Evaluation of Different Ion Pairing Reagents for LC/UV and LC/MS Analysis of Oligonucleotides (agilent.com)

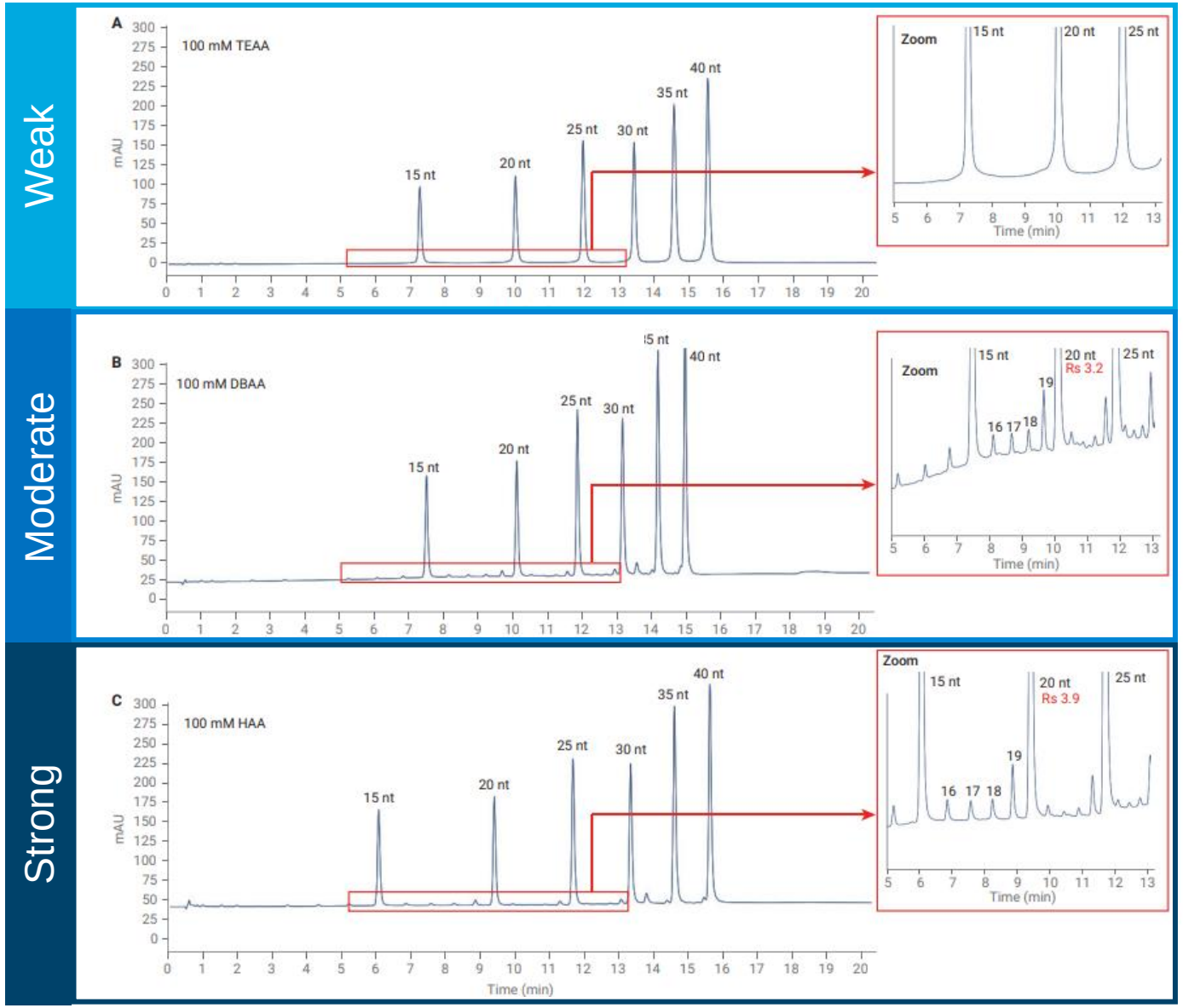
Donegan, M.; Nguyen, J. M.; Gilar, M. Effect of Ion-Pairing Reagent Hydrophobicity on Liquid Chromatography and Mass Spectrometry Analysis of Oligonucleotides. *Journal of Chromatography A* **2022**, *1666*, 462860. https://doi.org/10.1016/j.chroma.2022.462860.

Method Optimization

Ion-Pairing Reagents

Although the main 15 to 40 nt peaks were clearly separated using TEAA, short-mer contaminants were not well resolved. DBAA and HAA were able to clearly resolve short-mers such as the 16 to 19 nt peaks. HAA showed a superior resolution of 3.9 between the 19 and 20 nt peaks, compared to a resolution of 3.2 when using DBAA

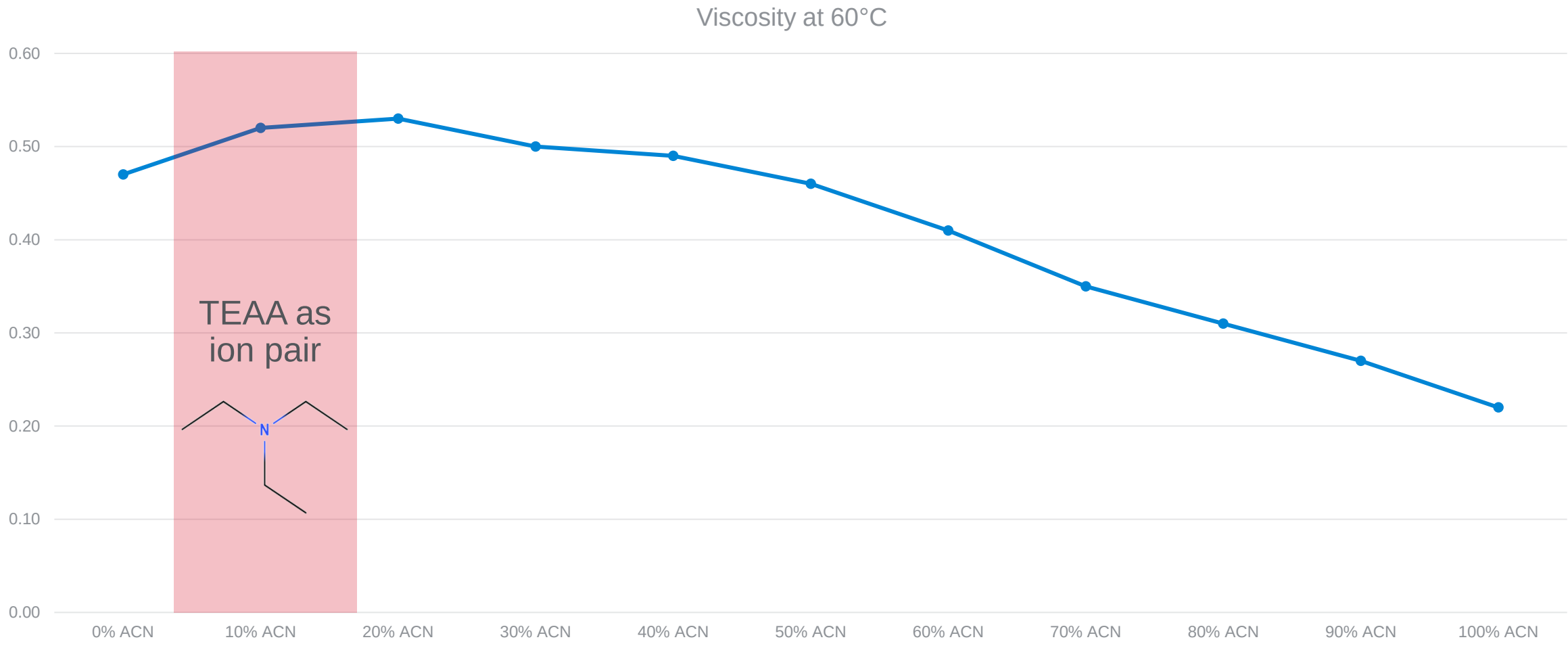
Column:	AdvanceBio Oligonucleotide, 2.1 x 150 mm
Flow rate:	0.6 mL/min
Sample:	ssDNA oligo-dT
Temperature:	60 °C
Injection:	5 µL
Detection:	UV at 260 nm



