Thinking Outside the C18 Box

Agilent
Trusted Answers

Modes of HPLC

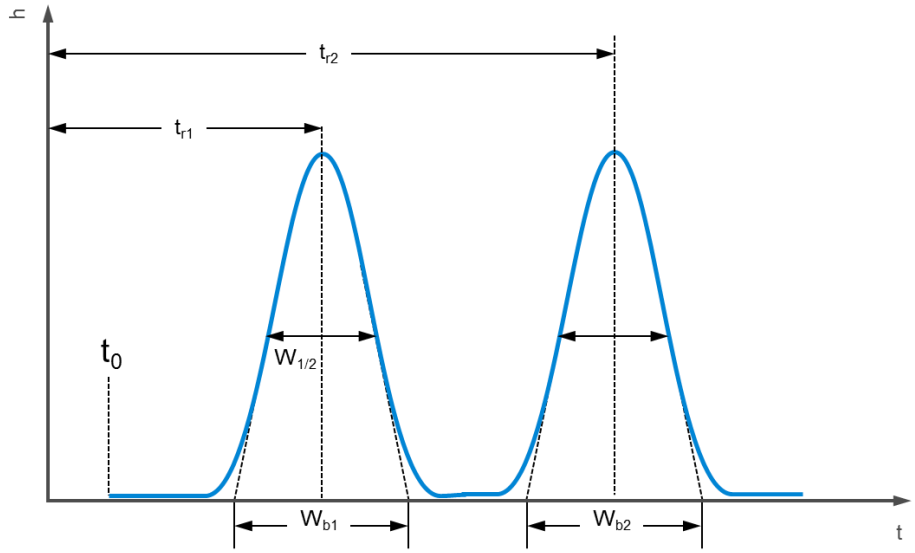
- Reversed-phase
  - C18, C8, other non-polar phases
  - Phenyl, PFP, other more polar phases
- Ion Pairing
- HILIC
  - Silica
  - Amide
  - Zwitterionic
  - Hydroxyl-based
- Chiral
- Normal phase
  - Silica (Rx-SIL)
  - Aminopropyl
- Ion Exclusion (Hi-Plex)
  - Sulfonated polystyrene/divinylbenzene
- Ion Exchange (Bio IEX)
  - Anion exchange
  - Cation exchange
- Size Exclusion/Gel Permeation (AdvanceBio SEC) (PLgel)

Retention Factor

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

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.

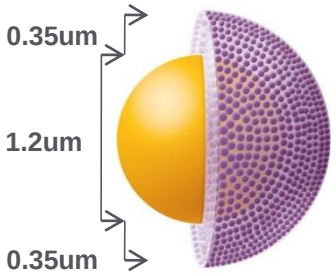
Pore Dewetting or Phase Collapse

- Alkyl phases such as C8 or C18 can exhibit poor retention or reproducibility of retention in low organic mobile phases
- Phenomenon known as pore dewetting or phase collapse
- Onset can be unpredictable
- A method robustness issue often mistaken as a column or lot issue
- See Przybyciel and Majors, *LCGC* **20**(6), 516-523 (2002).

Agilent InfinityLab Poroshell Phases

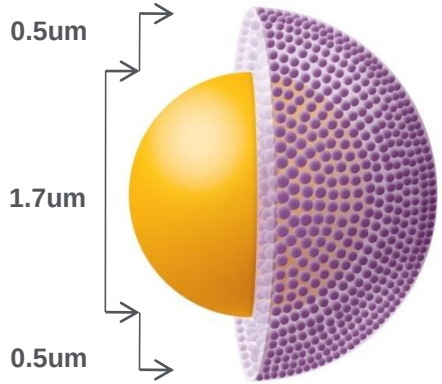
Best all around	Best for low pH mobile phases	Best for high and mid pH mobile phases	Best for alternative selectivity	Best for more polar compounds	HILIC for polar compounds
Poroshell 120 **EC-C18** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **SB-C18** 2.7 µm	Poroshell **HPH-C18** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **Phenyl-Hexyl** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **SB-Aq** 2.7 µm	Poroshell 120 **HILIC** 1.9 µm, 2.7 µm, 4 µm
Poroshell 120 **EC-C8** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **SB-C8** 2.7 µm	Poroshell **HPH-C8** 2.7 µm, 4 µm	Poroshell 120 **Bonus-RP** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **EC-CN** 2.7 µm	Poroshell 120 **HILIC-Z** 2.7 µm
			Poroshell 120 **PFP** 2.7 µm		Poroshell 120 **HILIC-OH5** 2.7 µm

Poroshell 120 Particle Sizes



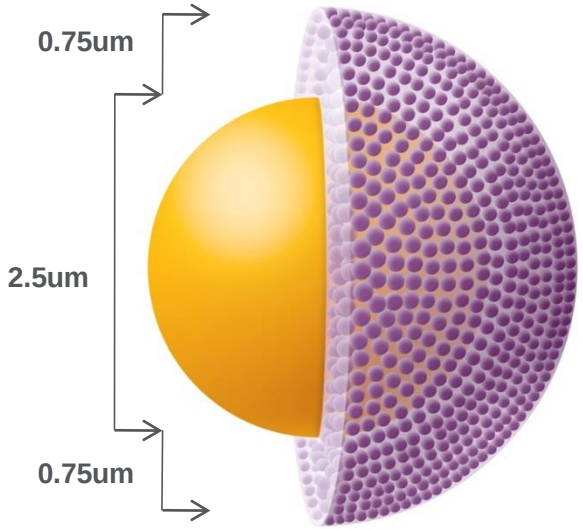
InfinityLab Poroshell 120
1.9 µm

Highest UHPLC performance



InfinityLab Poroshell 120
2.7 µm

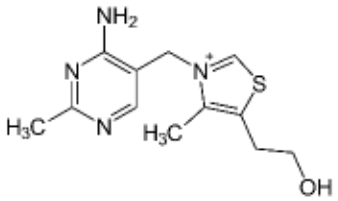
UHPLC performance at lower pressure



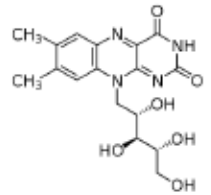
InfinityLab Poroshell 120
4 µm

Improved HPLC performance

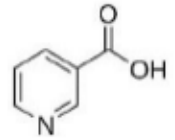
Water Soluble Vitamins



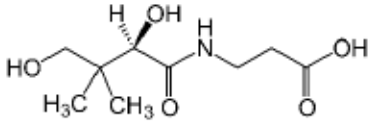
Thiamine, B1



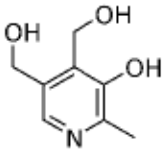
Riboflavin, B2



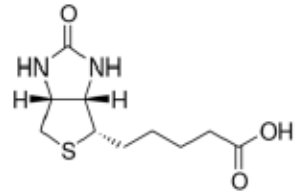
Nicotinic Acid, B3



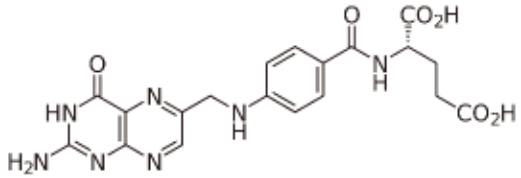
Pantothenic Acid, B5



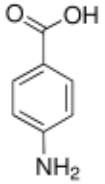
Pyridoxine, B6



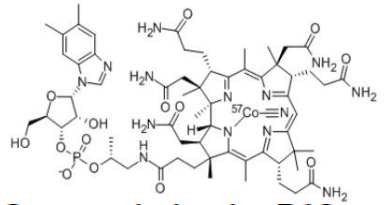
Biotin, B7



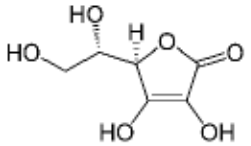
Folic Acid, B9



PABA, B10



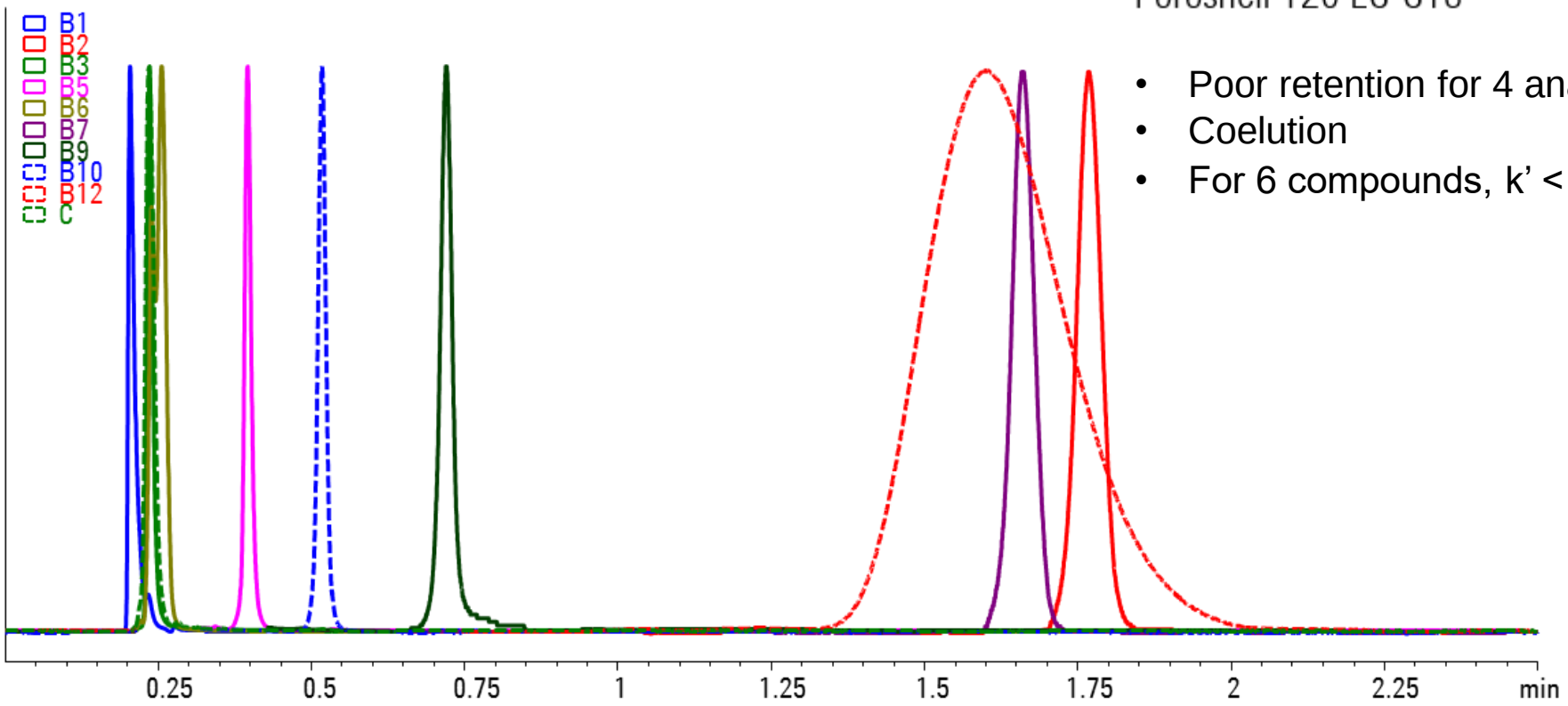
Cyanocobalamin, B12



Ascorbic Acid, C

Water Soluble Vitamins
C18 at low pH

A: 20 mM NaH_2PO_4 pH 2.5
B: CH_3CN, 10% B isocratic
0.5 mL/min, 30 C, 210 nm
2.1 x 50 mm, 2.7 µm
Poroshell 120 EC-C18

