How Do I Choose?

A guide to HPLC column selection

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Columns and Supplies Technical Support
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Agilent InfinityLab

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Overview

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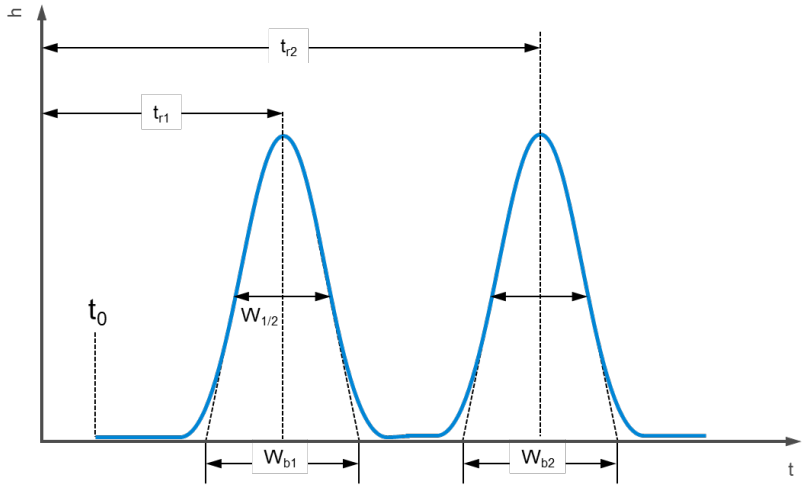
How do I choose?

- Efficiency
  - Particle size
  - Column length
- Selectivity
  - Bonded phase
  - Mobile phase
- Retention
  - Polar bonded phase
  - HILIC



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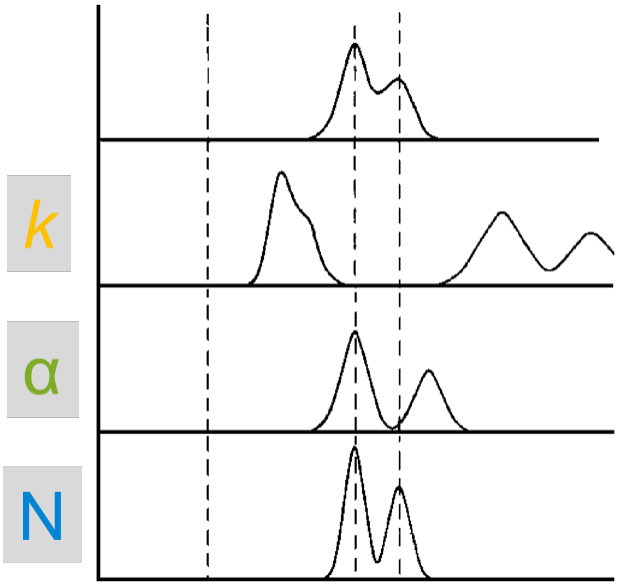
Resolution Equation

$$R_s = \underbrace{\frac{1}{4}\sqrt{N}}_{\text{Efficiency}} \cdot \underbrace{\left(\frac{\alpha - 1}{\alpha}\right)}_{\text{Selectivity}} \cdot \underbrace{\left(\frac{k}{1+k}\right)}_{\text{Retention}}$$



Improve resolution by improving any of these parameters:

- Efficiency describes the separation power of the column.
- Selectivity has the highest influence on the resolution. Small changes in selectivity can lead to big changes in resolution.
- Retention has only a significant influence at small k values.

Efficiency and Retention Factor

$$N \propto \frac{L}{\mathrm{d}_p}$$

Parameters influencing column **efficiency**:

- Column length (increasing column length increases efficiency)
- Particle size (decreasing particle size increases efficiency)

$$k = \frac{(t_R - t_0)}{t_0}$$

t_R = retention time for sample peak

t_0 = retention time for unretained peak

The **retention factor** measures the period of time that the sample component resides in the stationary phase relative to the time it resides in the mobile phase. It is calculated from the retention time divided by the time for an unretained peak.

Agilent's Small Molecule LC Columns

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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 LC

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

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

Higher overall retention, especially for early eluters, accepts larger amounts of strong solvent during injection

Scalable phases that range from UHPLC to HPLC to research scale prep

Scalable from UHPLC to HPLC to Prep-LC with higher retention.

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 non-polar	Polaris C18-Ether, C18 Amide, NH2

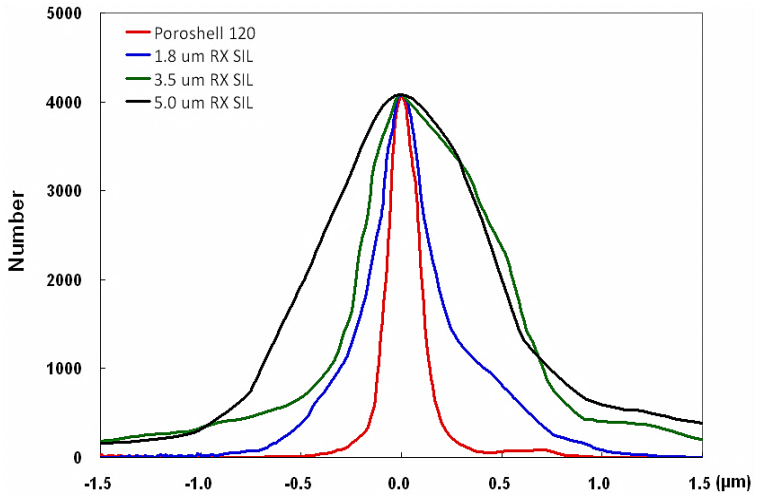
Unique chemistries that help to solve non-standard applications from HPLC to Prep.

Making a Poroshell Particle

Strict monitoring of every production step ensures column performance and quality

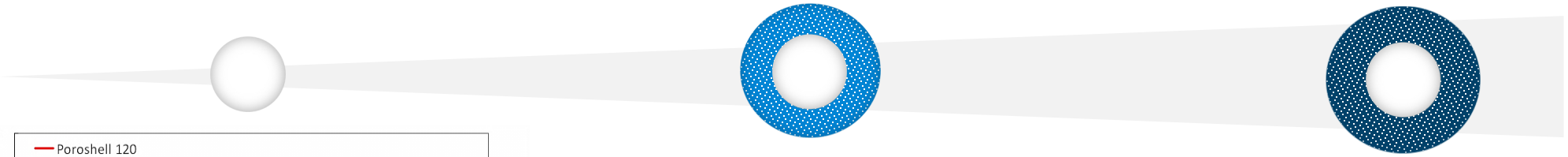
Step 1: Make the solid core

Poroshell 120 column cores have a very smooth surface and a uniform particle size which contributes to a tight overall particle size distribution. As a result, you get a more tightly packed column bed and therefore a better lifetime.



Step 2: Apply the porous shell

In contrast to other manufacturers, Agilent applies the porous shell in one single step. This unique single-step process delivers better column-to-column reproducibility.



Step 3: Apply the bonded phase

Most of the Poroshell chemistries are bonded in a single step. This further increases batch-to-batch reproducibility and method scalability from 1.9 to 2.7 to 4 µm.

Poroshell Particles

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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)

Column Type Particle	Traditional HPLC 4–5 µm	UHPLC 2.7 µm (SPP) / < 2 µm (FPP)				Low Dispersion UHPLC < 2 µm			
Recommended product (Max pressure / bar)	**1. 4 µm Poroshell (600)** 2. 3.5 and 5 µm ZORBAX (400) 3. 3 and 5 µm Pursuit, Polaris, HC/TC (400)	**1. 2.7 µm Poroshell (600)** 2. 1.8 µm ZORBAX RRHT (600)				**1. 1.9 µm Poroshell (1300)** 2. 1.8 µm ZORBAX RRHD (1200)			
Column length (mm)	50–300	Short: 30–50		Long: 100–150		Short: 30–50		Long: 100–150	
Column id (mm)	3.0–4.6	2.1	3.0–4.6	2.1	3.0–4.6	2.1	3.0	2.1	3.0
1300 bar Low Dispersion UHPLC – High Speed Pump (1290 Infinity II)	H/I								
1300 bar Low Dispersion UHPLC – Flexible Pump (1290 Infinity II)	H/I					V			
800 bar UHPLC – Quaternary Pump (1260 Infinity II Prime)	H/I					V		P	P
600 bar UHPLC – Binary Pump (1260 Infinity II)		V				V+P	V+P	V+P	P
600 bar UHPLC – Quaternary Pump (1260/1220 Infinity II)		V	V	V		V+P	V+P	V+P	V+P
400 bar HPLC (1100, 1260/1220 Infinity II VL)		V	V	V+P	P	V+P	V+P	V+P	V+P

400 bar = 6000 psi
600 bar = 9000 psi
1200 bar = 17000 psi
1300 bar = 19000 psi

Limitations
V – System volume (dispersion/delay)
P – Pressure limits
V+P – System volume and pressure
H/I – if instrument is used for HPLC methods / ISET emulation

Recommended
Acceptable
Limited Configurations
Not Recommended

Particle Size and Dimension: P120

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

Legend

decision

1st choice

alternative

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

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

3 Delay and dispersion volume. E.g., 0.17 mm ID tubing or bigger + 10 mm classic flow cell, valves, long tubing connections, old mixer design

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

Scalability

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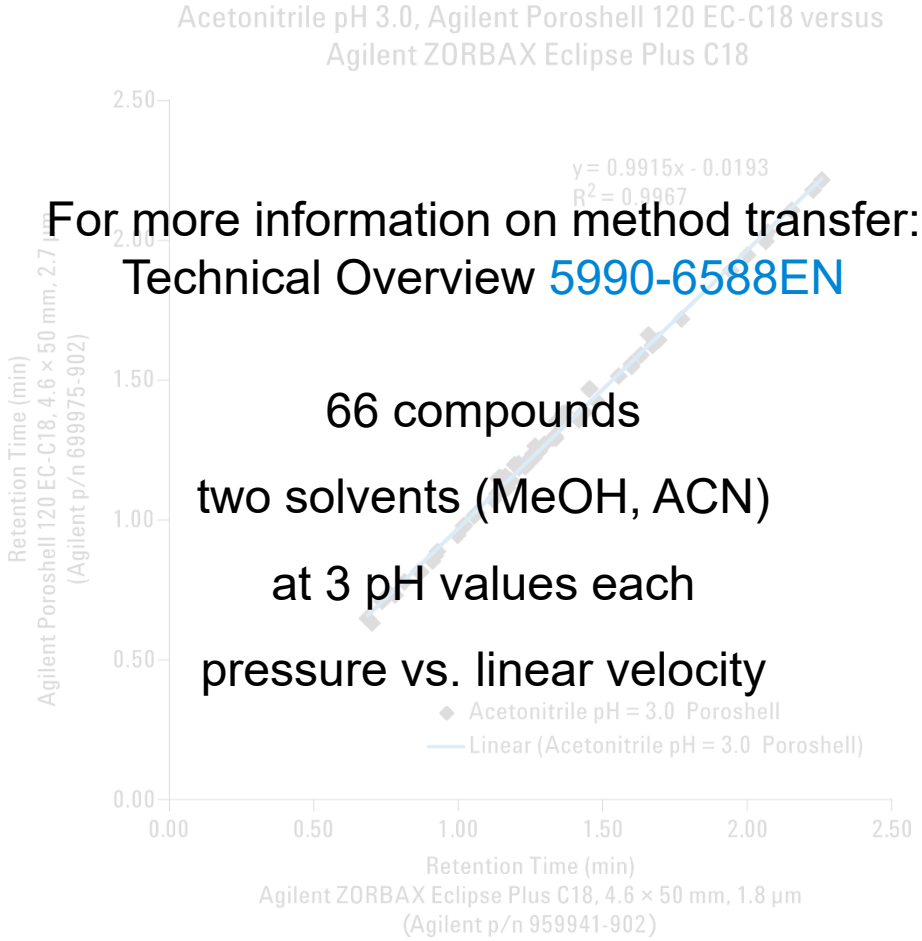
Method Transferability Across Product Families

