How Do I Determine the Best Chromatography for my Sample

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LC columns and Consumables Technical Support
January 20, 2021

Agilent InfinityLab

Agilent

Outline for Talk
Getting started

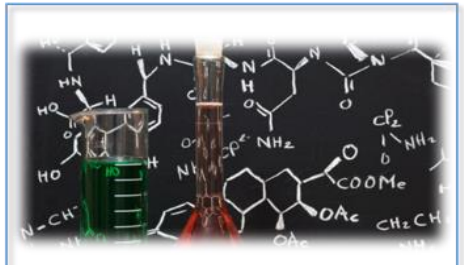
- Sample type and define the objective
- Particle options
  - Totally porous vs superficially porous
- Agilent column chemistries
  - Column chemistries C18 phases and more
- Alternate Selectivity
  - Effect on resolution
- Hilic, CS C18, and chiral chemistries
- Role of instruments
  - Instrument and column compatibility
  - Other instrument considerations

Sample Types


Pharma


BioPharma / Biotech


Fine Chemicals


Universities


Agricultural Biochemistry


Food & Health Supplements

Small molecule separations

Examples:

- Environmental pollutants
- Contaminants in food (pesticides)
- Food toxins
- Drugs of abuse

Large molecule separations

Examples:

- Biologics
  - Therapeutic proteins
  - mAbs (monoclonal antibodies)
- Synthetic polymers
  - Polystyrene
  - Polyethylene
- Natural polymers
  - Starch
  - Dextran

Types of Liquid Chromatography

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Separation achieved using a physical or chemical characteristic of molecule in solution

LC Modes	Mechanism	Small Molecules	Large Molecules
Normal phase (NP)	Polar particle	✓	
Reversed phase (RP)	Nonpolar particle	✓✓✓✓	✓
Ion exchange (IEX)	Charged particles, +ve or -ve	✓	✓
Size exclusion gel permeation (SEC/GPC)	Porous particles with defined pore size	✓	✓
Affinity	Exploits biological interactions – antigen-antibody		✓
Chiral	Shape recognition	✓	
Hydrophilic interaction (HILIC)	Similar to normal phases but uses aqueous/organic mobile phases	✓	✓
Hydrophobic interaction (HIC)	Similar to RP but uses aqueous mobile phases		✓

Reversed phase is the primary technique for small molecule LC
Multiple techniques are required to characterize a biomolecule

Define the Objective

- What is the sample type?
- How complex is the sample?
- What are the goals of the separation?
- Is high efficiency important?
- Is speed important?
- Any instrument considerations or limitations?

Particle Options

Comparison of two porous particles

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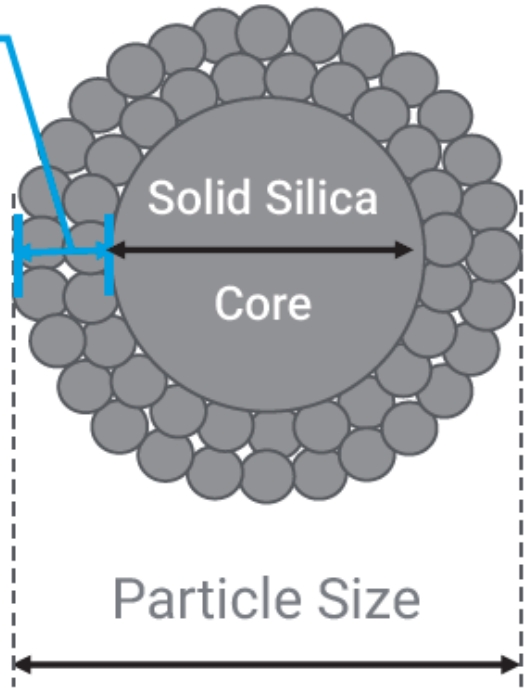
Novel superficially porous particle versus traditional totally porous particle

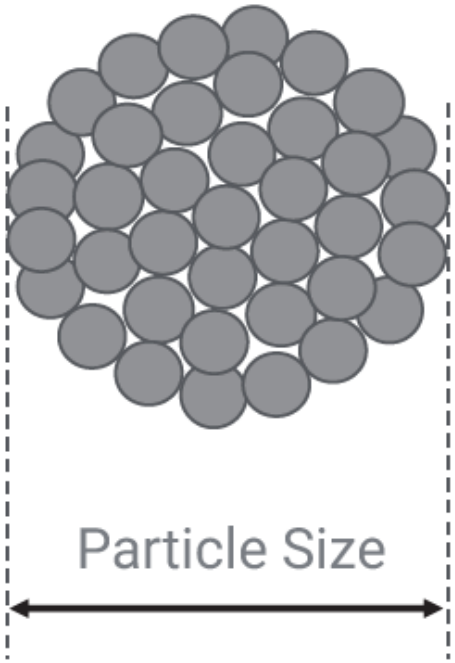
Porous Outer Layer

- Short analyte diffusion path in/out of the porous layer enables faster and higher-resolution separation.

Superficially Porous Poroshell



Totally Porous ZORBAX



- Totally porous particle offers improved retention, sample capacity and resistance to sample solvents.

- Higher chromatographic efficiencies.
- Faster and higher-resolution separations.

ZORBAX – Fully Porous Particles

Fully Porous Particle Column (ZORBAX)	Superficially Porous Particle Column (Poroshell)
Methods that require high sample loading	Faster, more efficient separations
Direct use in legacy fully porous particle methods	Newly developed or transferred methods
Direct scalability to prep-scale chemistries	Screening or method development
Some chemistries are unique to ZORBAX	High throughput methods

- Rapid trend to **superficially porous particles** due to their robustness and high efficiency
- More efficient Poroshell particles are typically the first choice for new methods
- In some cases, fully porous particles may still be considered

Why InfinityLab Poroshell?

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Performance

Poroshell columns deliver improved performance compared to totally porous materials with the same particle size, or similiar performance at significantly lower backpressures.

Compatibility

Poroshell 120 columns are ideal for the Agilent InfinityLab LC and LC/MSD instruments. Column ID tags enable convenient column tracking and reporting.

Scalability

Poroshell columns are available in 1.9, 2.7, and 4 µm pore size and simplify method transfer from HPLC to UHPLC to ultra-low dispersion UHPLC.

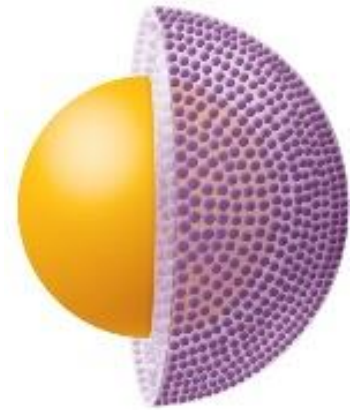
Robustness

Poroshell columns show excellent lifetime and are less demanding in sample preparation than fully-porous material with similar performance. This is due to bigger particle sizes and frits.

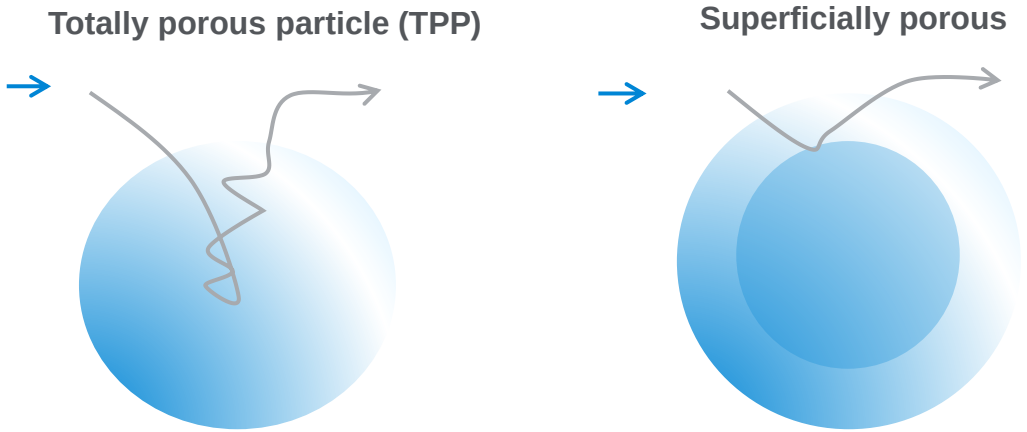
Selectivity

The stationary phase is the dominant factor for selectivity and, therefore, resolution. Poroshell offers a wide range of innovative chemistries to achieve selectivity differences.

Poroshell Technology – What Makes it Better?



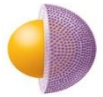
Poroshell is made of a solid core with a porous outer layer



- Analytes travel though the particle more efficiently
- High efficiency allows you to use a larger SPP (2.7 µm) for nearly equivalent performance to a smaller sub-2 µm (STM) TPP column
- Using a larger particle allows for lower backpressure compared to similar, efficient totally porous STM columns and flexible use on HPLC or UHPLC systems

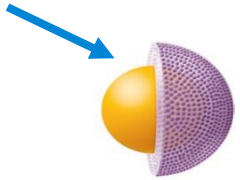
Which Particle Size is Best for Your Needs?

UHPLC



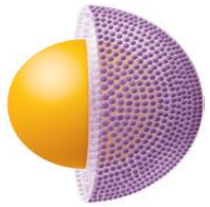
1.9 µm

UHPLC/
HPLC



2.7 µm

HPLC



4.0 µm

Highest UHPLC performance

- Pressure rating: 1300 bar
- Typical pressure: similar to sub-2 µm totally porous
- Efficiency: ~120% of sub-2 µm totally porous
- Ideal for: Agilent 1290 Infinity II LC System

UHPLC performance at lower pressures

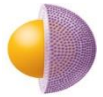
- Pressure rating: 600 bar
- Typical pressure: 50% of sub-2 µm totally porous
- Efficiency: ~90% of sub-2 µm totally porous
- Ideal for: Agilent 1260 Infinity II LC System or Agilent 1260 Infinity II Prime LC System

Improved HPLC performance

- Pressure rating: 600 bar
- Typical pressure: often <200 bar
- Efficiency: ~200% of 5 µm totally porous
- Ideal for: Agilent 1220 Infinity II LC System

Which Particle Size is Best for Your Needs?

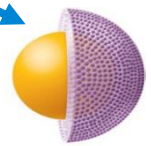
UHPLC



1.9 µm

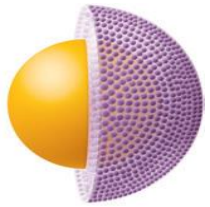
Most flexible/common particle size

UHPLC/ HPLC



2.7 µm

HPLC



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Improved HPLC performance

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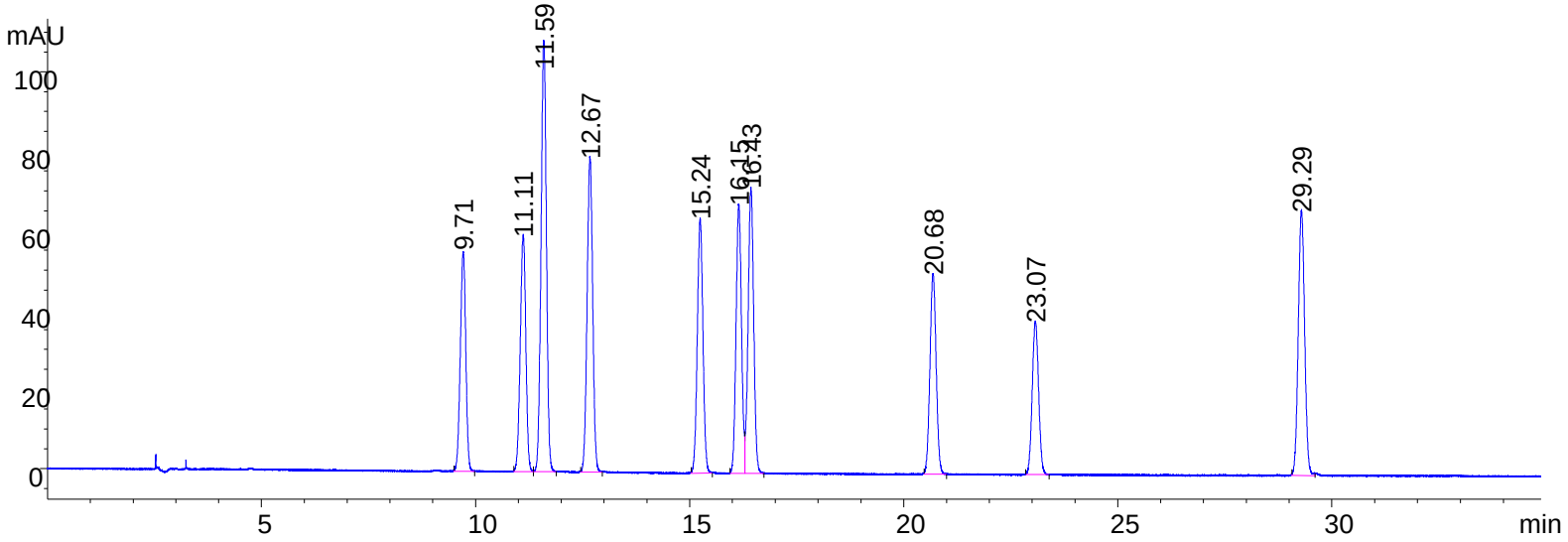
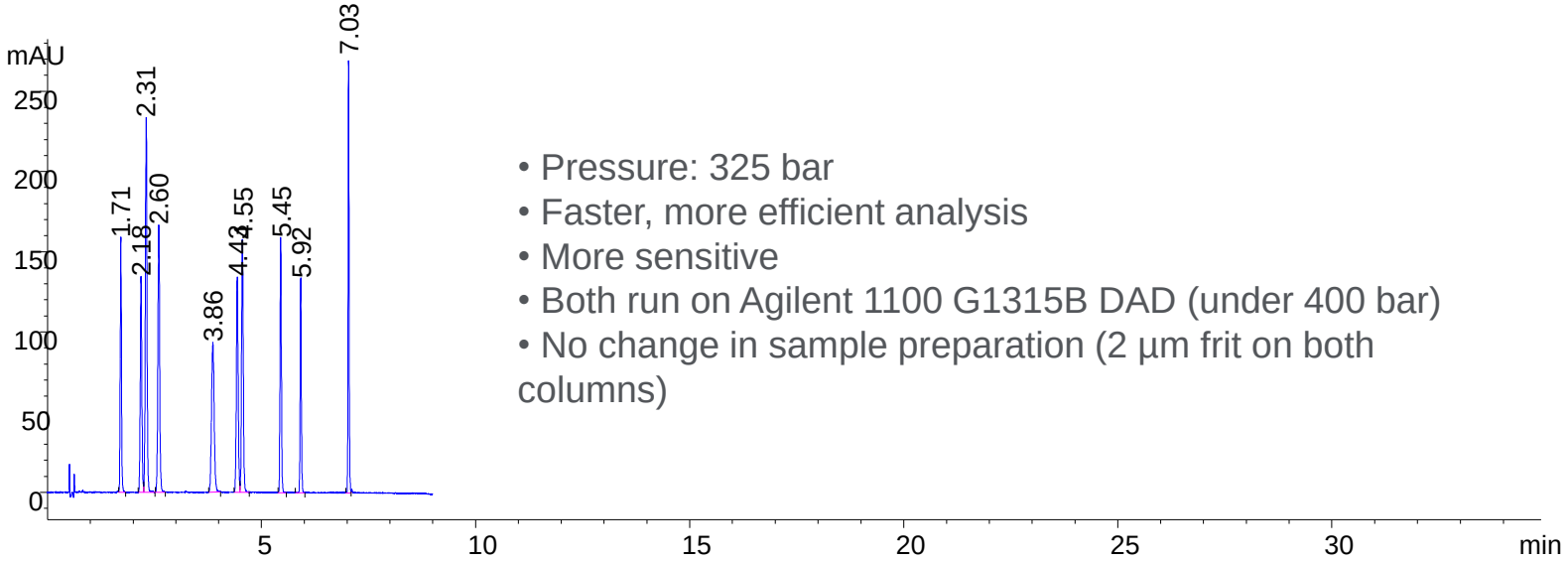
Column Choices – Which Particle Type to Choose?