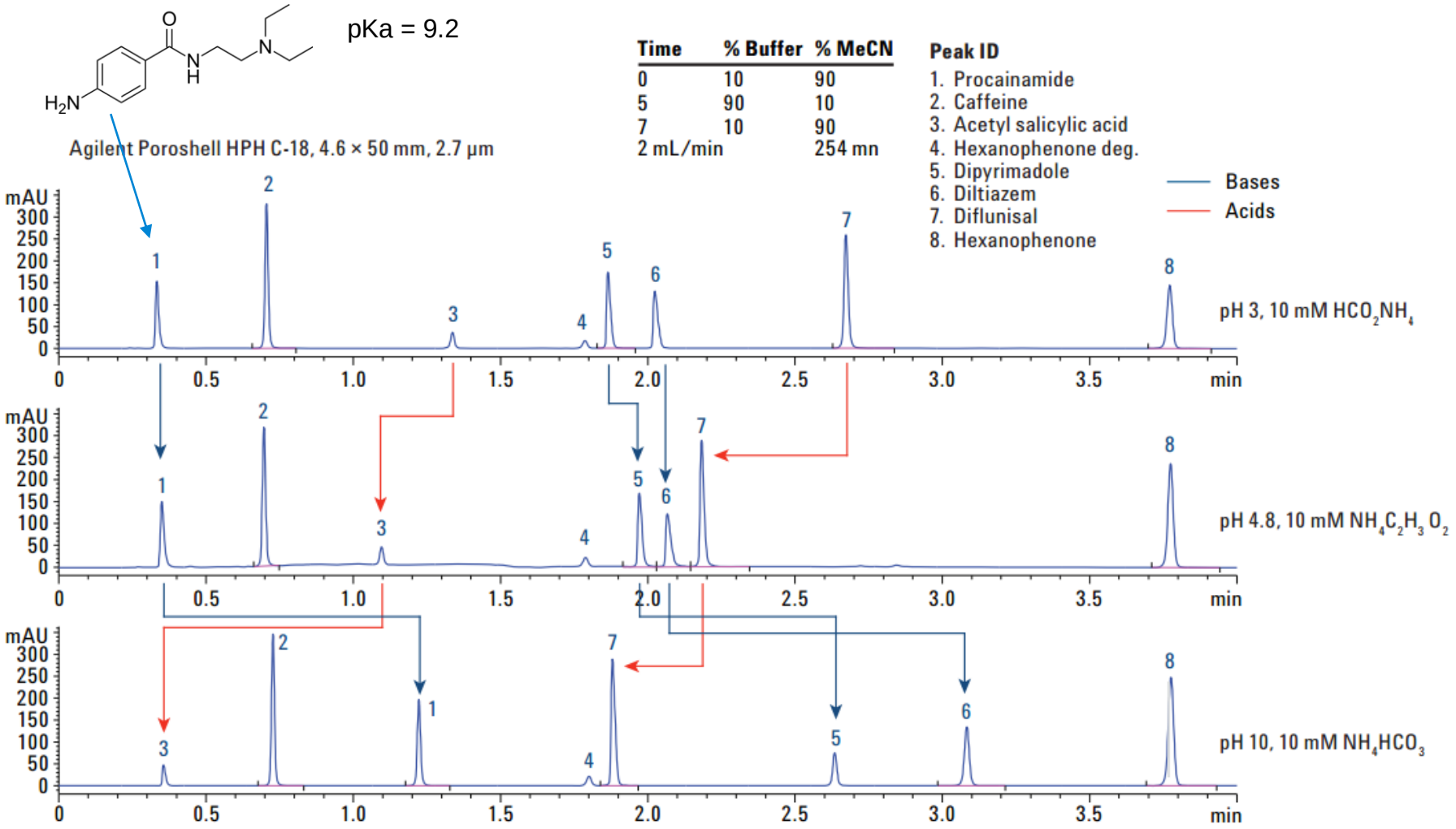


- Poor retention for 4 analytes
- Coelution
- For 6 compounds, k' < 2

Now What?

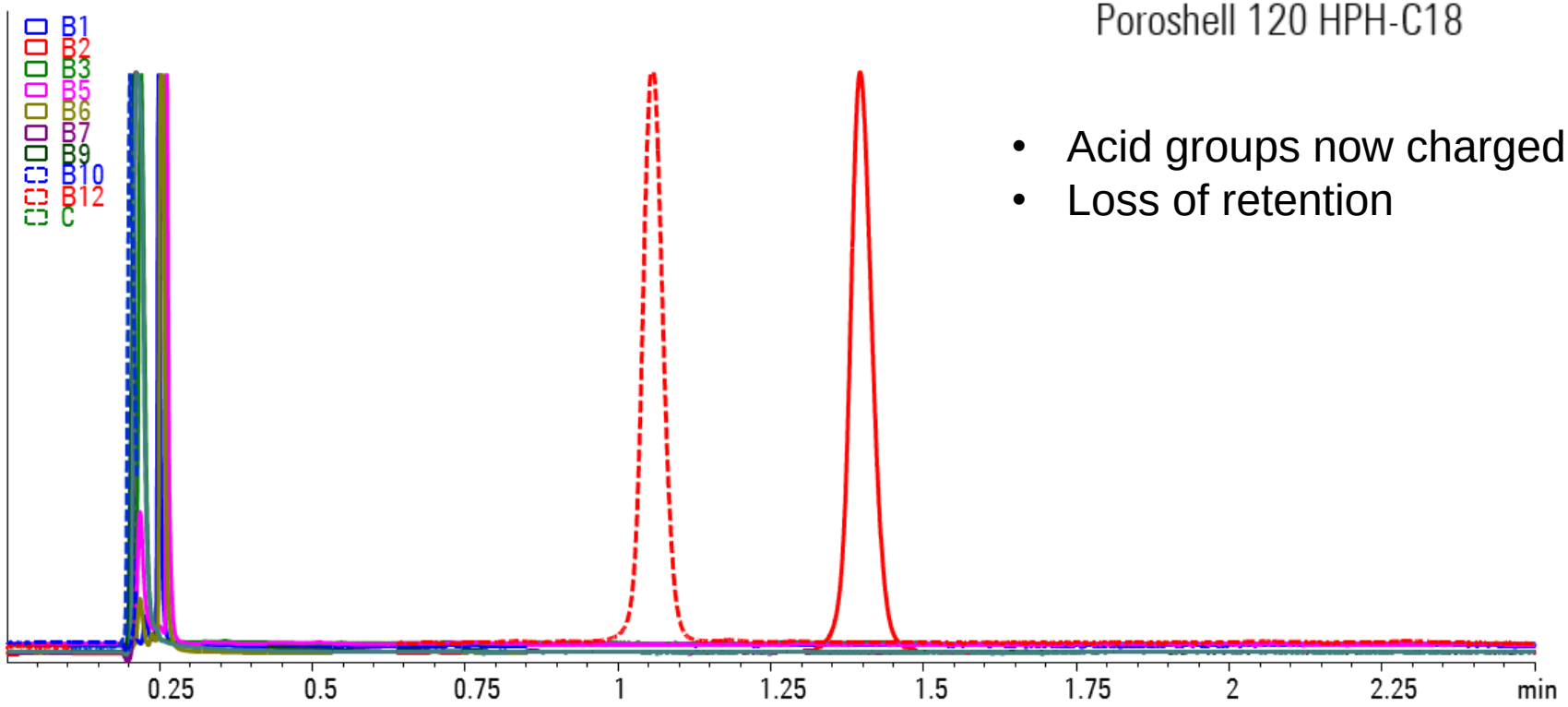
- **Adjust mobile phase pH**
- Ion-pair chromatography
- Alternate column choice
- HILIC

Why try higher pH?
Poroshell HPH-C8 or C18



Water Soluble Vitamins
C18 at pH 7.5

A: 20 mM Na_2HPO_4 pH 7.5
B: CH_3CN, 10% B isocratic
0.5 mL/min, 30 C, 210 nm
2.1 x 50 mm, 2.7 µm
Poroshell 120 HPH-C18



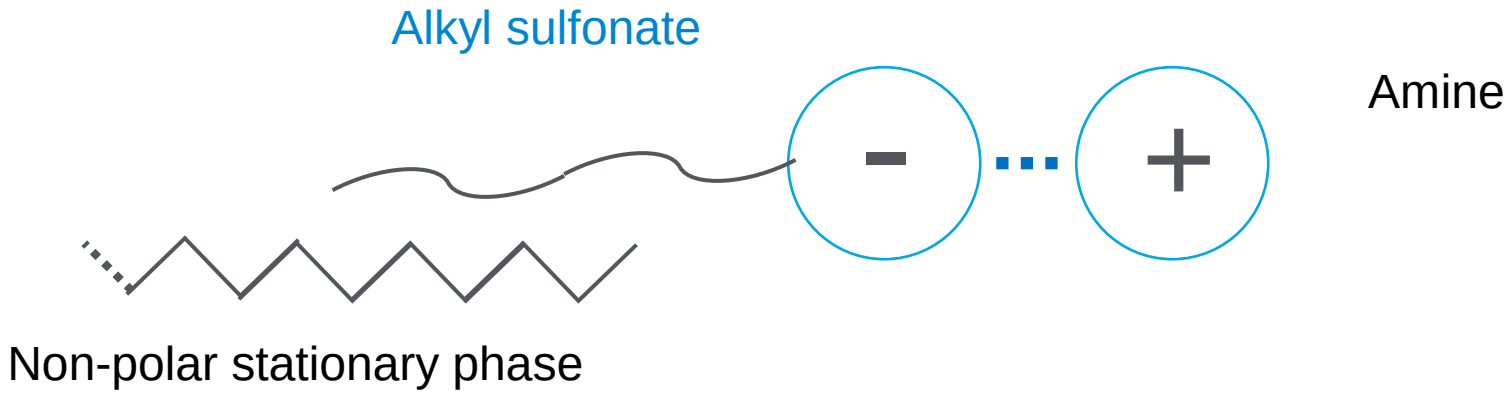
- Acid groups now charged
- Loss of retention

Now What?

- Adjust mobile phase pH
- **Ion-pair chromatography**
- Alternate column choice
- HILIC

Ion-Pair Chromatography

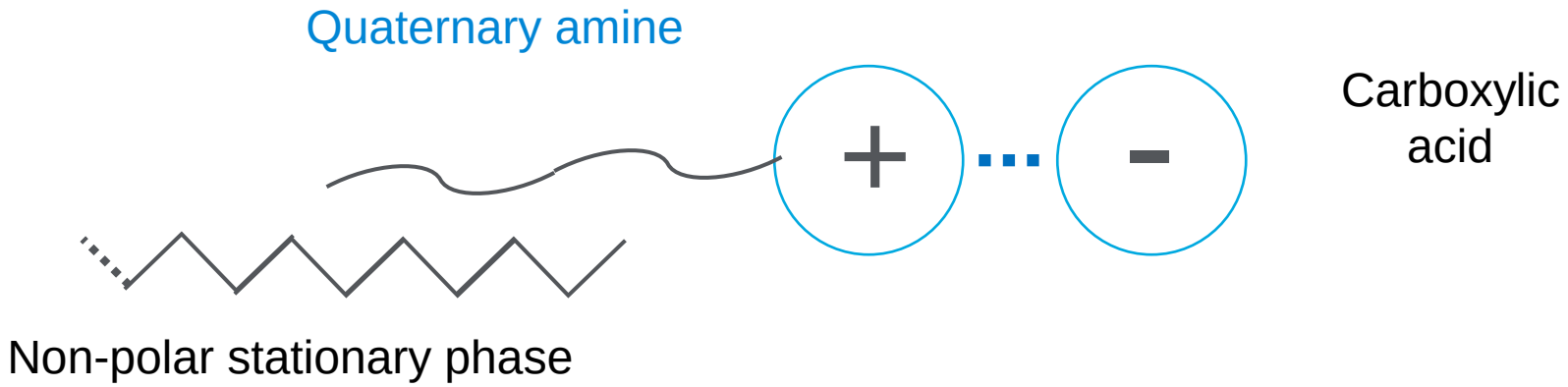
Similar to reversed-phase, but an ion-pairing reagent is added to the mobile phase



- *Non-polar alkyl chain will adsorb into the non-polar stationary phase*
- *Polar part of the ion-pairing reagent will "stick-out" into the mobile phase*

Ion-Pair Chromatography

Similar to reversed-phase, but an ion-pairing reagent is added to the mobile phase



- *Non-polar alkyl chain will adsorb into the non-polar stationary phase*
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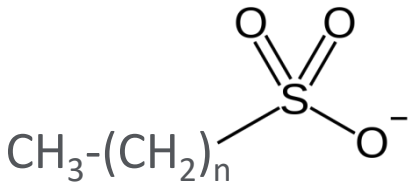
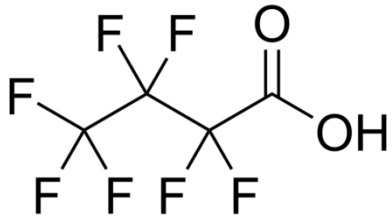
Some Common Ion-Pairing Reagents

Pairs with Cations

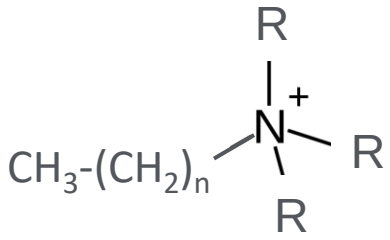


Alkyl sulfonates



Heptafluorobutyric acid (HFBA)

Pairs with Anions



Quaternary amines

Ion-Pair Chromatography

Suggested Experimental Conditions

Column: C8 or C18

Mobile Phase:

- Organic – often methanol
- Aqueous - Buffered with appropriate IP reagent
- Temperature controlled between 35° and 60°C

<u>Cations – bases</u>

Buffer: 25 – 50 mM phosphate, pH 2- 3

IP reagent: 10-100 mM heptane sulfonate

<u>Anions – acids</u>

Buffer: 25 – 50 mM phosphate, pH 6 – 7

IP reagent: 10-40 mM tetrabutyl ammonium phosphate

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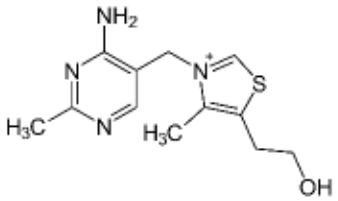
Water Soluble Vitamins