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

66 compounds

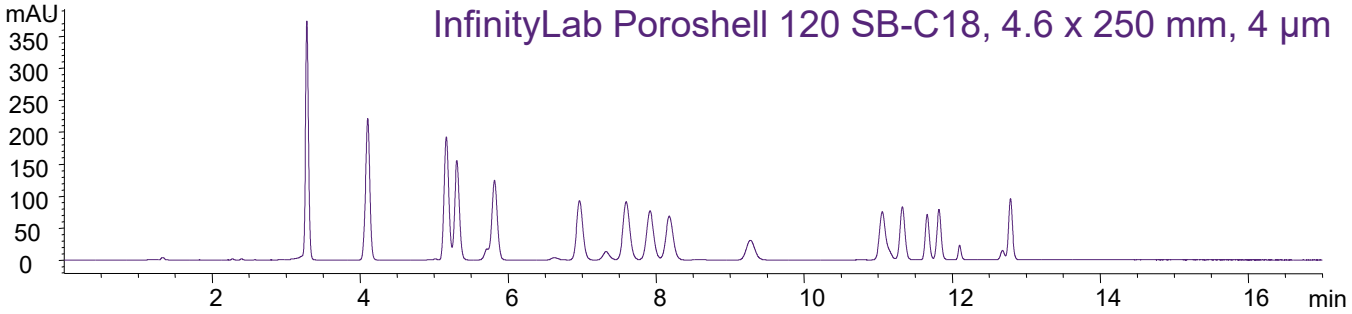
two solvents (MeOH, ACN)

at 3 pH values each

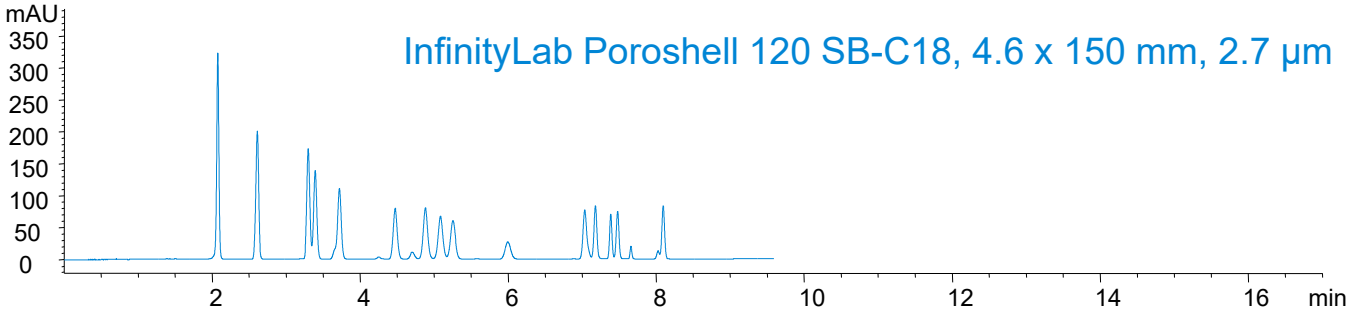
pressure vs. linear velocity

Scalability

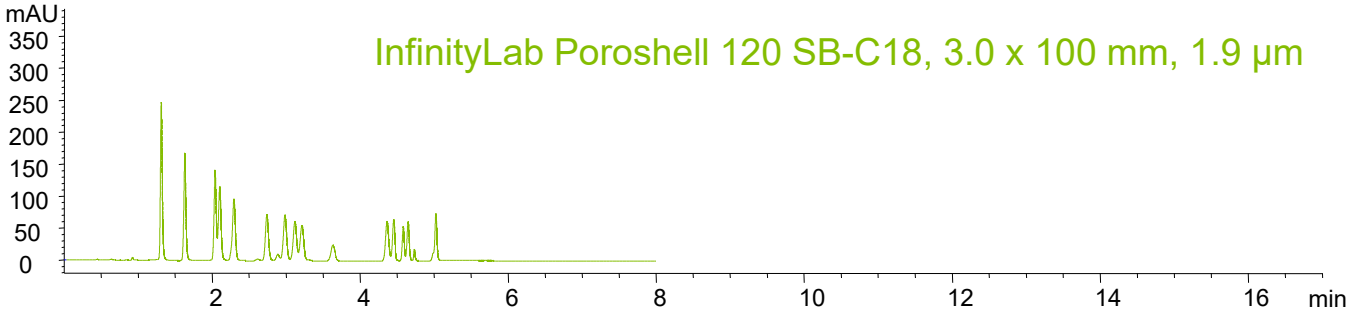
An example of scalability between particle sizes



HPLC (4 µm)	Value	Difference
Run time	14 min	--
Response / injection volume	80 mAU / µL	--
Solvent consumption	21 mL	--
Samples per 8 h day	**24**	**--**



UHPLC (2.7 µm)	Value	Difference
Run time	8.75 min	- 37.5%
Response / injection volume	113 mAU / µL	+ 41%
Solvent consumption	13.1 mL	- 37.5%
Samples per 8 h day	**48**	**+24**



LD UHPLC (1.9 µm)	Value	Difference
Run time	5.25 min	- 62.5%
Response / injection volume	295 mAU / µL	+ 269 %
Solvent consumption	3.36 mL	- 84 %
Samples per 8 h day	**80**	**+56**

Scalability

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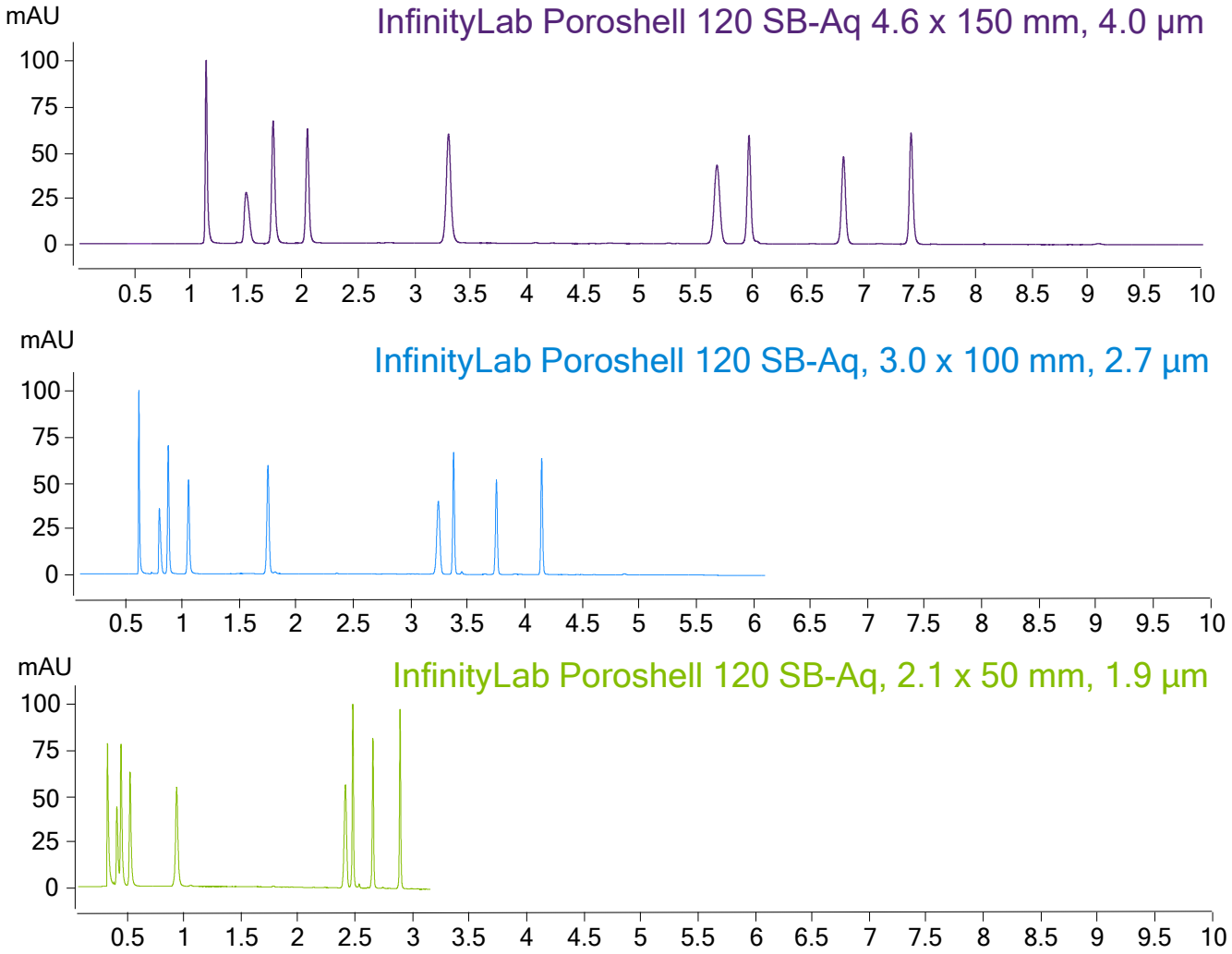
Scaling Water-Soluble Vitamins on InfinityLab Poroshell 120 SB-Aq



HPLC (4 µm)	Value	Difference
Run time	8 min	--
Response / injection volume	83.3 mAU / µL	--
Solvent consumption	12 mL	--
Samples per 8 h day	**48**	--

UHPLC (2.7 µm)	Value	Difference
Run time	4.5 min	- 44.8%
Response / injection volume	250 mAU/ µL	+200%
Solvent consumption	4.5 mL	-43.8%
Samples per 8 h day	**80**	**+ 32**

LD UHPLC (1.9 µm)	Value	Difference
Run time	3.1 min	- 61.3%
Response / injection volume	800 mAU / µL	+900%
Solvent consumption	1.55 mL	-87.1%
Samples per 8 h day	**145**	**+ 97**