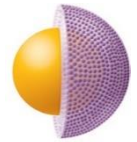
- Pressure: 325 bar
- Faster, more efficient analysis
- More sensitive
- Both run on Agilent 1100 G1315B DAD (under 400 bar)
- No change in sample preparation (2 µm frit on both columns)

Totally porous particle

ZORBAX Eclipse Plus C18
4.6 x 250 mm, 5 µm

Runtime: 35 minutes

Superficially porous particle



InfinityLab Poroshell 120 EC-C18
4.6 x 100 mm, 2.7 µm

Runtime: 9 minutes

A: 0.1% formic acid in water, B: ACN
Gradient: 8-33% ACN in 30 or 8 min
1 or 2 mL/min, 25 °C, 254 nm
Agilent application note: 5990-5572EN

The Agilent Column Families for Small Molecule LC Columns

Agilent InfinityLab

When to choose which product family

Special phases

HPLC	UHPLC	LD-UHPLC
5 µm, 3 µm	---	---

Features	Phases
High carbon load columns	Pursuit XRs, Pursuit XRs Ultra
Analytical to prep	Pursuit, Polaris
Alternative selectivity for polar and nonpolar	Polaris C18-Ether, C18 Amide, NH2

Unique chemistries that help to solve nonstandard applications from HPLC to prep

Agilent's Column Families for Small Molecule LC Columns

When to choose which product family

ZORBAX

HPLC	UHPLC	LD-UHPLC
5 µm, 3.5 µm	1.8 µm (RRHT)	1.8 µm (RRHD)

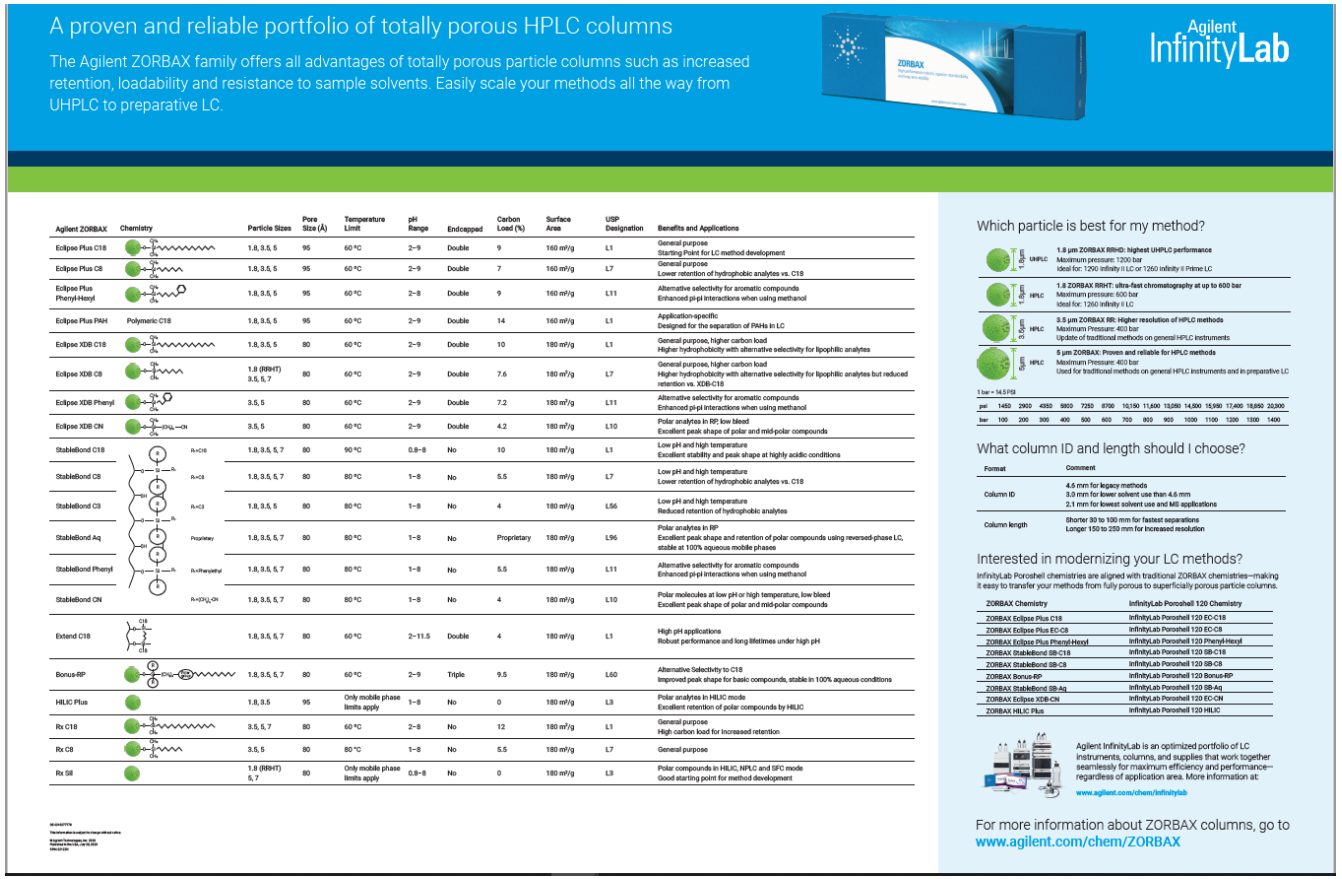
Features

Traditional, reliable columns that offer a vast amount of unique chemistries

Scalable phases that range from UHPLC to HPLC to Prep

Higher overall retention, accepts larger amounts of strong solvent during injection and often shows higher sample capacity.

Scalable from UHPLC to HPLC to Prep-LC with high sample capacity.



Zorbax Selectivity Overview
Pub No. 5994-2212EN

Agilent's Column Families for Small Molecule LC Columns

Agilent InfinityLab

When to choose which product family

InfinityLab Poroshell 120

HPLC	UHPLC	LD-UHPLC
4 µm	2.7 µm	1.9 µm

Features

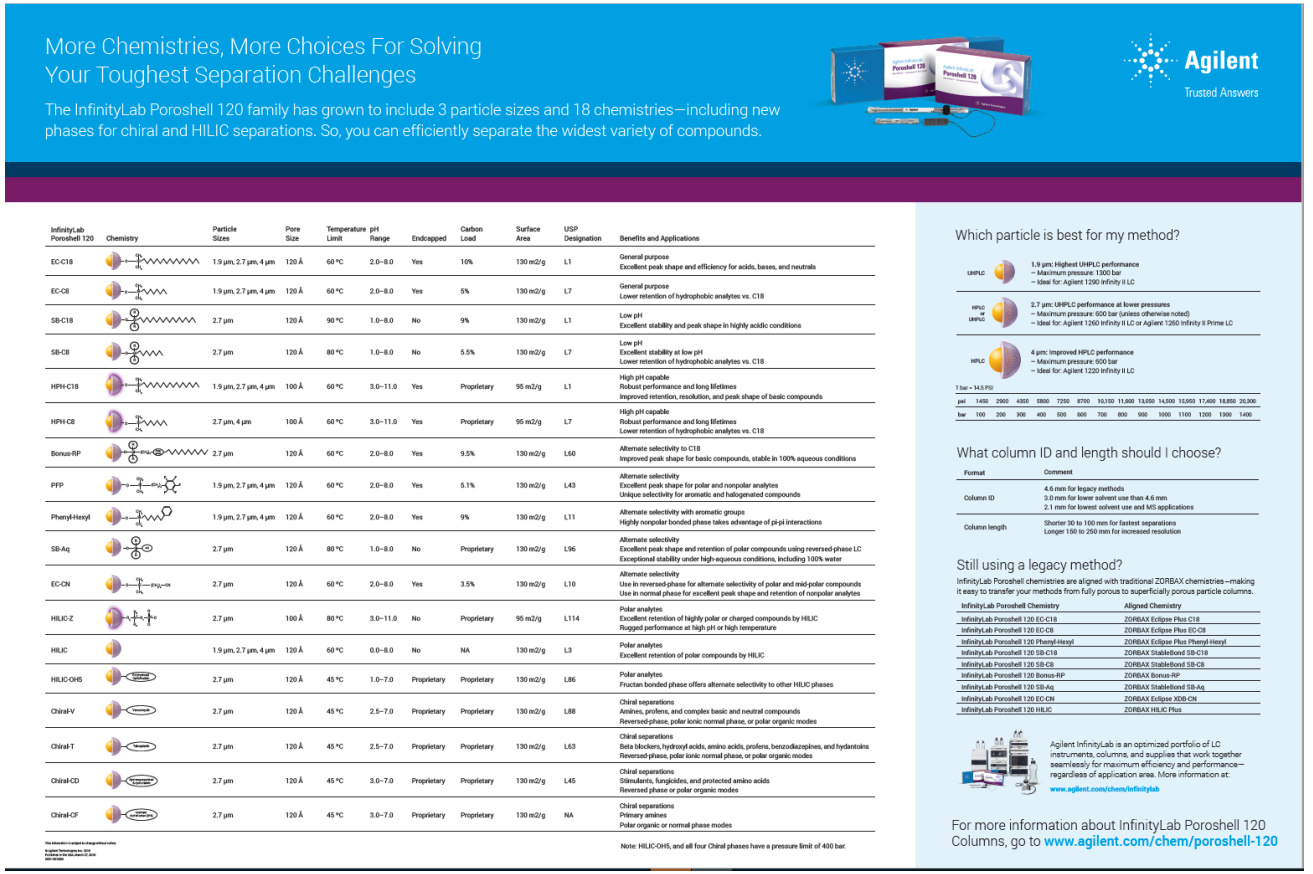
Modern column technology that offers higher performance at similar backpressure

or comparable performance at reduced backpressure

Designed in with Agilent LC instruments and supplies

Universal column platform with offerings for all separation modes, i.e RP, NP, HILIC, SFC as well as chiral separations

Modern, high-performance HPLC and UHPLC columns designed in for state-of-the-art instruments.



Poroshell Selectivity Overview
Pub No. 5991-9013EN

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Transferability ZORBAX to Poroshell

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Method transferability across product families

Traditional ZORBAX chemistries are aligned with InfinityLab Poroshell chemistries to offer simplified method transfer from fully porous particles to superficially porous particle columns.

InfinityLab Poroshell Chemistries	Aligned Chemistry
InfinityLab Poroshell 120 EC-C18	ZORBAX Eclipse Plus C18
InfinityLab Poroshell 120 EC-C8	ZORBAX Eclipse Plus C8
InfinityLab Poroshell 120 Phenyl-Hexyl	ZORBAX Eclipse Plus Phenyl-Hexyl
InfinityLab Poroshell 120 SB-C18	ZORBAX StableBond SB-C18
InfinityLab Poroshell 120 SB-C8	ZORBAX StableBond SB-C8
InfinityLab Poroshell 120 SB-Aq	ZORBAX StableBond SB-Aq
InfinityLab Poroshell 120 Bonus-RP	ZORBAX Bonus-RP
InfinityLab Poroshell 120 EC-CN	ZORBAX Eclipse XDB-CN
InfinityLab Poroshell 120 HILIC	ZORBAX HILIC-Plus

For more information on method transfer:
technical overview 5990-6588EN

The Poroshell 120 Family Offers Chemistries with Unique Selectivity

Agilent InfinityLab

InfinityLab Poroshell 120 offers a broad portfolio to suit your needs

For Nonpolar Analytes					
Best All Around	**Best for Low pH Mobile Phases**	**Best for High pH Mobile Phases**	**Best for Alternative Selectivity**	**Best for More Polar Analytes**	**Chiral**
EC-C18 1.9 µm, 2.7 µm, 4 µm	**SB-C18** 1.9 µm, 2.7 µm, 4 µm	**HPH-C18** 1.9 µm, 2.7 µm, 4 µm	**Bonus-RP** 2.7 µm	**SB-Aq** 1.9 µm, 2.7 µm, 4 µm	**Chiral-V** 2.7 µm
EC-C8 1.9 µm, 2.7 µm, 4 µm	**SB-C8** 2.7 µm	**HPH-C8** 2.7 µm, 4 µm	**PFP** 1.9 µm, 2.7 µm, 4 µm	**EC-CN** 2.7 µm	**Chiral-T** 2.7 µm
Phenyl-Hexyl 1.9 µm, 2.7 µm, 4 µm		**CS-C18** 2.7 µm		**HILIC** 1.9µm, 2.7 µm, 4 µm	**Chiral-CD** 2.7 µm
				HILIC-Z 1.9 µm, 2.7 µm, 4 µm	**Chiral-CF** 2.7 µm
				HILIC-OH5 2.7 µm	

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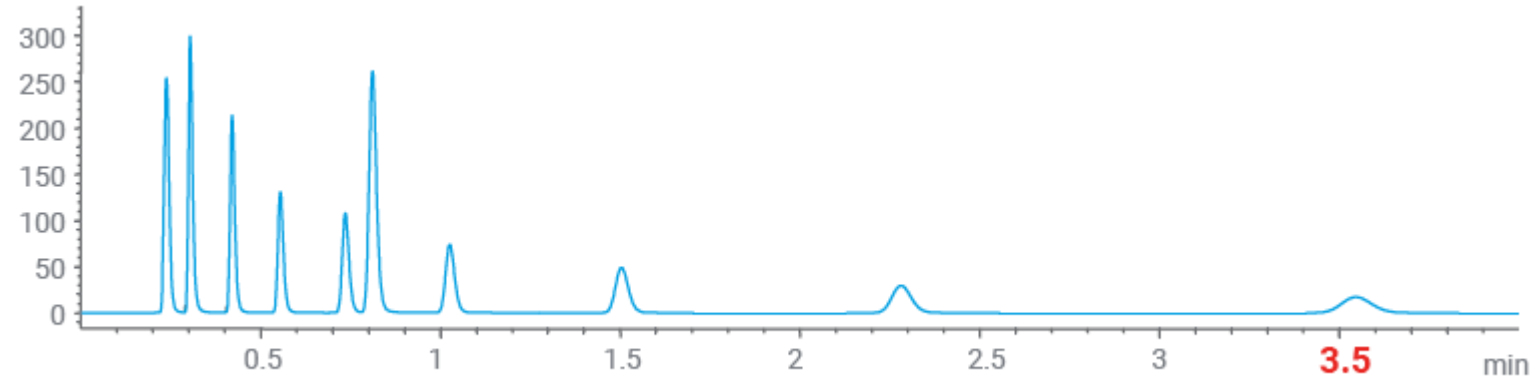
Phenyl-Hexyl 1.9 µm, 2.7 µm, 4 µm		**CS-C18** 2.7 µm		**HILIC** 1.9µm, 2.7 µm, 4 µm	**Chiral-CD** 2.7 µm
	For RP start here			**HILIC-Z** 1.9 µm, 2.7 µm, 4 µm	**Chiral-CF** 2.7 µm
				HILIC-OH5 2.7 µm	

Best All Round

Poroshell 120 EC C18 or EC C8

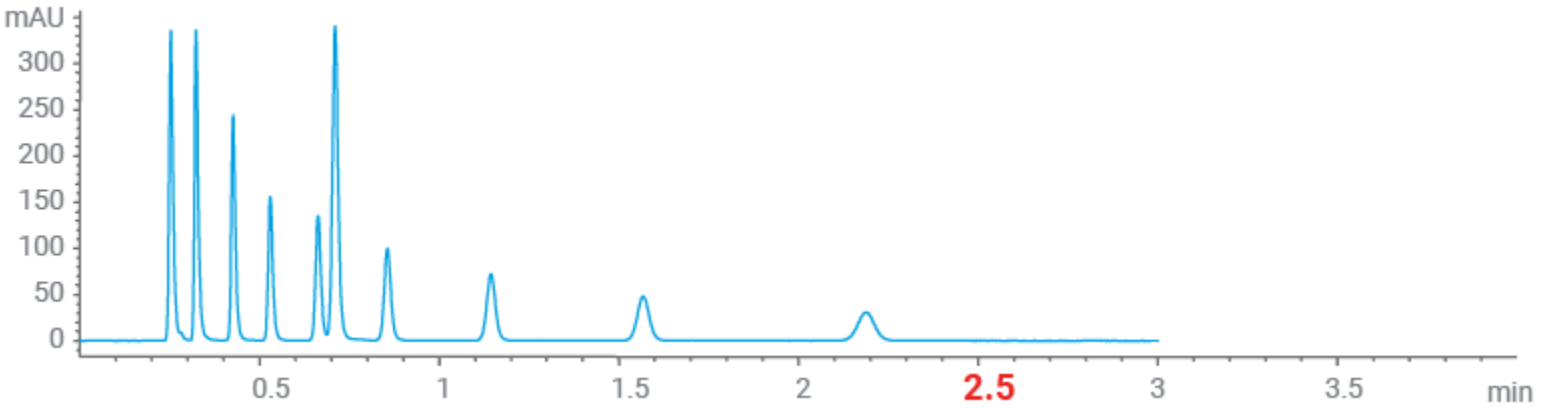
InfinityLab Poroshell 120 EC C18, 3.0 x 50 mm, 2.7 µm, p/n 699975-302



Conditions

Mobile phase:	60% ACN, 40% water
Flow rate:	0.85 mL/min
Temperature:	26 °C
Detection:	254 nm
Sample:	2 µL of RRLC checkout sample (p/n 5188-6529), alkylphenones

InfinityLab Poroshell 120 EC C8, 3.0 x 50 mm, 2.7 µm, p/n 699975-306



Need For Use Over a Wide pH Range

Poroshell HPH C18 and HPH C8

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Conditions

Flow rate:	2.0 mL/min
Temperature:	26 °C
UV detection:	254 nm

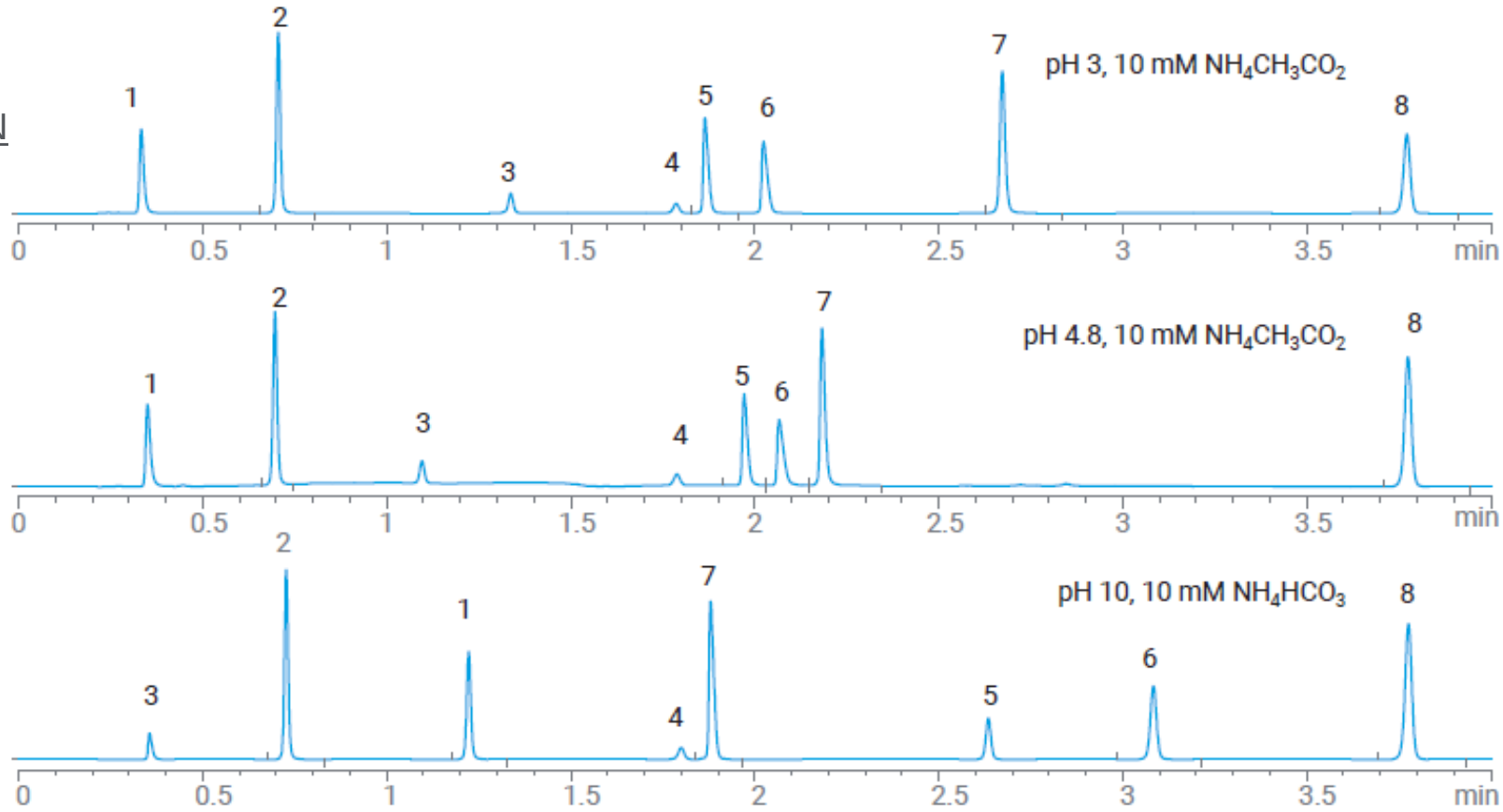
Gradient:

Time	%Buffer	%ACN
0	10	90
5	90	10
7	10	90

Sample:

1. Procainamide
2. Caffeine
3. Acetylsalicylic acid
4. Hexanophenone deg.
5. Dipyrimadole
6. Diltiazem
7. Diflunisal
8. Hexanophenone

InfinityLab Poroshell HPH-C18, 4.6 x 50 mm, 2.7 µm, p/n 699975-702



Polar Embedded Phase for Alternate Selectivity

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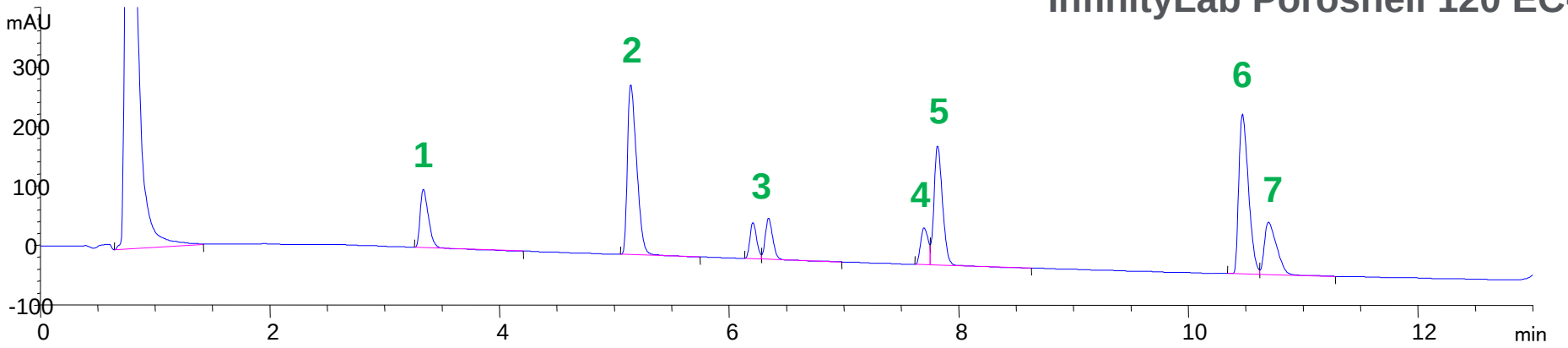
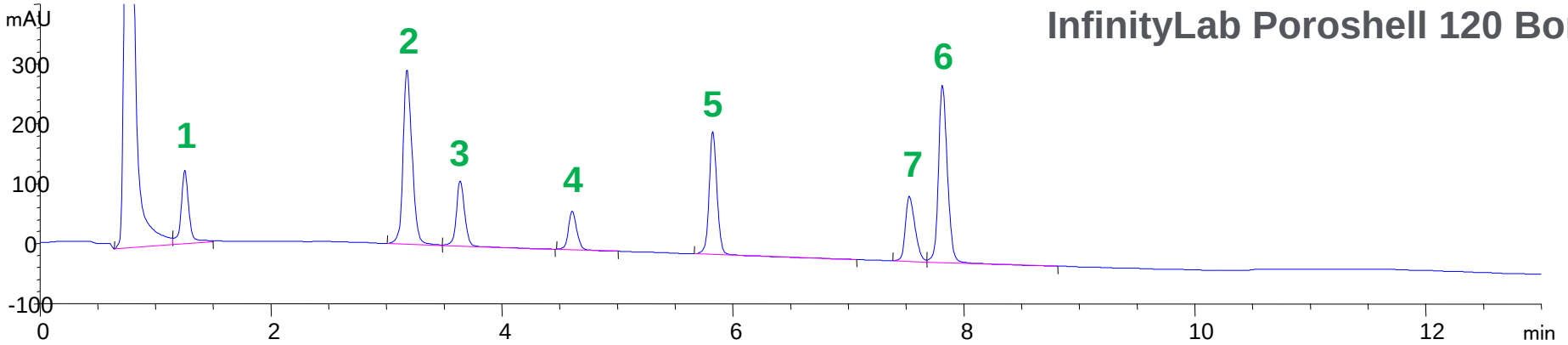
Beta blockers

1. Atenolol
2. Pindolol
3. Naldolol
4. Metoprolol
5. Acebutolol
6. Propranolol
7. Alprenolol

InfinityLab Poroshell 120 EC-C18



InfinityLab Poroshell 120 Bonus RP



Polar embedded group provides unique selectivity

0.35 mL/min, 10 mM pH 3.8 ammonium formate buffer and methanol, 10-70% methanol/12 min, 2.1 x 100 mm, 40 °C , DAD 260 nm

Chemistries with Unique Selectivity

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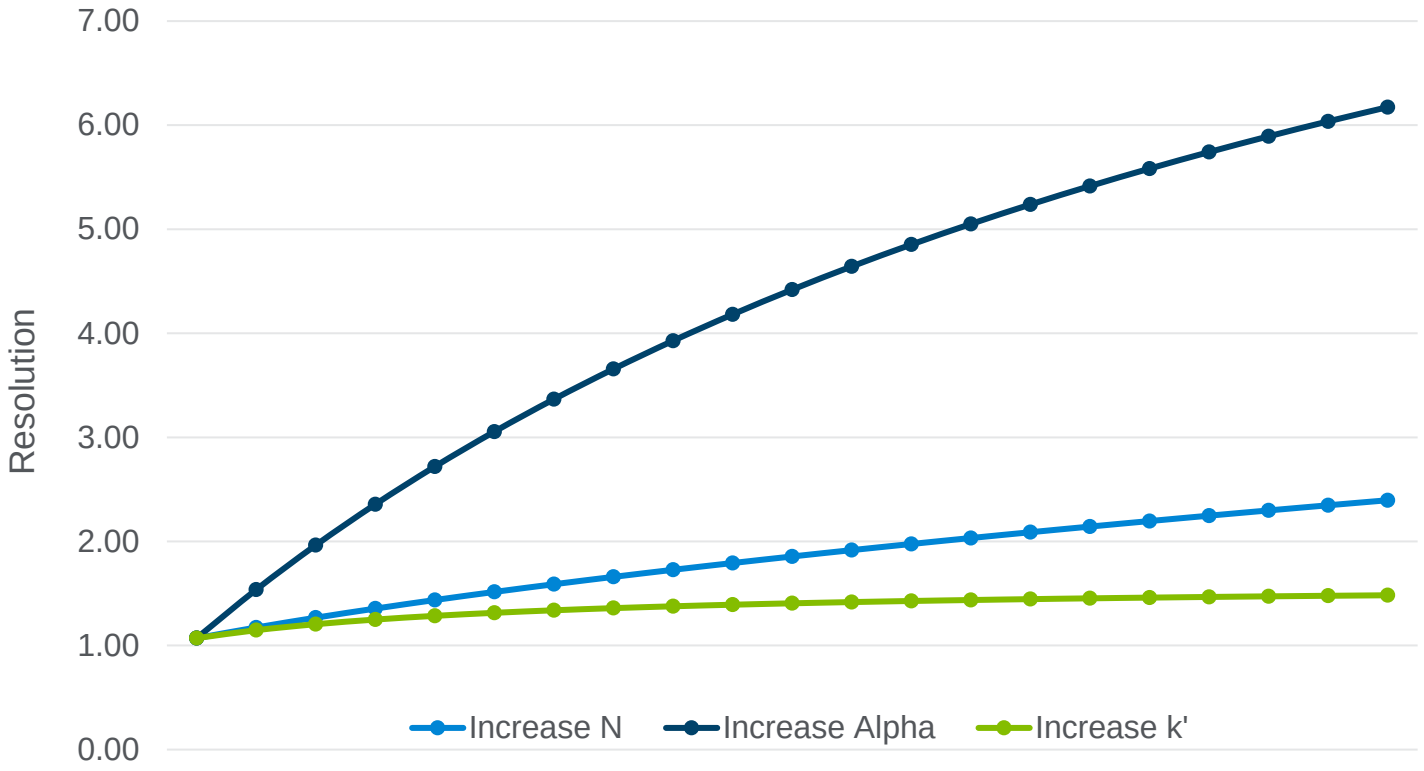
Selectivity impacts the resolution most

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right) \cdot \frac{k'}{k' + 1}$$

Chemistries with Unique Selectivity

Agilent InfinityLab

Selectivity impacts the resolution most

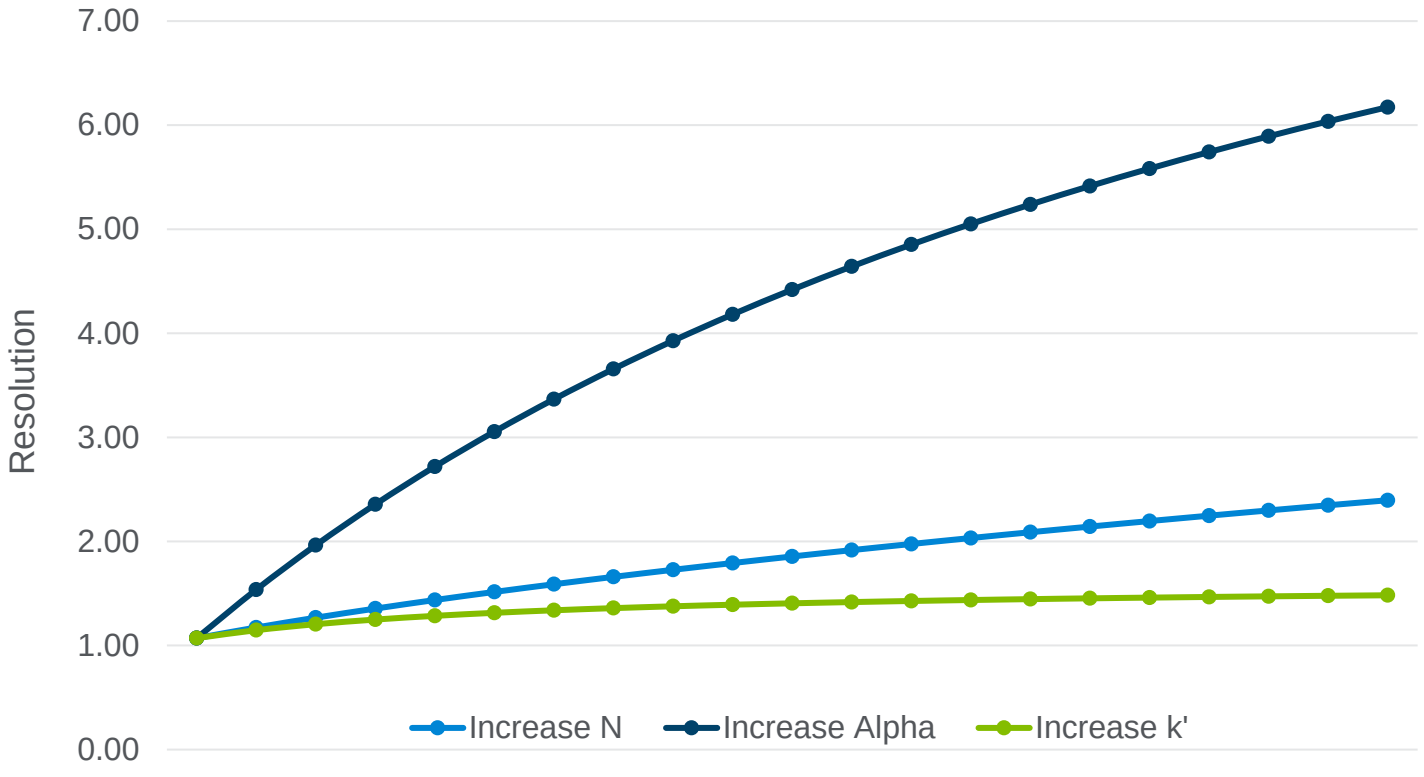


$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right) \cdot \frac{k'}{k' + 1}$$

Alpha	**1.10**	**1.35**	**1.60**	**1.85**	**2.1**
plates	5,000	10,000	15,000	20,000	25,000
k‘	2.0	4.5	7.0	9.5	12.0

Chemistries with Unique Selectivity

Selectivity impacts the resolution most



Alpha	1.10	1.35	1.60	1.85	2.1
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$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right) \cdot \frac{k'}{k' + 1}$$

Selectivity impacts resolution

- Stationary and mobile phase
- Temperature
- N is strongly influenced by alpha

Why Not to Use C18 chemistry

Some reasons for trying an alternative

C18 columns are a very common ‘go to’ column for HPLC methods and may be a suitable column choice for simple methods. **But** the C18 chemistry may not be the optimal choice.

For analysis of polar analytes, it is typically suggested that these sample types can be better separated on chemistries that have a greater polarity than C18.

Reasons to **try** another chemistry:

- Too much retention or selectivity with C18 for desired analysis time
- Polar analytes are not well retained with low or no organic modifier
- Polar analytes not well resolved even if retained
- A C18 method already in use is not rugged enough (revalidate)

- Screening different column chemistries is commonly advised when sample mixtures are complex

Why is Changing the Bonded Phase Effective?

- Differences in interactions between polar and nonpolar compounds
- Other types of interactions with a bonded phase can be exploited (for example, pi-pi interactions)
- These all change with the bonded phase
- Changing the bonded phase can improve selectivity/resolution
- Reduce analysis time

Orthogonal: Orthogonality in chromatography refers to alternative selectivity between separations.

12 Reverse Phase LC Chemistries

Agilent InfinityLab

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Best All Around	Best for Low pH Mobile Phases	Best for High pH Mobile Phases	Best for Alternative Selectivity	Best for More Polar Analytes	Chiral
EC-C18 1.9 µm, 2.7 µm, 4 µm	**SB-C18** 1.9 µm, 2.7 µm, 4 µm	**HPH-C18** 1.9 µm, 2.7 µm, 4 µm	**Bonus-RP** 2.7 µm	**SB-Aq** 1.9 µm, 2.7 µm, 4 µm	**Chiral-V** 2.7 µm
EC-C8 1.9 µm, 2.7 µm, 4 µm	**SB-C8** 2.7 µm	**HPH-C8** 2.7 µm, 4 µm	**PFP** 1.9 µm, 2.7 µm, 4 µm	**EC-CN** 2.7 µm	**Chiral-T** 2.7 µm
Phenyl-Hexyl 1.9 µm, 2.7 µm, 4 µm		**CS-C18** 2.7 µm		**HILIC** 1.9µm, 2.7 µm, 4 µm	**Chiral-CD** 2.7 µm
				HILIC-Z 1.9 µm, 2.7 µm, 4 µm	**Chiral-CF** 2.7 µm
				HILIC-OH5 2.7 µm	

12 Reverse Phase LC Chemistries

Agilent InfinityLab

InfinityLab Poroshell 120 offers a broad portfolio to suit your needs

Best All Around	Best for Low pH Mobile Phases	Best for High pH Mobile Phases	Best for Alternative Selectivity	Best for More Polar Analytes	Chiral
EC-C18 1.9 µm, 2.7 µm, 4 µm	**SB-C18** 1.9 µm, 2.7 µm, 4 µm	**HPH-C18** 1.9 µm, 2.7 µm, 4 µm	**Bonus-RP** 2.7 µm	**SB-Aq** 1.9 µm, 2.7 µm, 4 µm	**Chiral-V** 2.7 µm
EC-C8 1.9 µm, 2.7 µm, 4 µm	**SB-C8** 2.7 µm	**HPH-C8** 2.7 µm, 4 µm	**PFP** 1.9 µm, 2.7 µm, 4 µm	**EC-CN** 2.7 µm	**Chiral-T** 2.7 µm
Phenyl-Hexyl 1.9 µm, 2.7 µm, 4 µm			**CS-C18** 2.7 µm	**HILIC-Z** 1.9 µm, 2.7 µm, 4 µm	**Chiral-CD** 2.7 µm
				HILIC-OH5 2.7 µm	**Chiral-CF** 2.7 µm

Very orthogonal

Control Selectivity with Choice of Phases

Poroshell 120 columns

Agilent InfinityLab

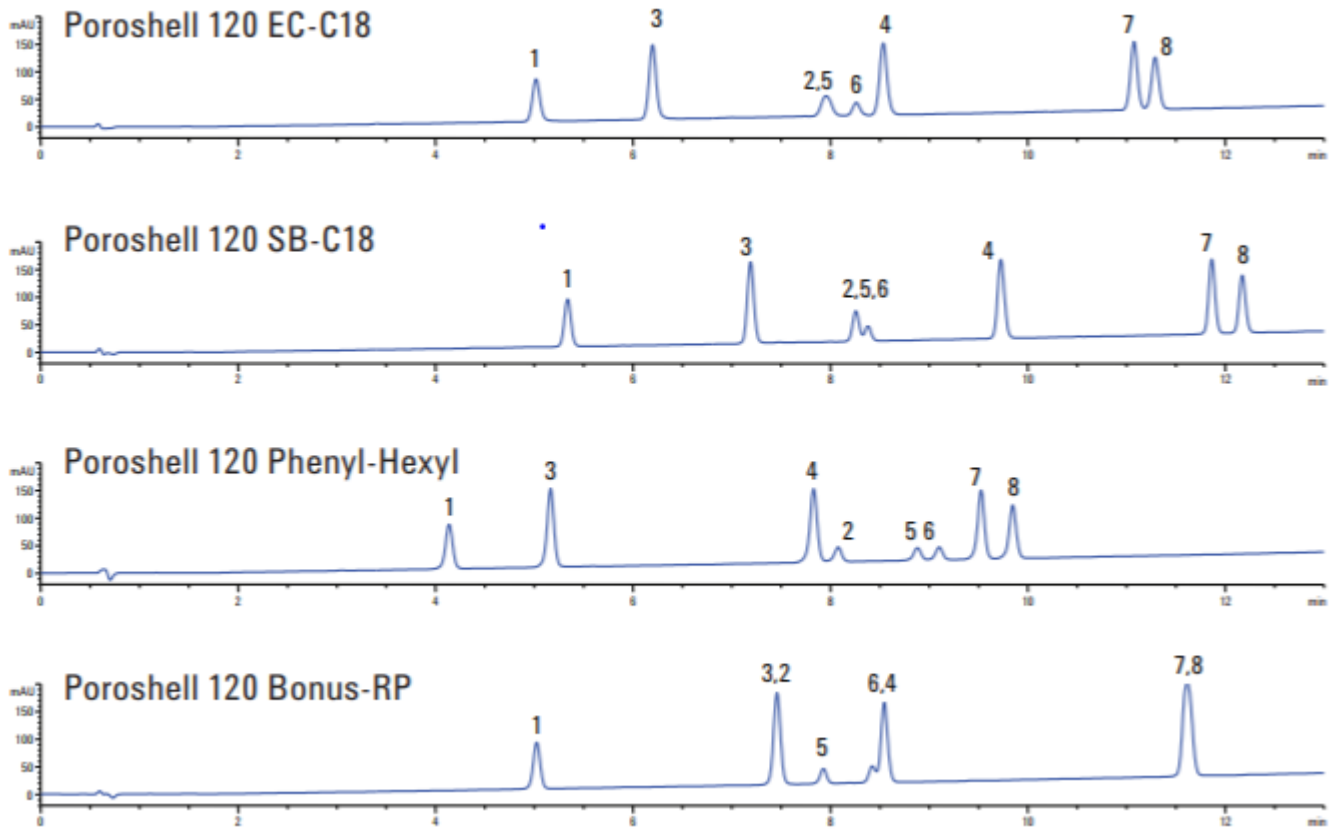
InfinityLab Poroshell 120 columns, 2.1 x 100 mm, 2.7 µm