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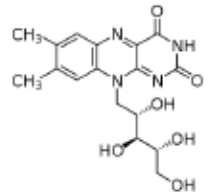

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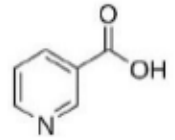

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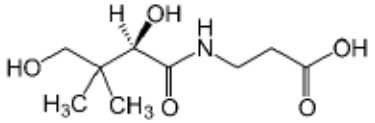

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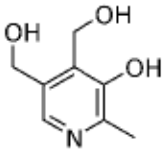
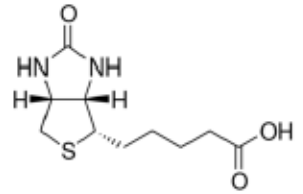


Pyridoxine, B6



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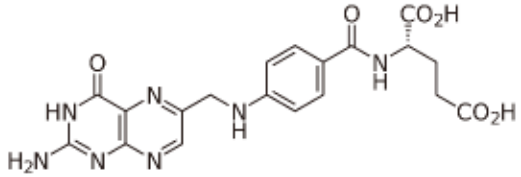

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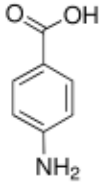
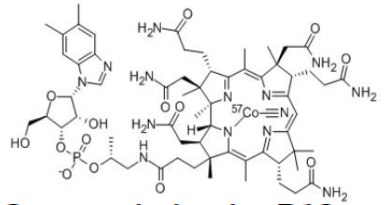
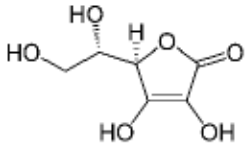


PABA, B10

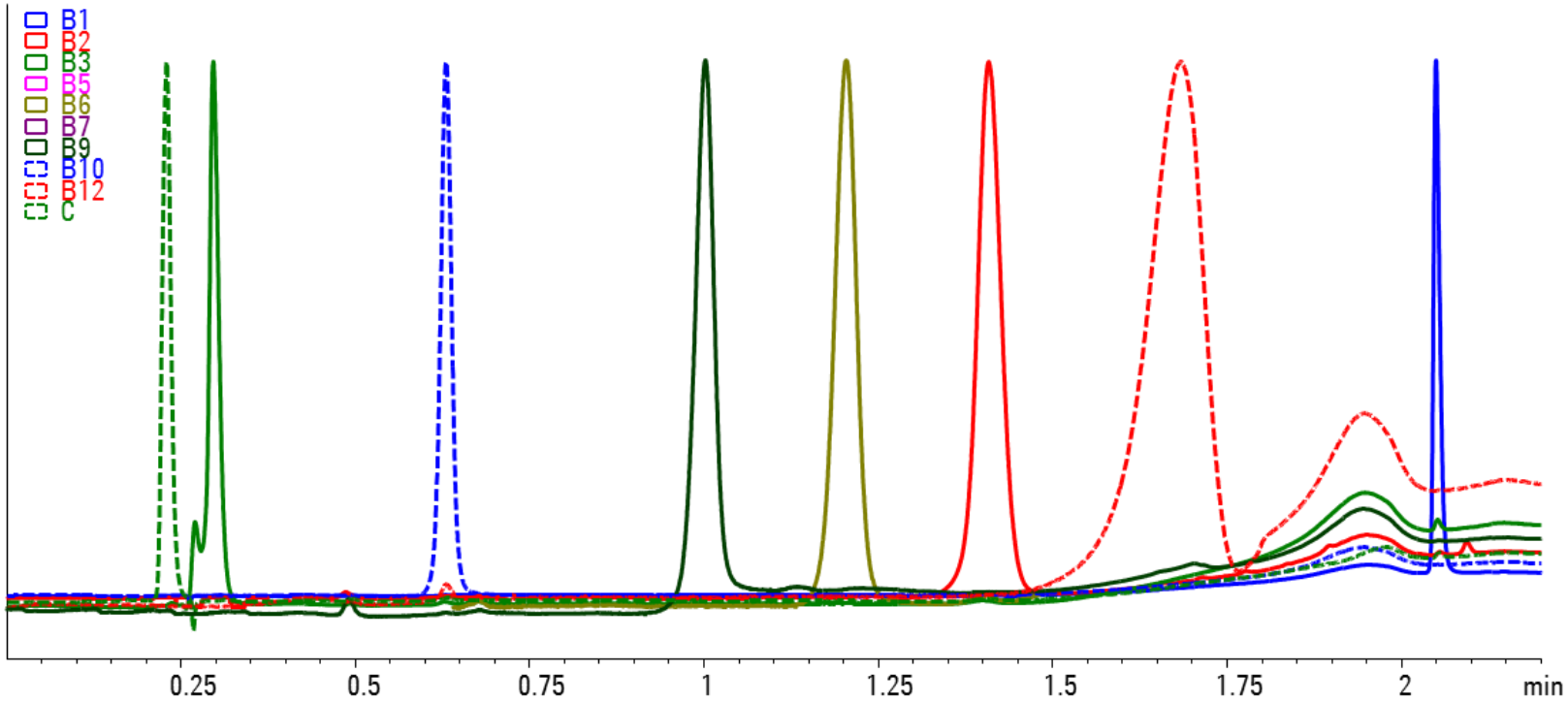


Cyanocobalamin, B12



Ascorbic Acid, C

Water Soluble Vitamins
Ion Pair Conditions



EC-C18
A: 1.5 g sodium 1-heptanesulfonate + 0.2 mL triethylamine + 7.5 mL acetic acid + 992.5 mL water
B: CH3CN
0.5 mL/min,10% B for 1 minute, then 10-40% B in 1 minute
injection volume: varies according to signal strength
TCC: 30 C
260, 8 nm Ref Off, 8 nm slit, 80 Hz

The ion pairing reagent increased retention for most compounds
- 6 compounds have k' > 2
- B5 and B7 could not be detected due to low signal and high background noise at 210 nm (not detectable at 260 nm)

Ion-Pair Chromatography Limitations

- Higher level of complexity than RP, so generally chosen only if needed
- Requires careful control of IP reagent, pH, temperature
- Gradient methods are more difficult than RP
- Equilibration is much slower than RP
- Column dedicated to IP
- IP reagent in the injection solvent
- IP reagents not desirable for MS detection

Now What?

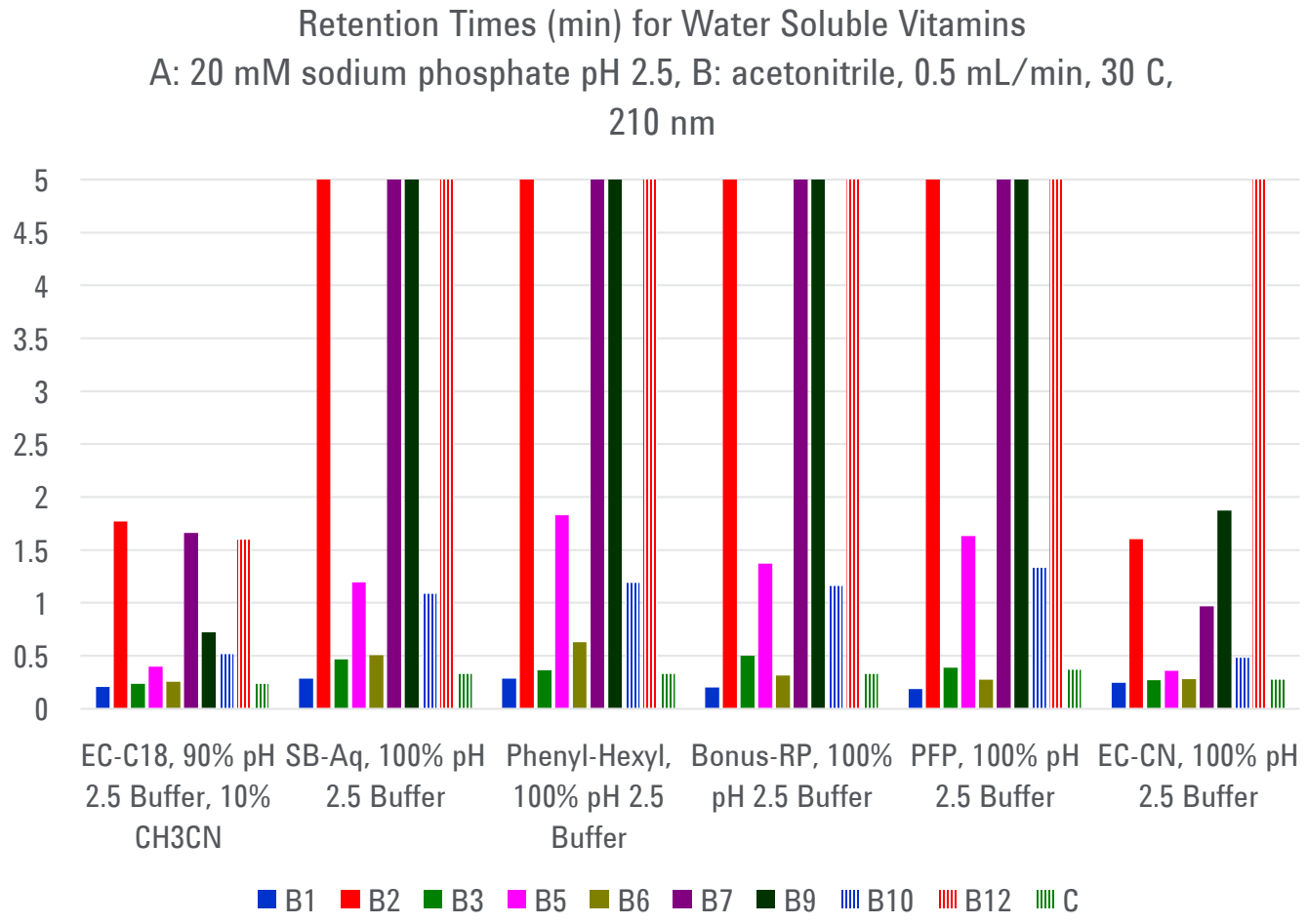
- Adjust method conditions
- Ion-pair chromatography
- **Alternate column choice**
- HILIC

Phase Choices

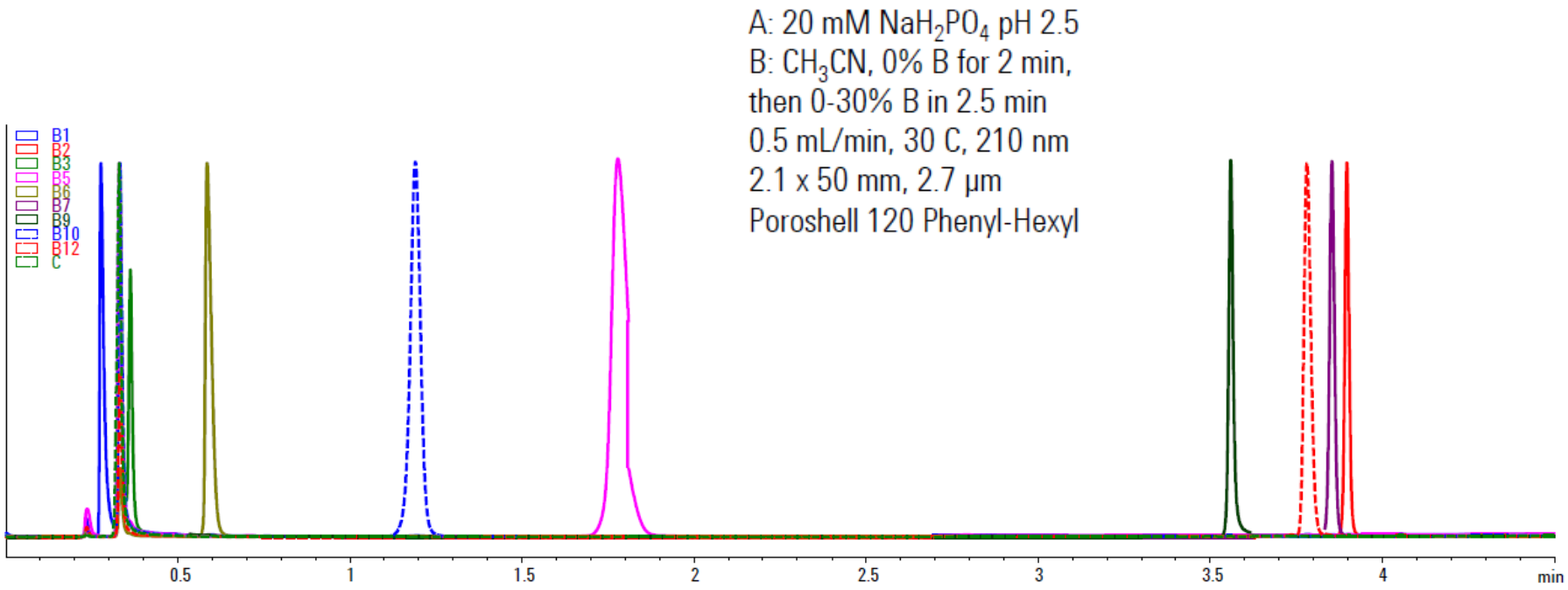
Best all around	Best for low pH mobile phases	Best for high and mid pH mobile phases	Best for alternative selectivity	Best for more polar compounds	HILIC for polar compounds
Poroshell 120 **EC-C18** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **SB-C18** 2.7 µm	Poroshell **HPH-C18** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **Phenyl-Hexyl** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **SB-Aq** 2.7 µm	Poroshell 120 **HILIC** 1.9 µm, 2.7 µm, 4 µm
Poroshell 120 **EC-C8** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **SB-C8** 2.7 µm	Poroshell **HPH-C8** 2.7 µm, 4 µm	Poroshell 120 **Bonus-RP** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **EC-CN** 2.7 µm	Poroshell 120 **HILIC-Z** 2.7 µm
			Poroshell 120 **PFP** 2.7 µm		Poroshell 120 **HILIC-OH5** 2.7 µm