Ion-Pair Reversed-Phase Chromatography and Agilent 1260 Infinity II Prime LC

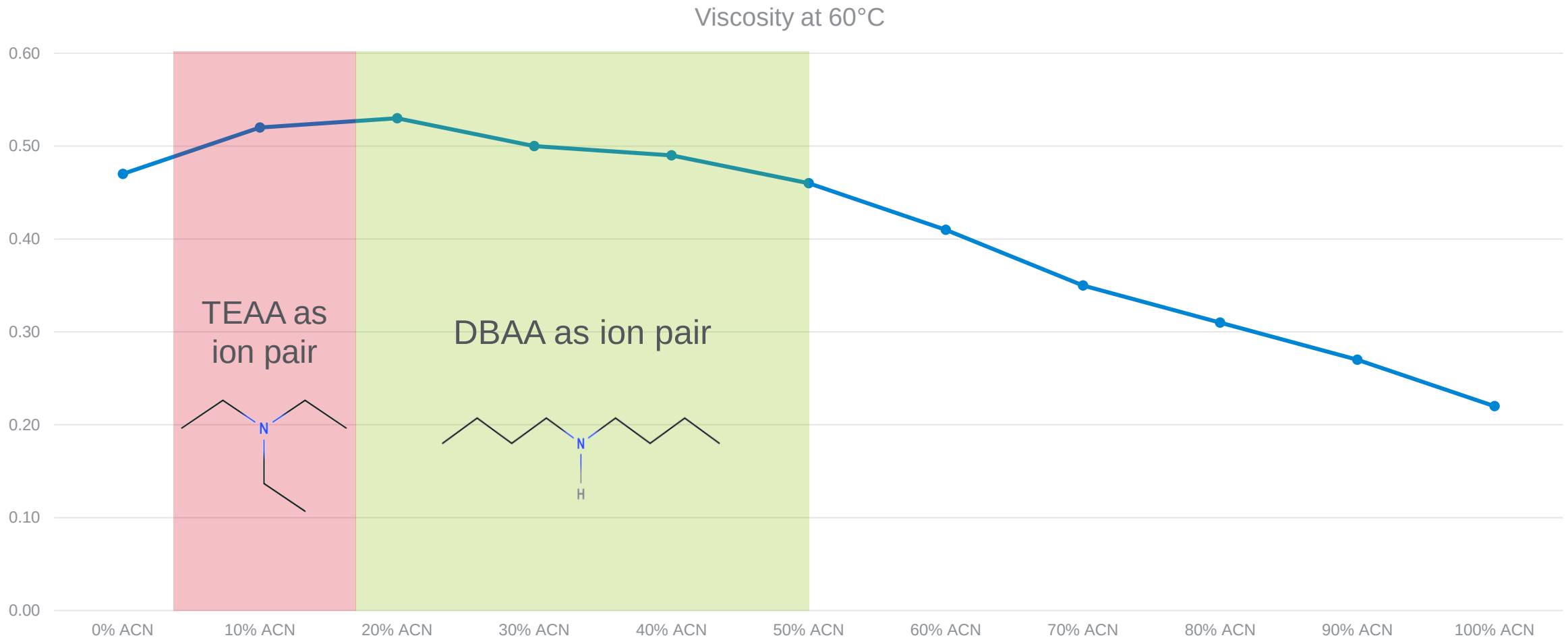
Method Optimization

Ion-Pairing Reagents



Method Optimization

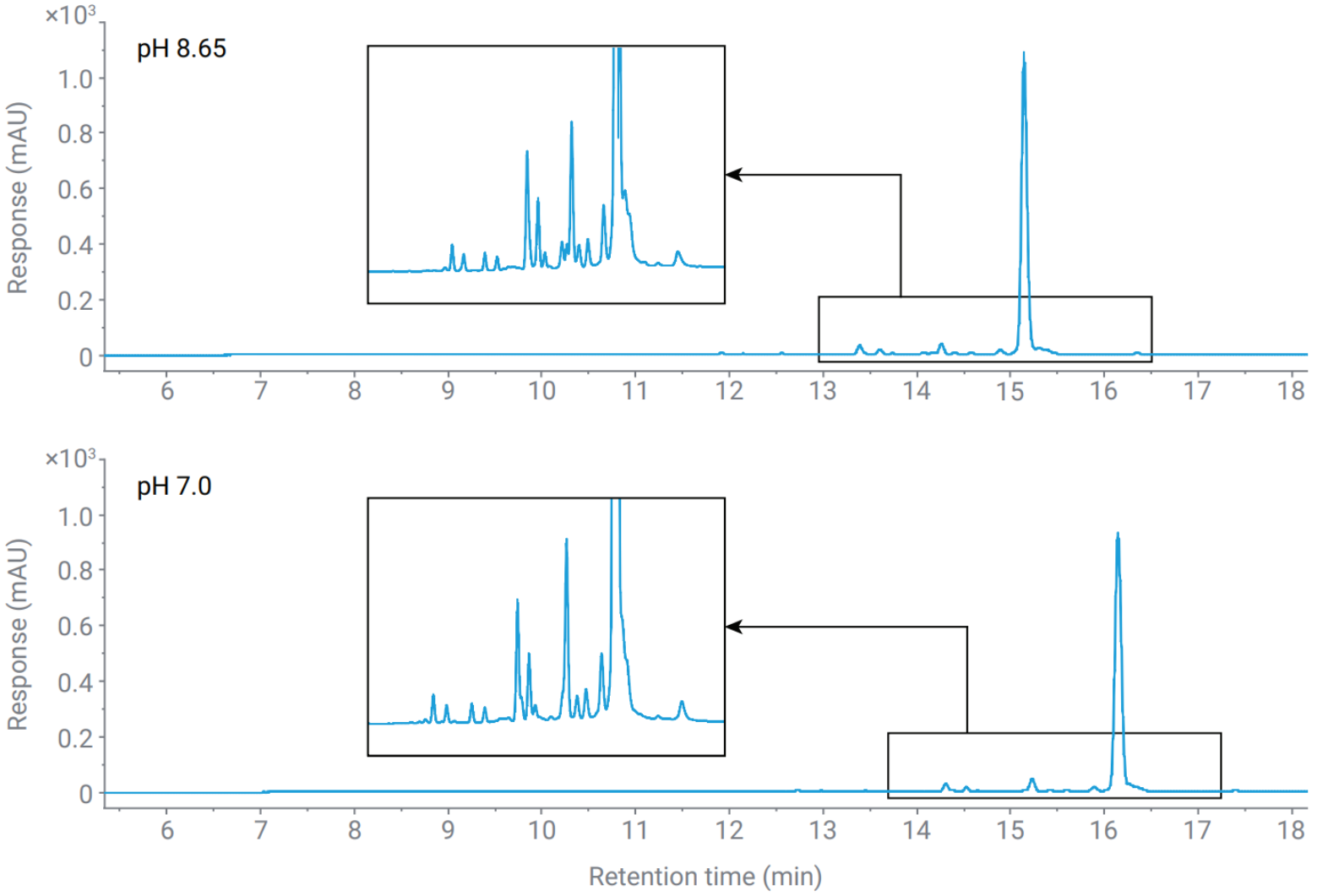
Ion-Pairing Reagents



Method Optimization

The Role of pH

At elevated pH there is better resolution of the crude 22-mer oligo from closely related impurities that elute before and after the target product.



Superficially Porous Columns for Semi-Preparative Purification of Synthetic Oligonucleotides

Method Optimization

Mobile Phase Best Practices

Ion-pairing buffers should be made daily for best resolution and reproducibility

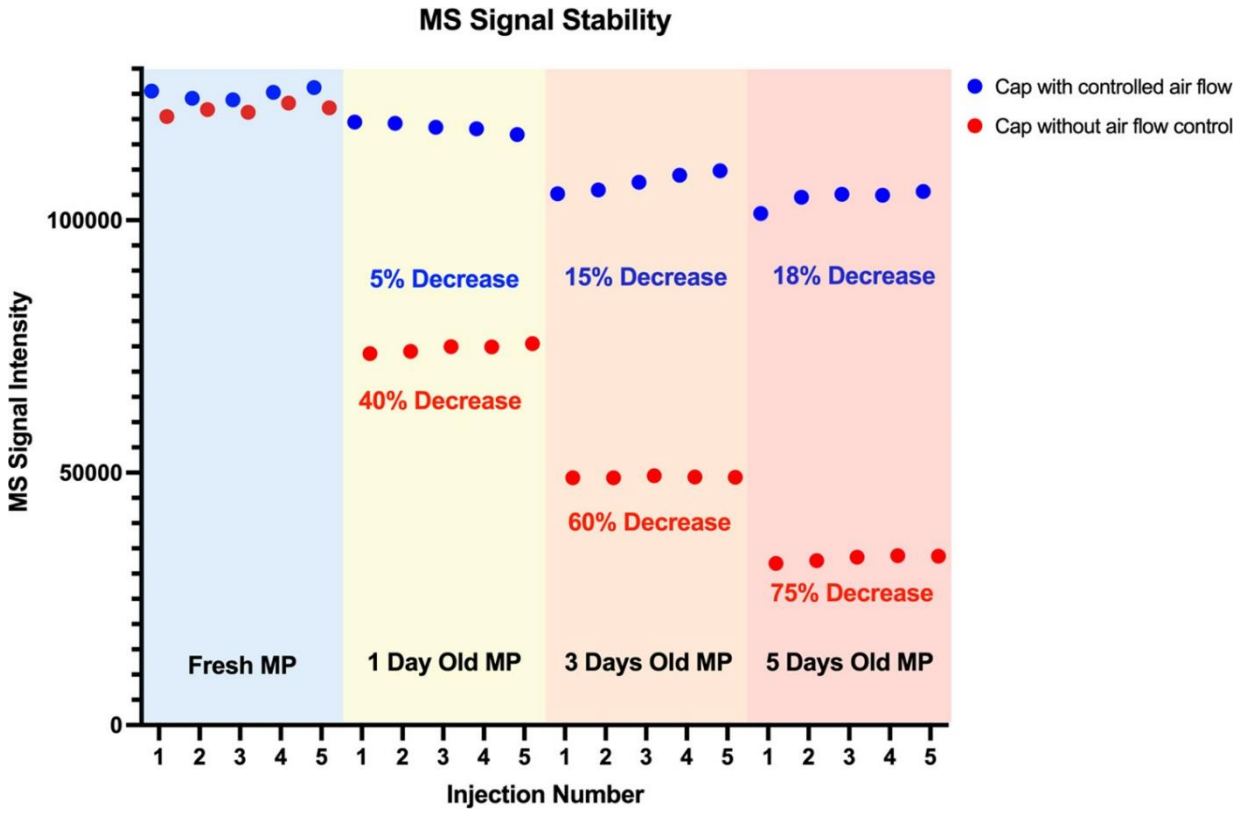
Buffers lose efficacy quickly in aqueous solutions