Conditions

Flow rate:	0.4 mL/min
Mobile phase:	A: 0.1% formic acid in H2O
	B: MeOH + 0.1% formic acid
Temperature:	25 °C
Detection:	260 nm
Gradient:	40-80 and MeOH/14 min

Sample: 8 steroids

1. Hydrocortisone
2. B Estradiole
3. Andostadiene 3,17 dione
4. Testosterone
5. Ethyestradione
6. Estrone

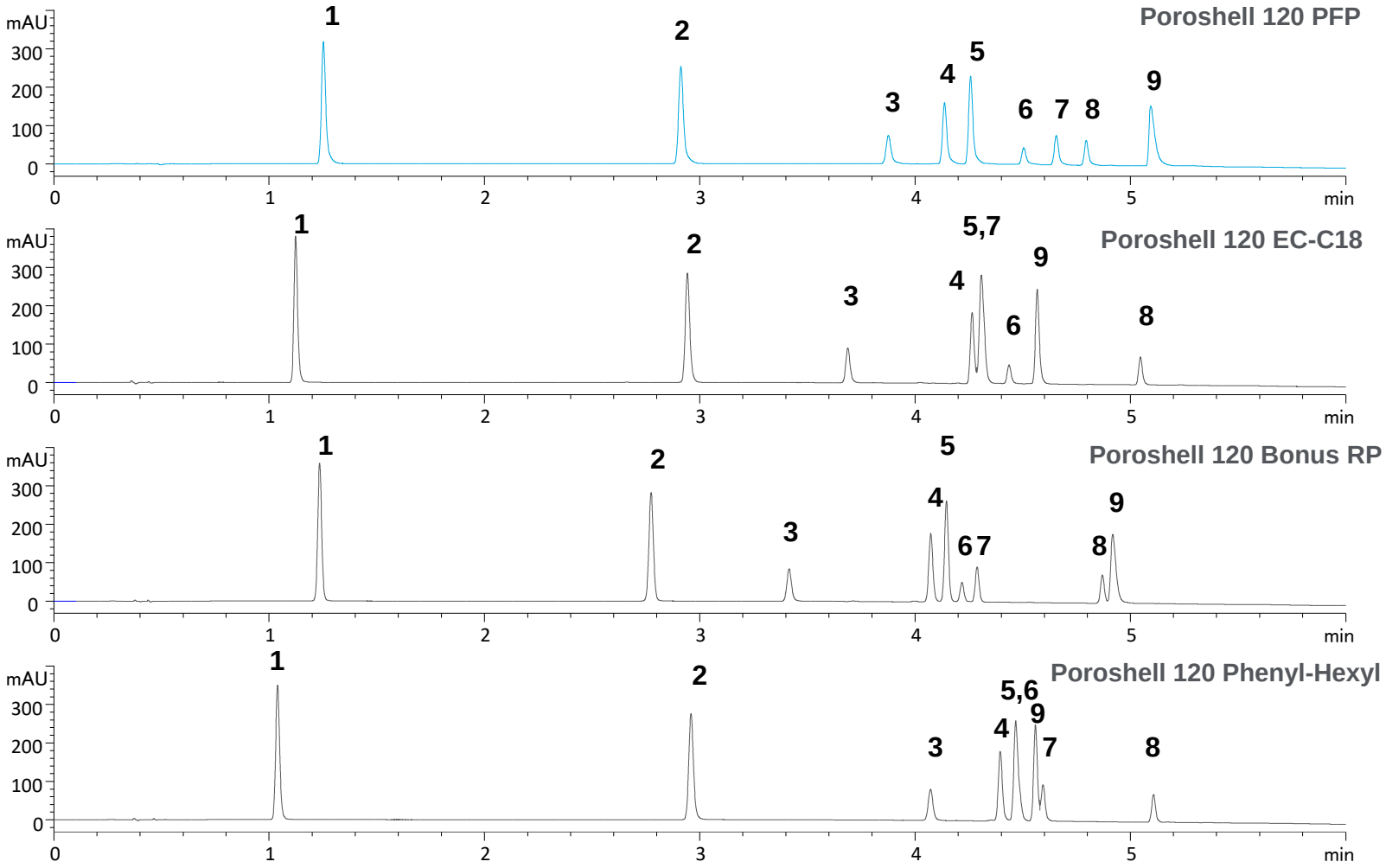


Agilent publication number: 5990-5951EN

Chemistries with Unique Selectivity

Agilent InfinityLab

The influence of stationary phase on selectivity and resolution is dominant

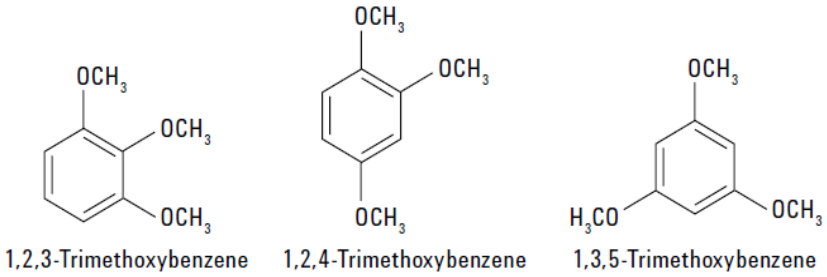


Time	% Organic
0	8
6	100
7	100
8	8

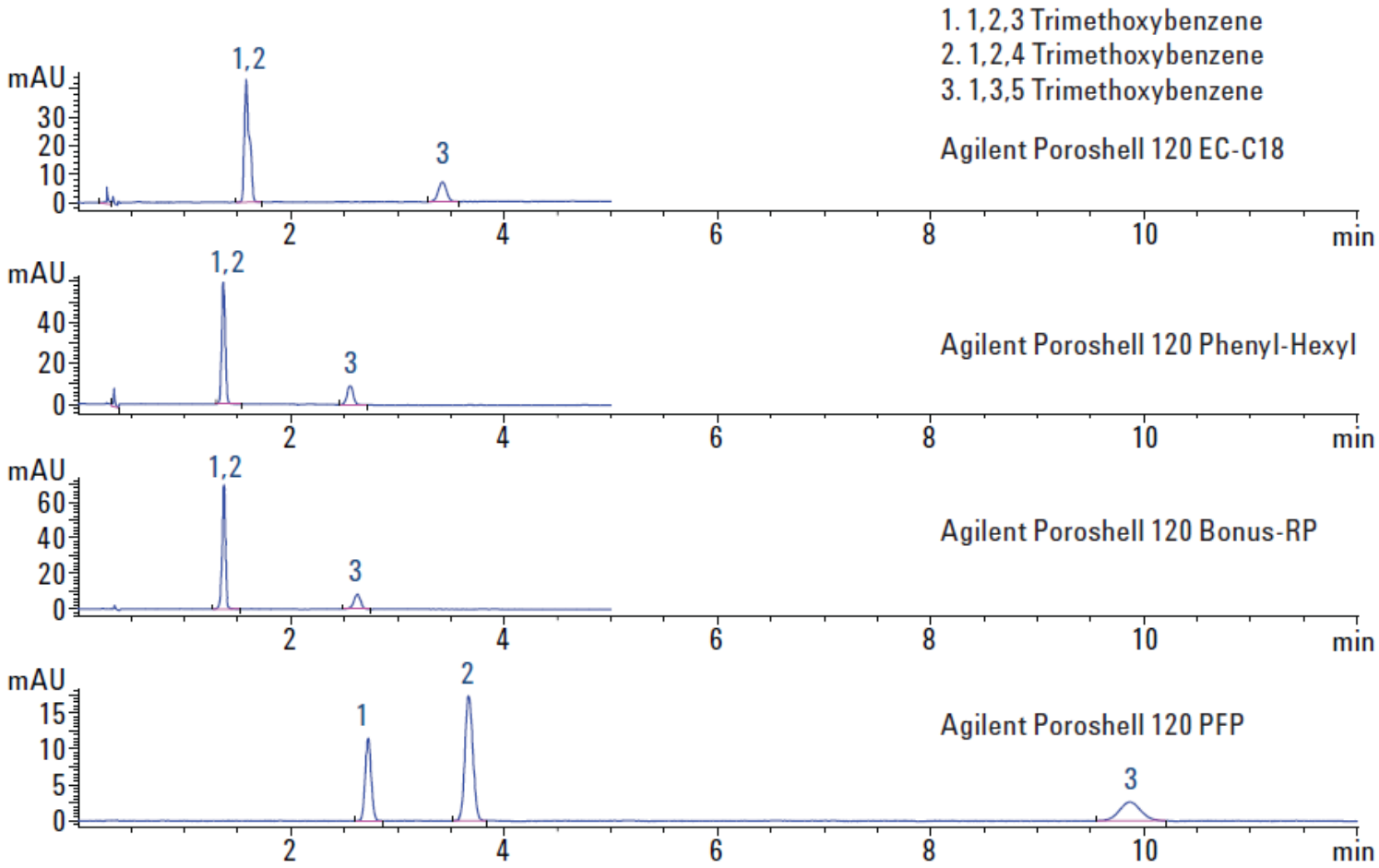
2mL/min 254 nm

Compounds
1. APAP, 2. Phenacetin, 3. Piroxicam,
4. Tolmetin, 5. Ketoprofen, 6. Naproxen,
7. Sulindac, 8. Diclofenac, 9. Diflunisal

Importance of Alternate Selectivity Chemistries



- Three compounds
  - Same molecular weight
  - Only differ by positional location of the functionality



InfinityLab Poroshell 120 columns, 4.6 x 50 mm, 2.7 µm
70:30 – MeOH/H2O, 1.5 mL/min, 40 °C, 254 nm

The Poroshell 120 Family of Chemistries

Agilent InfinityLab

InfinityLab Poroshell 120 offers a broad portfolio to suit your needs

Best All Around	Best for Low pH Mobile Phases	Best for High pH Mobile Phases	Best for Alternative Selectivity	Best for More Polar Analytes	Chiral
EC-C18 1.9 µm, 2.7 µm, 4 µm	**SB-C18** 1.9 µm, 2.7 µm, 4 µm	**HPH-C18** 1.9 µm, 2.7 µm, 4 µm	**Bonus-RP** 2.7 µm	**SB-Aq** 1.9 µm, 2.7 µm, 4 µm	**Chiral-V** 2.7 µm
EC-C8 1.9 µm, 2.7 µm, 4 µm	**SB-C8** 2.7 µm	**HPH-C8** 2.7 µm, 4 µm	**PFP** 1.9 µm, 2.7 µm, 4 µm	**EC-CN** 2.7 µm	**Chiral-T** 2.7 µm
Phenyl-Hexyl 1.9 µm, 2.7 µm, 4 µm		**CS-C18** 2.7 µm		**HILIC** 1.9µm, 2.7 µm, 4 µm	**Chiral-CD** 2.7 µm
	Charged surface C18		HILIC chemistries	**HILIC-Z** 1.9 µm, 2.7 µm, 4 µm	**Chiral-CF** 2.7 µm
				HILIC-OH5 2.7 µm	

What is HILIC and When Should it be Considered?

Agilent InfinityLab

HILIC complements RPLC

Reversed-Phase LC		Hydrophilic Interaction LC (HILIC)
Nonpolar stationary phase (for example, C18)	Polarity	Polar stationary phase (for example, silica)
Polar mobile phase H_2O/CH_3OH, H_2O/CH_3CN	Mobile phase	Polar mobile phase CH_3CN/H_2O
Decrease retention by decreasing polarity of mobile phase CH_3CN ↑ = retention ↓	Gradient	Decrease retention by increasing polarity of mobile phase ddH2O ↑ = retention ↓
Polar to nonpolar	Elution order	Nonpolar to polar

Agilent

HILIC Column Chemistries

Agilent InfinityLab

InfinityLab Poroshell 120 HILIC column options

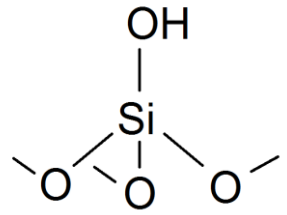
Best for More Polar Analytes
HILIC 1.9µm, 2.7 µm, 4 µm
HILIC-Z 1.9 µm, 2.7 µm, 4 µm
HILIC-OH5 2.7 µm

For more information on method development for HILIC, see Agilent technical note 5991-9271EN

HILIC Column Chemistries

InfinityLab Poroshell 120 HILIC column options

Best for More Polar Analytes
HILIC 1.9µm, 2.7 µm, 4 µm
HILIC-Z 1.9 µm, 2.7 µm, 4 µm
HILIC-OH5 2.7 µm



HILIC

- Bare silica chemistry
- For very simple mixtures

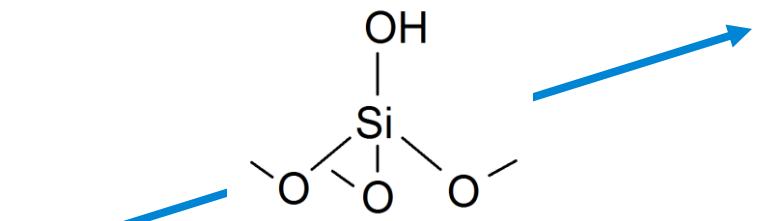
For more information on method development for HILIC, see Agilent technical note 5991-9271EN

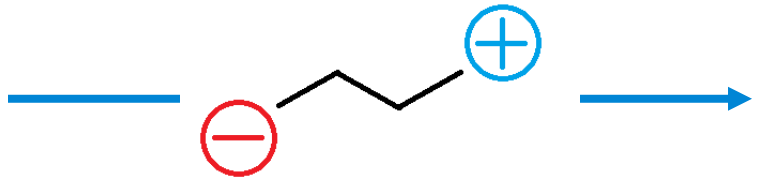
HILIC Column Chemistries

Agilent InfinityLab

InfinityLab Poroshell 120 HILIC column options

Best for More Polar Analytes
HILIC 1.9µm, 2.7 µm, 4 µm
HILIC-Z 1.9 µm, 2.7 µm, 4 µm
HILIC-OH5 2.7 µm





HILIC

- Bare silica chemistry
- For very simple mixtures

HILIC-Z

- Proprietary zwitterionic chemistry, high pH stable
- **The most modern and robust column – start method development here**
- PEEK-lined version available

For more information on method development for HILIC, see Agilent technical note 5991-9271EN

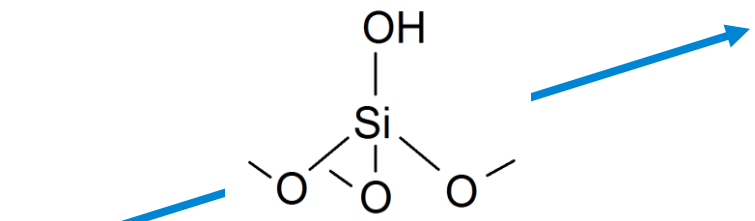
HILIC Column Chemistries

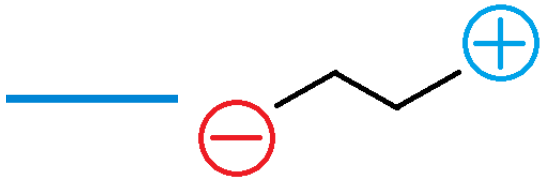
Agilent InfinityLab

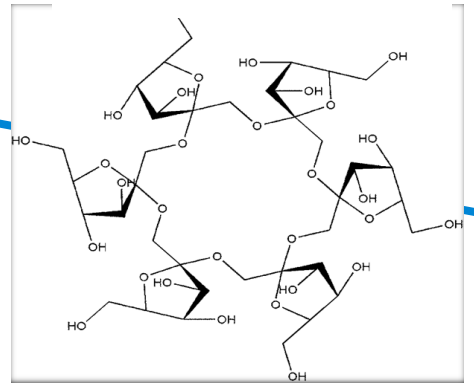
InfinityLab Poroshell 120 HILIC column options