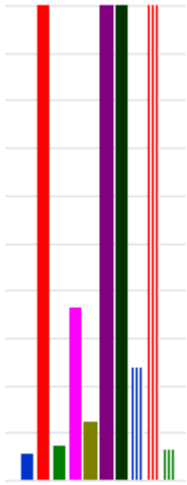
These phases can be used with high aqueous mobile phases to improve retention of highly polar analytes in RPLC mode

Water Soluble Vitamins
Alternative Phases



Water Soluble Vitamins
Phenyl-Hexyl

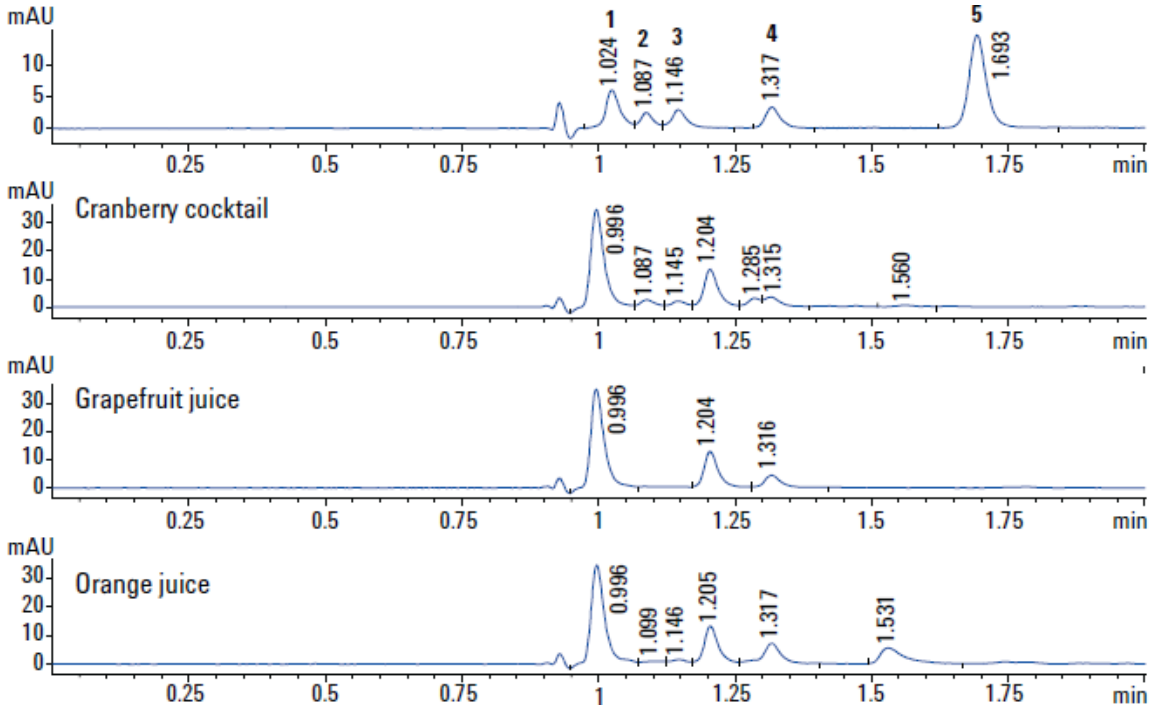




Phenyl-Hexyl, 100% pH 2.5 Buffer

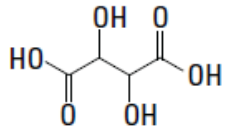
- Phenyl-Hexyl has the best retention
- 7 compounds have k'> 2;
- C18 analysis had only 4 compounds with k' > 2

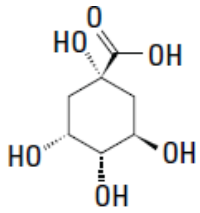
Aliphatic Acids
SB-Aq



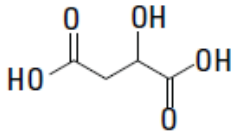
Peak ID

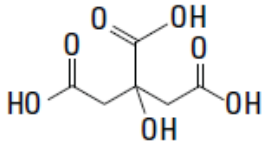
1. Tartaric acid
2. Quinic acid
3. Malic acid
4. Citric acid
5. Fumaric acid

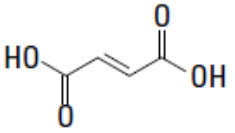

Tartaric acid


Quinic acid

Column:	Agilent Poroshell 120 SB-Aq, 3 × 100 mm, 2.7 µm (p/n 685975-314)
Eluent:	100 mM Potassium phosphate buffer, pH 2.5
Injection volume:	5 µL
Flow rate:	0.5 mL/min
Temperature:	50 °C
Detector:	DAD, at 226 nm


Malic acid


Citric acid


Fumaric acid

5991-1992EN

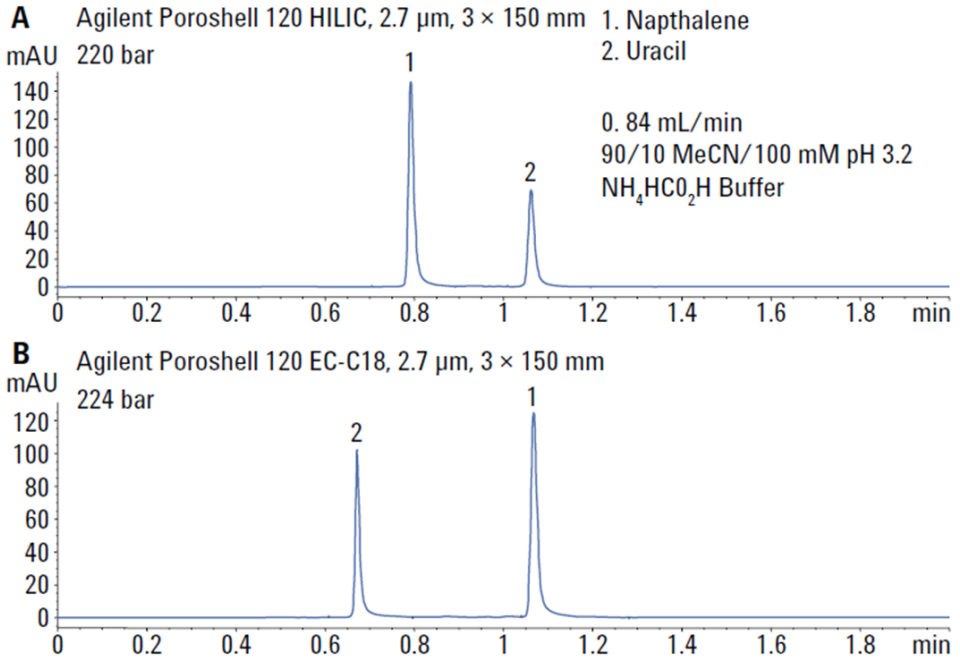
What Now?

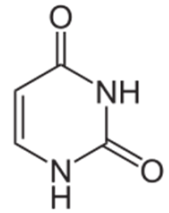
- Adjust method conditions
- Ion-pair chromatography
- Alternate column choice
- **HILIC**

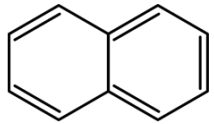
What is HILIC?

Hydrophilic **I**nteraction **Li**quid **C**hromatography

- Retains hydrophilic compounds
- Polar stationary phase: silica, amino, diol/hydroxyl-based, zwitterionic, etc.
- Uses an organic and water mobile phase with a buffer

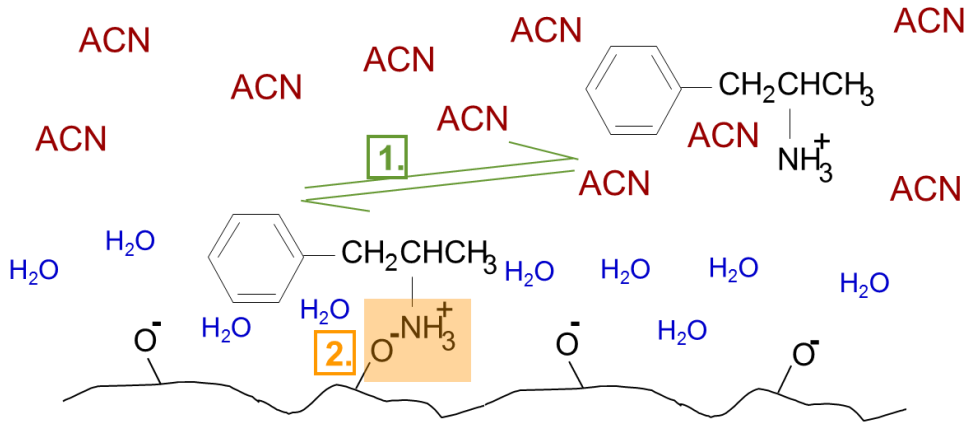






HILIC Mechanism

- Retains moderate to highly polar analytes
  - A water layer is adsorbed onto the polar silica surface, creating a liquid/liquid extraction system
  - Polar analytes can **partition into and out of the water layer**, with more polar analytes having a stronger interaction (1)
  - Charged polar analytes can also undergo **ion exchange with the silica surface** (2)

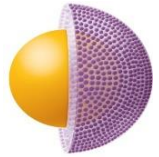


1. Partitioning in and out of adsorbed water layer
2. Ion exchange with silanols

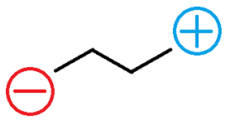
Key points to note:

- Water is the strong solvent
- Elution is least polar to most polar, opposite of RPLC.
- Gradients run from **high organic to high aqueous** (10% aqueous to 50% aqueous is a common scouting gradient)
- Reversed-phase solvents (ACN/Water)
  - MeOH, EtOH, IPA can also be used
- Typically uses a buffer like ammonium formate or ammonium acetate
- Higher buffer concentration increases solvent strength, improves peak shape, and can change selectivity slightly

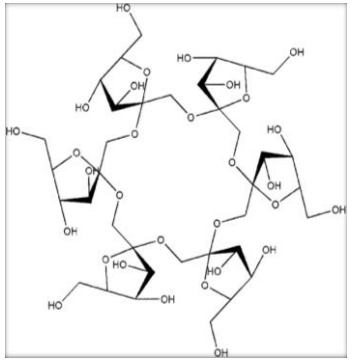
InfinityLab Poroshell 120 HILIC Columns - Specifications



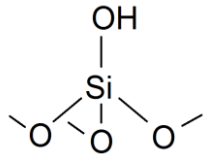
InfinityLab Poroshell Column	Bonded phase	Particle	Particle Size	Pressure Limit	Pore Size	Temp Limit	pH Range	Dimensions (ID in mm)	Dimensions (Length in mm)
HILIC-Z	Proprietary zwittionic chemistry	Hybrid Poroshell superficially porous particle	2.7µm	600 bar	100 Å	80°C	2-12	2.1 3.0 4.6	5 (guard) 50 100 150
								PEEK-lined version available	
HILIC-OH5	Poly-hydroxy fructan chemistry	Poroshell superficially porous particle	2.7µm	400 bar	120 Å	45°C	1-7	2.1 3.0 4.6	50 100 150
HILIC	Bare-silica (unbonded)	Poroshell superficially porous particle	1.9µm 2.7µm 4µm	1300 bar (1.9) 600 bar (2.7) 600 bar (4)	120 Å	60°C	0-8	2.1 3.0 4.6	5 (guard) 50 100 150



HILIC-Z
Proprietary Zwitterionic chemistry



HILIC-OH5
Poly-hydroxy fructan chemistry



HILIC
Bare Silica chemistry

HILIC Method Development: Common LC Parameters

Type of stationary phase

- Vary retention mechanism and selectivity
- 3 phases on Agilent InfinityLab Poroshell 120 2.7 µm particles
  - HILIC-Z, HILIC-OH5, HILIC