- Alkylamines oxidize and form aggregates
- HFIP is volatile

Bottle head assemblies that control airflow, such as the Agilent Stay Safe Caps, can extend the mobile phase lifetime

Standard cap with two open holes

Agilent InfinityLab Stay Safe cap



[Agilent InfinityLab Stay Safe Caps and Accessories]

[Agilent InfinityLab Stay Safe Caps Reduction of Solvent Evaporation]

Guimaraes, G. J.; Saad, J. G.; Annavarapu, V.; Bartlett, M. G. Mobile Phase Aging and Its Impact on Electrospray Ionization of Oligonucleotides. *Journal of the American Society for Mass Spectrometry* **2023**, *34* (12), 2691–2699. https://doi.org/10.1021/jasms.3c00264.

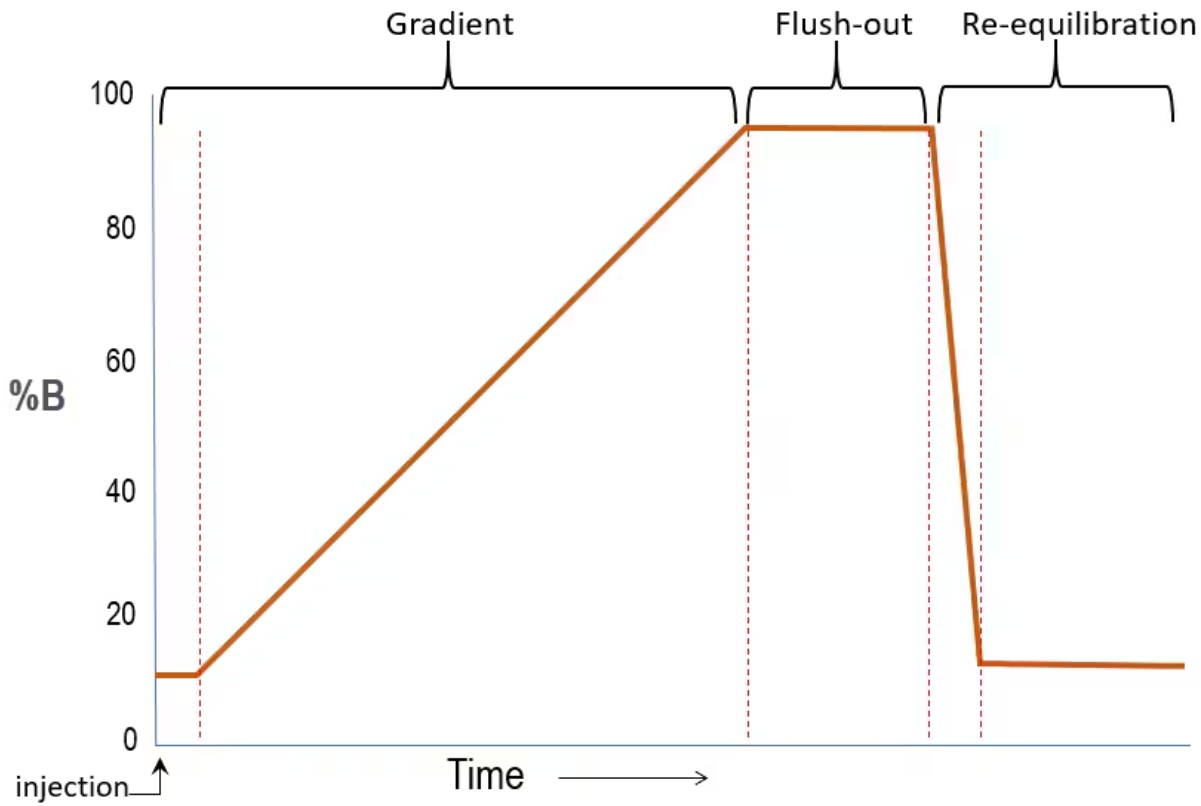
Method Optimization

Method Optimization	
Ion-Pairing Reagent	• Amines with longer alkyl chains can increase oligo hydrophobicity and increase retention • Increasing the concentration of ion-pairing buffer can increase retention
Gradient Steepness	• A steeper gradient can shorten method • A shallower gradient improves resolution
Temperature	• Increase temperature reduces secondary oligo structures • Reduces mobile phase viscosity and secondary column interactions → improved peak shape

Method Optimization

Gradient

- A good starting point when developing a method is a scouting gradient.
- Recommended starting conditions are 5–95% mobile phase B
- Gradient length is dependent on the column length

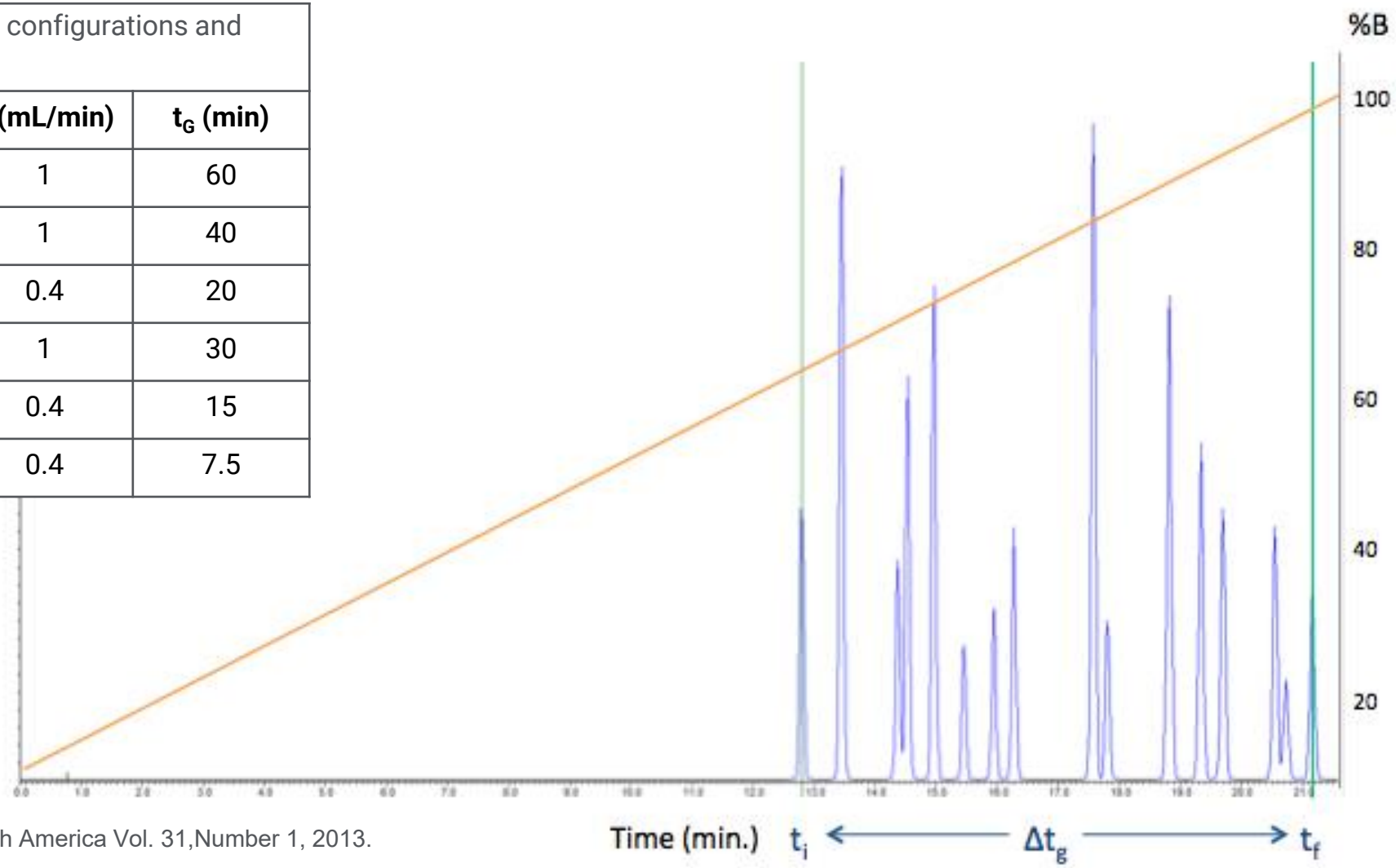


"Making to the most of a Gradient Scouting Run" LCGC North America Vol. 31,Number 1, 2013.

