A Look at *Column Choices*

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Applications Engineer

Columns and Supplies Technical Support

Overview

What to consider when choosing a column?

- Column chemistry
  - Silica surface
  - Bonded phase
  - End-capping
- Particle size options
  - Totally porous
  - Superficially porous
- Method conditions
- Column lifetime

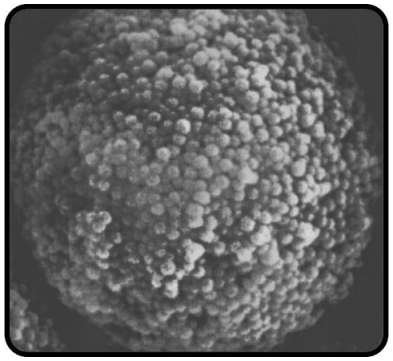
Silica Column Characteristics

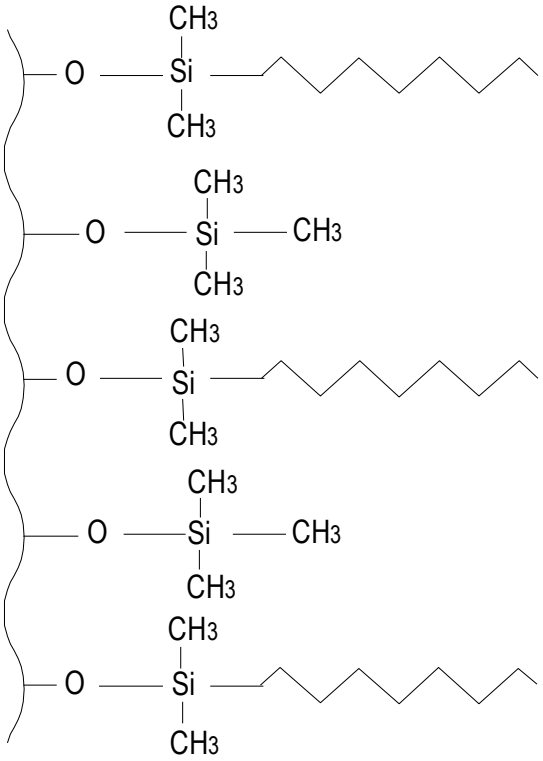