Best for More Polar Analytes

HILIC 1.9µm, 2.7 µm, 4 µm
HILIC-Z 1.9 µm, 2.7 µm, 4 µm
HILIC-OH5 2.7 µm







HILIC

- Bare silica chemistry
- For very simple mixtures

HILIC-Z

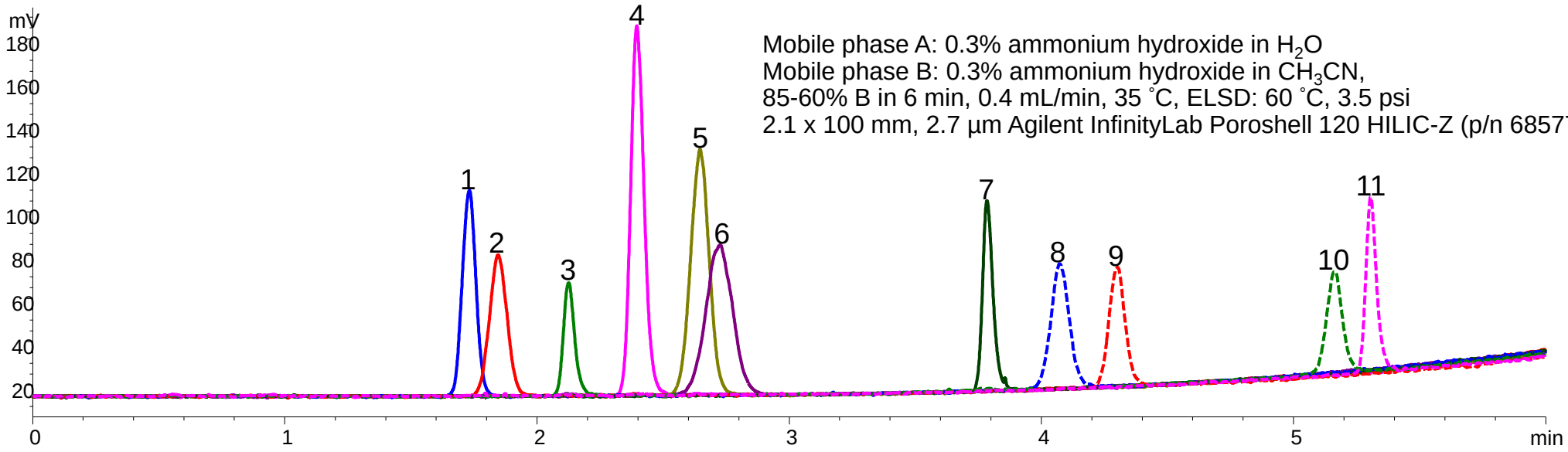
- Proprietary zwitterionic chemistry, high pH stable
- **The most modern and robust column – start method development here**
- PEEK-lined version available

HILIC-OH5

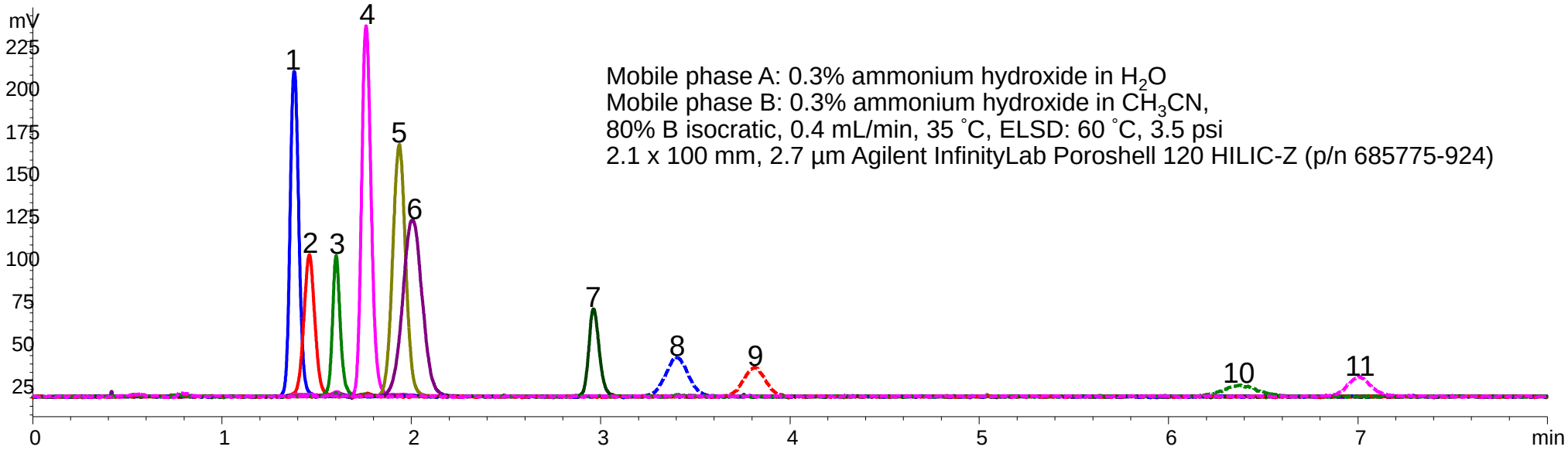
- Brushed fructan chemistry
- Alternative selectivity

For more information on method development for HILIC, see Agilent technical note 5991-9271EN

Separation of 11 Sugars on Poroshell 120 HILIC-Z



Mobile phase A: 0.3% ammonium hydroxide in H_2O
Mobile phase B: 0.3% ammonium hydroxide in CH_3CN,
85-60% B in 6 min, 0.4 mL/min, 35 ˚C, ELSD: 60 ˚C, 3.5 psi
2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z (p/n 685775-924)



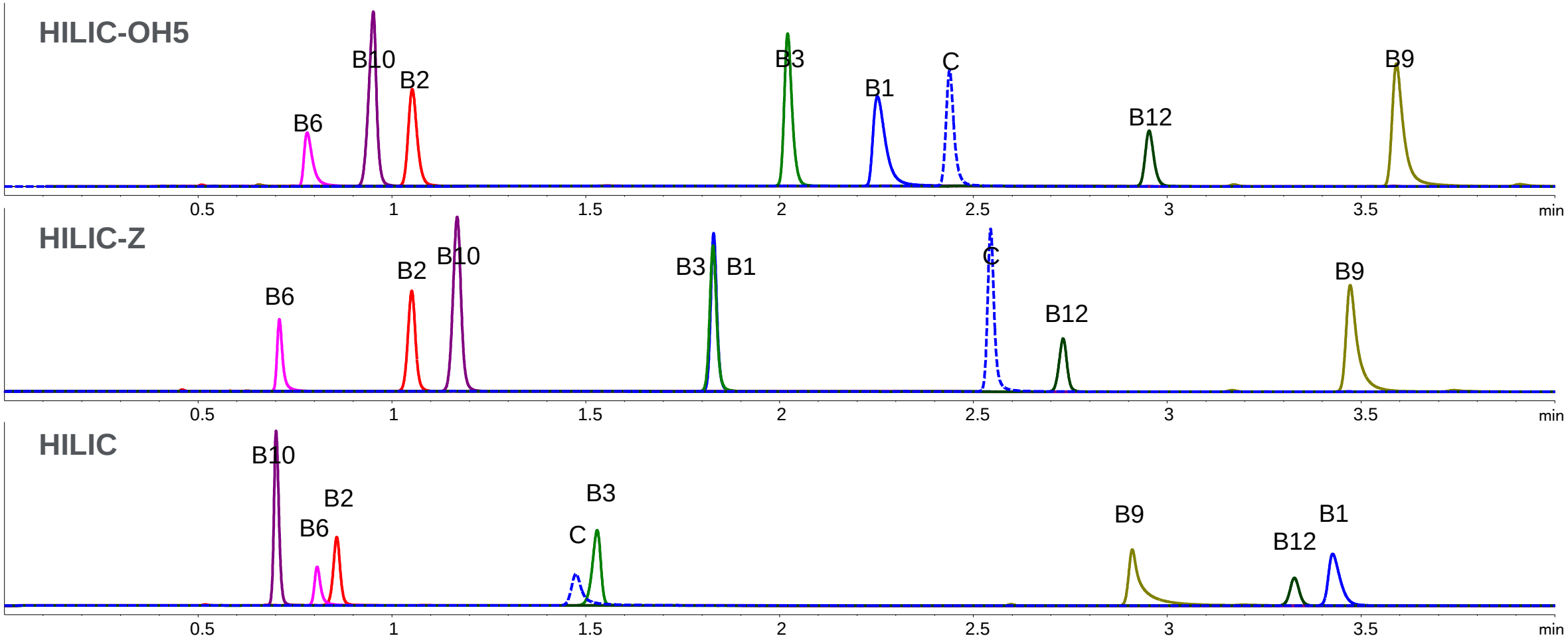
Mobile phase A: 0.3% ammonium hydroxide in H_2O
Mobile phase B: 0.3% ammonium hydroxide in CH_3CN,
80% B isocratic, 0.4 mL/min, 35 ˚C, ELSD: 60 ˚C, 3.5 psi
2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z (p/n 685775-924)

1. Xylose
2. Arabinose
3. Fructose
4. Mannose
5. Glucose
6. Galactose
7. Sucrose
8. Maltose
9. Lactose
10. Maltotriose
11. Raffinose

Agilent publication number: 5991-8984EN

Selectivity Options with Poroshell 120 HILIC Columns

Water soluble vitamins



Columns used: 2.1 x 100 mm, 2.7 µm; A: 100 mM ammonium acetate + 0.5% acetic acid (pH ~4.6) in H_2O, B: CH_3CN, 0.5 mL/min, 87% B for 1 min, 87-50% B in 4 min, 3 min re-equilibration, 1 µL injection of individual vitamin standards (0.1-0.4 mg/mL each), 40 ˚C, 260 nm, 80 Hz

How Do I Determine the Best Chromatography for my Sample

InfinityLab Poroshell 120 CS-C18

Built using Agilent innovation

Hybrid endcapped C18 phase

+

SPP particle modified to have a charged surface

=

InfinityLab Poroshell 120 CS-C18 for basic analytes with low ionic strength mobile phases

- High pH stable
- Alternate C18 selectivity

- Better peak shape for basic compounds
- Formic acid compatibility
- Reduced operating pressures
- Increased speed of analysis

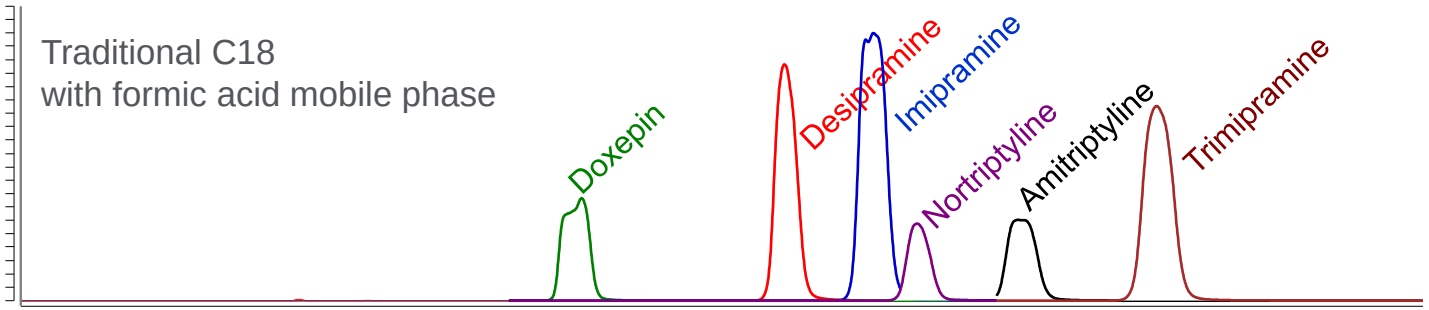
Column dimensions

- 2.1, 3, 4.6 mm id x 50, 100, 150 mm length
- PEEK-lined options
- Corresponding Fast Guards

PEEK-lined column options are rare in the reversed phase column market and help with challenging metal sensitive compounds.

Agilent InfinityLab Poroshell 120 CS-C18

Better peak shape than traditional C18 for analytes with FA mobile phase

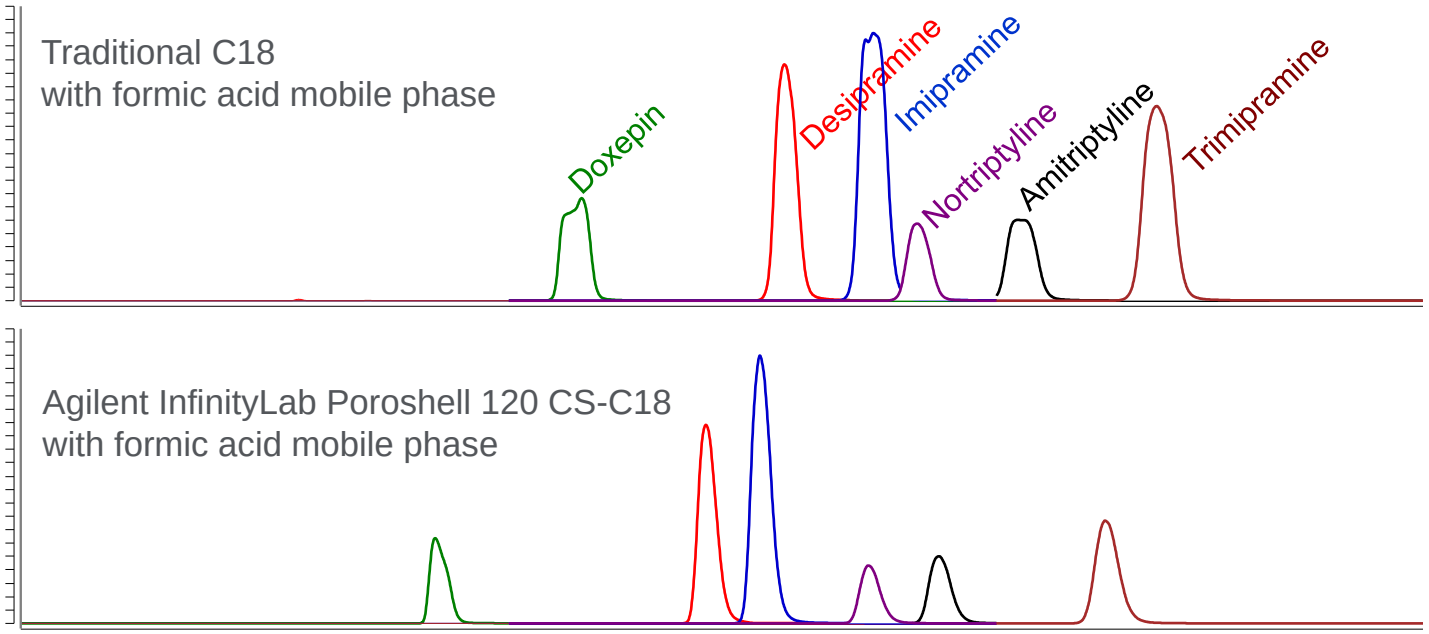


A: 0.1% formic acid or 0.2% trifluoroacetic acid in water; B: acetonitrile; 0.4 mL/min; isocratic: %B varies; 2.1 x 100 mm columns, 1 µL injection, 30 °C, LC/MS: ESI+, dMRM; sample: 5 µg/mL of doxepin, desipramine, imipramine, nortriptyline, amitriptyline, trimipramine