Method Optimization

Gradient

Table 1: Column volumes for various column configurations and recommended scouting gradient times				
L (mm)	**d_c (mm)**	**V_m (mL)**	**F (mL/min)**	**t_G (min)**
250	4.6	2.5	1	60
150	4.6	1.7	1	40
150	2.1	0.37	0.4	20
100	4.6	1.2	1	30
100	2.1	0.24	0.4	15
50	2.1	0.12	0.4	7.5

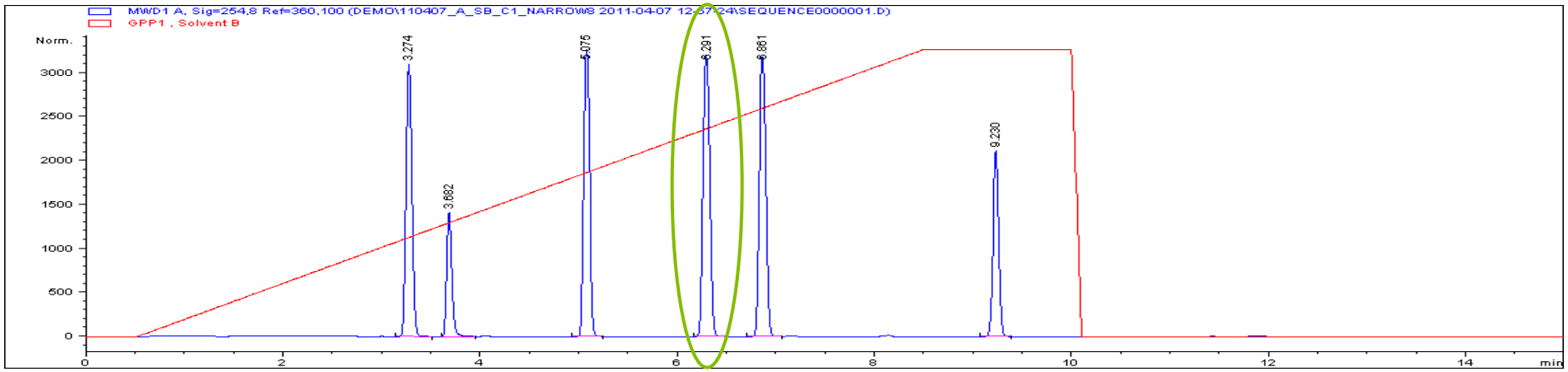


"Making to the most of a Gradient Scouting Run" LCGC North America Vol. 31,Number 1, 2013.

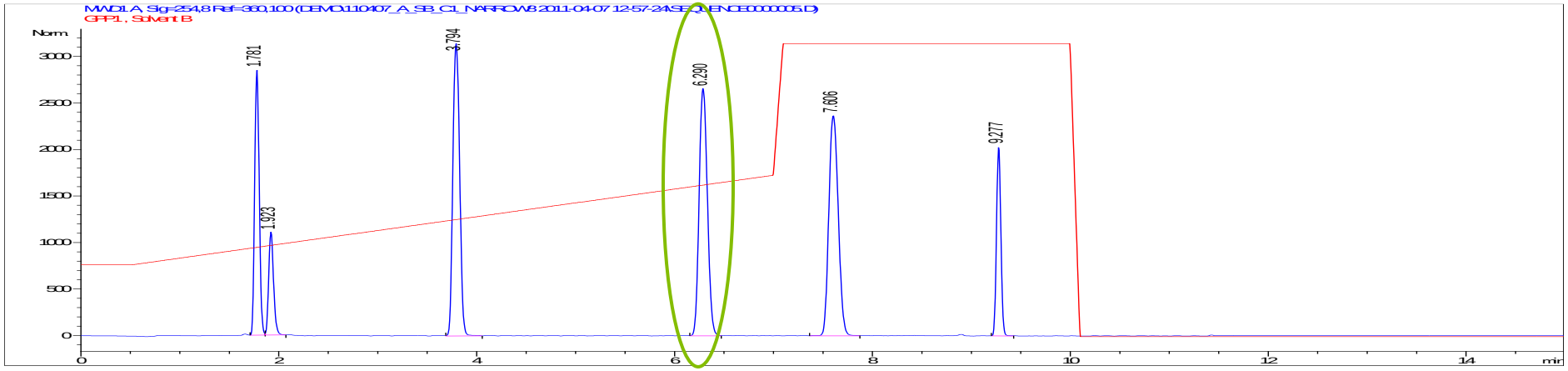
Method Optimization

Gradient

General Scouting Gradient



Optimized Gradient

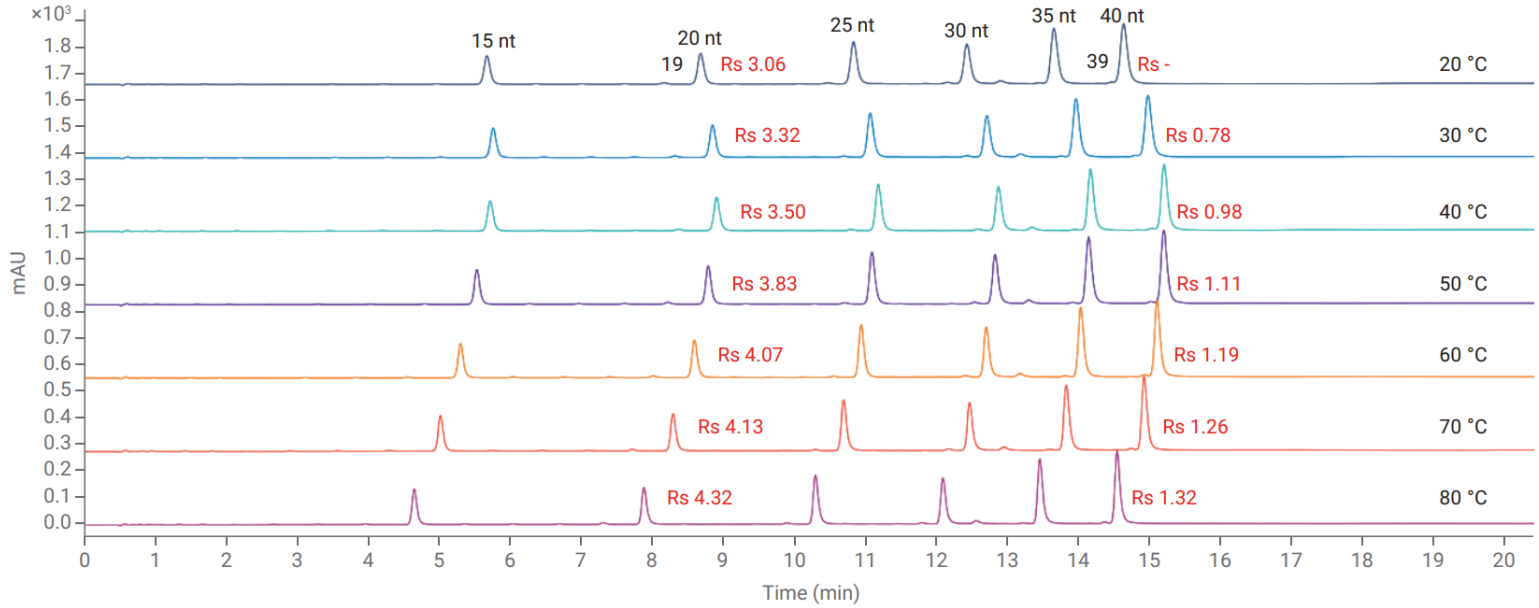


Method Optimization

Method Optimization	
Ion-Pairing Reagent	• Amines with longer alkyl chains can increase oligo hydrophobicity and increase retention • Increasing the concentration of ion-pairing buffer can increase retention
Gradient Steepness	• A steeper gradient can shorten method • A shallower gradient improves resolution
Temperature	• Increase temperature reduces secondary oligo structures • Reduces mobile phase viscosity and secondary column interactions → improved peak shape

Method Optimization

Temperature



- Temperature can influence the thermodynamic properties of solvents and solutes, such as pressure, viscosity, and diffusivity.
- It can also affect the adsorption and desorption kinetics of solutes on the stationary phase, which can affect retention times and peak shapes.
- **Elevated temperatures also help denature impurities in synthetic oligonucleotides, which include self-associating components.**