Surface area

- Pore size
- Particle size

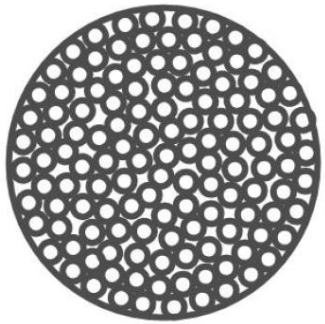
Surface chemistry

- Bonding
- Silanols





Pore Size



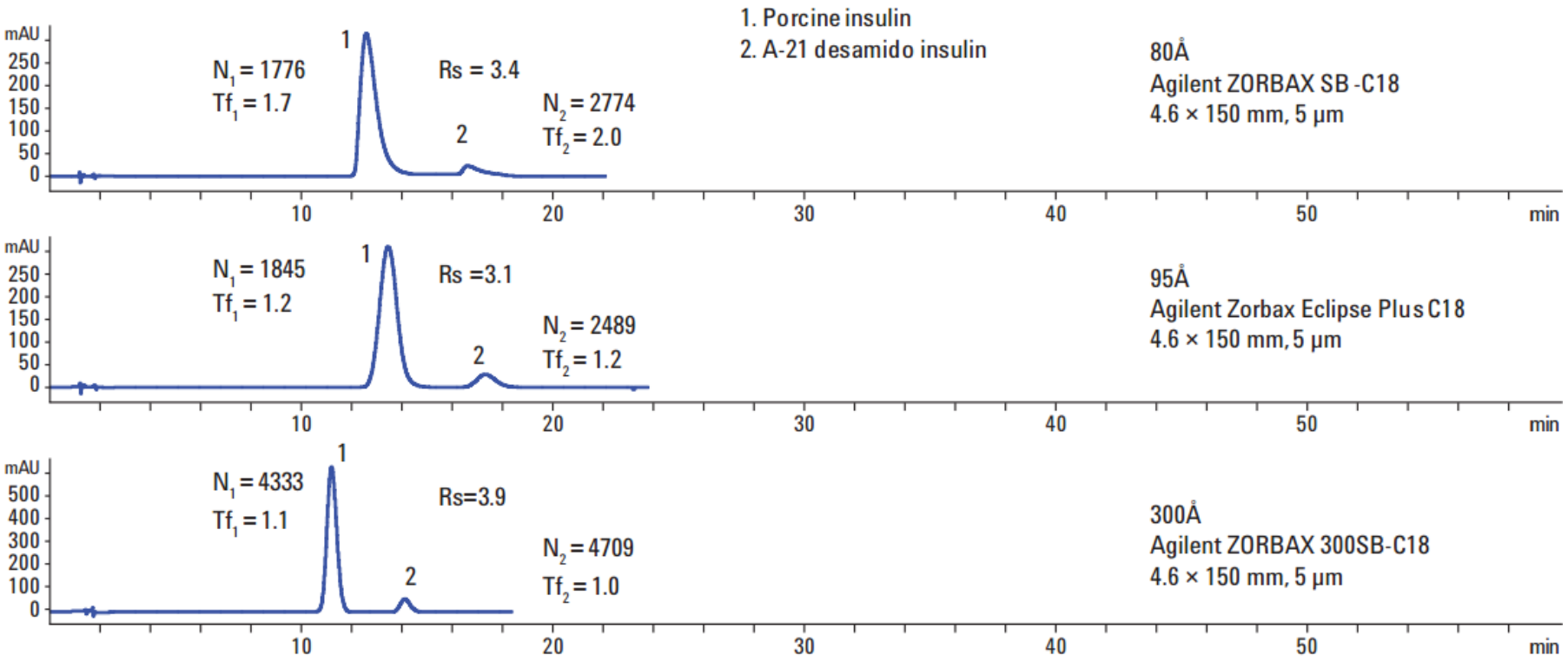
Small molecules

- 80 – 120 Å
- Maximizes loading and retention

Peptides, proteins, other large biomolecules

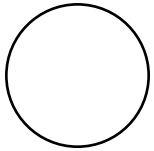
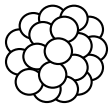
- 120 Å (Peptides)
- 300 Å to 450 Å (or higher)
- Maintain high efficiency