Mobile phase pH

- Controls ionization of silica and analytes
- Compounds are more retained in their charged state
  - Acids should be run at high pH, bases at low pH

Temperature

- Increasing temperature will decrease retention
- Increasing temperature will increase column efficiency
- Decreasing temperature can improve selectivity

HILIC Method Development: Mobile Phase Considerations

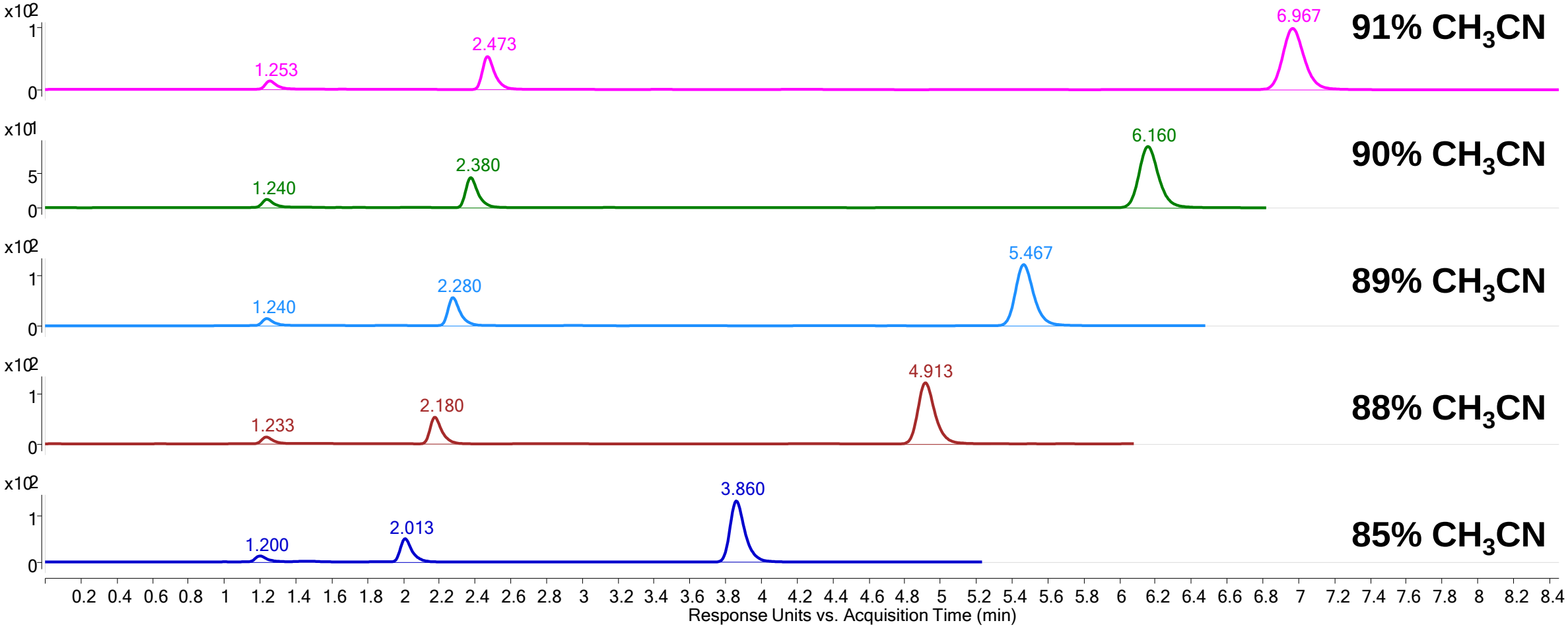
Organic solvent concentration

- Solvent strength in HILIC mode:
  - *THF < Acetone < CH_3CN < IPA < EtOH < MeOH < H_2O*
- H_2O must be present — *need > 3% H_2O*
- **Ionic strength of buffer**
- Concentration of (salt) buffer increases strength
- Different anions and cations may can also affect analyte retention

Type of buffer

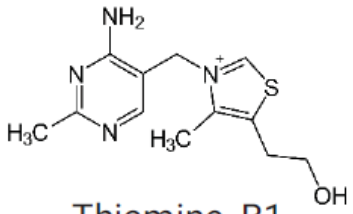
- Acetates, formates good, soluble in CH_3CN—also MS friendly
- *Phosphate salts* have low CH_3CN solubility

Less CH_3CN Makes a HILIC Mobile Phase Stronger, Causing Less Retention

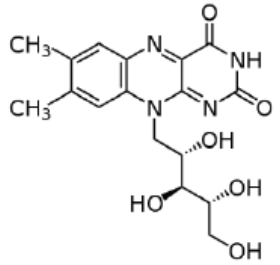


Column used was 2.1 x 150 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z (PEEK lined); A: 100 mM pH 3 Ammonium Formate in Water, B: Acetonitrile, x % B, isocratic elution, 0.25 mL/min, 30 °C, 1 uL injection of toluene, cytosine, uracil QC mixture, 254 nm

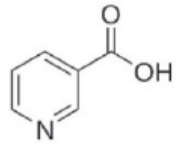
Water Soluble Vitamins



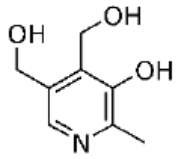
Thiamine, B1



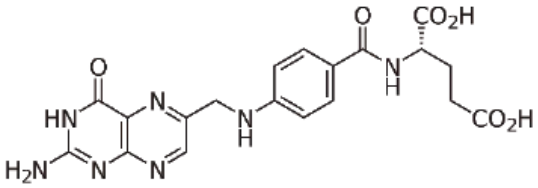
Riboflavin, B2



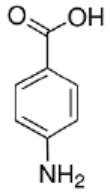
Nicotinic acid, B3



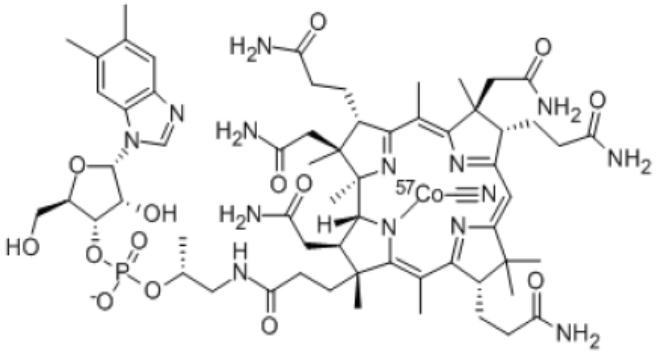
Pyridoxine, B6



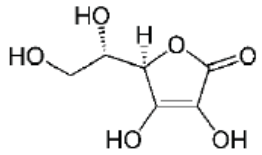
Folic acid, B9



PABA, B10

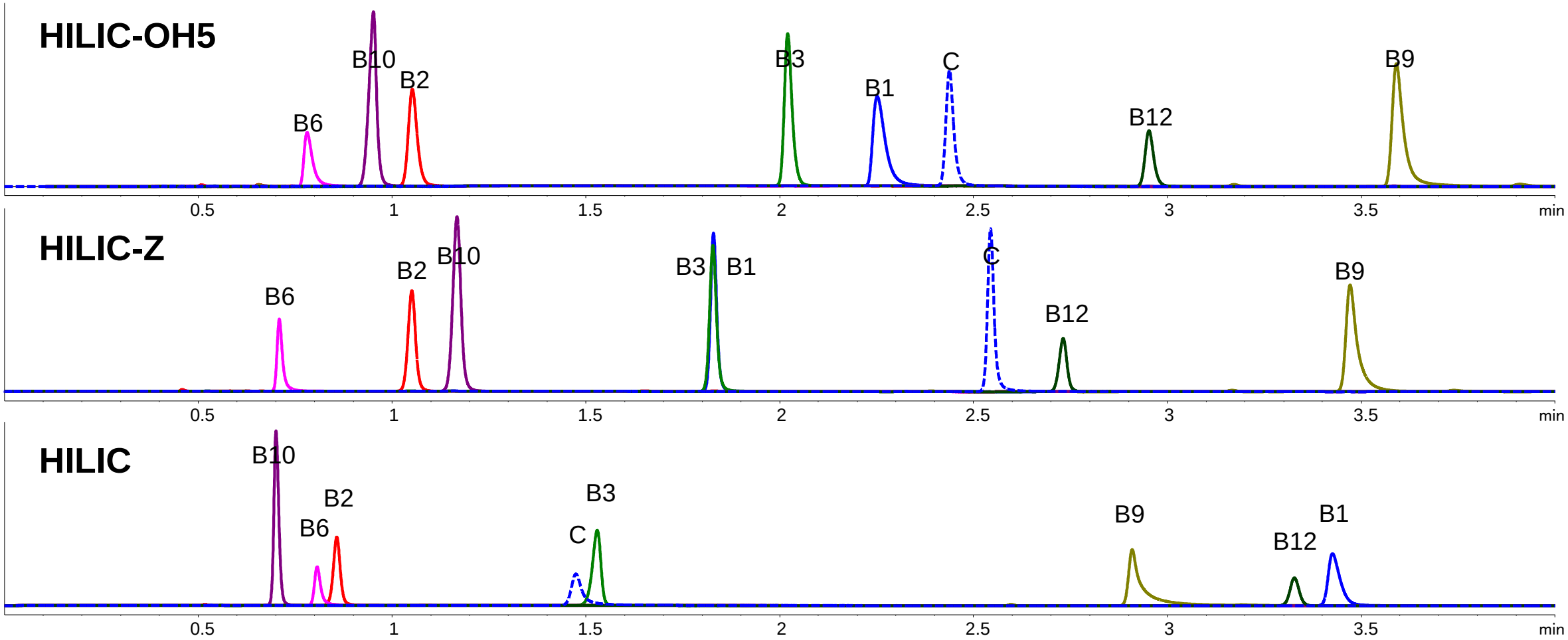


Cyanocobalamin, B12



Ascorbic acid, C

Water Soluble Vitamins by HILIC

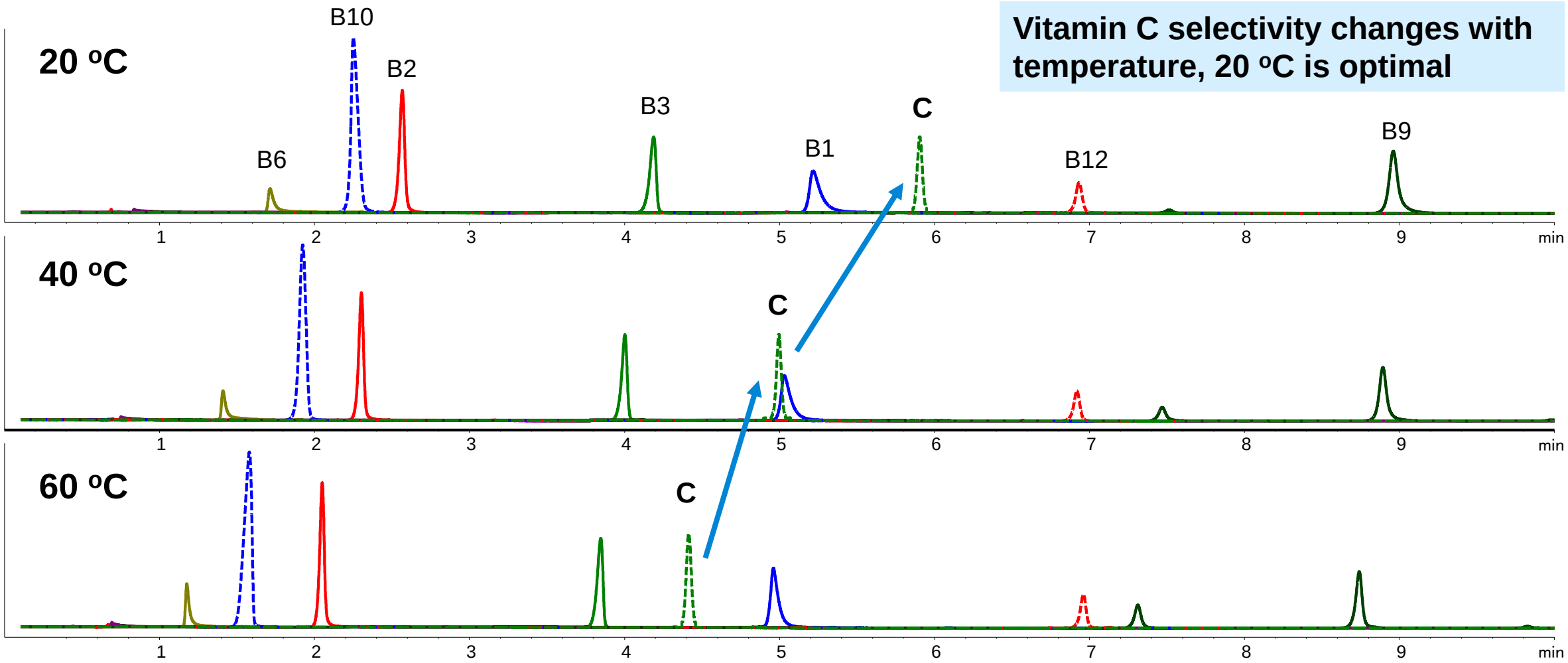


Columns used were 2.1 x 100 mm, 2.7 µm; A: 100 mM Ammonium Acetate + 0.5% Acetic Acid (pH ~4.6) in H_2O, B: CH_3CN, 0.5 mL/min, 87% B for 1 min, 87-50% B in 4 min, 3 min re-equilibration, 1 µL injection of individual vitamin standards (0.1-0.4 mg/mL each), 40 °C, 260 nm, 80 Hz