Increase Throughput with Ultrafast Separations

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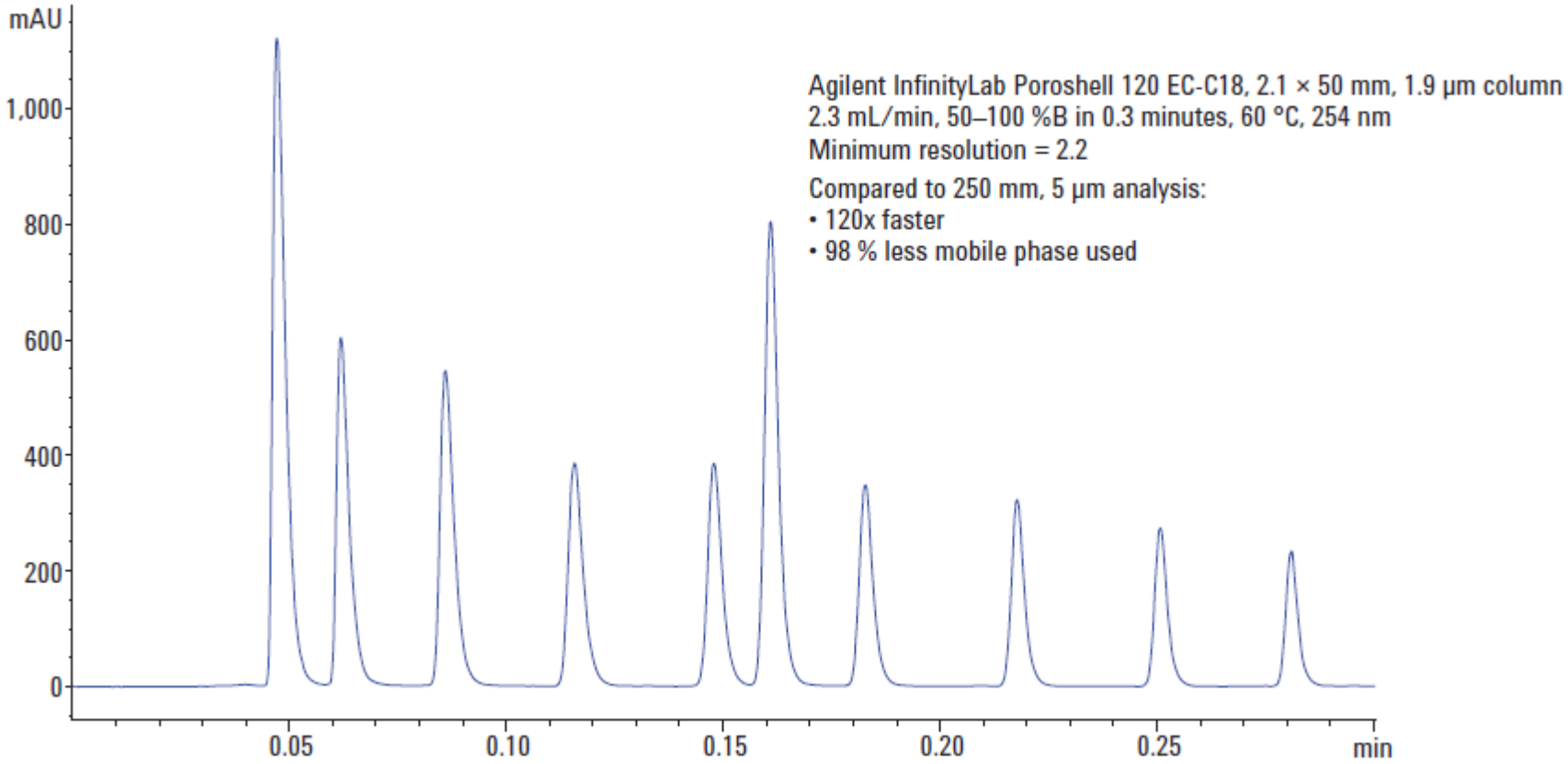
Modern columns help to increase the number of samples measured per day

High throughput UHPLC
at 1150 bar and 60 °C



Increase Throughput with Ultrafast Separations

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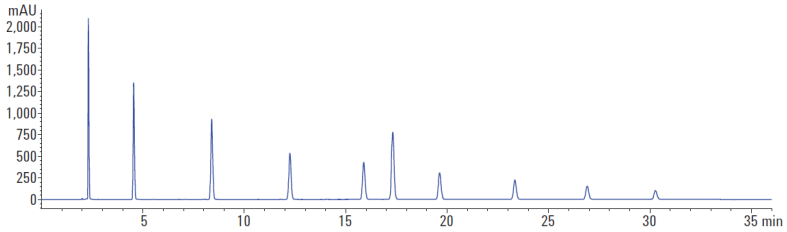
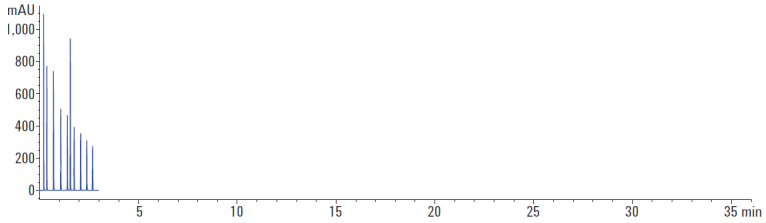
Increase the amount of samples analyzed per day

Traditional HPLC



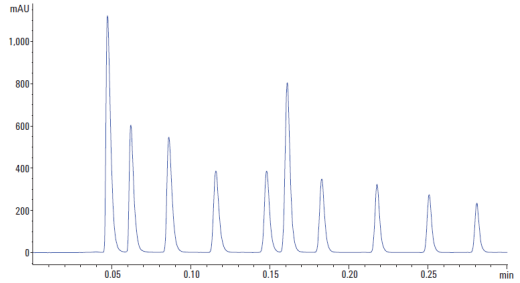
- ZORBAX Eclipse Plus C18, 5 µm
- 36 min runtime

UHPLC



- Poroshell 120 EC-C18, 1.9 µm
- 3 min runtime (12 x faster)
- 96% less solvents used
- 95% less sample injected

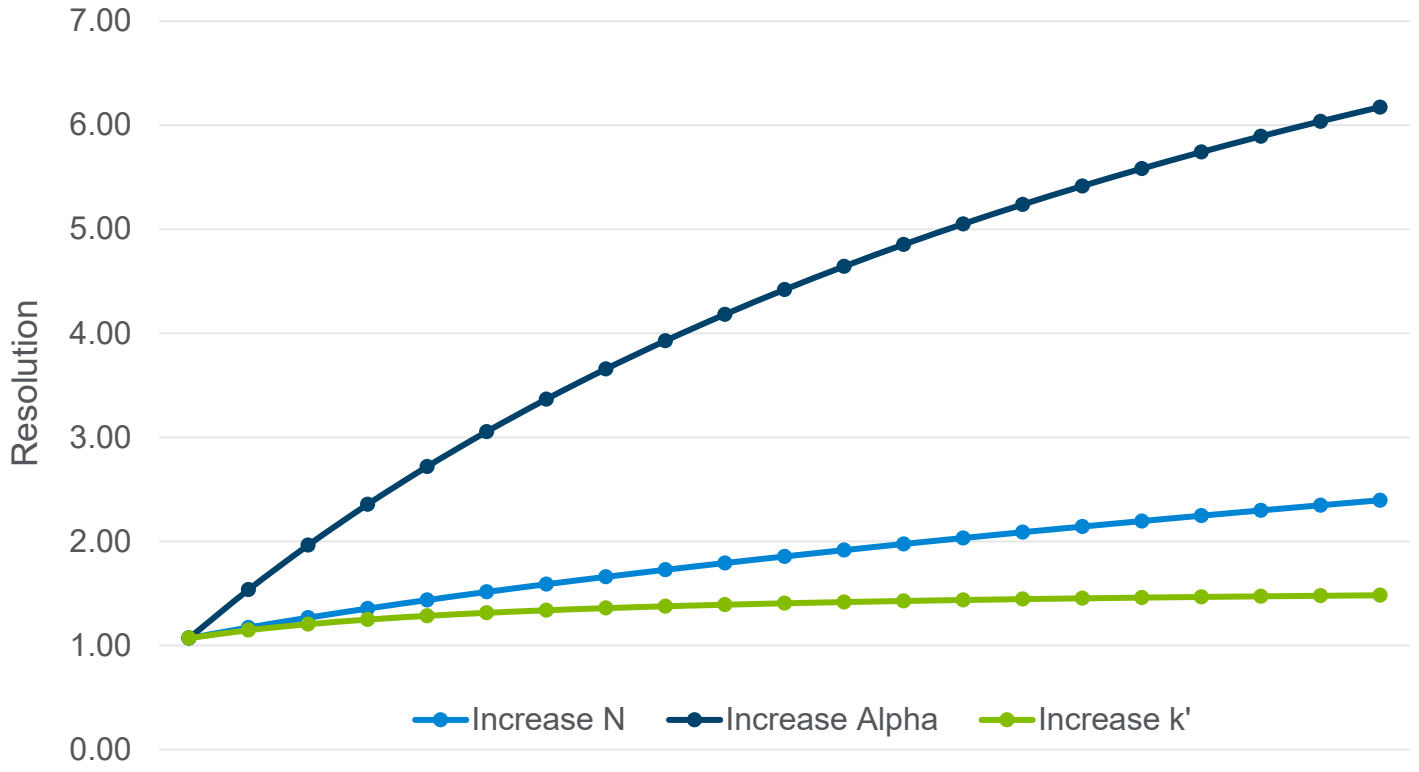
Ultrafast UHPLC



- Poroshell 120 EC-C18, 1.9 µm
- 0.3 min runtime (120 x faster)
- 98% less solvent

Selectivity

Selectivity impacts the resolution most



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

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

Selectivity impacts resolution

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

The InfinityLab Poroshell 120 Portfolio

Agilent InfinityLab

Agilent Poroshell columns are designed for multiple separation modes

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 [A] 1.9 µm, 2.7 µm, 4 µm	**SB-C18** [A] 1.9 µm, 2.7 µm, 4 µm	**HPH-C18** [A] 1.9 µm, 2.7 µm, 4 µm	**Bonus-RP** [A,B] 2.7 µm	**SB-Aq** [A,B] 1.9 µm, 2.7 µm, 4 µm	**Chiral-V** [A,C,D] 2.7 µm
EC-C8 [A] 1.9 µm, 2.7 µm, 4 µm	**SB-C8** [A] 2.7 µm	**HPH-C8** [A] 2.7 µm, 4 µm	**PFP** [A,B,D] 1.9 µm, 2.7 µm, 4 µm	**EC-CN** [A,B,C,D] 2.7 µm	**Chiral-T** [A,C,D] 2.7 µm
Phenyl-Hexyl [A] 1.9 µm, 2.7 µm, 4 µm		**CS-C18** [A] 2.7 µm		**HILIC** [C,D,E] 1,9 µm, 2.7 µm, 4 µm	**Chiral- CD** [A,C,D] 2.7 µm
				HILIC-Z [C,D,E] 1.9 µm, 2.7 µm, 4 µm	**Chiral-CF** [A,C,D] 2.7 µm
				HILIC- OH5 [C,D,E] 2.7 µm	

Legend
- [A] reversed phase
- [B] can be operated at 100% aqueous
- [C] Normal phase
- [D] SFC
- [E] HILIC