Pore Size

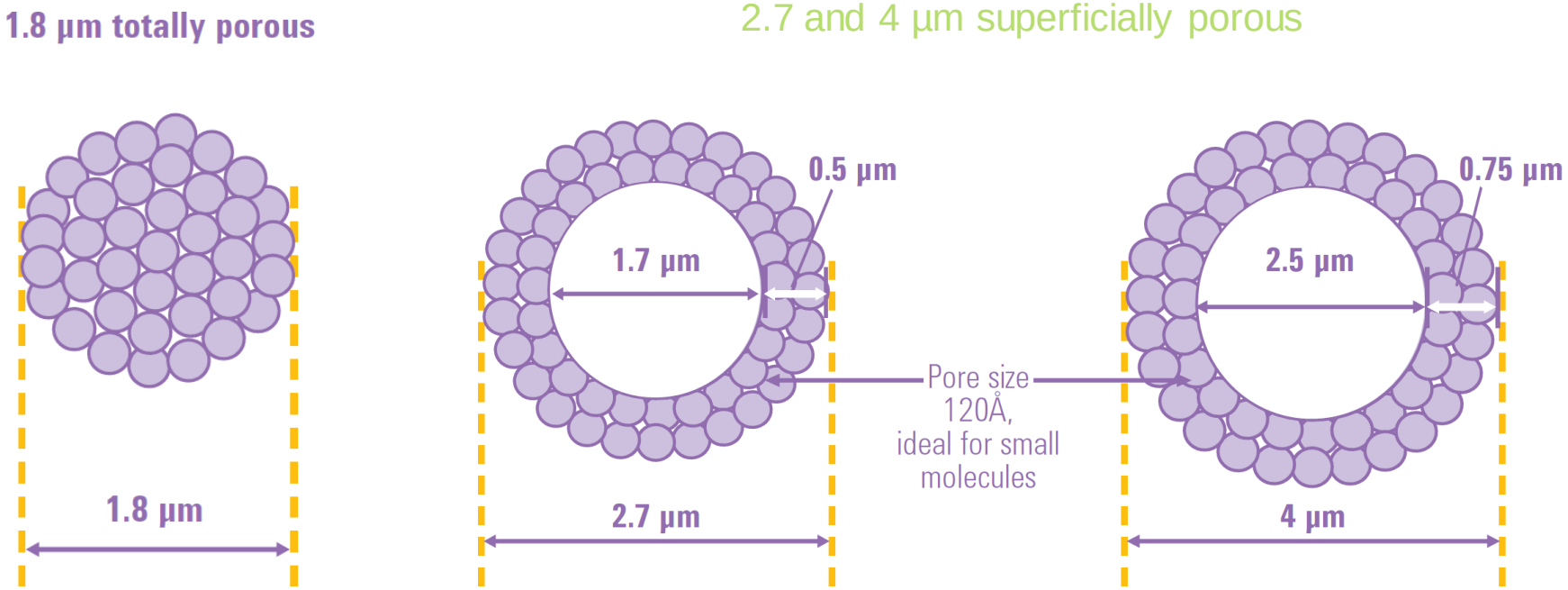


Chromatograms on 4.6 × 150 mm, 5 µm columns with different pore size.

Particle Technologies

Year(s) of Acceptance	Particle Size	Most Popular Nominal Size	Plates / 15cm
1950's		100µm	200
1967		57µm (pellicular)	1,000
1972		10µm	6,000
1985		5µm	12,000
1992		3.5µm	22,000
2003		≤2µm	>30,000
2007/2008		2.7 µm	32,000

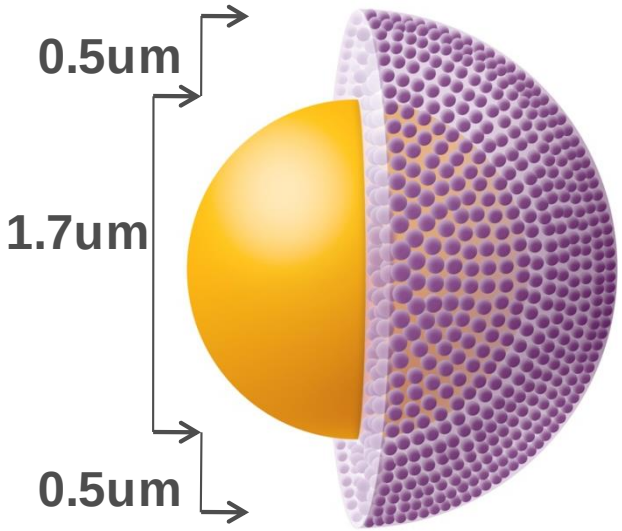
Particle Technologies



Superficially Porous Column Technologies

Poroshell 120 columns:

- Efficiency ≈ 90% of sub-2 µm
- Pressure ≈ 40-50% of sub-2 µm
- N ≈ 2X 3.5 µm (totally porous)
- d_p = 2.7µm
- 2 µm frit to reduce clogging
- P_{limit} = 600 bar for HPLC or UHPLC
- Particles
  - 1.7 µm solid core
  - 0.5 µm diffusion path
  - 2.7 µm total diameter