Agilent publication number: 5994-2095EN

Agilent InfinityLab Poroshell 120 CS-C18

Better peak shape than traditional C18 for analytes with FA mobile phase

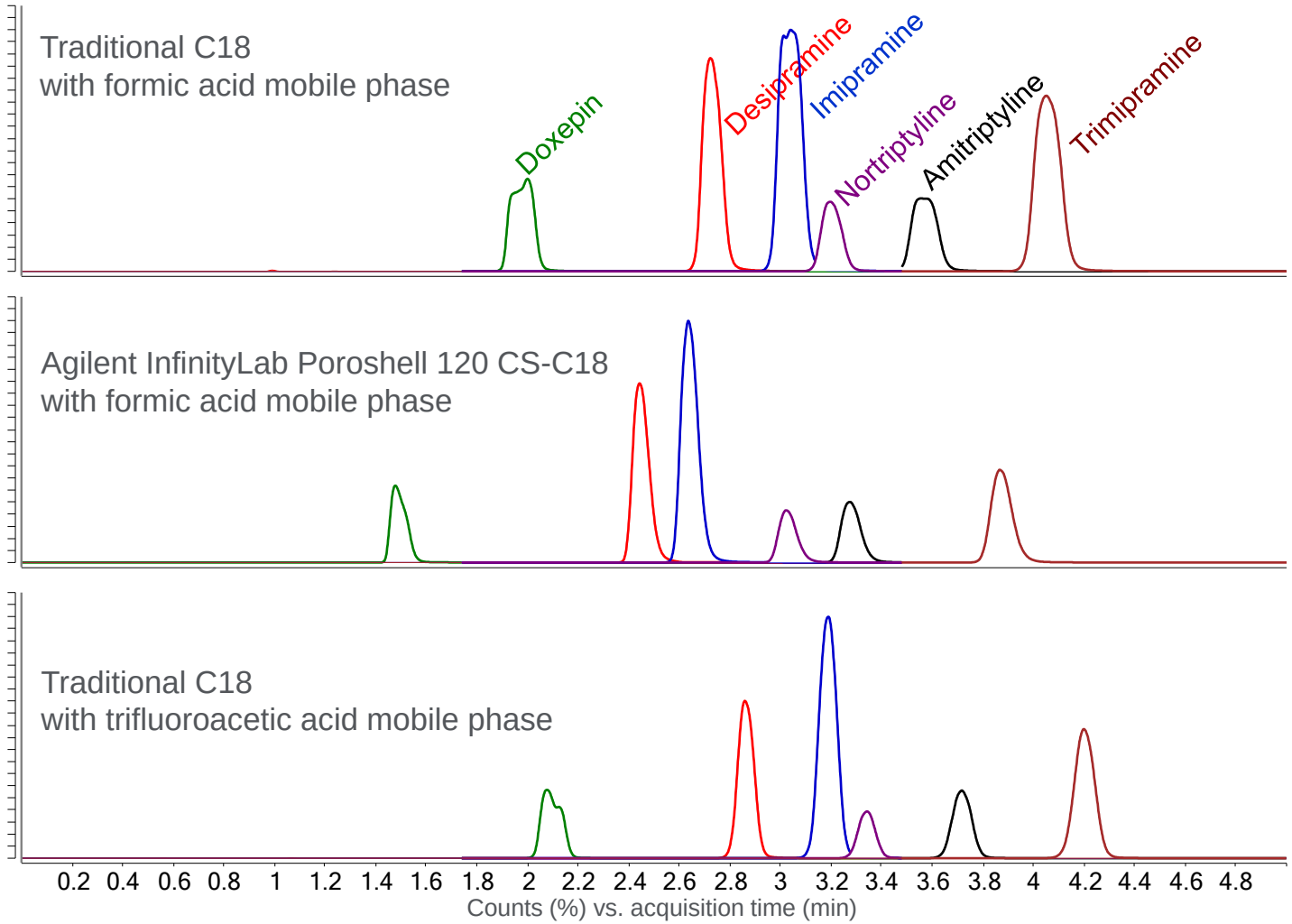


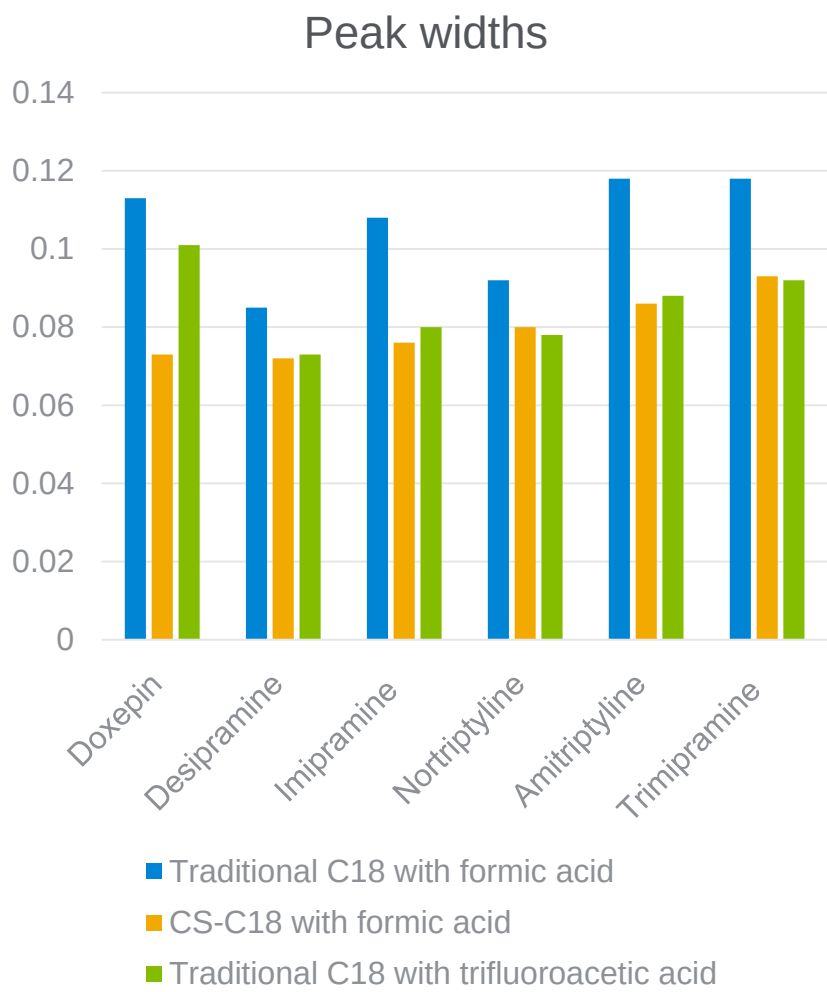
A: 0.1% formic acid or 0.2% trifluoroacetic acid in water; B: acetonitrile; 0.4 mL/min; isocratic: %B varies; 2.1 x 100 mm columns, 1 µL injection, 30 °C, LC/MS: ESI+, dMRM; sample: 5 µg/mL of doxepin, desipramine, imipramine, nortriptyline, amitriptyline, trimipramine

Agilent publication number: 5994-2095EN

Agilent InfinityLab Poroshell 120 CS-C18

Better peak shape than traditional C18 for analytes with FA mobile phase

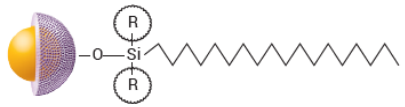
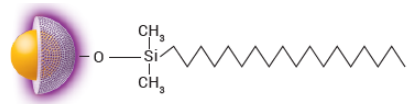
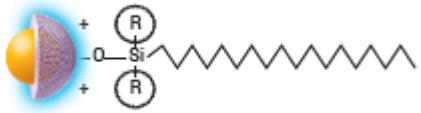




A: 0.1% formic acid or 0.2% trifluoroacetic acid in water; B: acetonitrile; 0.4 mL/min; isocratic: %B varies; 2.1 x 100 mm columns, 1 µL injection, 30 °C, LC/MS: ESI+, dMRM; sample: 5 µg/mL of doxepin, desipramine, imipramine, nortriptyline, amitriptyline, trimipramine

Agilent publication number: 5994-2095EN

Choosing Between C18s

InfinityLab Poroshell 120	Chemistry	Pore Size	Endcapped	Carbon Load	Surface Area	Best For
EC-C18 1.9 µm, 2.7 µm, 4 µm		120 Å	Yes	10%	130 m^2/g	General purpose Excellent peak shape and efficiency for acids, bases, neutrals
SB-C18 1.9 µm, 2.7 µm, 4 µm		120 Å	No	9%	130 m^2/g	Low pH Excellent stability and peak shape in highly acidic conditions
HPH-C18 1.9 µm, 2.7 µm, 4 µm		100 Å	Yes	Proprietary	95 m^2/g	Capable of high pH Robust performance and long lifetimes
CS-C18 2.7 µm		100 Å	Yes	Proprietary	95 m^2/g	Alternate selectivity Improved peak shape and sample capacity for basic compounds with low ionic strength mobile phases Capable of high pH

The Poroshell 120 Family

19 chemistries with unique selectivity

Agilent InfinityLab

InfinityLab Poroshell 120 offers a broad portfolio to suit your needs

Best All Around	Best for Low pH Mobile Phases	Best for High pH Mobile Phases	Best for Alternative Selectivity	Best for More Polar Analytes	Chiral
EC-C18 1.9 µm, 2.7 µm, 4 µm	**SB-C18** 1.9 µm, 2.7 µm, 4 µm	**HPH-C18** 1.9 µm, 2.7 µm, 4 µm	**Bonus-RP** 2.7 µm	**SB-Aq** 1.9 µm, 2.7 µm, 4 µm	**Chiral-V** 2.7 µm
EC-C8 1.9 µm, 2.7 µm, 4 µm	**SB-C8** 2.7 µm	**HPH-C8** 2.7 µm, 4 µm	**PFP** 1.9 µm, 2.7 µm, 4 µm	**EC-CN** 2.7 µm	**Chiral-T** 2.7 µm
Phenyl-Hexyl 1.9 µm, 2.7 µm, 4 µm		**CS-C18** 2.7 µm		**HILIC** 1.9µm, 2.7 µm, 4 µm	**Chiral-CD** 2.7 µm
				HILIC-Z 1.9 µm, 2.7 µm, 4 µm	**Chiral-CF** 2.7 µm
				HILIC-OH5 2.7 µm	

Why Do Chiral Separations

- Most small molecule drugs on the market today are either racemates or enantiomerically pure.
- Enantiomers: Same chemical and physical properties, but can have **very different behavorial properties.**
- It is important to characterize each enantiomer.

InfinityLab Poroshell 120 Chiral Chemistries

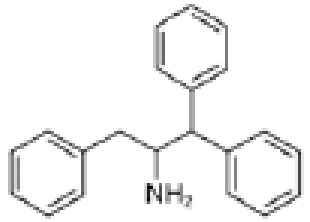
Column Chemistry	Chiral Selector (bonded chemistry)	Typical LC Mode	Typical Applications
InfinityLab Poroshell 120 Chiral-CF	Derivatized cyclofructan (CF6)	Polar Organic (PO)	Primary amines
		Normal Phase (NP)	Primary amines
InfinityLab Poroshell 120 Chiral-CD	Hydroxypropylated-β-cyclodextrin	Reversed Phase (RP)	Stimulants, fungicides, t-boc amino acids
		Polar Organic (PO)	Complex molecules
InfinityLab Poroshell 120 Chiral-V	Vancomycin (macrolide antibiotic)	Polar Ionic (PI)	Basic pharmaceuticals (various)
		Reversed Phase (RP)	Amines, profens
		Polar Organic (PO)	Complex neutral molecules
InfinityLab Poroshell 120 Chiral-T	Teicoplanin (macrolide antibiotic)	Polar Ionic (PI)	Beta blockers, hydroxyl acids
		Reversed Phase (RP)	Amino acids, hydroxyl acids, profens
		Polar Organic (PO)	Hydantoins, benzodiazepines

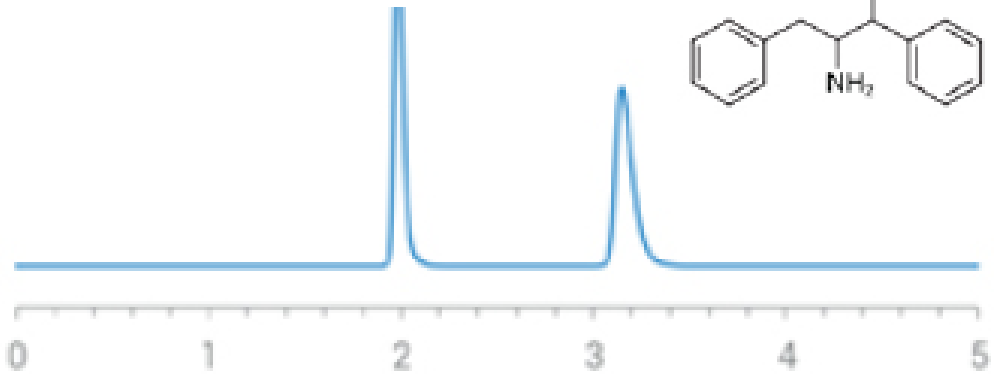
Application Examples
InfinityLab Poroshell chiral columns

Agilent InfinityLab

1-benzyl-2,2,diphenylethylamine

Retention (min): 1.98/3.15 (Peak1/Peak 2)
Resolution: 9.47



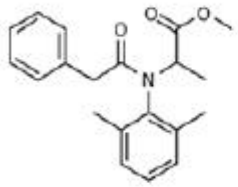


Method Conditions

Column:	InfinityLab Poroshell 120 Chiral-V (10 cm x 4.6 mm, 2.7 µm)
Mobile phase:	100/0.1 wt %: Methanol/Ammonium Trifluoroacetate
Flow Rate:	1.0 mL/min
Temperature:	Ambient (23 °C)
Injection Volume:	1.0 µL
Detection:	UV 220 nm

Benalaxyl – fungicide

Retention (min): 1.64/1.87 (Peak1/Peak 2)
Resolution: 3.13



Method Conditions

Column:	InfinityLab Poroshell 120 Chiral-CD (10 cm x 4.6 mm, 2.7 µm)
Mobile phase:	30/70: Acetonitrile/15 mM Ammonium Formate (pH 3.6)
Flow Rate:	1.0 mL/min
Temperature:	Ambient (23 °C)
Injection Volume:	1.0 µL
Detection:	UV 220 nm

InfinityLab Chiral Applications Compendium

Publication number: 5991-8450EN

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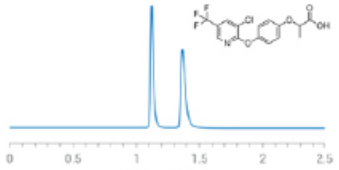
Fungicides

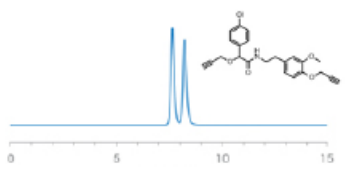
Haloxyfop



Retention (min): 1.12/1.37 (Peak1/Peak 2)
Resolution: 4.48

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-T (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 100/0.3 wt %: Methanol/Ammonium Trifluoroacetate
Flow Rate: 1.0 mL/min
Temperature: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 220 nm

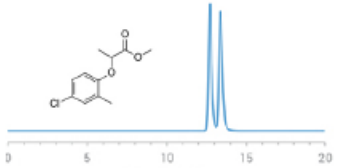
Mandipropamid



Retention (min): 7.65/8.22 (Peak1/Peak 2)
Resolution: 1.87

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-V (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 30/70: Methanol/15 mM Ammonium Formate (pH 3.6)
Flow Rate: 0.5 mL/min
Temperature: 45 °C
Injection Volume: 1.0 µL
Detection: UV 230 nm

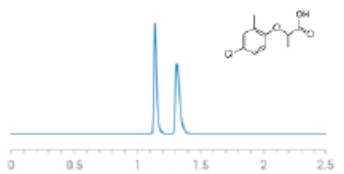
Mecoprop methyl ester



Retention (min): 12.73/13.39 (Peak1/Peak 2)
Resolution: 1.74

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-T (15 cm x 4.6 mm, 2.7 µm)
Mobile phase: 30/70: Methanol/50 mM Ammonium Formate (pH 3.6)
Flow Rate: 0.5 mL/min
Temperature: 45 °C
Injection Volume: 1.0 µL
Detection: UV 230 nm

Mecoprop

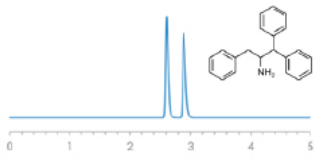


Retention (min): 1.14/1.31 (Peak1/Peak 2)
Resolution: 3.59

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-T (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 100/0.3 wt %: Methanol/Ammonium Trifluoroacetate
Flow Rate: 1.0 mL/min
Temperature: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 220 nm

Amines

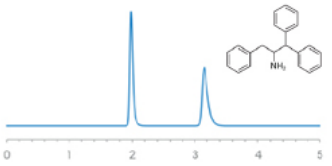
1-benzyl-2,2-diphenylethylamine



Retention (min): 2.61/2.89 (Peak1/Peak 2)
Resolution: 3.51

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-CD (15 cm x 4.6 mm, 2.7 µm)
Mobile phase: 30/70: Acetonitrile/15 mM Ammonium Formate (pH 3.6)
Flow Rate: 1.0 mL/min
Temperature: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 220 nm

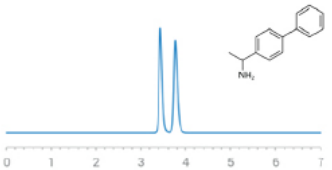
1-benzyl-2,2-diphenylethylamine



Retention (min): 1.98/3.15 (Peak1/Peak 2)
Resolution: 9.47

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-V (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 100/0.1 wt %: Methanol/Ammonium Trifluoroacetate
Flow Rate: 1.0 mL/min
Temperature: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 220 nm

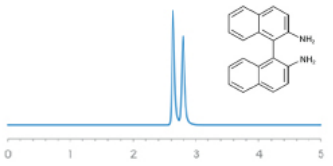
1,1'-binaphthyl-2,2'-diamine



Retention (min): 3.44/3.77 (Peak1/Peak 2)
Resolution: 2.11

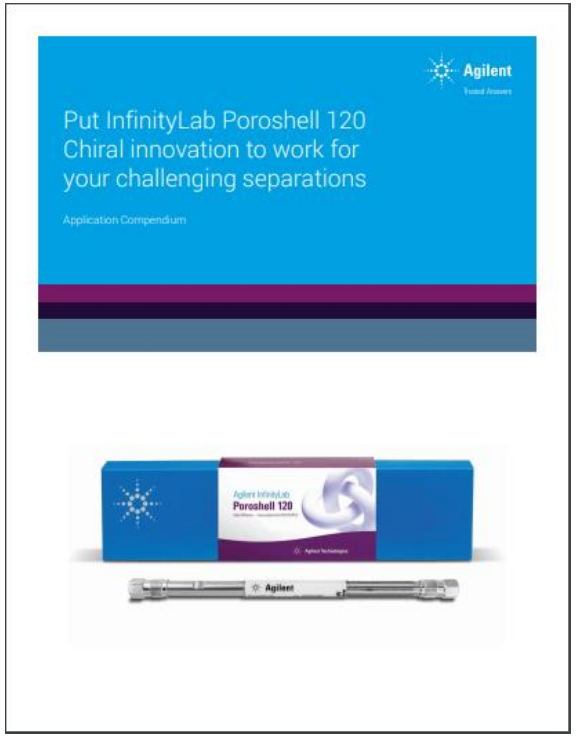
Method Conditions
Column: InfinityLab Poroshell 120 Chiral-CD (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 90/10/0.3/0.2: Acetonitrile/Methanol/Trifluoroacetate/TEA
Flow Rate: 1.0 mL/min
Temperature: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 254 nm

1,1'-binaphthyl-2,2'-diamine



Retention (min): 2.64/2.79 (Peak1/Peak 2)
Resolution: 1.31

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-V (10 cm x 4.6 mm, 2. 7µm)
Mobile phase: 90/10: Methanol/15 mM Ammonium Formate (pH 3.6)
Flow Rate: 1.0 mL/min
Temperature: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 220 nm



Role of the LC Instrument

Instrument considerations

Column compatibility with LC instruments

Extra column volume

Proper connections and fittings

Importance of cell volume

Data collection rate

Full Compatibility

Agilent InfinityLab

Designed along with your LC instruments for highest performance

SPP Particle	For	Maximum Pressure	Typical Pressure	Efficiency	Target System
1.9 µm	Highest UHPLC performance	1300 bar	Similar to sub-2 µm totally porous	~120% of sub-2 µm totally porous	1290 Infinity II
2.7 µm	UHPLC performance at lower pressures	600 bar/ 1000 bar	50% of sub-2 µm totally porous	~90% of sub-2 µm totally porous	1290 Infinity II 1260 Infinity II
4 µm	Improved HPLC performance	600 bar	Typically <200 bar	~200% of 5 µm totally porous	1260 Infinity II VL 1220 Infinity II (VL)

Particle Size	ID	Optimum Flow
1.9 µm	2.1 mm	0.4 – 0.5 mL/min
	3.0 mm	0.8 – 1 mL/min
2.7 µm	2.1 mm	0.4 – 0.5 mL/min
	3.0 mm	0.8 – 1 mL/min
	4.6 mm	1.5 – 2 mL/min
4 µm	3.0 mm	0.5 – 0.75 mL/min
	4.6 mm	1 – 1.25 mL/min

Column length	Recommended Use
50	High speed
100	High resolution
>=150	Ultra-high resolution