Ion-Pair Reversed-Phase Chromatography and Agilent 1260 Infinity II Prime LC

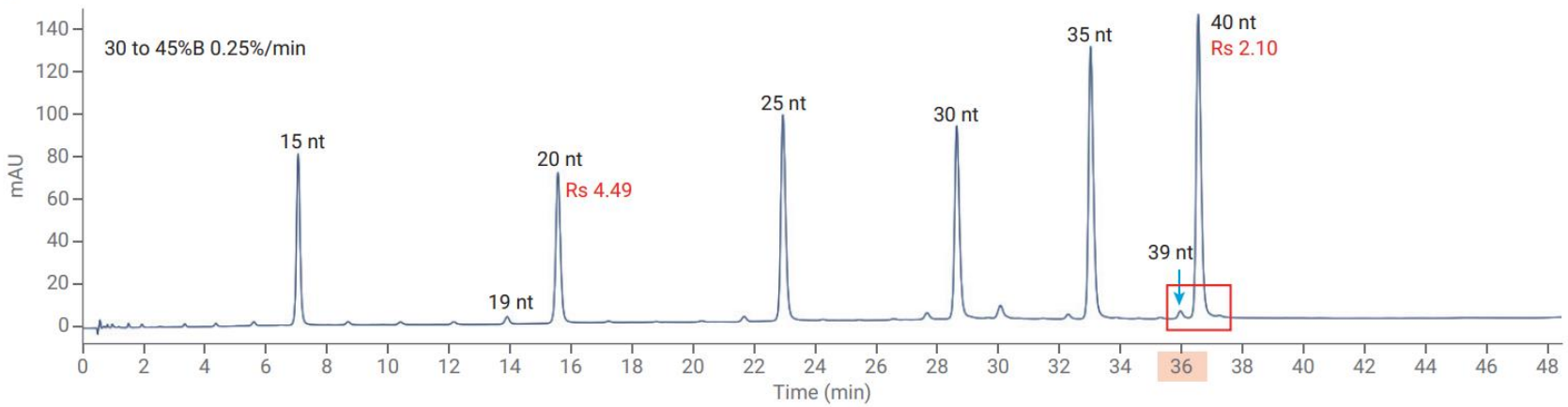
An Introduction to IP-RP Oligonucleotide Separations

Analytical Analysis

Analytical Analysis

Optimized Method

By using the strong ion-pairing reagent HAA, and a shallow gradient of +0.25 %B/min, the 40 nt ssDNA peak and its n-1 short-mer were separated with a resolution of 2.1.



Column:	AdvanceBio Oligonucleotide, 2.1 x 150 mm, 2.7 µm
Mobile phase A:	100 mM HAA in water, pH 7
Mobile phase B:	100 mM HAA in acetonitrile, pH 7
Gradient:	30 to 45 %B in 60 min
Flow rate:	0.6 mL/min
Sample:	ssDNA oligo-dT
Temperature:	60 °C
Injection:	5 µL
Detection:	UV at 260 nm

Ion-Pair Reversed-Phase Chromatography and Agilent 1260 Infinity II Prime LC

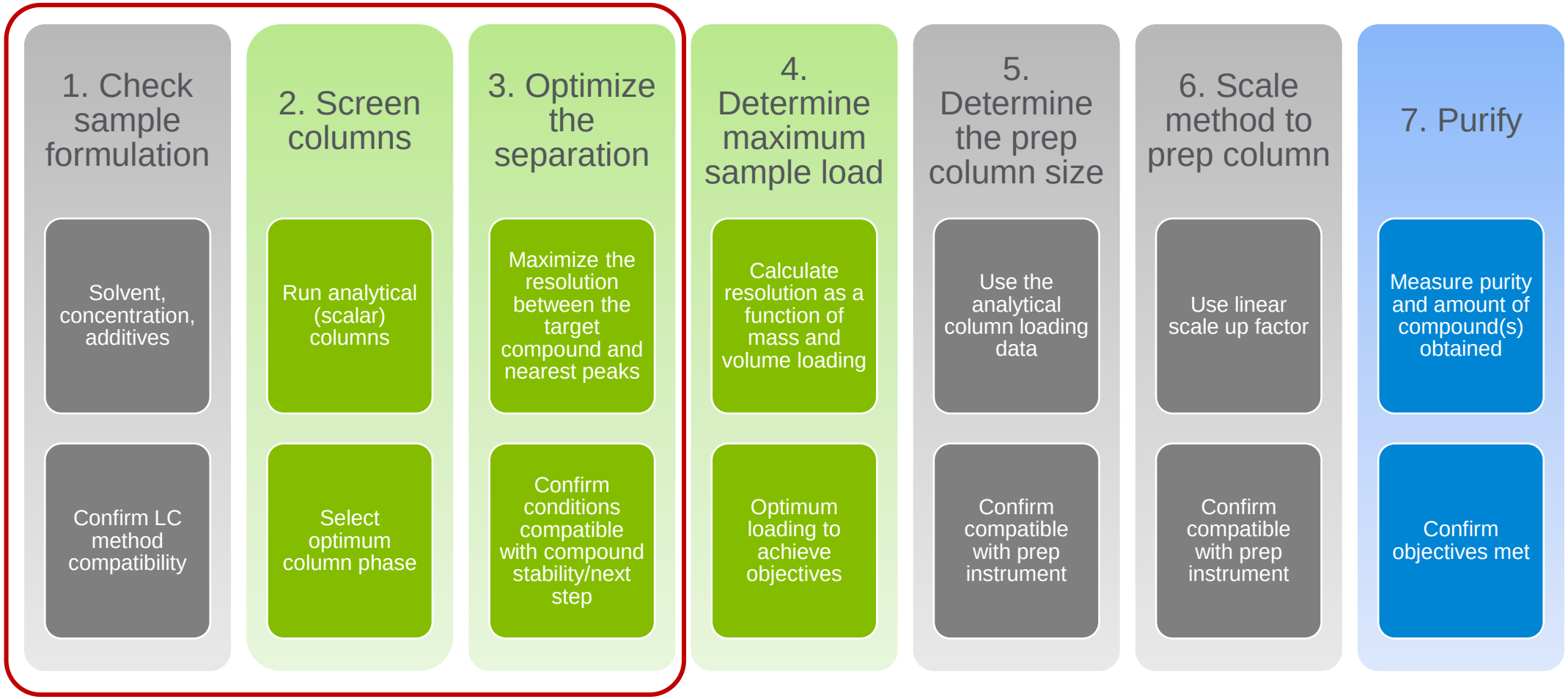
An Introduction to IP-RP Oligonucleotide Separations

Purification

Method Optimization

Purification

Steps in developing a purification method



Method Optimization

Purification

1. Check sample formulation
2. Screen columns
3. Optimize the separation
4. Determine maximum sample load
5. Determine the prep column size
6. Scale method to prep column
7. Purify

Maximize sample load

- Column load is a critical factor for effective purification
- Particle size, pore size, analyte type/size, and column chemistry can all impact load-ability

		Loading (mg) per 50 mm bed depth (based on 5% capacity)											
		PLRP-S 100 Å			PLRP-S 300 Å			PLRP-S 1000 Å			PLRP-S 4000 Å		
ID (mm)	Vol (mL)	25 mer	50 mer	75 mer	25 mer	50 mer	75 mer	25 mer	50 mer	75 mer	25 mer	50 mer	75 mer
2.1	0.17	0.4	0.3	0.1	0.3	0.2	0.3	0.1	0.1	0.2	0.1	0.1	0.1
4.6	0.83	2.0	1.3	0.5	1.3	1.2	1.2	0.7	0.7	0.9	0.3	0.3	0.4
7.5	2.2	5.2	3.3	1.3	3.4	3.1	3.3	1.9	1.8	2.3	0.8	0.9	1.1
25	24.5	58	37	15	37	34	36	21	20	25	9	10	12
50	98	232	148	58	149	137	145	83	80	101	36	38	50
100	393	927	591	234	597	550	581	334	318	404	145	153	198

Dynamic Binding Capacity of Oligonucleotides on PLRP-S Columns and Stationary Phases