Comparing Efficiency and Pressure with Different Types of Columns

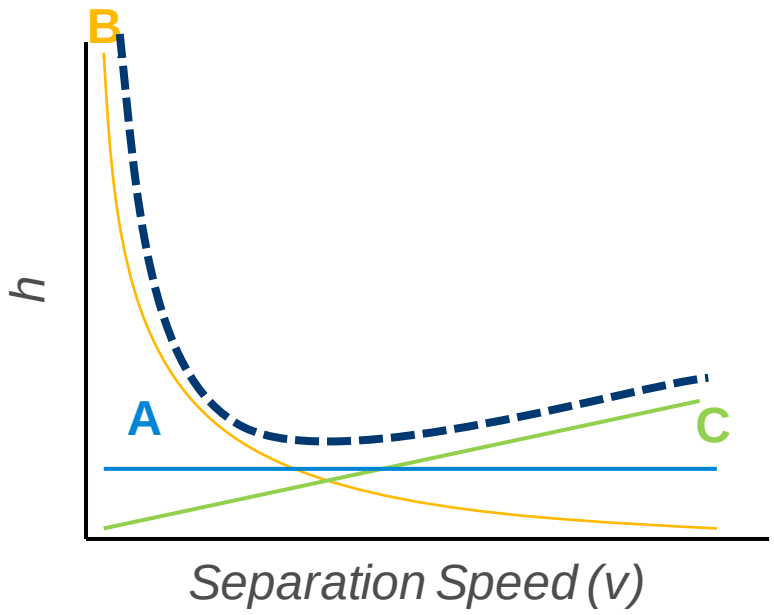
Particle Size/Type	Pressure	Efficiency	LC Compatibility
5 µm Totally Porous	80 bar	5,000	All 400 bar instruments
3.5 µm Totally Porous	123 bar	7,800	All 400 bar instruments
2.7 µm Poroshell 120	180 bar	12,000	All LCs/UHPLCs (up to 600 bar)
1.8 µm Totally Porous	285 bar	12,500	All LCs/UHPLCs (up to 1200 bar)

Columns: 4.6 x 50mm, Mobile Phase: 60% ACN:40% Water Flow Rate: 2 mL/min

Efficiency Improvement with Superficially Porous Particles

van Deemter equation:

$$h = A + B/\nu + C \cdot \nu$$

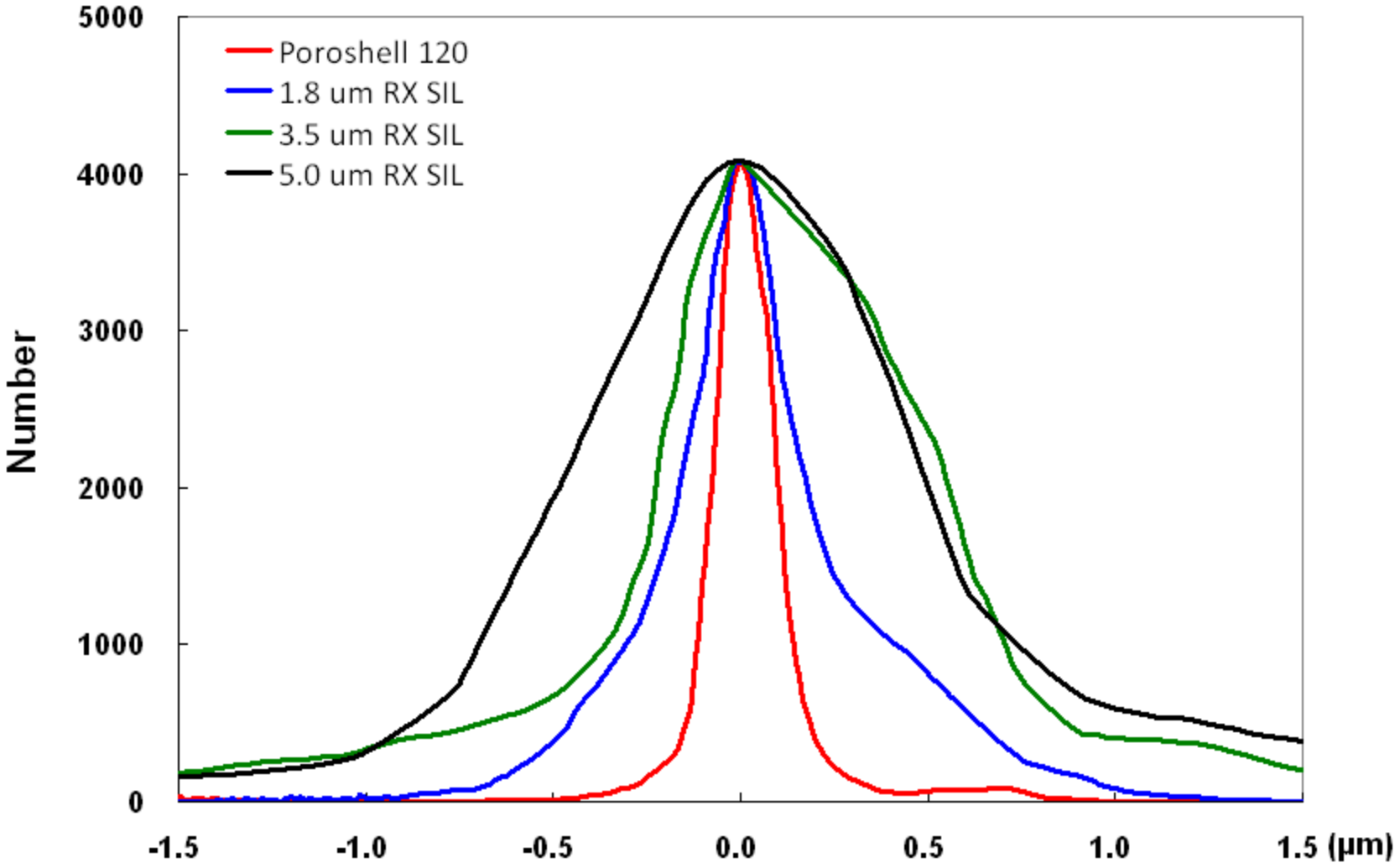


Lower *h* = higher efficiency!

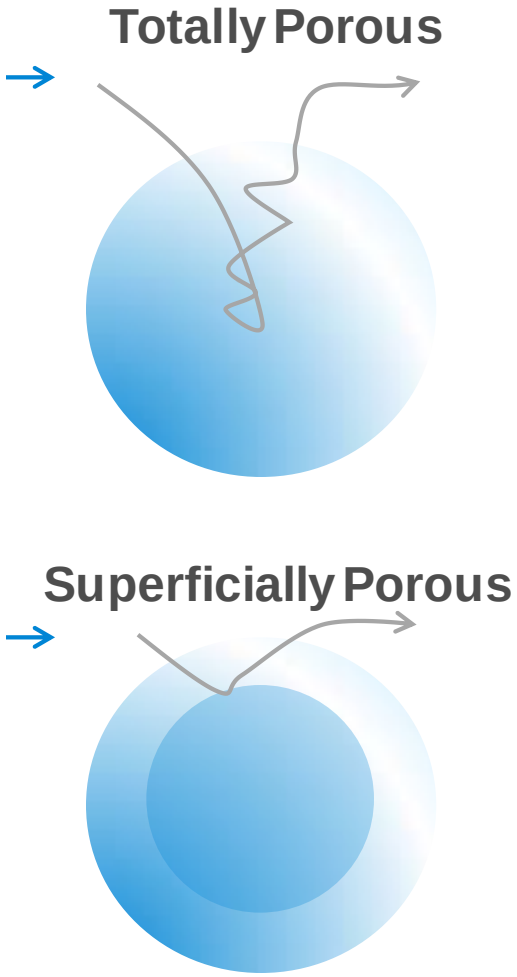
- **A term – eddy diffusion and flow distribution**
  - particle size & packing quality important
  - narrow particle size distribution
- **B term – longitudinal diffusion**
  - Slightly lower due to longitudinal diffusion reduction
- **C term – mass transfer**
  - shorter diffusion paths
  - better with superficially porous particles
  - more effect on large molecules