Full Compatibility

Agilent InfinityLab

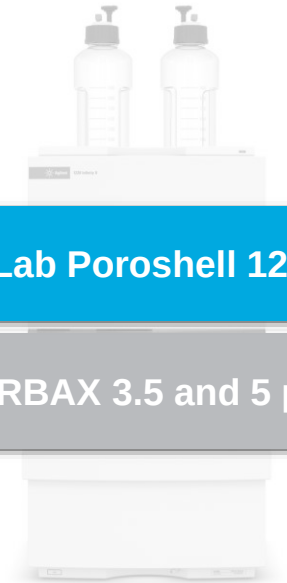
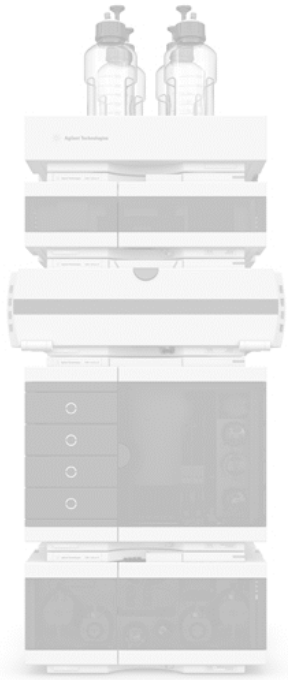
Separation efficiency

	<400 bar	400-800 bar	800-1300 bar
			
	HPLC	**UHPLC**	**Low dispersion UHPLC**
Pressure	Low	Moderate	High

Full Compatibility

Agilent InfinityLab

Separation efficiency

<400 bar	400-800 bar	800-1300 bar
InfinityLab Poroshell 120, 4 µm ZORBAX 3.5 and 5 µm		
		
HPLC	**UHPLC**	**Low dispersion UHPLC**
Pressure: Low	Pressure: Moderate	Pressure: High

Full Compatibility

Agilent InfinityLab

Separation efficiency

<400 bar	400-800 bar	800-1300 bar
InfinityLab Poroshell 120, 4 µm	InfinityLab Poroshell 120, 2.7 µm	
ZORBAX 3.5 and 5 µm	ZORBAX RRHT, 1.8 µm	
HPLC	UHPLC	Low dispersion UHPLC
Pressure: Low	Pressure: Moderate	Pressure: High

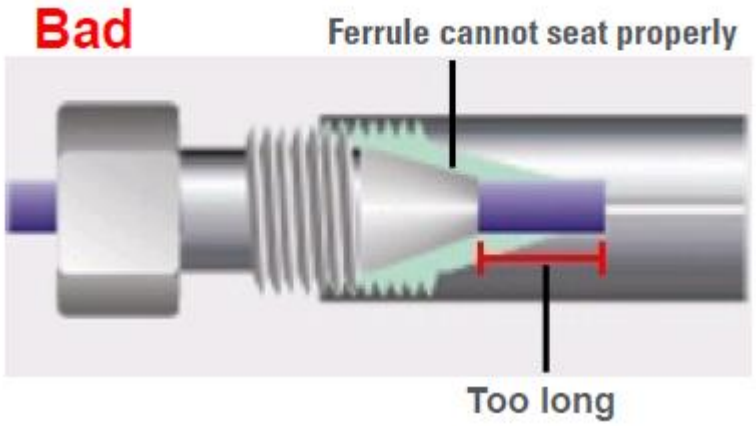
Full Compatibility

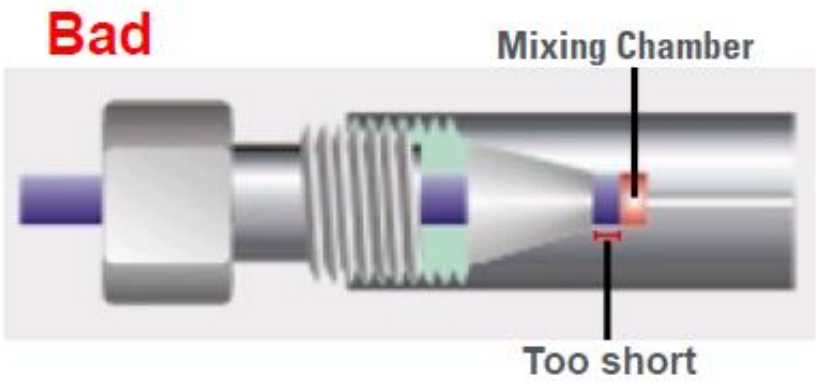
Agilent InfinityLab

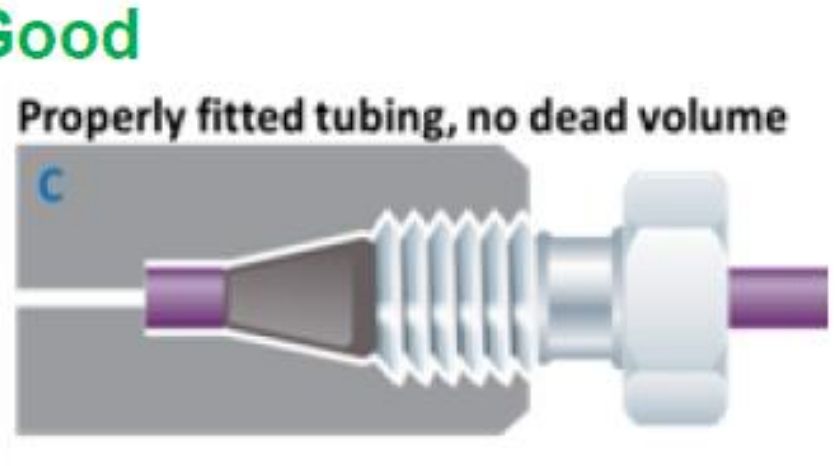
Separation efficiency

<400 bar	400-800 bar	800-1300 bar
InfinityLab Poroshell 120, 4 µm	InfinityLab Poroshell 120, 2.7 µm	Infinity Lab Poroshell 120, 1.9 µm
ZORBAX 3.5 and 5 µm	ZORBAX RRHT, 1.8 µm	ZORBAX RRHD, 1.8 µm
HPLC	UHPLC	Low dispersion UHPLC
Pressure: Low	Pressure: Moderate	Pressure: High

Making Correct Connections



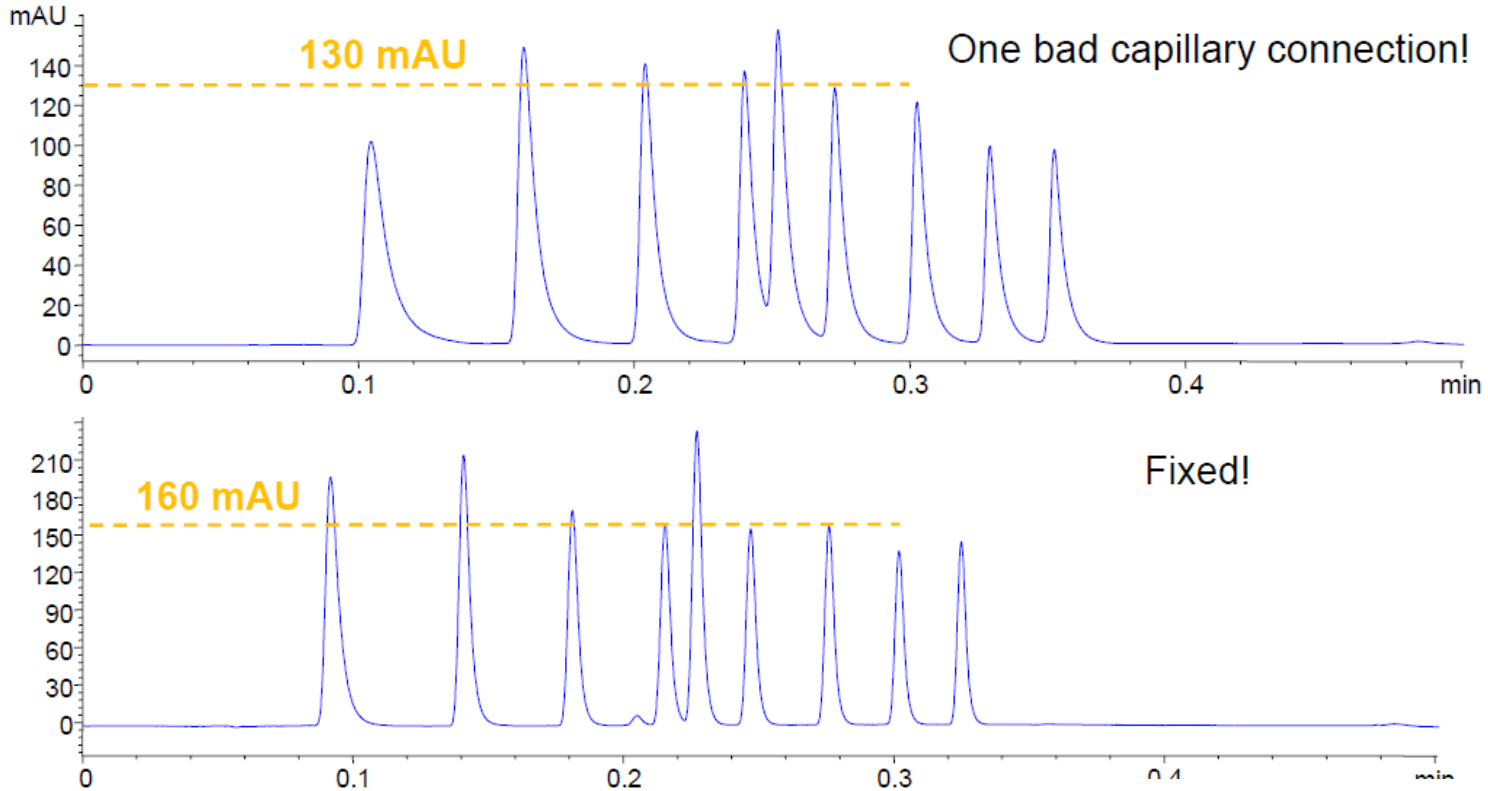




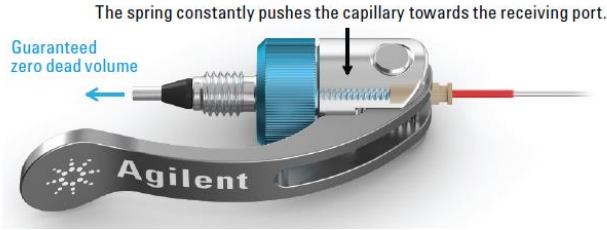
Poor fitting connections

- Will broaden or split peaks, or cause tailing
- Will typically affect all peaks, but especially early eluting peaks
- Can cause carryover

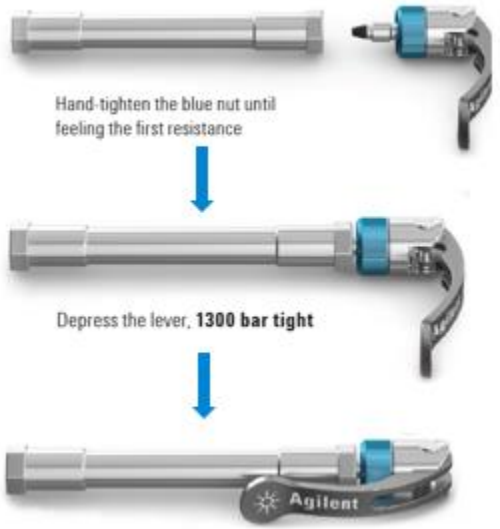
Importance of Correct Connections



Quick Turn



Quick Connect



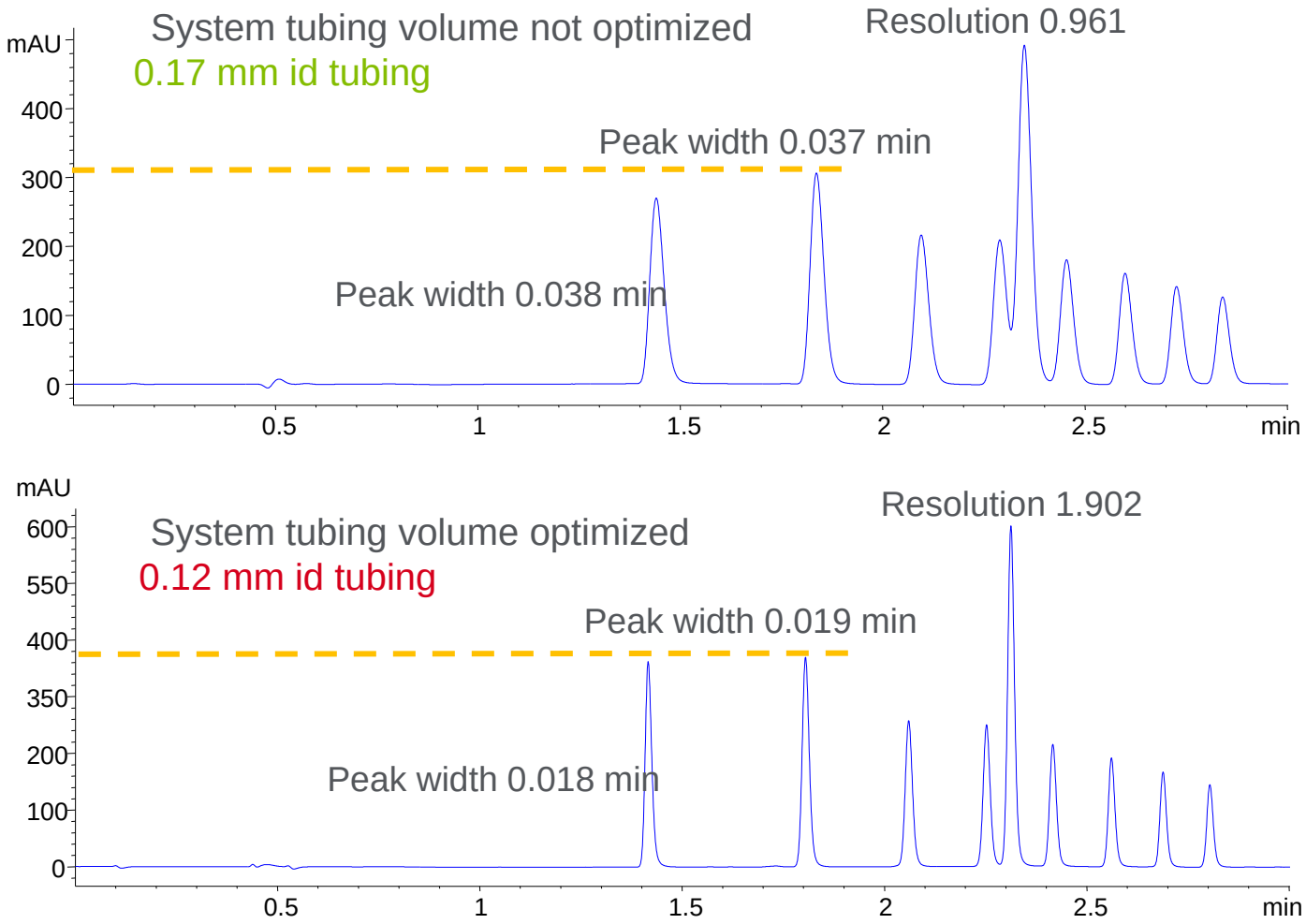
Correct connection every time

Compatible to 1300 bar

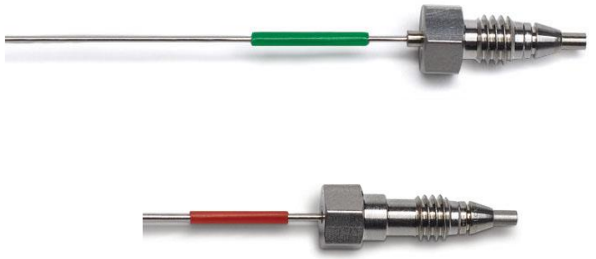
Agilent technical note: Agilent InfinityLab UHPLC fittings

Publication number: 5991-5525EN

Optimizing Connecting Tubing Volume for UHPLC Columns



Length	10 mm	50 mm	100 mm	150 mm
Tubing id	Volume	Volume	Volume	Volume
0.17 mm (green)	0.227 µL	1.1 µL	2.27 µL	3.3 µL
0.12 mm (red)	0.113 µL	0.55 µL	1.13 µL	1.65 µL



Resolution and Importance of Flow Cell Volume

Differences in detector flow cell volume can affect N and R_s

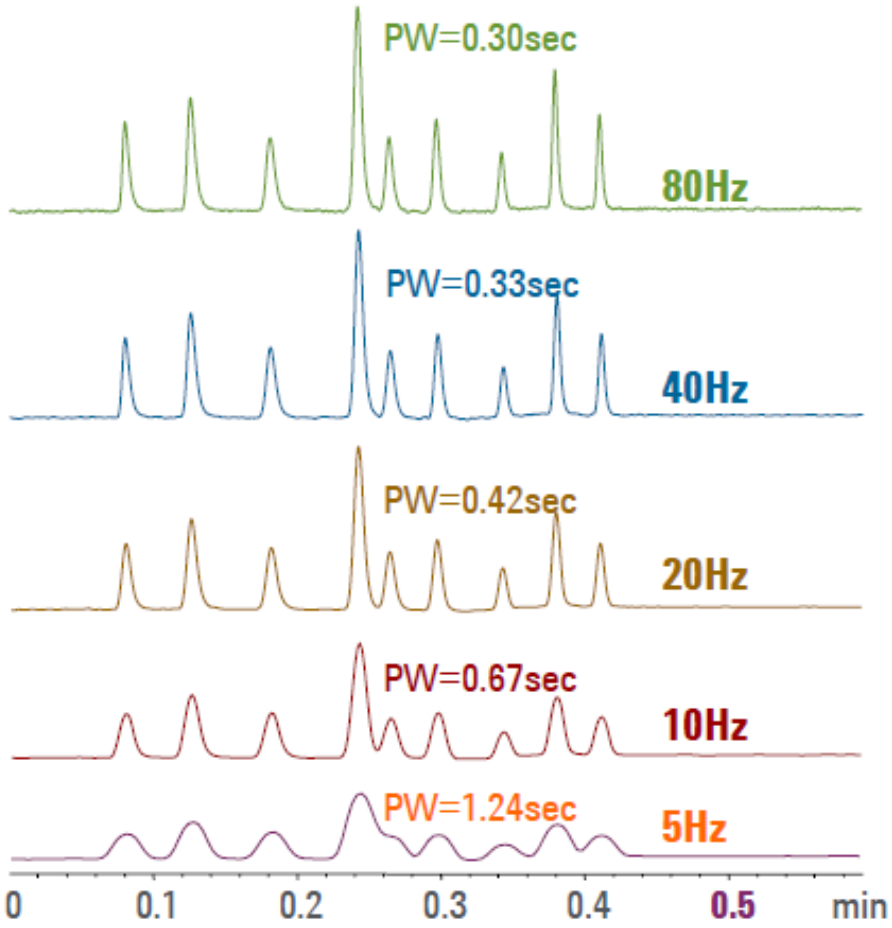
Scenario: Agilent ZORBAX Rapid Resolution column: 75 mm, 3.5 µm; flow rate: 1mL/min; k = 3

Flow Cell Volume	Band Broadening* (4.6 mm)	Band Broadening* (2.1 mm**)
1.7 µL	0.3%	6%
8 µL	6%	138%
14 µL	19%	423%

*Versus 8571 theoretical plates (HPLC Calculations Assistant, Version 2.1, Savant Audiovisuals)
**Flow rate, 0.2 mL/min

Maintaining Resolution at High Analysis Speed

Importance of data collection rate for narrow peaks



80Hz versus 10Hz (20Hz) Data Rate

- Peak Width: – 55% (– 30%)
- Resolution: + 90% (+ 30%)
- Peak Capacity: + 120% (+ 40%)
- App. Column Eff.: + 260% (+ 70%)

Data Rate	Peak Width	Resolution	Peak Capacity
80 Hz	0.300	2.25	60
40 Hz	0.329	2.05	55
20 Hz	0.416	1.71	45
10 Hz	0.666	1.17	29
5 Hz	1.236	0.67	16

Sample: Phenones Test Mix
Column: Zorbax SB-C18, 4.6x30, 1.8um
Gradient:: 50-100%ACN in 0.3min
Flow Rate: 5ml/min

Full Compatibility

Designed to seamlessly integrate into the InfinityLab family

Part of the InfinityLab family

Agilent **InfinityLab** products are designed to provide the best efficiency in your liquid chromatography workflow, regardless of application area. When relying on Agilent **InfinityLab instruments**, **LC columns** and **supplies** be assured that every part works together seamlessly.