Method Optimization

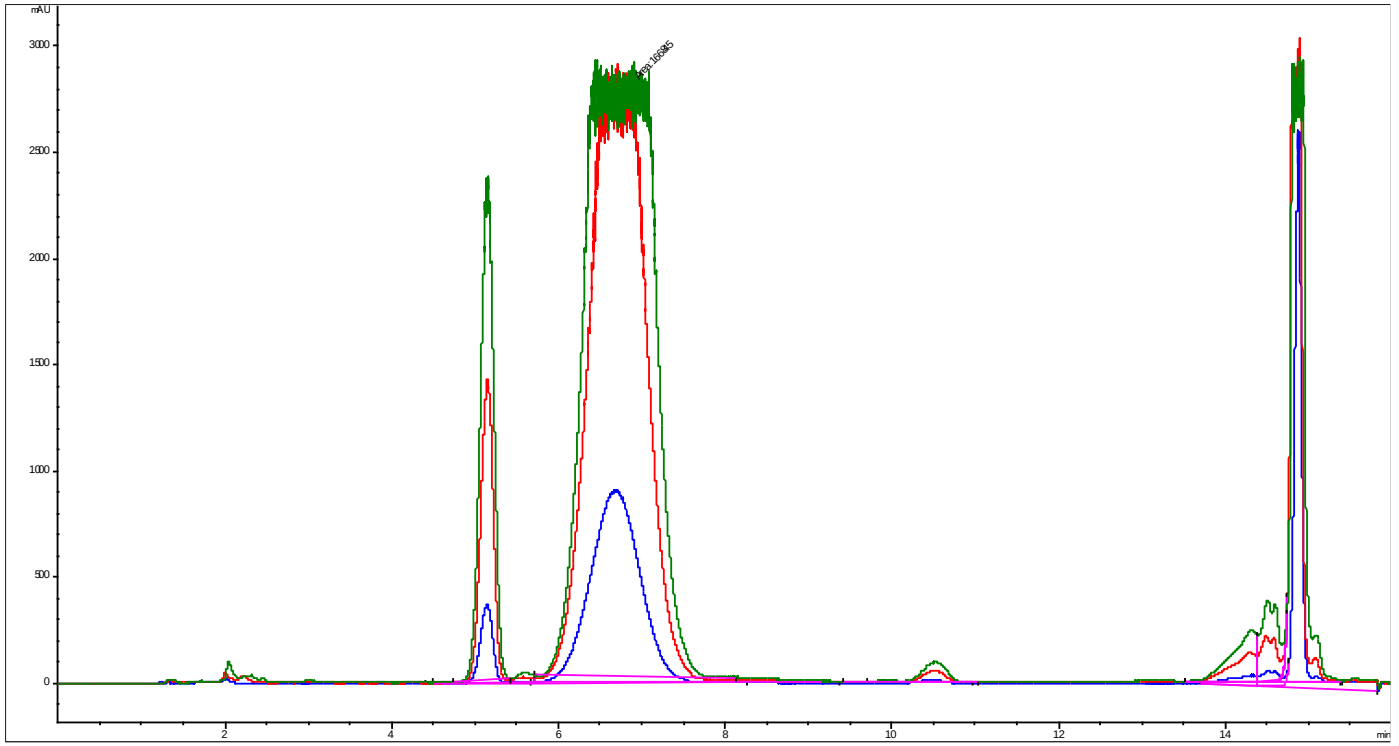
Purification

1. Check sample formulation
2. Screen columns
3. Optimize the separation
4. Determine maximum sample load
5. Determine the prep column size
6. Scale method to prep column
7. Purify

Maximize sample load

In preparative LC, it is a common practice to overload the column to increase throughput. Loading studies can be performed to determine suitable sample amounts.

- Indiscriminate overloading can cause coelution with impurities or the analyte to precipitate out of solution.
- Performing these studies on an analytical column can conserve valuable sample while minimizing solvent consumption and waste.



Method Optimization

Purification

1. Check sample formulation
2. Screen columns
3. Optimize the separation
4. Determine maximum sample load
5. Determine the prep column size
6. Scale method to prep column
7. Purify

Prep column selection

Select the smallest column suitable for your purification:

- Smaller columns are less expensive
- They reduce run time, fraction volume and dry down time
- Less solvent will be consumed

A larger column can be selected if it will give you a significant loading advantage:

- Minimize number of injections and increases throughput
- Only justified if the increase in loading capacity is greater than the increase in flow rate

General Guidelines*			
Column ID	Recommended injection volume	Easy Separation (α >1.5)	Difficult Separation (1.2 < α < 1.5)
4.6 mm	10 µL	3 – 15 mg	0.5 – 3mg
21.2 mm	750 µL	70 – 400 mg	20 – 70 mg
30 mm	1500 µL	140 – 800 mg	40 – 140 mg
50 mm	2500 µL	400 – 2000 mg	100 – 400 mg

Method Optimization

Purification

1. Check sample formulation
2. Screen columns
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5. Determine the prep column size
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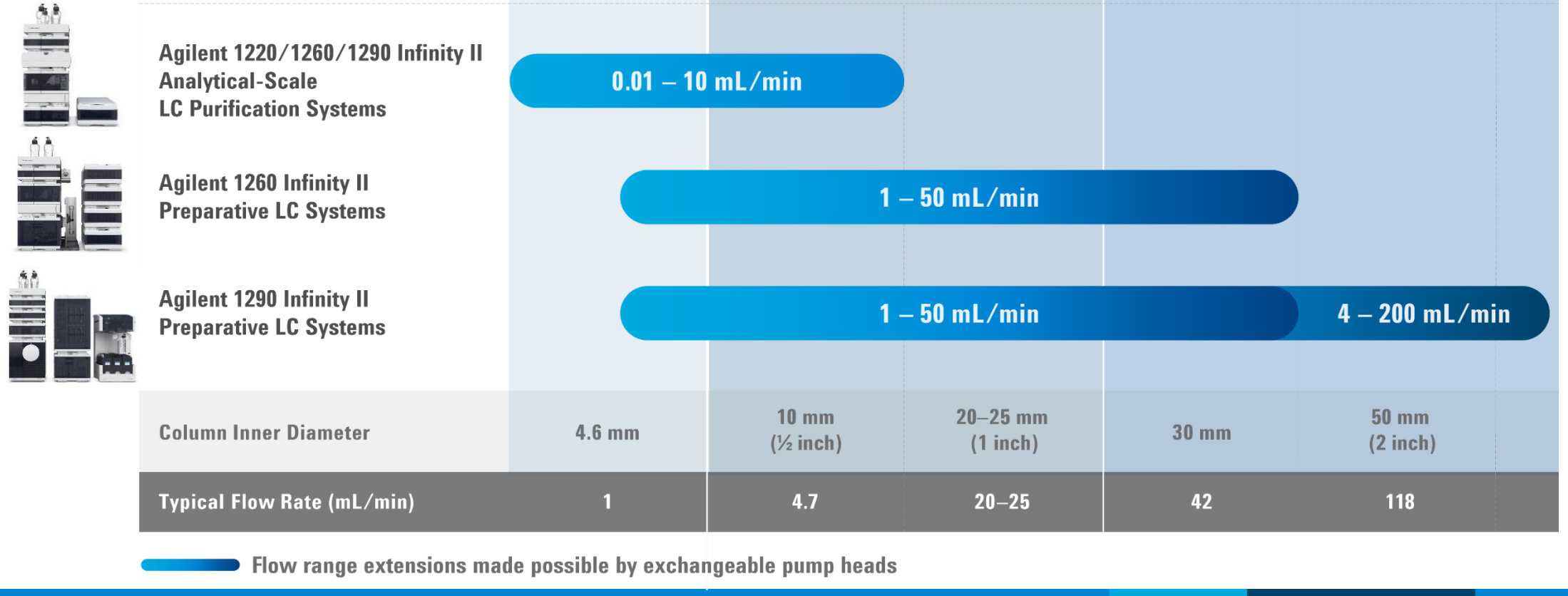
7. Purify

Prep column selection



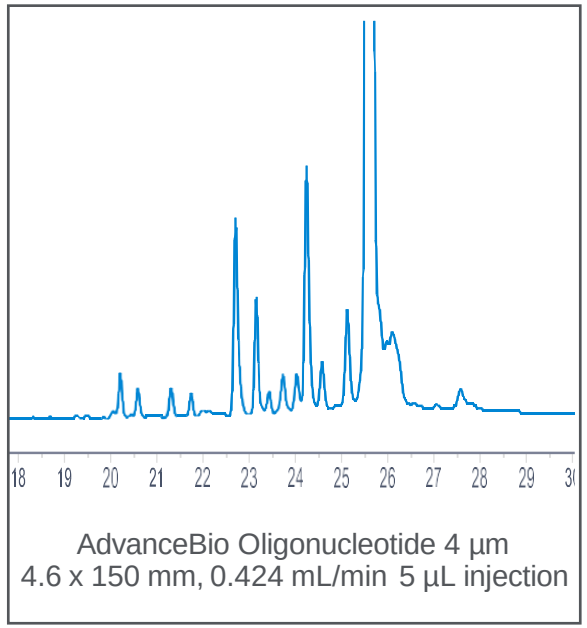
Method Optimization

Purification

1. Check sample formulation
2. Screen columns
3. Optimize the separation
4. Determine maximum sample load
5. Determine the prep column size
6. Scale method to prep column
7. Purify

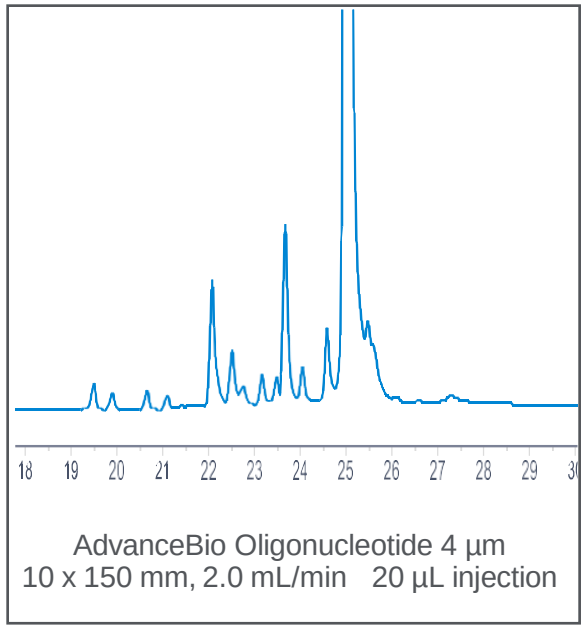
Scale method

Analytical



AdvanceBio Oligonucleotide 4 µm
4.6 x 150 mm, 0.424 mL/min 5 µL injection

Preparative



AdvanceBio Oligonucleotide 4 µm
10 x 150 mm, 2.0 mL/min 20 µL injection

Method Optimization

Purification

1. Check sample formulation
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3. Optimize the separation
4. Determine maximum sample load
5. Determine the prep column size
6. Scale method to prep column
7. Purify