Comparison of Particle Size Distributions

Totally Porous and Poroshell 120 Particles



Analyte Mass Transfer Improvements through Lower Diffusion



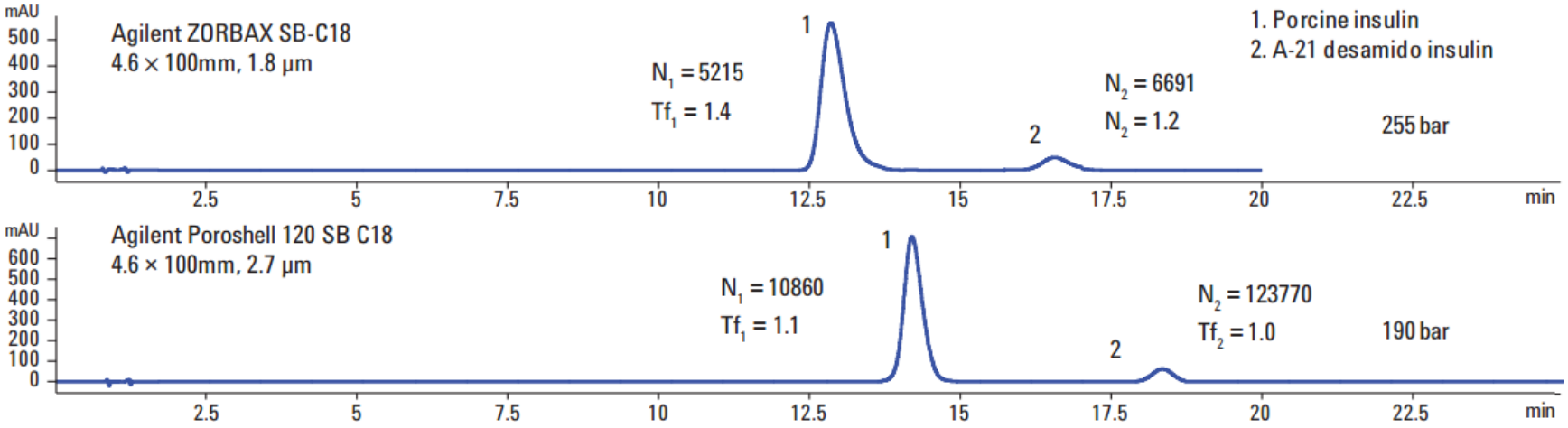
- **Totally porous particles**
  - **diffusion throughout particle**
- **Poroshell 120**
  - **diffusion limited to outer shell**

van Deemter equation:

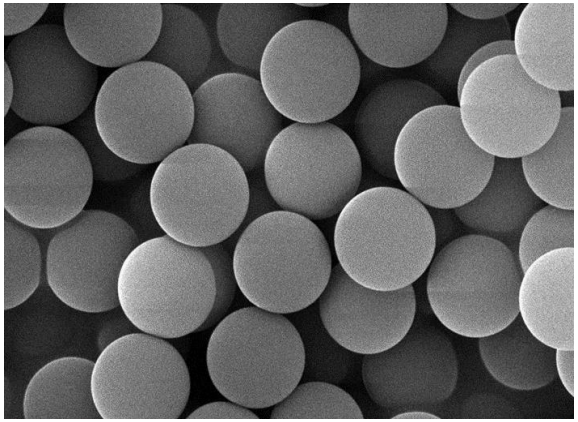
$$h = A + B/\nu + C \cdot \nu$$

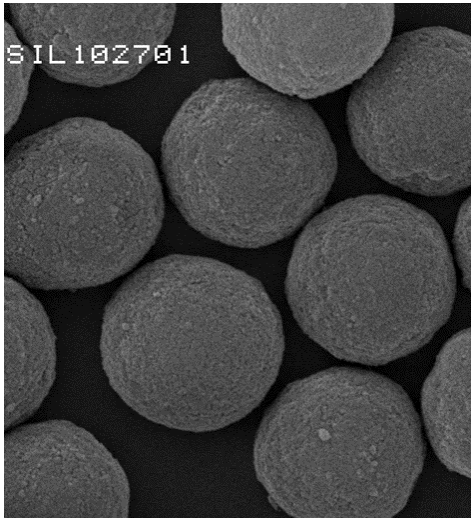
- **Results:**
  - **Lower C term**
  - **Higher efficiency**
- **And**
  - **Higher flow rate with**
  - **Minimal impact on efficiency**