InfinityLab LC columns

InfinityLab supplies and parts

InfinityLab StaySafe caps

InfinityLab LC series

InfinityLab accessories

Agilent Resources for Support

- Resource page http://www.agilent.com/chem/agilentresources
  - InfinityLab Poroshell 120 brochure – https://www.agilent.com/cs/library/brochures/5991-8750EN_InfinityLab_Poroshell120_brochure.pdf
  - Quick reference guides, product catalogs
  - Online selection tools, “How-to” videos
  - Column user guides - https://www.agilent.com/en-us/support/liquid-chromatography/kb005965
  - Tech support: http://www.agilent.com/chem/techsupport
- InfinityLab LC Supplies catalog (5991-8031EN)
- Agilent University http://www.agilent.com/crosslab/university
- YouTube – Agilent Channel
- Your local product specialists
- Subscribe to Agilent Peak Tales podcasts at peaktales.libsyn.com

Contact Agilent Chemistries and Supplies Technical Support

Available in the USA and Canada 8-5 all time zones

1-800-227-9770 option 3, option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

Thank you for attending

Any questions?

Additional Resources and Information on Agilent LC Columns

Brochures

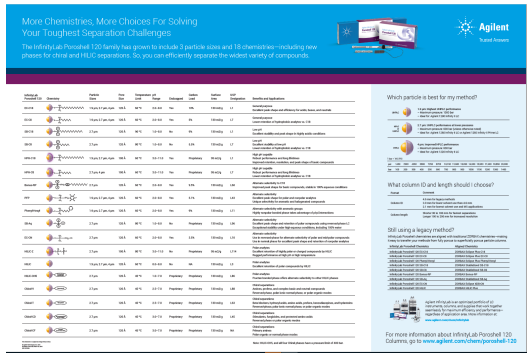
Poroshell 120 Portfolio brochure

5991-8750EN

Poroshell 120 ordering guide

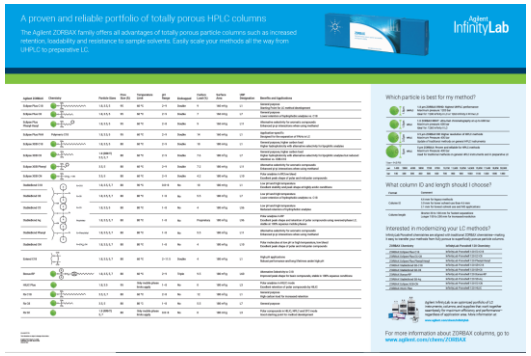
5991-9123EN

Posters



Poroshell 120 selectivity overview

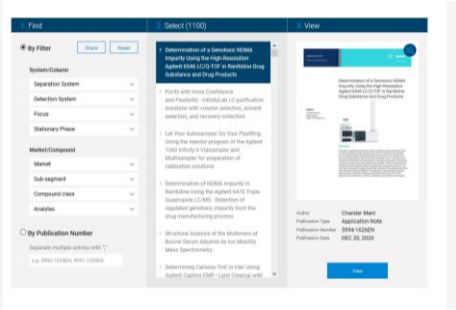
5991-9013EN



ZORBAX selectivity overview

5994-2212EN

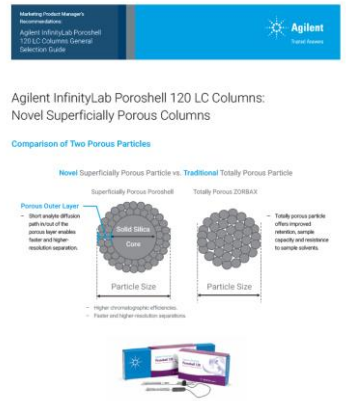
Application Notes



Agilent Application Finder

>200 Poroshell 120 application notes

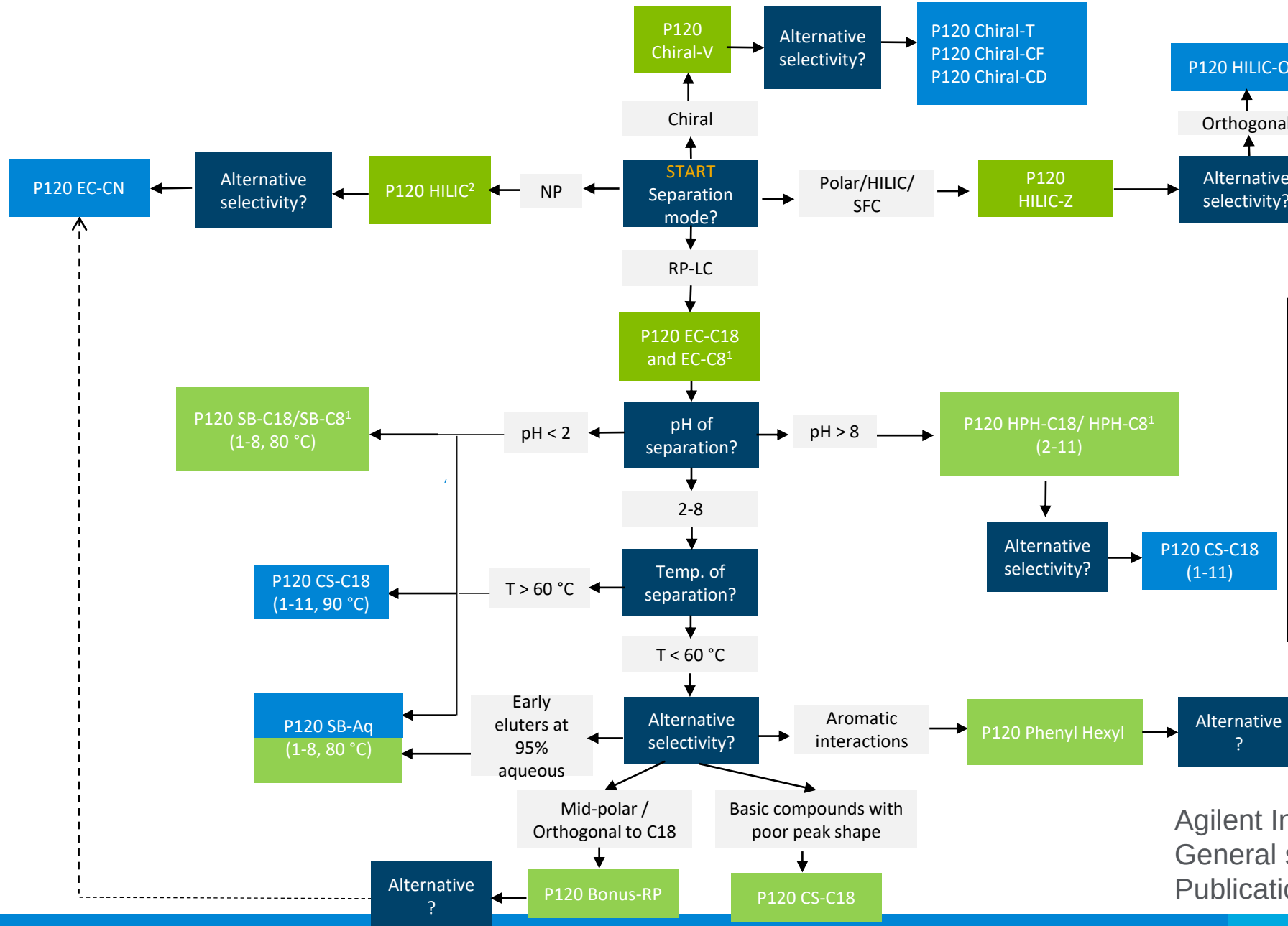
Selection Guide



Poroshell 120 general selection guide

5994-1982EN

HPLC Chemistry Selection: Poroshell 120



Legend

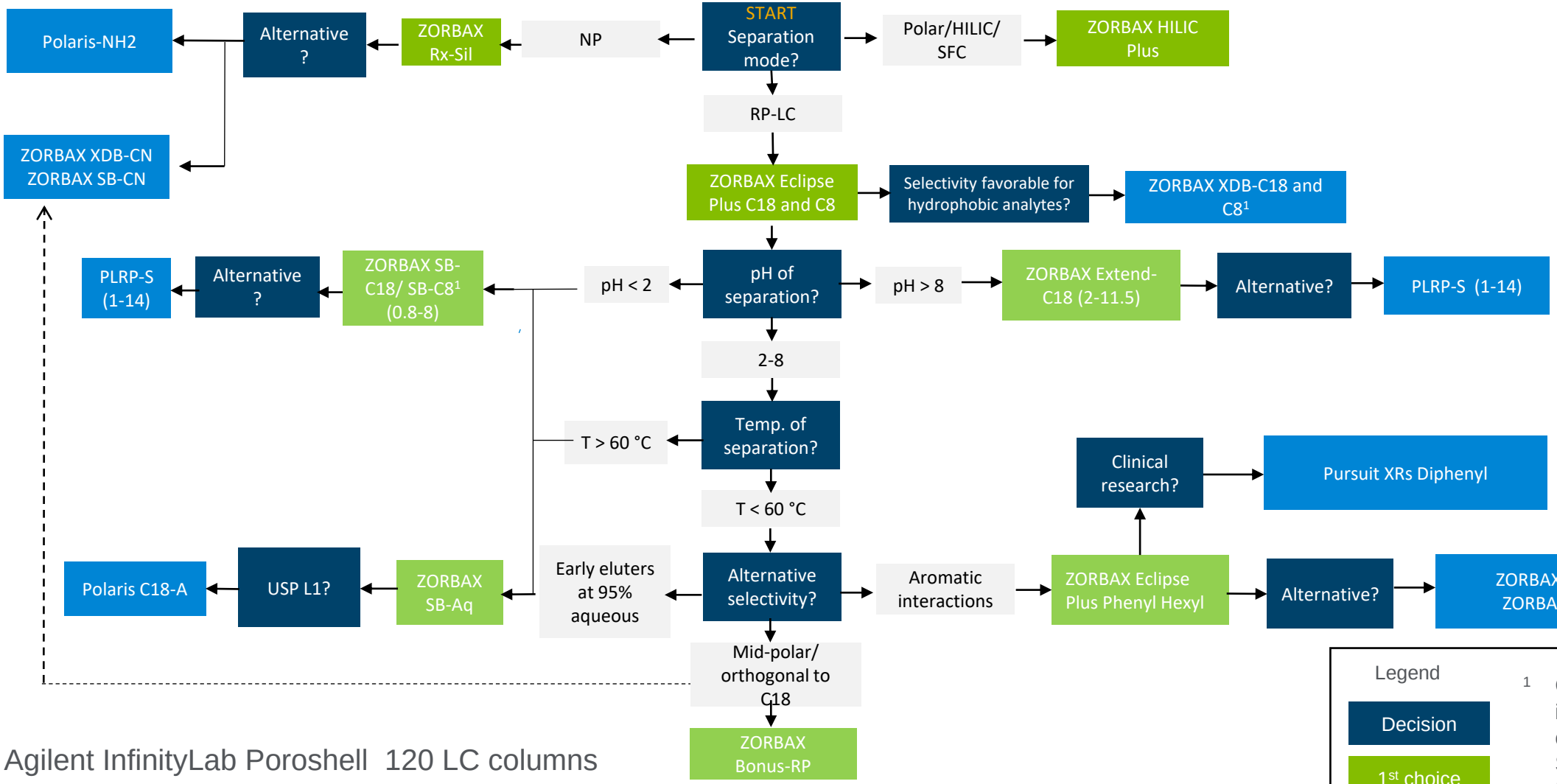
- Decision
- 1st choice
- Alternative

1 C8 phases have nearly identical selectivity compared to C18. Suitable for faster analysis times when sufficient resolution and retention is achieved.

2 Bare silica column. Shipped in RP solvents. For NP use, please properly flush out shipping solvent before.

Agilent InfinityLab Poroshell 120 LC columns
General selection guide
Publication number 5994-1982EN

ZORBAX, Pursuit, Polaris, PLRP-S



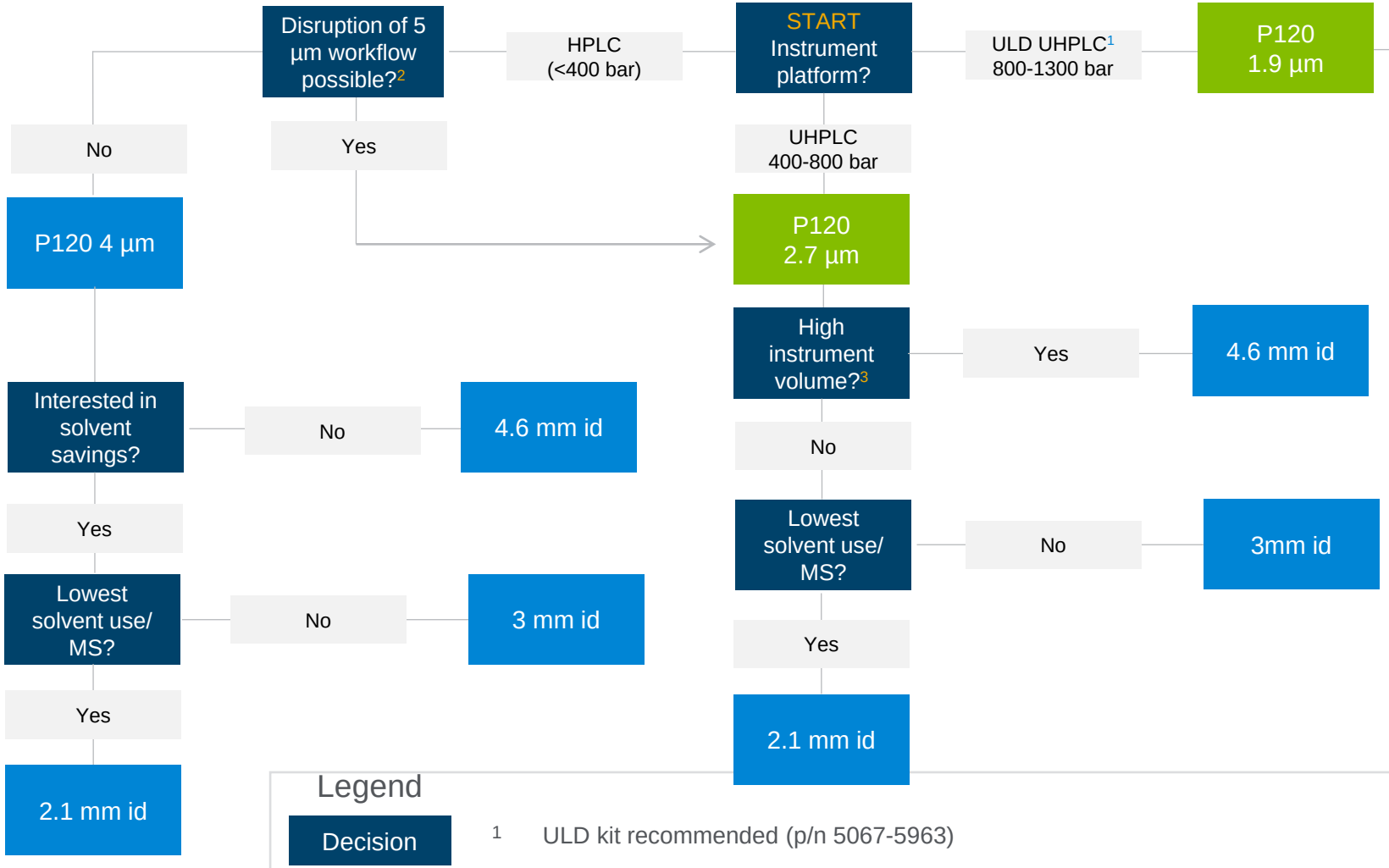
Agilent InfinityLab Poroshell 120 LC columns
General selection guide
Publication number 5994-1982EN

Legend

- Decision
- 1st choice
- Alternative

[1] C8 phases have nearly identical selectivity compared to C18. Suitable for faster analysis times when sufficient resolution and retention is achieved.

Particle Size and Dimension: Poroshell 120



Particle Size	ID	Optimum Flow
1.9 µm	2.1 mm	0.4 – 0.5 mL/min
	3.0 mm	0.8 – 1 mL/min
2.7 µm	2.1 mm	0.4 – 0.5 mL/min
	3.0 mm	0.8 – 1 mL/min
	4.6 mm	1.5 – 2 mL/min
4 µm	3.0 mm	0.5 – 0.75 mL/min
	4.6 mm	1 – 1.25 mL/min

Column Length	Recommended Use
50	High speed
100	High resolution
>=150	Ultrahigh resolution

Legend

Decision

1st choice

Alternative

1 ULD kit recommended (p/n 5067-5963)

2 Not possible with regulated gradient methods, not recommended for lab technicians that lack experience with UHPLC

3 Delay and dispersion volume. For example, 0.17 mm id tubing or bigger + 10 mm classic flow cell, valves, long tubing connections, old mixer design