Chemistries with Unique Selectivity

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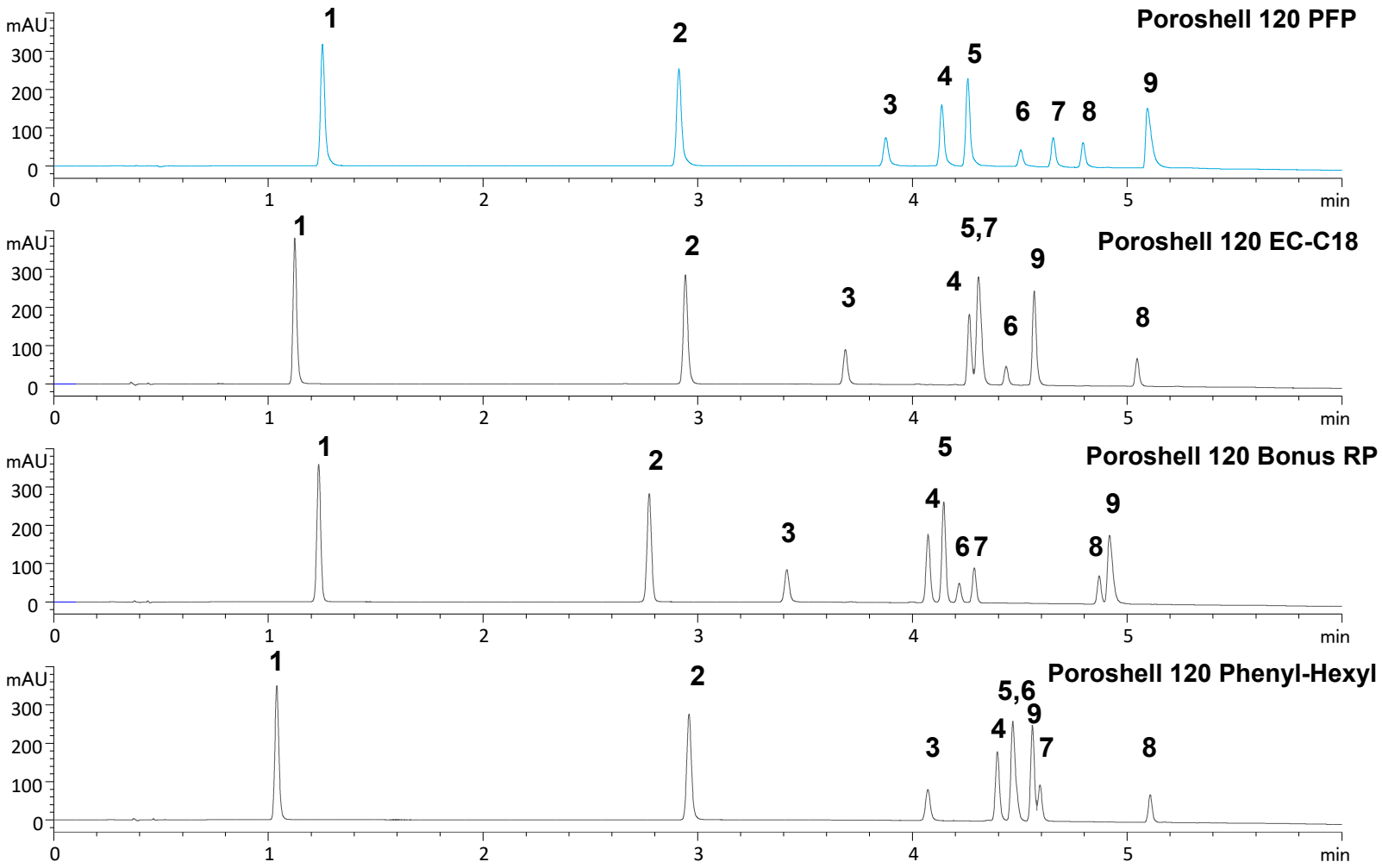
The influence of stationary phase on selectivity and resolution



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

Stationary Phase Selection Guide

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Starting Recommendation

Poroshell 120 EC-C18

Change Selectivity Slightly	Change Selectivity Significantly	For Many Early Eluters	No retention at 98+% Aqueous in RP	Acidic Solvents (pH < 2)	Basic Solvents (pH >6)
1. Poroshell Phenyl-Hexyl 2. Polaris C18-A 3. Poroshell EC-C8	1. Poroshell Bonus-RP 2. Poroshell PFP 3. Pursuit XRs Diphenyl	1. Poroshell SB-Aq 2. Poroshell PFP 3. Poroshell HILIC-Z	1. Poroshell HILIC-Z 2. Poroshell PFP 3. Poroshell HILIC-OH5	1. Poroshell SB-C18 2. Poroshell SB-Aq 3. PLRP-S 4. Poroshell SB-C8	1. Poroshell HPH-C18 2. PLRP-S 3. Poroshell CS-C18

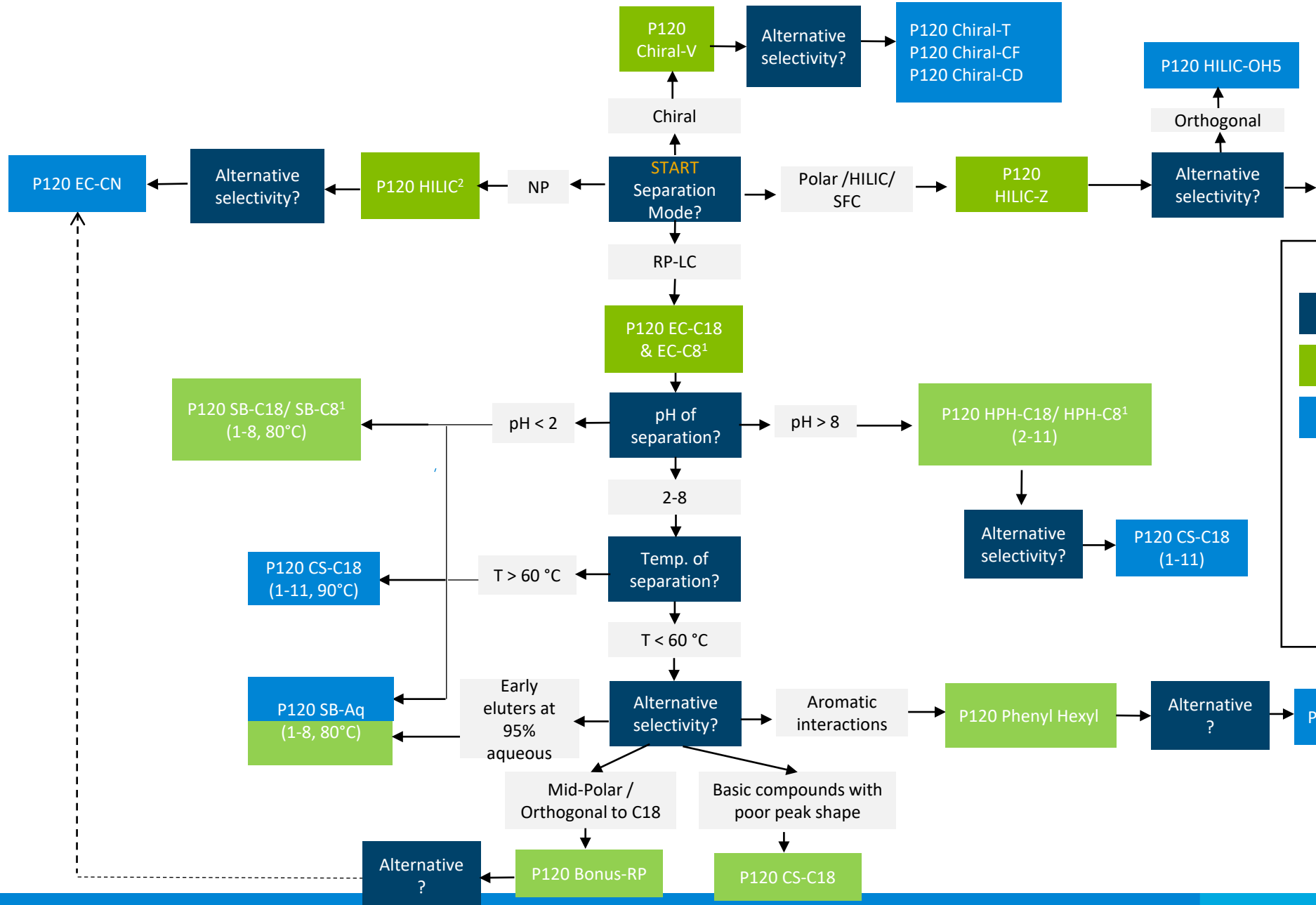
Top 3 to keep around (covers 95% of analyses)		
Poroshell EC-C18	Poroshell HILIC-Z	Poroshell PFP

Recommended Solvent A (Weak)	Solvent B (Strong)
1. 0.1% Formic Acid (pH ~2.7) 2. 10 mmol Ammonium Acetate (adj. pH 5) 3. 0.1% Ammonium Hydroxide (pH ~10) 4. 0.1% Trifluoroacetic acid (pH ~1.5, *no MS*) 5. 150 mmol Sodium Phosphate (adj. pH 3, *no MS*)	1. Acetonitrile 2. Methanol 3. Isopropanol 4. THF 5. Acetone

USP L1	USP L7
1. Poroshell 120 EC-C18 2. Polaris C18-A 3. Polaris C18-Ether 4. Pursuit XRs C18	1. Poroshell 120 EC-C8 2. Polaris C8-A
USP L3	**USP L8**
1. Poroshell 120 HILIC 2. ZORBAX Rx-Sil 3. Pursuit XRs Si	1. Polaris NH2 2. ZORBAX NH2
	USP L11
	1. Poroshell Phe-Hex 2. Pursuit XRs Diphenyl

Sugars (RI or ELSD)	Normal Phase	Chiral
1. Poroshell HILIC-Z 2. Hi-Plex H 3. Hi-Plex Ca 4. Polaris NH2	1. Poroshell HILIC 2. Poroshell EC-CN 3. Polaris NH2	1. Poroshell Chiral-V 2. Poroshell Chiral-T 3. Poroshell Chiral-CD 4. Poroshell Chiral-CF

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.