Temperature Used to Optimize a HILIC Separation

Water Soluble Vitamins on Agilent InfinityLab Poroshell 120 HILIC-OH5

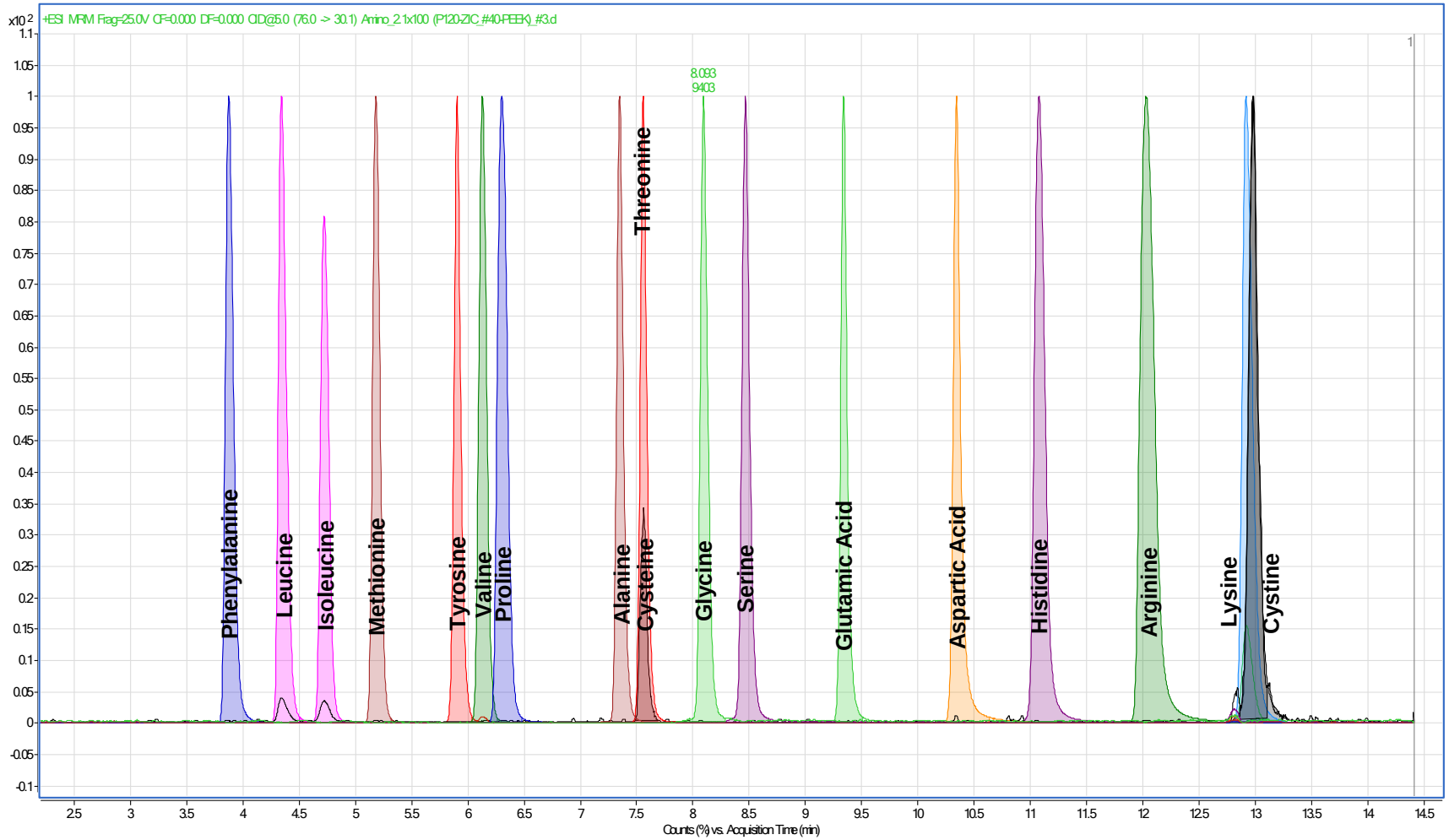
Vitamin C selectivity changes with temperature, 20 ºC is optimal



Agilent InfinityLab Poroshell 120 HILIC-OH5 2.1 x 100 mm, 2.7 µm; A: 100 mM Ammonium Acetate (no pH adjustment) in H_2O, B: CH_3CN, 0.5 mL/min, 95-60%B in 10 min, 3 min re-equilibration, 1 µL injection of individual vitamin standards (0.1-0.4 mg/mL each), 20/40/60 ºC, 260 nm, 80 Hz

Excellent retention, peak shape and sensitivity with HILIC-Z

Underivatized Amino Acids by LC/MS



InfinityLab Poroshell HILIC-Z 2.1 x 100mm, 2.7µm

A: 20mM ammonium formate in H20, pH3
B: 9/1 ACN/H20 with 20mm ammonium formate, pH3

Gradient: 100%B to 70% B over 10 min, return to 100% B
Flow rate: 0.8 ml/min
Temp: 30deg
MS Detection: Agilent MS-QQQ, MS2 SIM mode

HILIC Analyses Perform Best with Weak Injection Solvents

B Vitamins on HILIC with Isocratic Elution

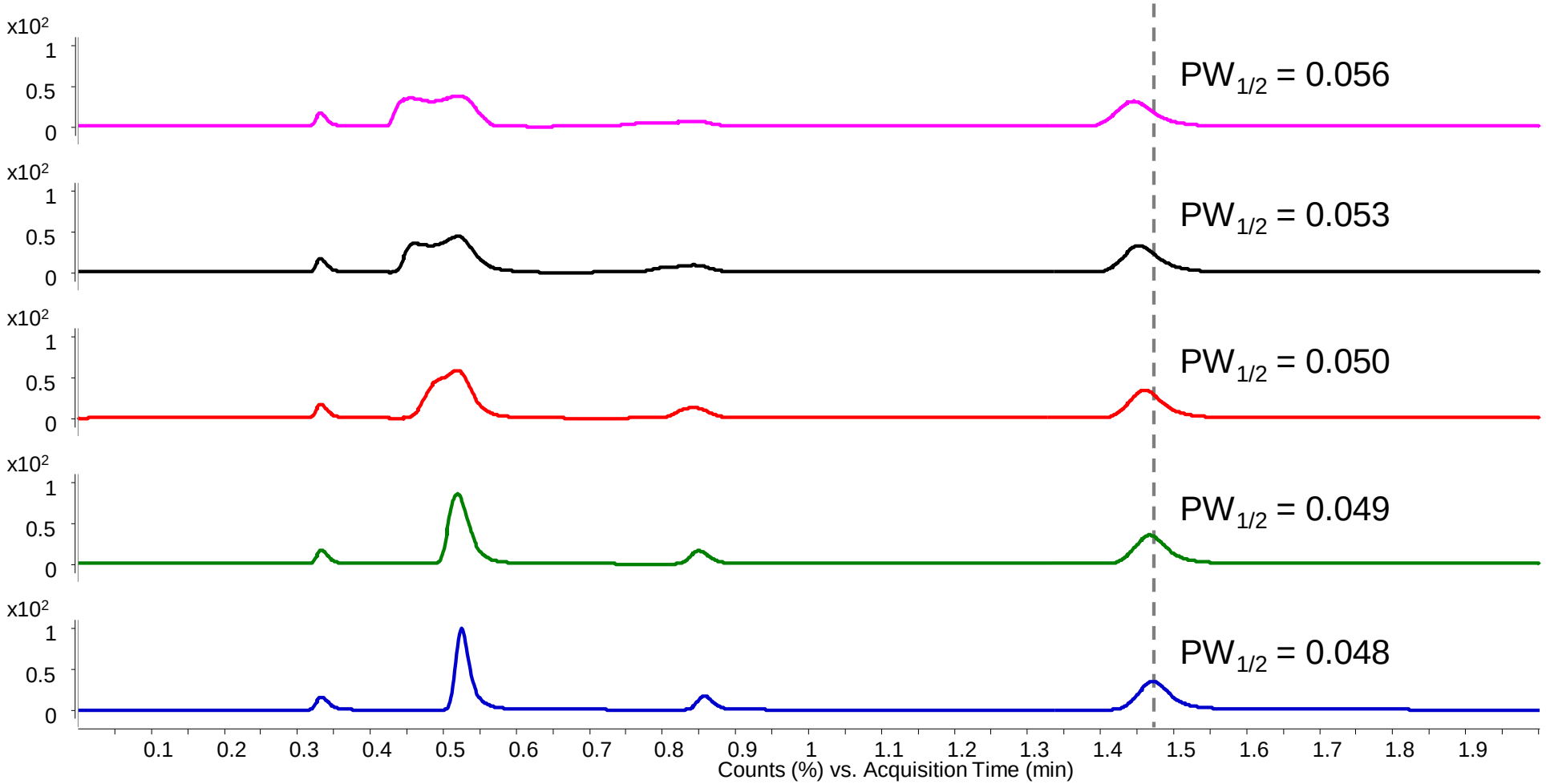
1 µL injection in H_2O

1 µL injection in H_2O/CH_3CN (3:1)

1 µL injectionin H_2O/CH_3CN (1:1)

1 µL injection in H_2O/CH_3CN (1:3)

1 µL injection in CH_3CN



Agilent ZORBAX RRHD HILIC Plus 2.1 x 50 mm, 1.8 µm; Mobile Phase: acetonitrile / 100 mM Ammonium Formate pH 3.2 in water (9:1), isocratic elution, 0.4 mL/min, 1 µL injection of 5.7 µg/mL each of 4-aminobenzoic acid, nicotinamide, riboflavin, nicotinic acid ; 25 °C, MS Source: ESI+, 200 °C, 10 L/min,, 30 psi, 4000 V; SIM: 138, 123, 377, 124

Advantages of HILIC

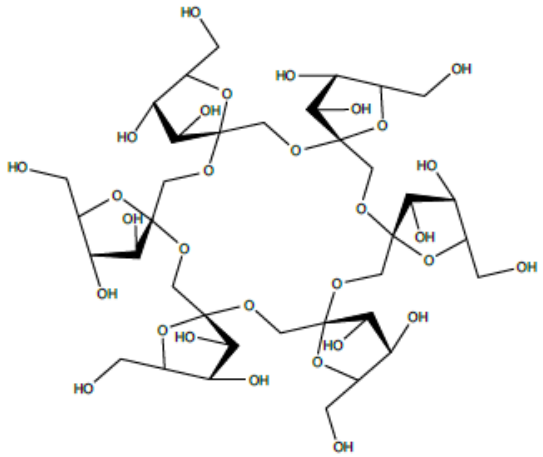
- Retains polar analytes where reversed-phase methods may not
- Offers alternative selectivity to RPLC mode
- Can retain cations, anions, and polar neutrals in a single run
- Can improve peak shape for basic compounds
- Uses a standard LC system and common reversed-phase solvents
- Uses low viscosity mobile phases with high organic content
  - Fast methods with high flow rates
  - Longer columns for higher efficiency at lower pressures
- Enhanced detection sensitivity with MS compatible methods, in both positive and negative modes
- Can directly inject ACN extracts from C18 SPE cartridges

Agilent InfinityLab Poroshell Phases

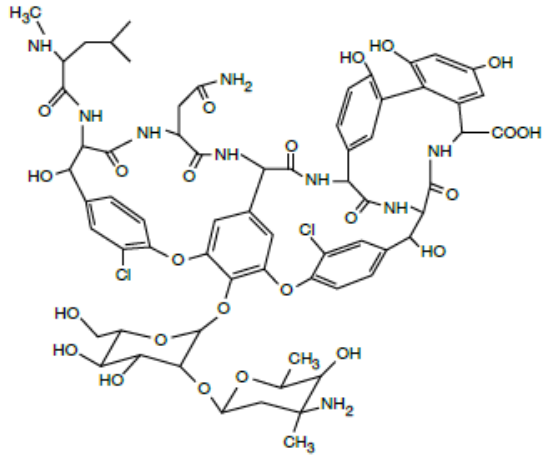
Best all around	Best for low pH mobile phases	Best for high and mid pH mobile phases	Best for alternative selectivity	Best for more polar compounds	HILIC for polar compounds	Chiral phases
Poroshell 120 **EC-C18** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **SB-C18** 2.7 µm	Poroshell **HPH-C18** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **Phenyl-Hexyl** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **SB-Aq** 2.7 µm	Poroshell 120 **HILIC** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **Chiral-CF** 2.7 µm
Poroshell 120 **EC-C8** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **SB-C8** 2.7 µm	Poroshell **HPH-C8** 2.7 µm, 4 µm	Poroshell 120 **Bonus-RP** 1.9 µm, 2.7 µm, 4 µm	Poroshell 120 **EC-CN** 2.7 µm	Poroshell 120 **HILIC-Z** 2.7 µm	Poroshell 120 **Chiral-CD** 2.7 µm
			Poroshell 120 **PFP** 2.7 µm		Poroshell 120 **HILIC-OH5** 2.7 µm	Poroshell 120 **Chiral-V** 2.7 µm
						Poroshell 120 **Chiral-T** 2.7 µm