Scale method

Flow-rate transfer

$$f_{p,P} = f_{a,A} \frac{d_P^2}{d_A^2} \frac{p_A}{p_P}$$

d_A	Diameter of analytical column
d_P	Diameter of preparative column
$f_{a,A}$	Actual flow in analytical system
$f_{p,P}$	Proposed flow in preparative system
p_A	Column particle size in analytical system
p_P	Column particle size in preparative system

Column load

$$V_{inj,P} = V_{inj,A} \frac{d_P^2}{d_A^2} \frac{L_P}{L_A}$$

d_A	Diameter of analytical column
d_P	Diameter of preparative column
L_A	Length of analytical column
L_P	Length of preparative column
$V_{inj,P}$	Injection volume for analytical system
$V_{inj,A}$	Injection volume for preparative system

Gradient transfer

$$\frac{t_{D,A} + t_{I,A}}{t_{c,A}} = \frac{t_{D,P} + t_{I,P}}{t_{c,P}}$$

$t_{D,A}$	Dwell time of analytical system
$t_{I,A}$	Initial hold of analytical system generic gradient
$t_{c,A}$	Column pass time in analytical system
$t_{D,P}$	Dwell time of preparative system
$t_{I,P}$	Initial hold of preparative system gradient
$t_{c,P}$	Column pass time in preparative system

Principles and Practical Aspects of Preparative Liquid Chromatography

Method Optimization

Purification

1. Check sample formulation
2. Screen columns
3. Optimize the separation
4. Determine maximum sample load
5. Determine the prep column size
6. Scale method to prep column
7. Purify

Agilent preparative scaling calculator

	Analytical Method	Preparative Method
System Description		
System name		
Minimum flow rate (mL/min)		
Maximum flow rate (mL/min)		
Dwell volume (mL)		
Injector cycle time (min)		
Solvent A		
Solvent B		
Column Information		
Phase		
Particle size (µm)*	2.7	4.0
ID (mm)*	4.6	4.6
Length (mm)*	150	150
Flow rate (mL/min)*	1	0.68
Injection volume (µL)	5	5
Compound concentration (mg/mL)	1	
Override preparative flow rate	Yes / No	
Desired amount of purified compound (mg)		
Current mass on column (mg)	0.01	0.01
Estimated column capacity (mg)	14.96	14.96
Column void volume (mL)	1.5	1.5

Analytical Gradient				Preparative Gradient			
Time (min)	Flow (mL/min)	%A	%B	Time (min)	Flow (mL/min)	%A	%B
0	1	95	5	0	0.7	95	5
4		95	5	5.9		95	5
34		65	35	50.4		65	35
40		0	100	59.3		0	100
45		0	100	66.7		0	100
50		95	5	74.1		95	5
60		95	5	88.9		95	5

Add row | Delete row

[Preparative LC Scaling Calculator | Agilent]

Method Optimization

Purification

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2. Screen columns
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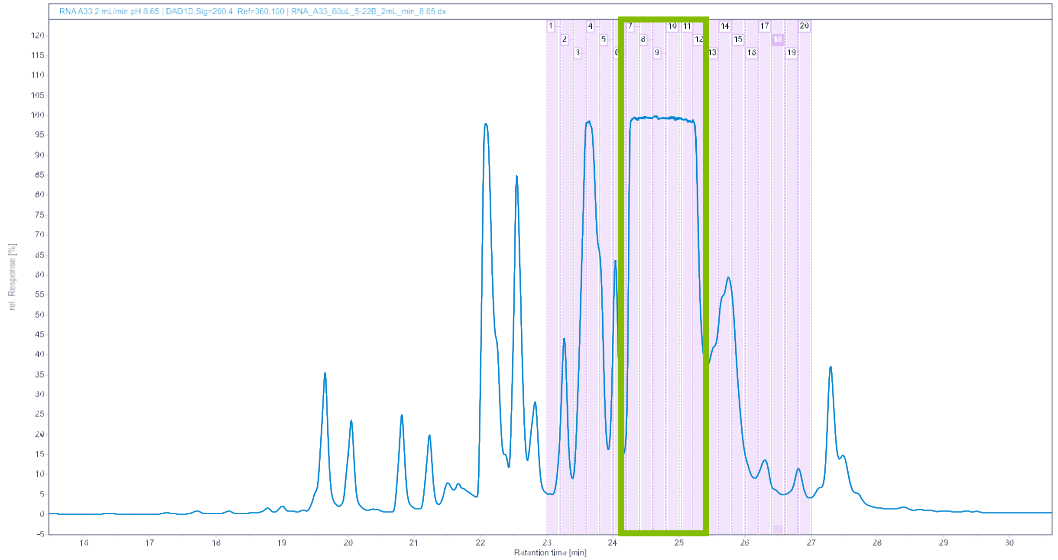
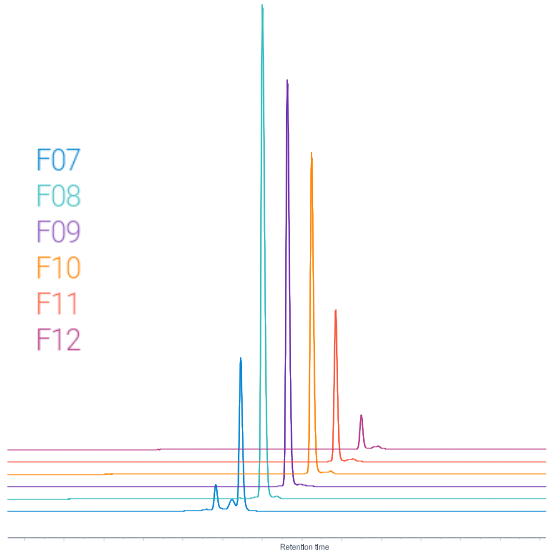
Purify





Fraction	Product Purity	Peak Yield
F07	65.2%	9.4%
F08	99.2%	33.4%
F09	98.9%	25.9%
F10	97.9%	20.2%
F11	76.8%	9.0%
F12	46.9%	2.0%

The figure above shows an optimized semi-prep separation of a crude oligo sample run at 60°C on an AdvanceBio Oligo 10x150mm column. Utilizing the gradient to focus the sample at the head of the column, a quad injection, of 160 µL (3.2 mg) total is loaded onto the column. The fractions were collected and analyzed on an analytical column to demonstrate purity and yield in the figure below.

Method Optimization

Purification

On demand webinar

Mark Powell
Application Engineer
Agilent Technologies, Inc.

Title: Scale Up with Confidence: Column Selection for Preparative HPLC

In preparative HPLC method development, our goal is the separation and isolation of a valuable compound. Throughput, yield, and purity of the compound are the important parameters for preparative HPLC. In this webinar, we will discuss the process of scaling up from an analytical to a preparative HPLC method. We will also look at column considerations to increase sample throughput using superficially porous particles in preparative LC columns to drive speed and efficiency.