RP = reversed phase
NP = normal phase

Choosing Between C18s

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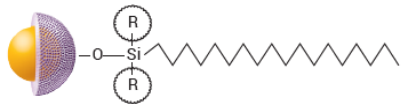
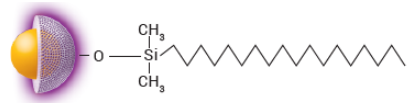
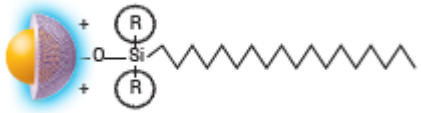
InfinityLab Poroshell 120	Chemistry	Pore Size	Endcapped	Carbon Load	Surface Area	Best For
EC-C18 1.9 µm, 2.7 µm, 4 µm		120 Å	Yes	10%	130 m2/g	General Purpose Excellent peak shape and efficiency for acids, bases, neutrals
SB-C18 1.9 µm, 2.7 µm, 4 µm		120 Å	No	9%	130 m2/g	Low pH Excellent stability and peak shape in highly acidic conditions
HPH-C18 1.9 µm, 2.7 µm, 4 µm		100 Å	Yes	Proprietary	95 m2/g	High pH capable Robust performance and long lifetimes
CS-C18 2.7 µm		100 Å	Yes	Proprietary	95 m2/g	Alternate selectivity Improved peak shape and sample capacity for basic compounds with low ionic strength mobile phases High pH capable

A pH Change Can Strongly Affect Selectivity

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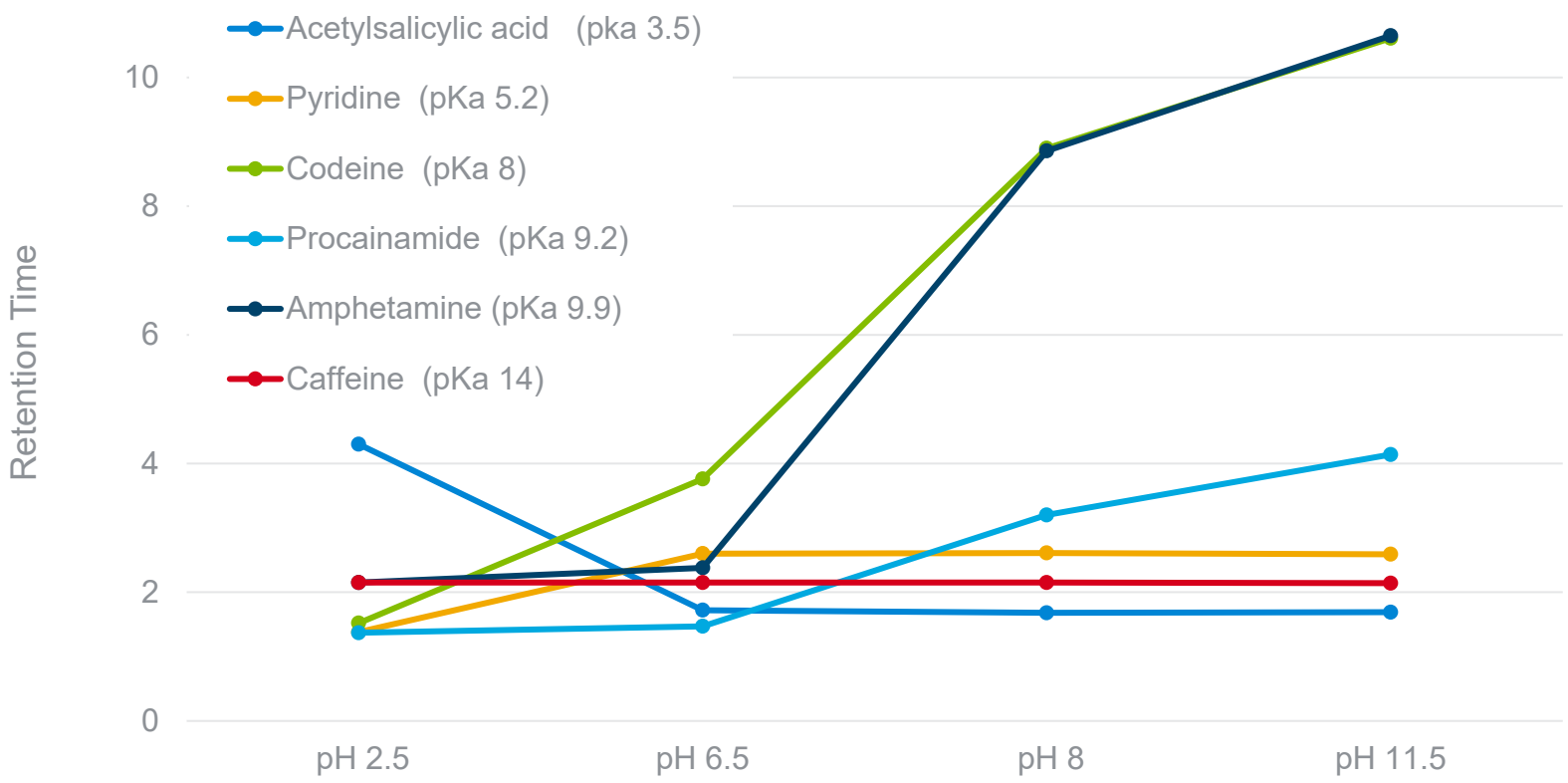
Mobile phase pH is a powerful method development tool for separating ionizable compounds

- In RPLC mode, ionizable analytes are more retained in their neutral state
- **Acids** are more retained at low pH
- **Bases** are more retained at high pH
- **Neutrals** are not impacted by mobile phase pH



Mobile phase: 45% Methanol, 55% 20 mM Phosphate Buffer

Selectivity Can be Controlled by Changing pH

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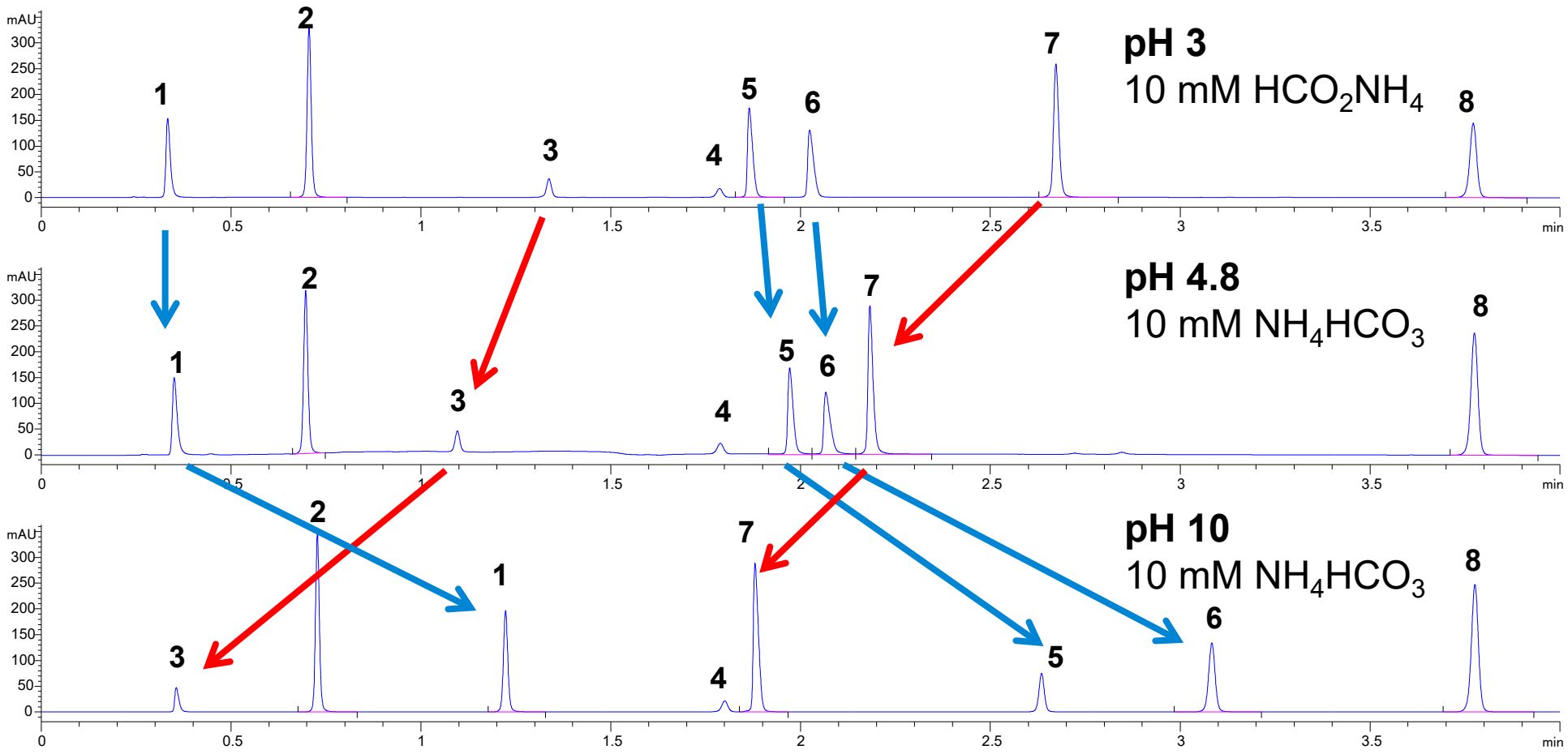
Agilent InfinityLab Poroshell HPH-C18 4.6 x 50 mm, 2.7 µm