Poroshell 120 Chiral Chemistries

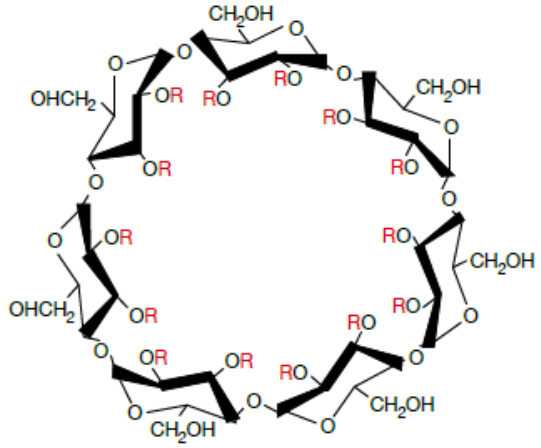
InfinityLab Poroshell 120 Chiral-CF
(Cyclofructan CF-6)



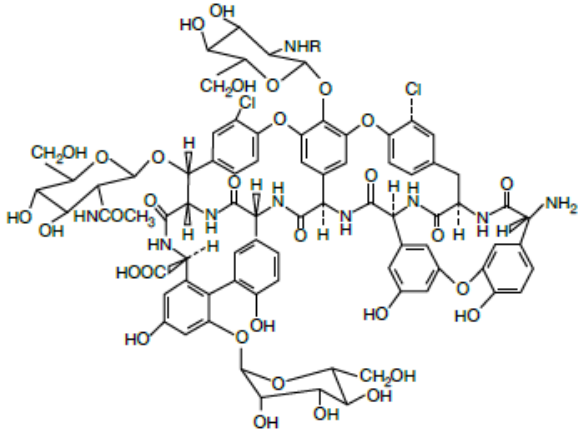
InfinityLab Poroshell 120 Chiral-V
(Vancomycin)



InfinityLab Poroshell 120 Chiral-CD
(Hydroxypropylated beta-cyclodextrin)



InfinityLab Poroshell 120 Chiral-T
(Teicoplanin)



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

Modes of Separation used with Infinity Lab Chiral Columns

Polar Ionic Mode

Methanol with acid or base or volatile salt < 0.2 % wt. (MeOH + HOAc + TEA)

Non-aqueous mobile phase; fast, MS detection; for ionizable molecules – any acid or base

Dominant interactions: Ionic interaction, hydrogen bonding

Example: MeOH with 0.2 wt% ammonium formate

Reversed Phase Mode

Methanol/Water/Buffer,

MS compatible, ideal for manufacturing QC, bioanalysis for all types of molecules

Example: 30/70 MeOH/20 mM ammonium formate (pH 4)

Polar Organic Mode

Acetonitrile/Methanol/Ethanol/Isopropanol+ HOAc + TEA

Dominant interactions: Hydrogen bonding, dipole-dipole

Example: 60/40/0.3/0.2 ACN/MeOH/acetic acid/TEA

Normal Phase

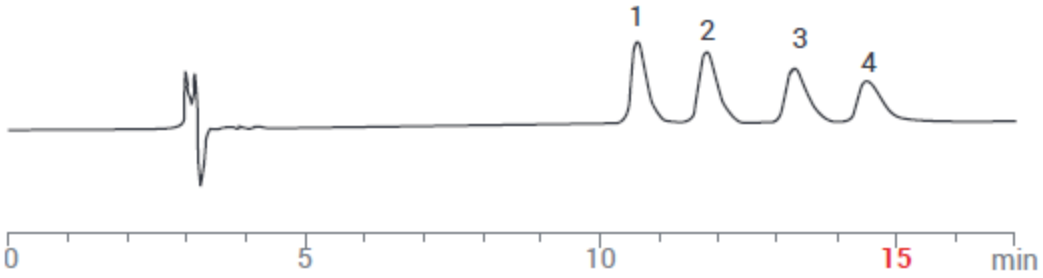
Heptane (or Hexane)/methanol or ethanol

Example: 60/40/0.3/0.2 ACN/MeOH/acetic acid/TEA

Fast, High Efficiency Chiral Separations

Traditional Chiral Separation—totally porous particle

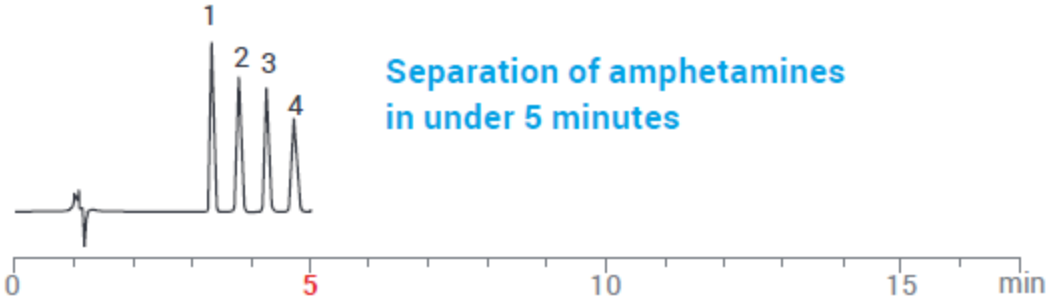
Chirobiotic V2 (250 x 4.6 mm, 5 µm)



1. D-(+)-Amphetamine, 2. L-(-)-Amphetamine, 3. D-(+)-Methamphetamine
4. L-(-)-Methamphetamine 100/0.1/0.02 MeOH/HOAc/NH_4OH with a
1.0 mL/min flow rate at room temperature and UV at 220 nm

Agilent InfinityLab Poroshell 120 Chiral Separation—superficially porous particle

InfinityLab Poroshell 120 Chiral-V (100 x 4.6 mm, 2.7 µm)



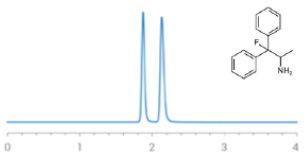
Separation of amphetamines in under 5 minutes

1. D-(+)-Amphetamine, 2. L-(-)-Amphetamine, 3. D-(+)-Methamphetamine
4. L-(-)-Methamphetamine 100/0.1/0.02 MeOH/HOAc/NH_4OH with a
1.0 mL/min flow rate at room temperature and UV at 220 nm

Chiral Applications Compendium
Publication number: **5991-8450EN**

Amines

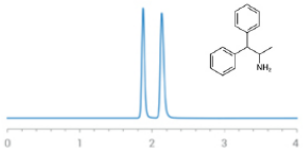
1,1-diphenyl-1-fluoro-2-aminopropane



Retention (min): 1.48/1.67 (Peak1/Peak 2)
Resolution: 3.20

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 230 nm

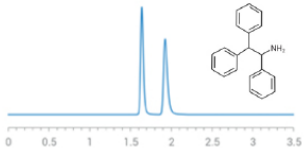
1,1-diphenyl-2-aminopropane



Retention (min): 1.87/2.13 (Peak1/Peak 2)
Resolution: 3.33

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 230 nm

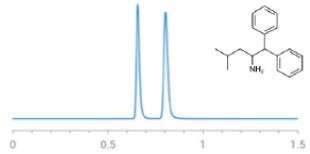
1,2,2-triphenylethylamine



Retention (min): 1.64/1.93 (Peak1/Peak 2)
Resolution: 3.88

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

2-amino-4-methyl-1,1-diphenylpentanepropane

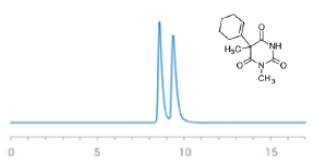


Retention (min): 0.66/0.80 (Peak1/Peak 2)
Resolution: 4.49

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

Anesthetics

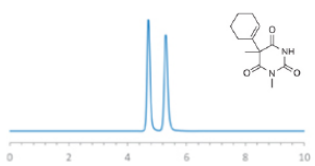
Hexobarbital



Retention (min): 4.63/4.99 (Peak1/Peak 2)
Resolution: 1.54

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-T (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: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 220 nm

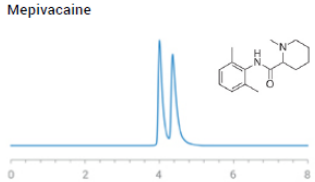
Hexobarbital



Retention (min): 4.70/5.29 (Peak1/Peak 2)
Resolution: 2.92

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: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 220 nm

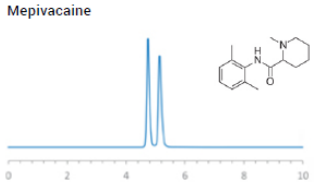
Mepivacaine



Retention (min): 4.01/4.36 (Peak1/Peak 2)
Resolution: 1.42

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-T (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 100/0.2/0.05: Methanol/Acetic Acid/Ammonium Hydroxide
Flow Rate: 1.0 mL/min
Temperature: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 220 nm

Mepivacaine

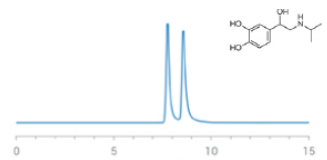


Retention (min): 4.75/5.14 (Peak1/Peak 2)
Resolution: 2.25

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-V (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 90/10: Methanol/15 mM Ammonium Formate (pH 3.6)
Flow Rate: 0.5 mL/min
Temperature: 30 °C
Injection Volume: 1.0 µL
Detection: UV 220 nm

Beta blockers

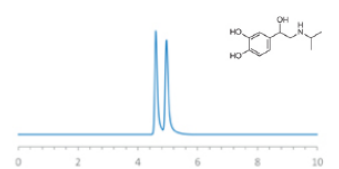
Isoproterenol



Retention (min): 7.76/8.56 (Peak1/Peak 2)
Resolution: 2.70

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-T (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 100/0.2/0.1: Methanol/Trifluoroacetate/Ammonium Hydroxide
Flow Rate: 0.7 mL/min
Temperature: 45 °C
Injection Volume: 1.0 µL
Detection: UV 230 nm

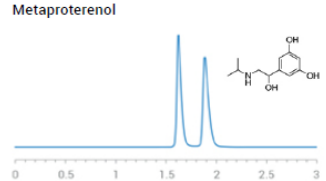
Isoproterenol



Retention (min): 4.60/4.95 (Peak1/Peak 2)
Resolution: 1.0

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-V (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 100/0.2/0.05: Methanol/Acetic Acid/Ammonium Hydroxide
Flow Rate: 0.5 mL/min
Temperature: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 220 nm

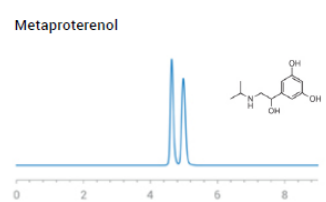
Metaproterenol



Retention (min): 1.62/1.89 (Peak1/Peak 2)
Resolution: 3.01

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-T (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 60/40/0.3/0.2: Acetonitrile/Methanol/Acetic Acid/TEA
Flow Rate: 3.5 mL/min
Temperature: 45 °C
Injection Volume: 1.0 µL
Detection: UV 230 nm

Metaproterenol



Retention (min): 4.63/4.98 (Peak1/Peak 2)
Resolution: 1.89

Method Conditions
Column: InfinityLab Poroshell 120 Chiral-V (10 cm x 4.6 mm, 2.7 µm)
Mobile phase: 100/0.2/0.05: Methanol/Acetic Acid/Ammonium Hydroxide
Flow Rate: 0.5 mL/min
Temperature: Ambient (23 °C)
Injection Volume: 1.0 µL
Detection: UV 230 nm

Summary

- What do you do when C18 does not work?
- Stick with reversed-phase but--
  - Adjust pH of mobile phase
  - Try a more polar bonded phase
- Consider HILIC
- Chiral phases
- Fast Poroshell methods make method development faster