Scale Up with Confidence: Column Selection for Preparative HPLC

An Introduction to IP-RP Oligonucleotide Separations

AdvanceBio Spin Columns

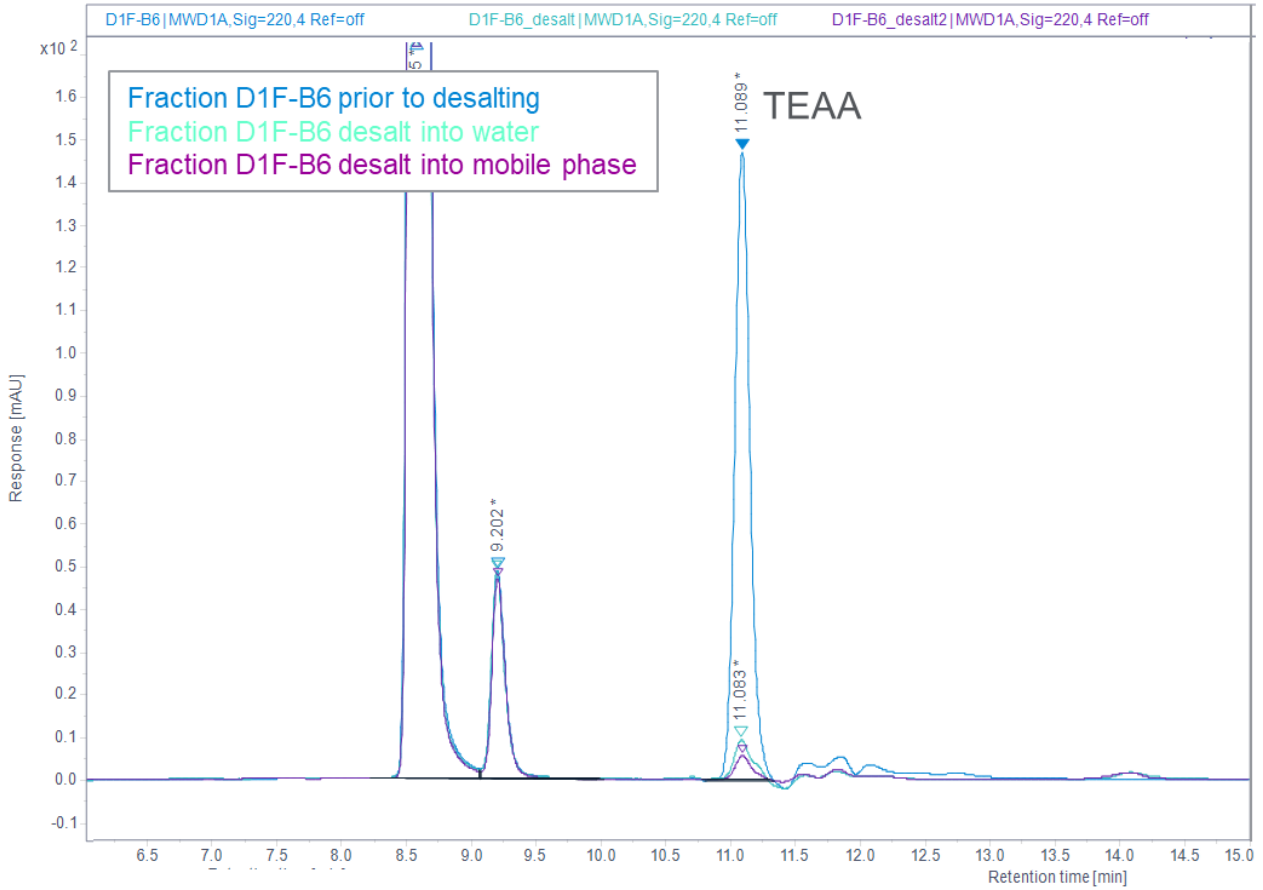
AdvanceBio Spin Columns

TEAA Removal

AdvanceBio Spin Columns are a rapid, convenient, straightforward technique that uses centrifugation with gel filtration for sample cleanup.

Results

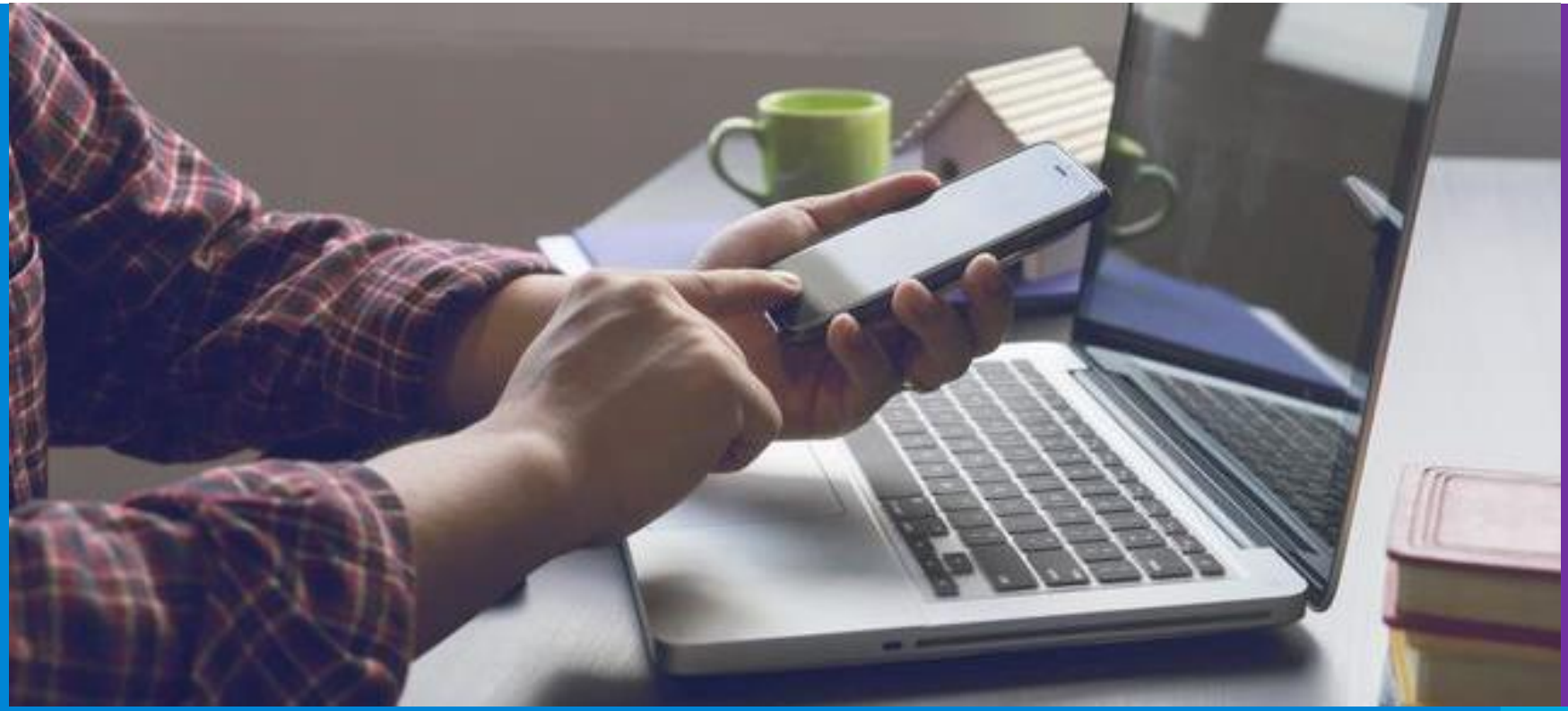
- Fraction desalted into water had TEAA 93.1% TEAA removal with 91.4% oligo recovery
- Fraction desalted into mobile phase had 96% TEAA removal with 92.8% oligo recovery



Quick and Efficient Sample Cleanup for Biomolecule Analysis

An Introduction to IP-RP Oligonucleotide Separations

Resources



Biocolumn Resources

Biocolumn Catalog: Agilent BioHPLC Columns and Consumables – Your Resource for Biomolecule Analysis

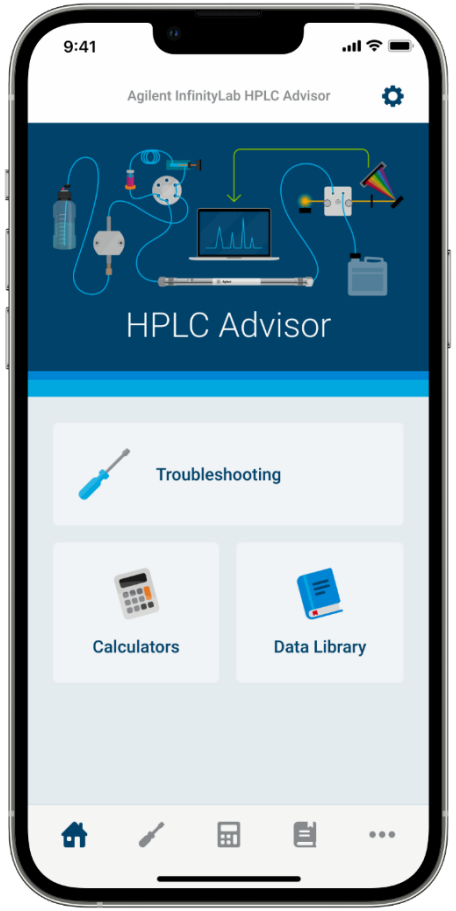
Biocolumn CQA Application Compendiums:

- Agilent Biocolumns Application Compendium - Critical Quality Attributes
- Agilent Biocolumns Application Compendium - Intact and Subunit Purity
- Agilent-NISTmAb_Application-Compendium-Intact Analysis
- Agilent-NISTmAb_Application-Compendium-Variant-Analysis
- Agilent Biocolumns Application Compendium - Aggregate/Fragment Analysis
- Agilent Biocolumns Application Compendium - Titer Determination

Biocolumn User Guides: Bio LC Column User Guides | Agilent

Agilent Resources for Support

- Resource page http://www.agilent.com/chem/agilentresources
  - Online selection tools, “How-to” videos
  - Column user guides - https://www.agilent.com/en-us/support/liquid-chromatography/kb005965
- HPLC Advisor app for troubleshooting and method calculators: HPLC Advisor app | Agilent
- Application Note Finder: Application Finder | Agilent
- Tech support: http://www.agilent.com/chem/techsupport
- Agilent University http://www.agilent.com/crosslab/university
- YouTube – Agilent Channel
- Your local product specialists



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