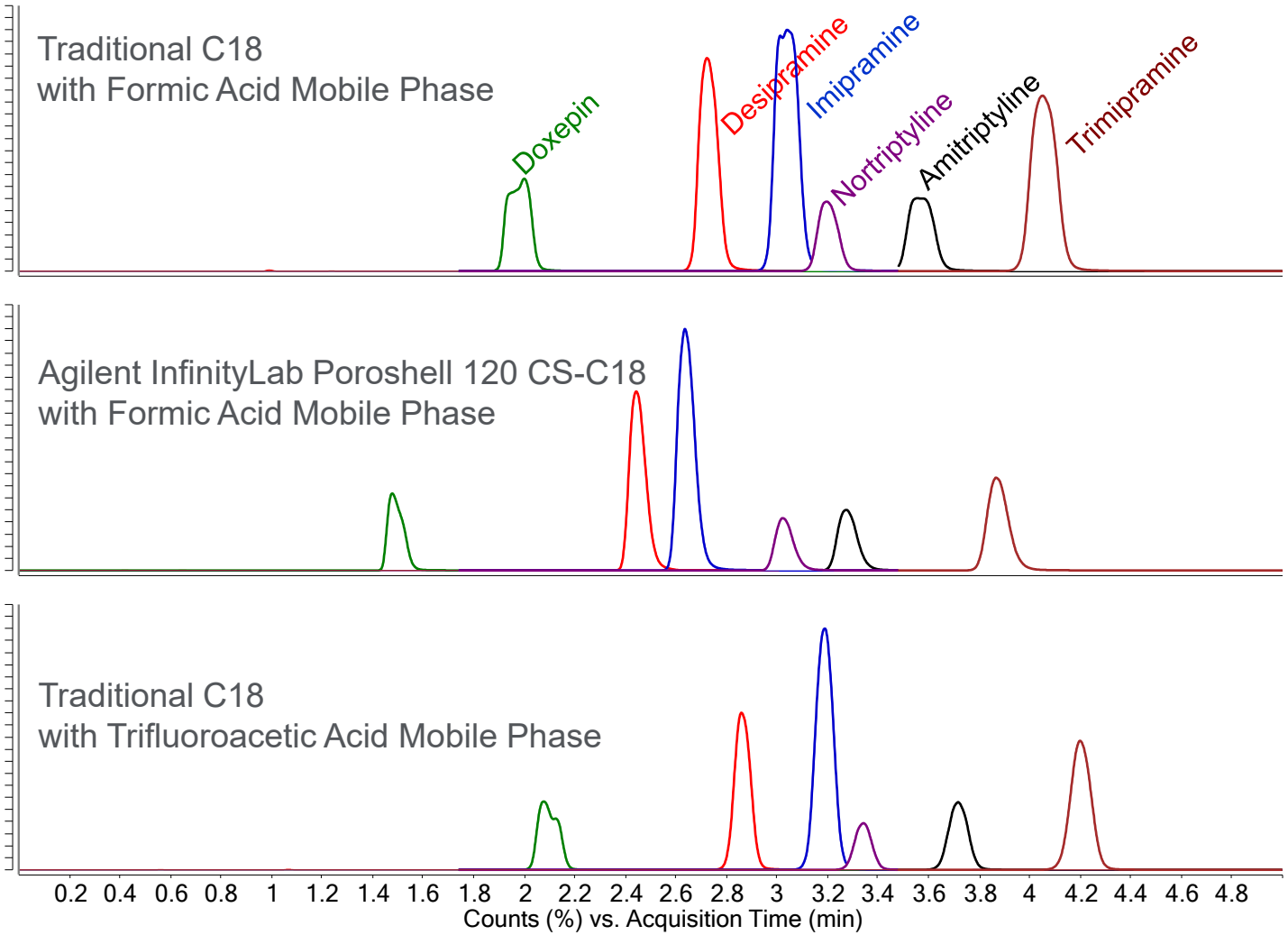


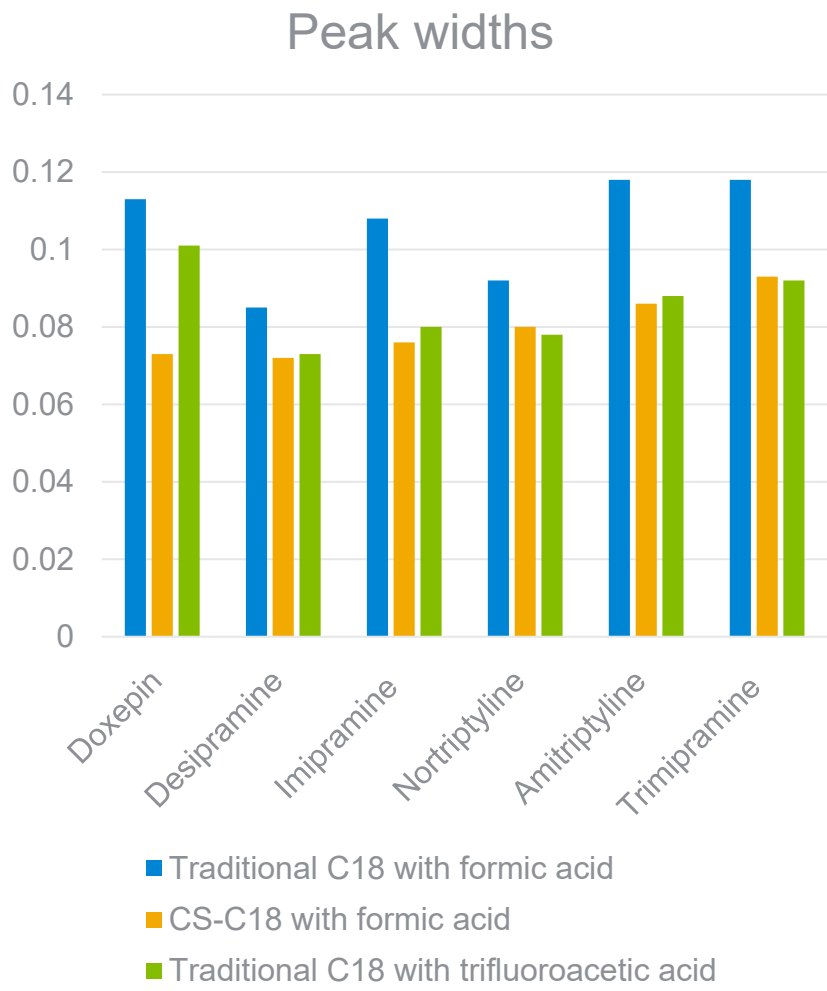
1. Procainamide
2. Caffeine
3. Acetyl Salicylic Acid
4. Hexanophenone Deg.
5. Dipyrimadole
6. Diltiazem
7. Diflunisal
8. Hexanophenone

Time	% Buffer	% MeCN
0	10	90
5	90	10
7	10	90
2 ml/min		254 mn

Acids
Bases

Agilent InfinityLab Poroshell 120 CS-C18 Gives Better Peak Shape for Basic Analytes with Formic Acid Mobile Phase than a Traditional C18





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

Read more: Agilent application note: 5994-2095EN

InfinityLab Poroshell 120 CS-C18

Agilent InfinityLab

Hybrid end-capped 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

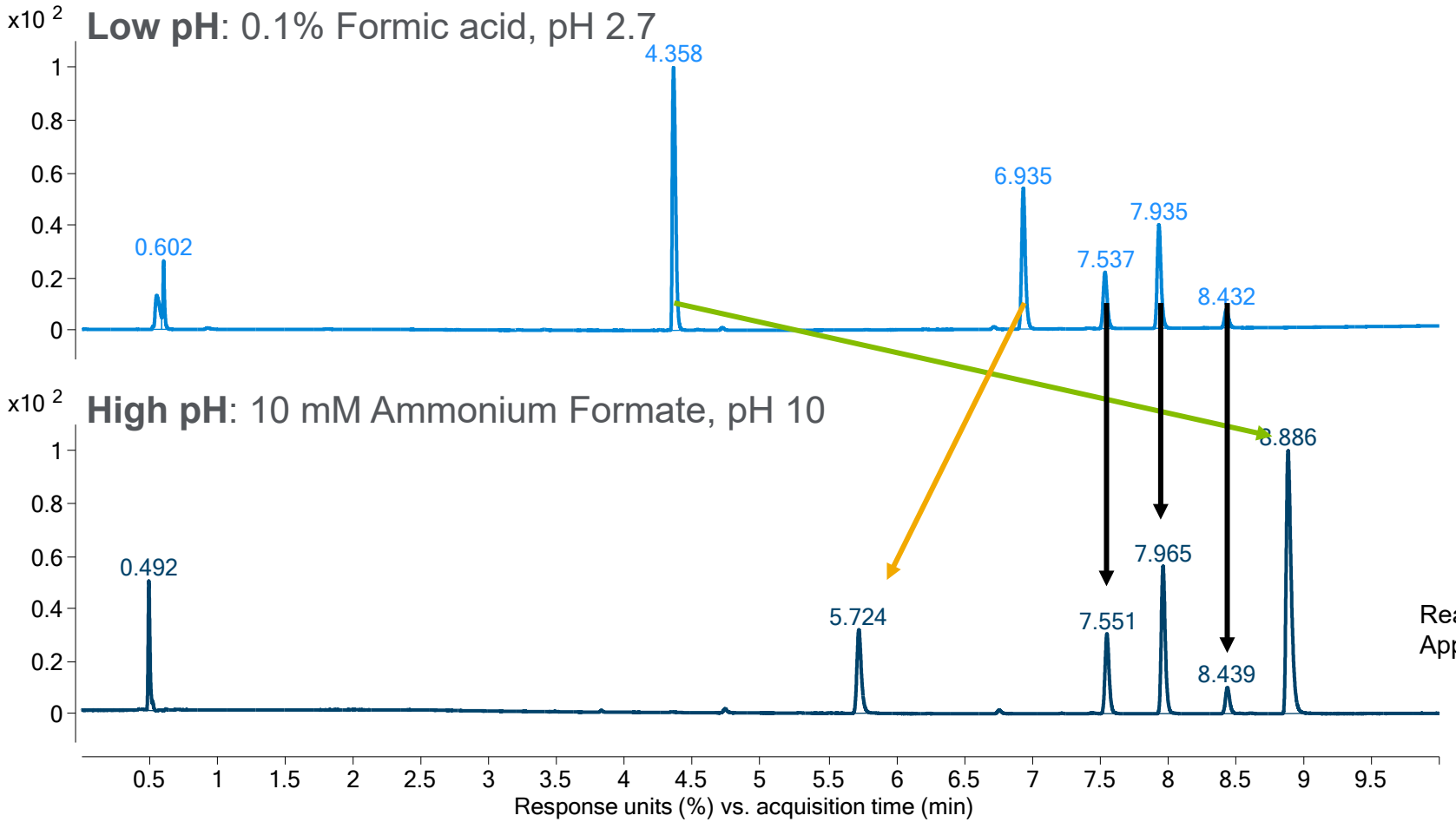
- 2.1, 3, 4.6 mm id x 50, 100, 150 mm length
- PEEK-lined options ★

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

A pH Change Can Strongly Affect Selectivity

Agilent InfinityLab

CS-C18 is another high-pH compatible L1 stationary phases

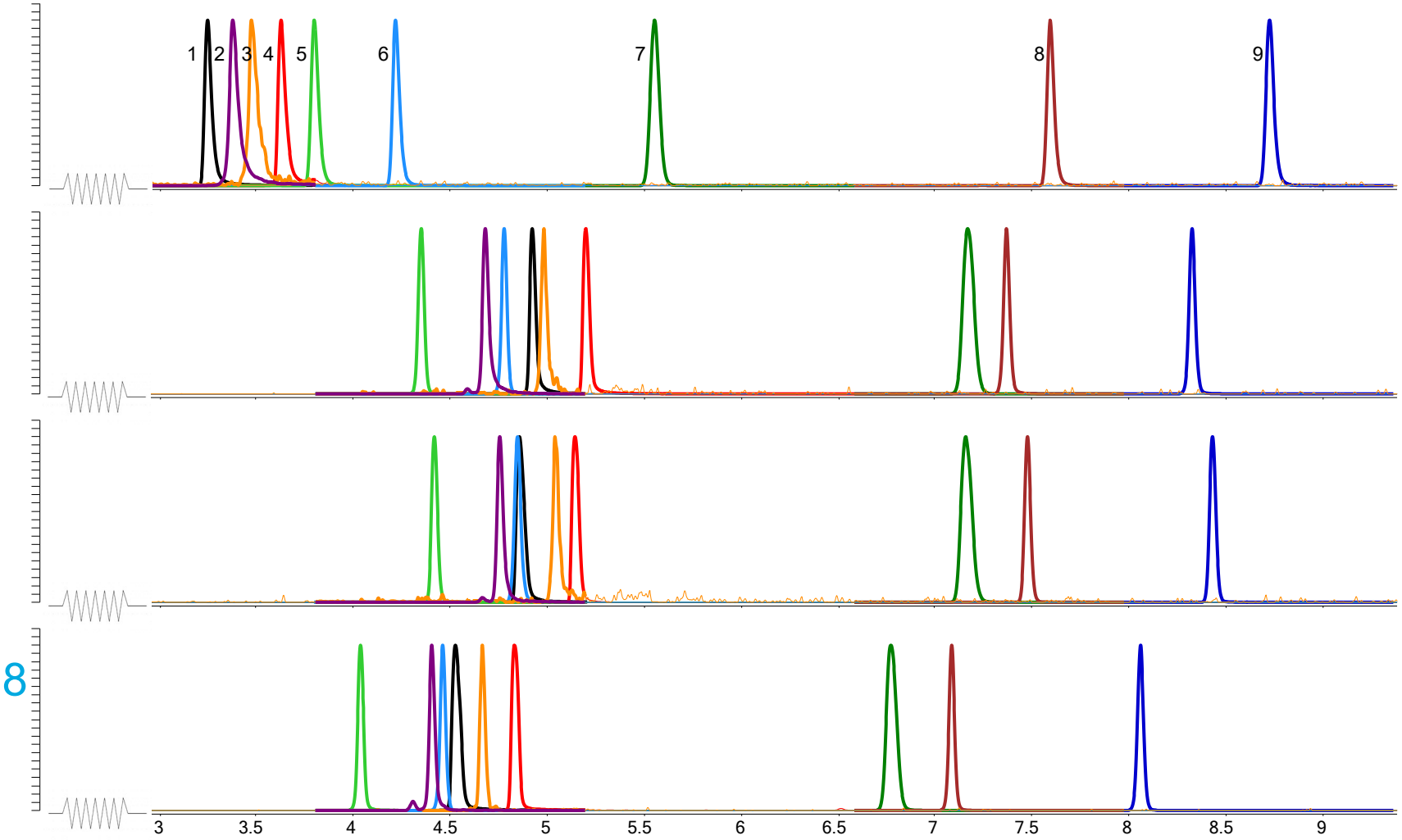


Read more:
Application Note 5994-2274EN

5-95% CH_3CN in 10 min, 4 min post run, mobile phase A varies, 0.4 mL/min, 2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 CS-C18, 30 °C, DAD: 254 nm, 80 Hz; Sample: uracil, amitriptyline, butyl paraben, dipropyl phthalate, acenaphthene