Contact Agilent Chemistries and Supplies Technical Support

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

Option 1 for GC/GCMS Columns and Supplies

Option 2 for LC/LCMS Columns and Supplies

Option 3 for Sample Prep Products, Filtration and QuEChERS

Option 4 for Spectroscopy Supplies

Available in the USA 8-5 all time zones

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

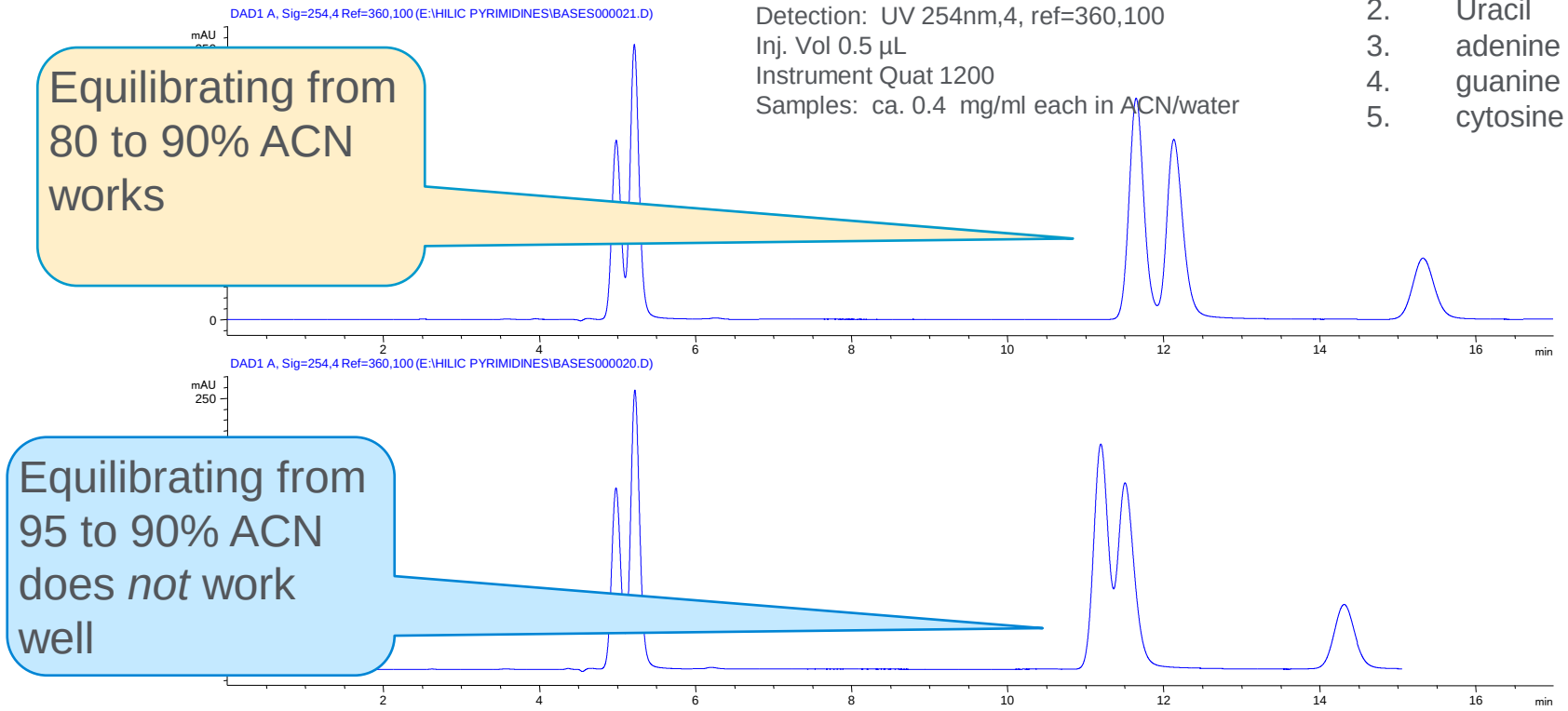
Equilibrate from high aqueous to low

Critical factor when changing mobile phases

ZORBAX Rx-Sil, 2.1 x 150, 5um

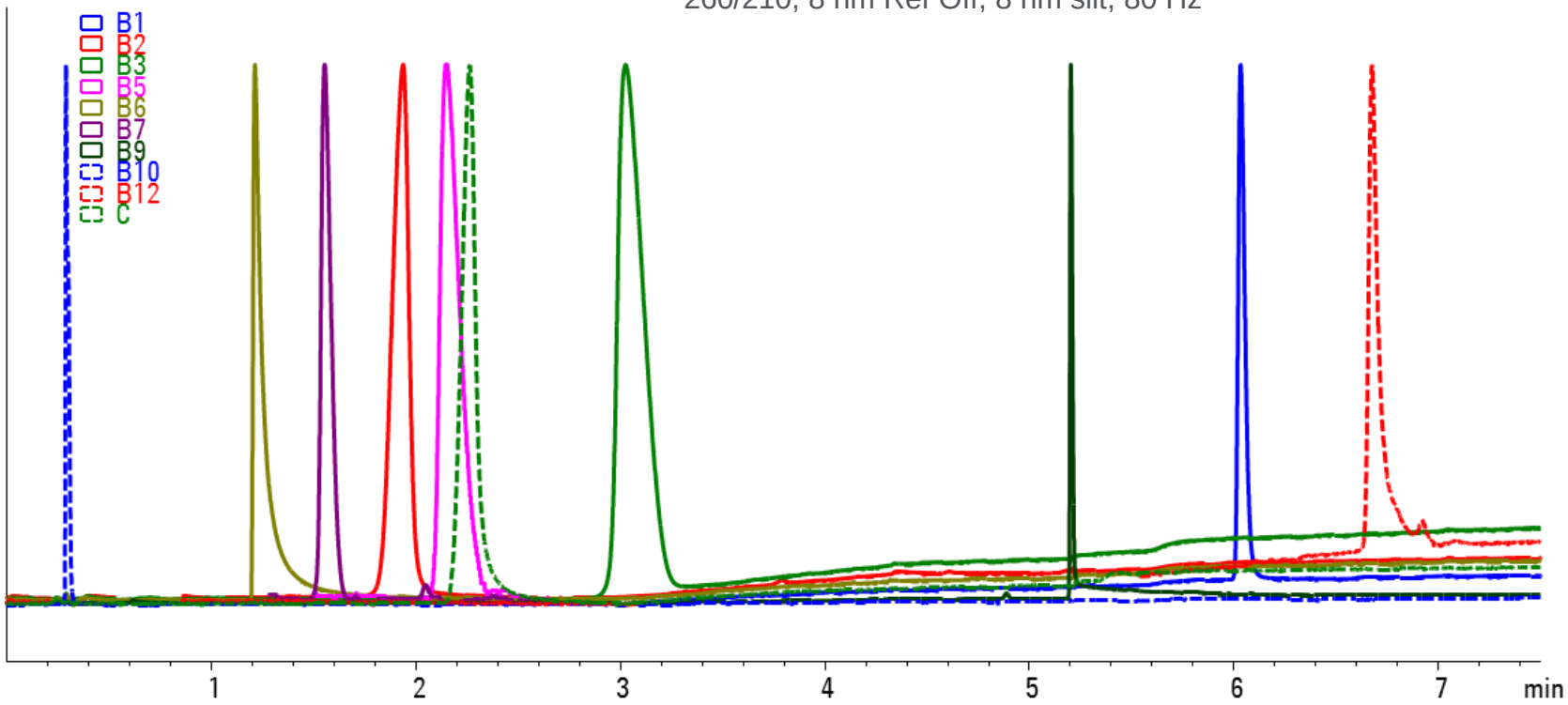
A: 25mM ammonium acetate with 2.5 mM ammonium formate
B: acetonitrile
10:90 A:B
Flow : 0.1 mL/min
Temp: 25 °C
Detection: UV 254nm,4, ref=360,100
Inj. Vol 0.5 µL
Instrument Quat 1200
Samples: ca. 0.4 mg/ml each in ACN/water

1. thymine
2. Uracil
3. adenine
4. guanine
5. cytosine

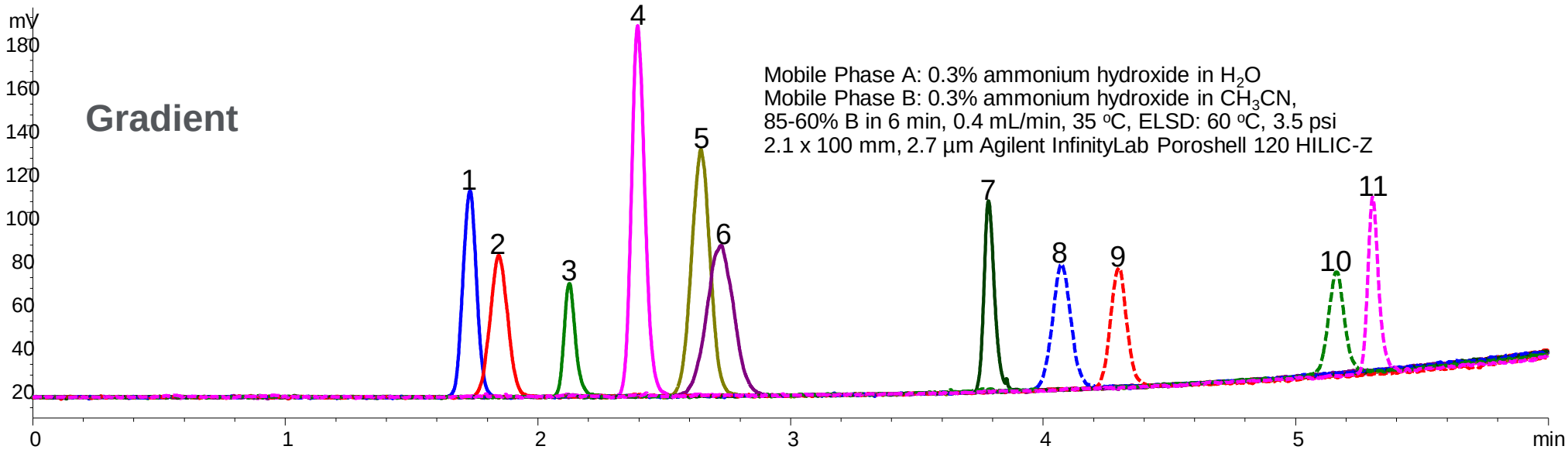


Water Soluble Vitamins
HILIC

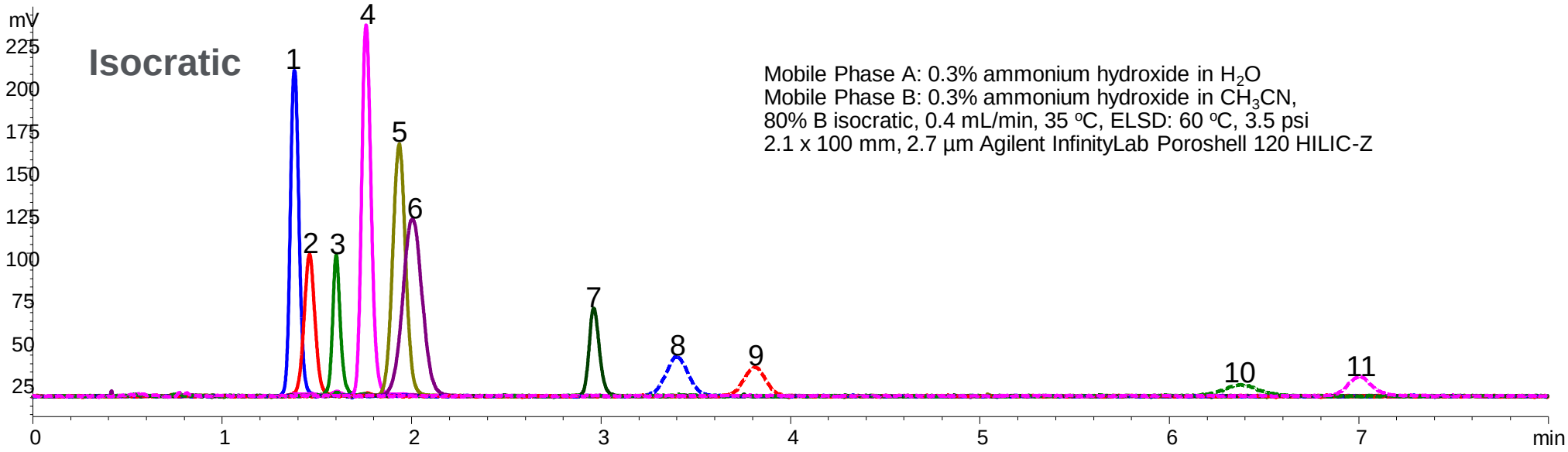
Poroshell 120 HILIC
A: 100 mM Ammonium Formate in H2O pH 3.0 with Formic Acid
B: CH3CN
0.5 mL/min, 97% B for 2.5 minutes, then 97-60%D in 5 minutes
injection volume: varies according to signal strength
TCC: 30 C
260/210, 8 nm Ref Off, 8 nm slit, 80 Hz



Sugar Analysis on Agilent InfinityLab Poroshell 120 HILIC-Z using ELSD



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



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

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