The Agilent InfinityLab Poroshell 120 CS-C18 Offers Alternative Selectivity to Other C18s to Facilitate Method development at Low pH

Agilent InfinityLab



Veterinary Drugs	
1	Ciprofloxacin
2	Oxytetracycline
3	Tetracycline
4	Enrofloxacin
5	Sulfamerazine
6	Sulfamethazine
7	Erythromycin
8	Penicillin-G
9	Oxacillin

Method Parameters:
A: 0.1% formic acid in water
B: acetonitrile
0.4 mL/min, 0-95% B in 15 min
0.05 µL injection
Sample: 0.1 mg/mL in water
Column: 30 °C, 2.1 x 100 mm, 2.7 µm
Detection: LC/MS, ESI+, dMRM

Agilent Application Note: 5994-2358EN

What is HILIC and When Should I Consider it?

Agilent InfinityLab

HILIC Complements RPLC

Reversed-phase LC		Hydrophilic interaction LC (HILIC)
Non-polar stationary phase (e.g., C18)	Polarity	Polar stationary phase (e.g., silica)
Polar mobile phase H_2O/CH_3OH, H_2O/CH_3CN	Mobile Phase	Polar mobile phase H_2O/CH_3CN
Decrease retention by decreasing polarity of mobile phase ddH2O ↓ = retention ↑ CH_3CN ↑ = retention ↓	Gradient	Retains hydrophilic (polar and ionized) compounds well and often reverses elution order vs RPLC ddH2O ↑ = retention ↓ CH_3CN ↓ = retention ↑
polar to non-polar	Elution Order	non-polar to polar

HILIC Method Development

Agilent InfinityLab

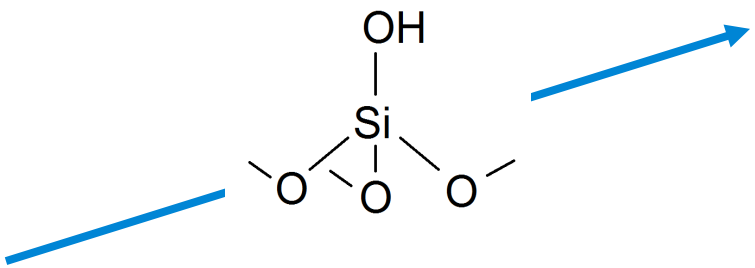
InfinityLab Poroshell 120 HILIC column options

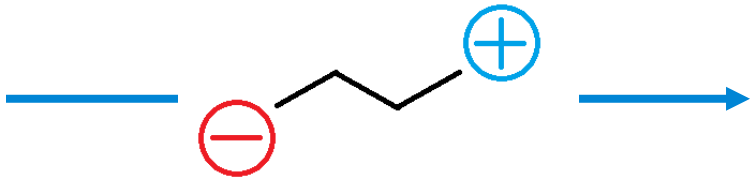
Best for polar analytes

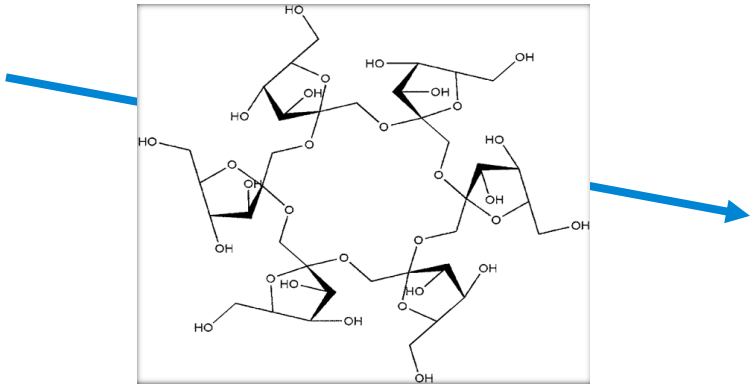
InfinityLab Poroshell
HILIC
1.9 µm, 2.7 µm, 4 µm

InfinityLab Poroshell
HILIC-Z
1.9 µm, 2.7 µm, 4 µm

InfinityLab Poroshell
HILIC-OH5
2.7 µm







HILIC

- Bare silica chemistry
- For very simple mixtures, low column bleed

HILIC-Z

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

HILIC-OH5

- Brushed fructan chemistry
- Alternative selectivity

HILIC Method Development

Agilent InfinityLab

Starting mobile phases

Mobile Phase A (Strong phase, H_2O):

- Typical buffer concentration: 5-30 mM
  - 10 - 20 mM is most common

Basic Analytes

- Ammonium Formate, pH 3
- Ammonium Acetate, pH 4–5

Acidic Analytes

- Ammonium Acetate, pH ~7
  - Ammonium acetate solution is near pH 7, before adjusting with other modifiers
  - Not a true buffer, but still commonly used at mid-pH
- Ammonium Acetate or Formate, pH 9–10
  - Can be formate or acetate because the ammonium ion is buffering
  - HILIC-Z only!

Sugars

- Ammonium Hydroxide, pH 10–11
  - HILIC-Z only!
- *Phosphate buffers are not recommended **

*Note: Phosphates have low solubility in high % ACN (1-30 mM). Always test solubility before running. Never run in >80% ACN to avoid precipitation.

Mobile Phase B (Weak phase, CH_3CN):

- Buffer concentration should match Mobile Phase A for improved reproducibility
- Adding 10% water in ACN generally recommended for improved solubility and faster re-equilibration
- Pure MeOH is too strong a solvent for most HILIC separations. Mixed with ACN in small quantities (<15%), it can be used to change selectivity slightly.

Example of mobile phase preparation:

Stock: 200 mM ammonium formate adjusted to pH 3 with formic acid

A: 900 mL water + 100 mL stock

B: 900 mL acetonitrile + 100 mL stock

Effect of pH on Retention of Acidic Compounds with HILIC

Agilent InfinityLab

Starting Mobile Phases

In HILIC mode, ionizable compounds are better retained when they are ionized

- Acids at high pH
- Bases at low pH

Once the analyte is fully ionized, retention should stabilize

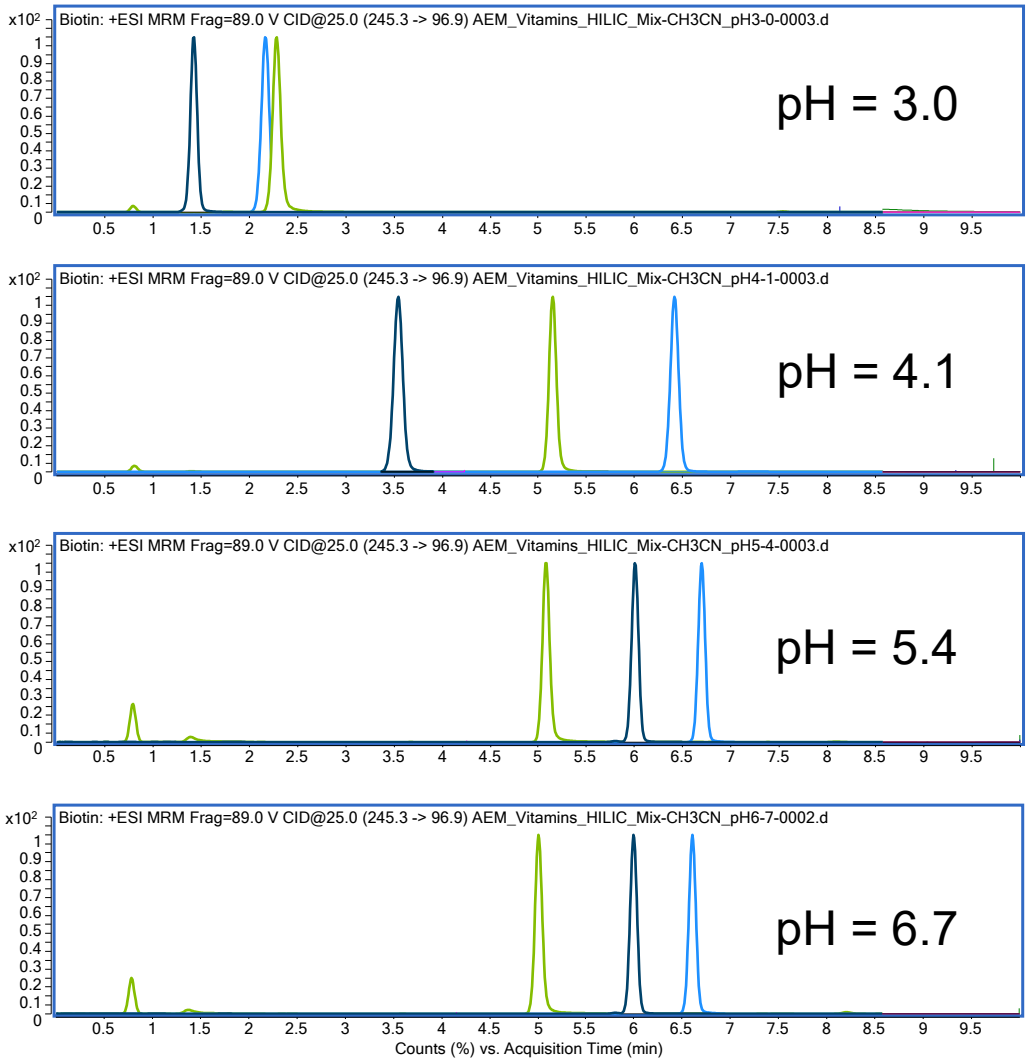
- Note: if other retention mechanisms are occurring, this may not be true

Biotin pKa = 4.5

Nicotinic acid pKa = 4.8

Pantothenic acid pKa = 4.3

Mobile Phase A: H_2O, B: CH_3CN, D: varies, 200 mM Ammonium Formate or Acetate; Flow Rate: 0.5 mL/min; Gradient: 95% B for 1 min, 95–65% B in 9 min, hold 5% D constant throughout analysis, 5 min post run; Injection: 0.5 µL of 13.3 µg/mL each in CH_3CN/H_2O 19:1; Column: 25 °C, 2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z; Detection: Ultivo TQ/MS ESI+ dMRM



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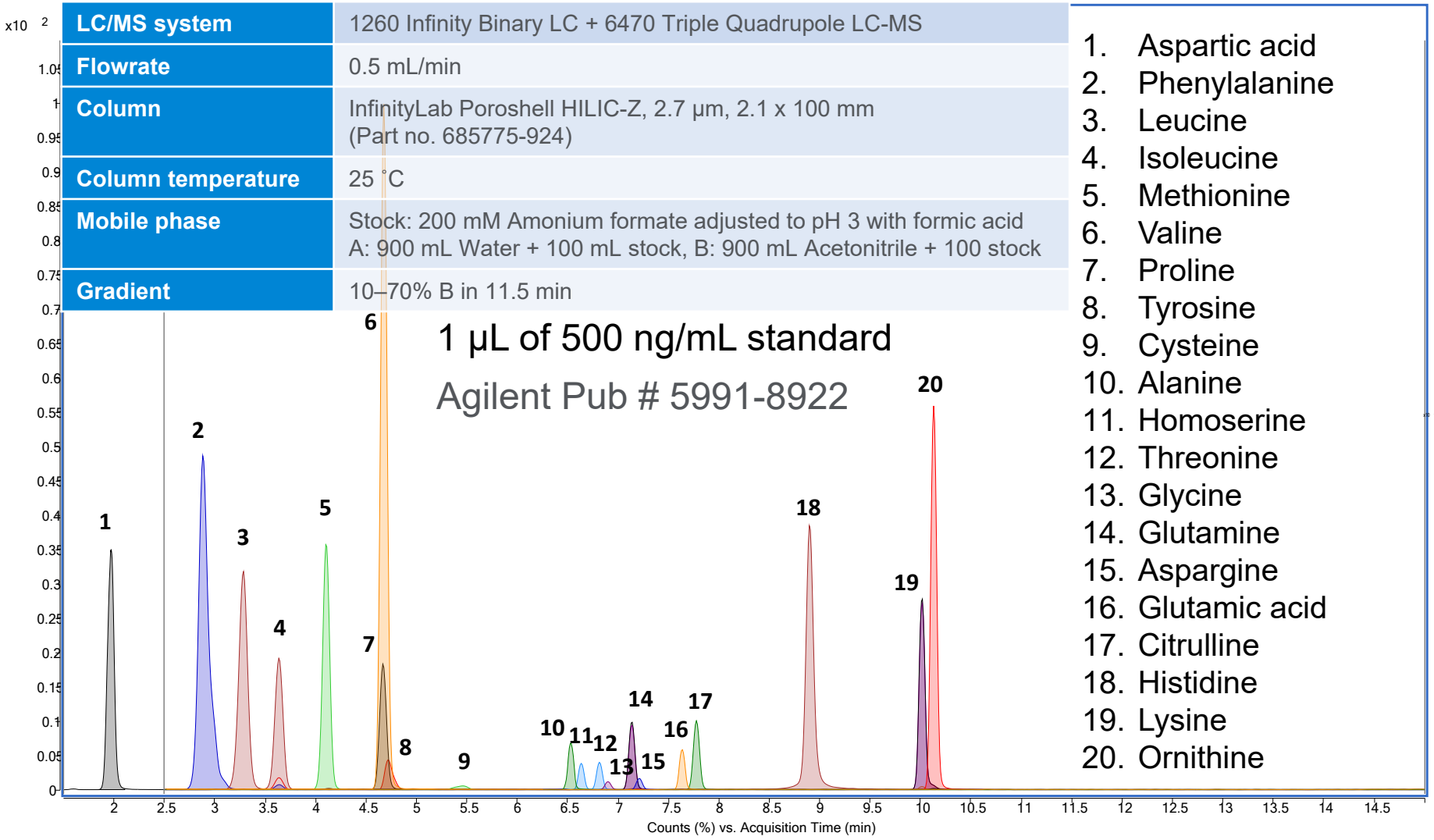
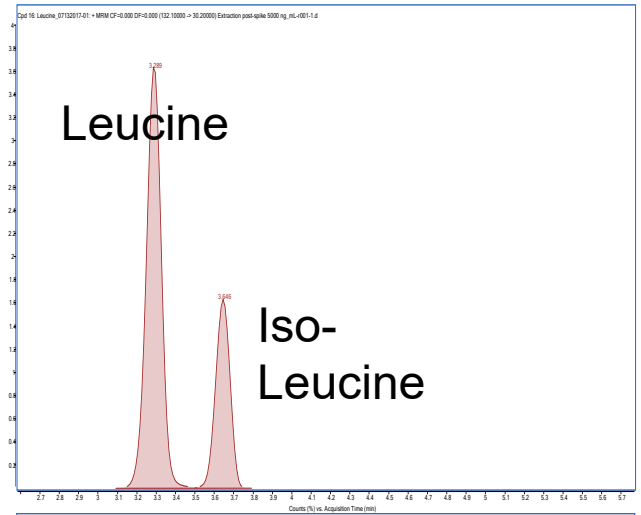
Analysis of Amino Acids (and Isobars) in Plant Tissue with LC-MS/MS

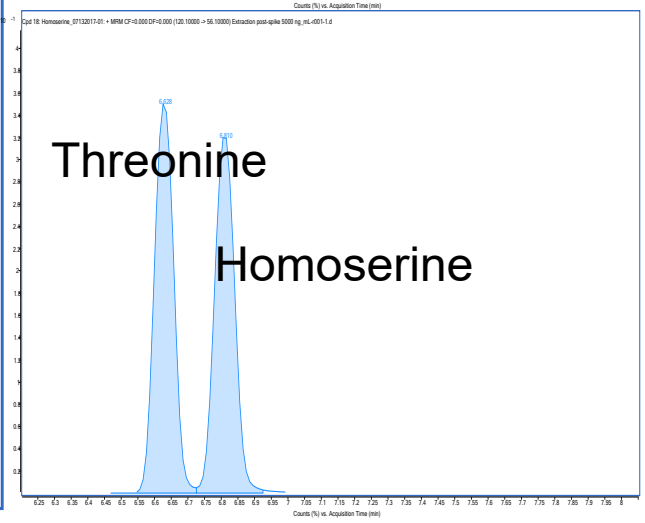
Agilent InfinityLab

LC/MS system	1260 Infinity Binary LC + 6470 Triple Quadrupole LC-MS
Flowrate	0.5 mL/min
Column	InfinityLab Poroshell HILIC-Z, 2.7 µm, 2.1 x 100 mm (Part no. 685775-924)
Column temperature	25 °C
Mobile phase	Stock: 200 mM Amonium formate adjusted to pH 3 with formic acid A: 900 mL Water + 100 mL stock, B: 900 mL Acetonitrile + 100 stock
Gradient	10–70% B in 11.5 min



1. Aspartic acid
2. Phenylalanine
3. Leucine
4. Isoleucine
5. Methionine
6. Valine
7. Proline
8. Tyrosine
9. Cysteine
10. Alanine
11. Homoserine
12. Threonine
13. Glycine
14. Glutamine
15. Aspargine
16. Glutamic acid
17. Citrulline
18. Histidine
19. Lysine
20. Ornithine





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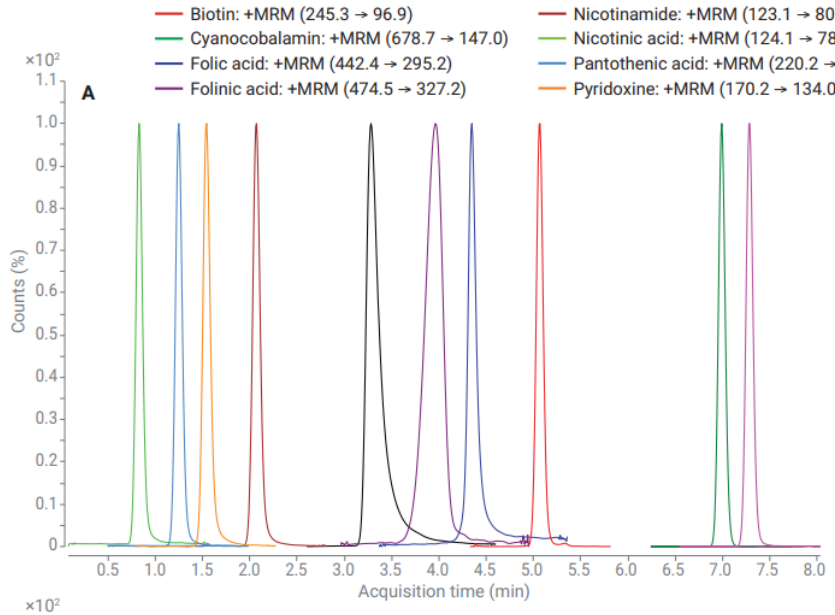
Reversed Phase versus HILIC



Mobile Phase A	H_2O
Mobile Phase B	CH_3CN
Mobile Phase D	200 mM ammonium acetate + 0.2% acetic acid, pH ~5.3
Flow Rate	0.5 mL/min
Gradient	0% B for 1 minute, 0 to 25% B in 8 minutes, hold 5% D constant throughout analysis, 3 minutes post run
Injection	0.5 µL of 0.4 µg/mL vitamin standard in H_2O
Column	25 °C, Agilent InfinityLab Poroshell 120 Phenyl-Hexyl, 2.1 × 100 mm, 2.7 µm
Detection	Agilent Ultivo TQ/MS ESI+ dMRM, DAD Sig = 260 nm, 80 Hz

Mobile Phase A	H_2O
Mobile Phase B	CH_3CN
Mobile Phase D	200 mM ammonium acetate (no pH adjustment), pH ~6.7
Flow Rate	0.5 mL/min
Gradient	95 to 65% B in 10 minutes, hold 5% D constant throughout analysis, 5 minutes post run
Injection	0.5 µL injection of 0.4 µg/mL vitamin standard in CH_3CN
Column	25 °C, Agilent InfinityLab Poroshell 120 HILIC-OH5, 2.1 × 100 mm, 2.7 µm
Detection	Agilent Ultivo TQ/MS ESI+ dMRM (parameters above), DAD Sig = 260 nm, 80 Hz

Full Compatibility

Designed to seamlessly integrate into the InfinityLab family

Part of the InfinityLab family

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

InfinityLab parts

InfinityLab columns

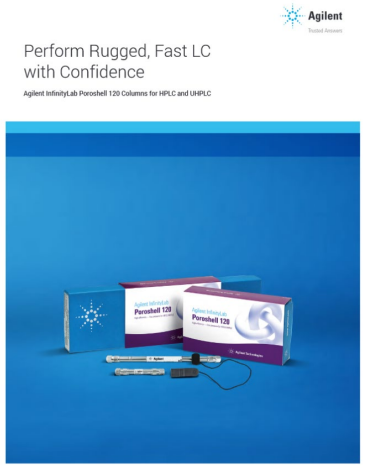
InfinityLab LC series

InfinityLab LC/MSD series

InfinityLab accessories

Agilent

Agilent Resources for Support



Poroshell 120 portfolio brochure

5991-8750EN

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