Pore Size and Efficiency



Making a Poroshell Particle





Make the solid core

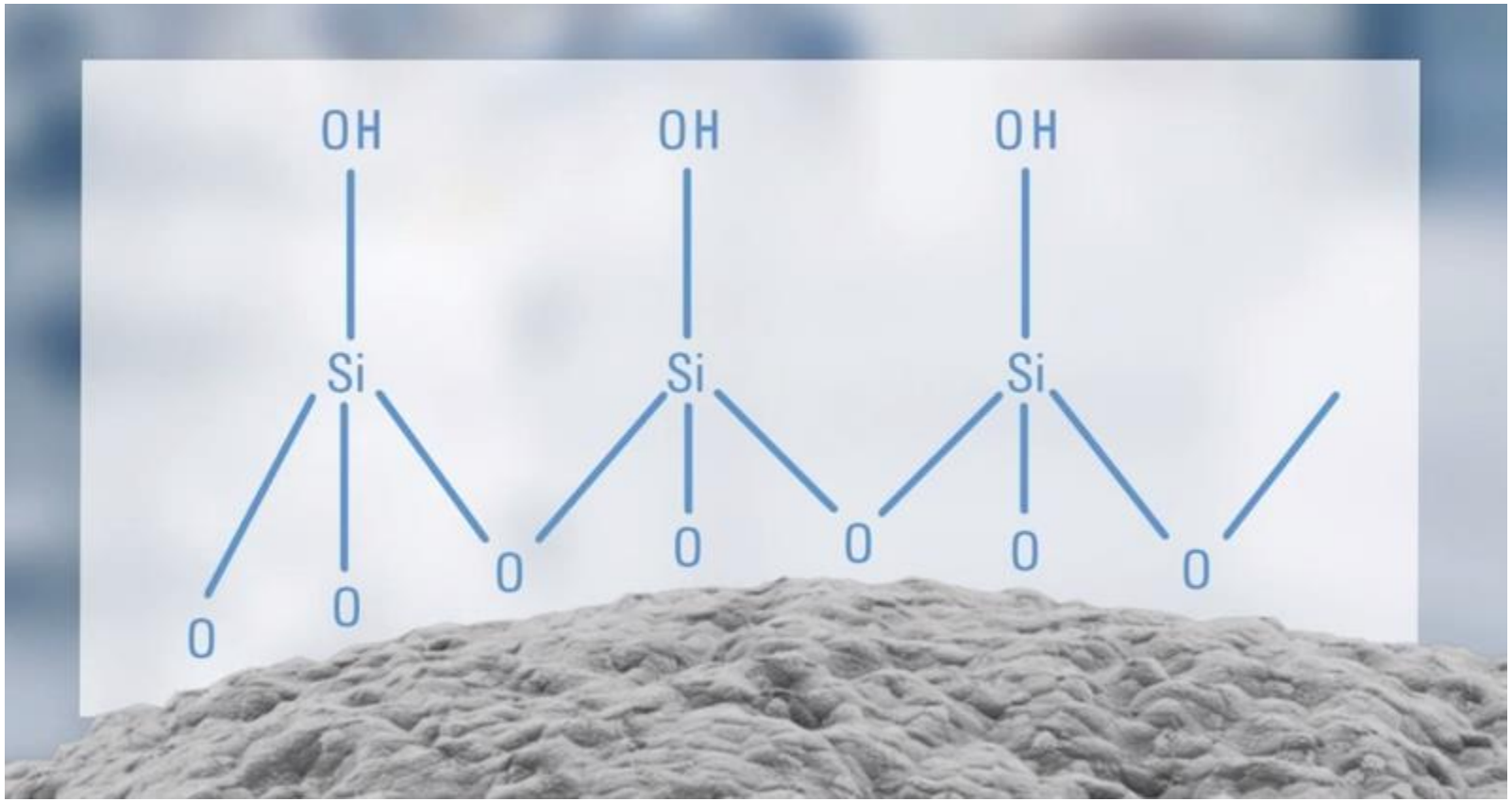
- Smooth surface
- Tight particle size distribution
- Tightly packed column bed
- Higher efficiency

Apply the porous shell

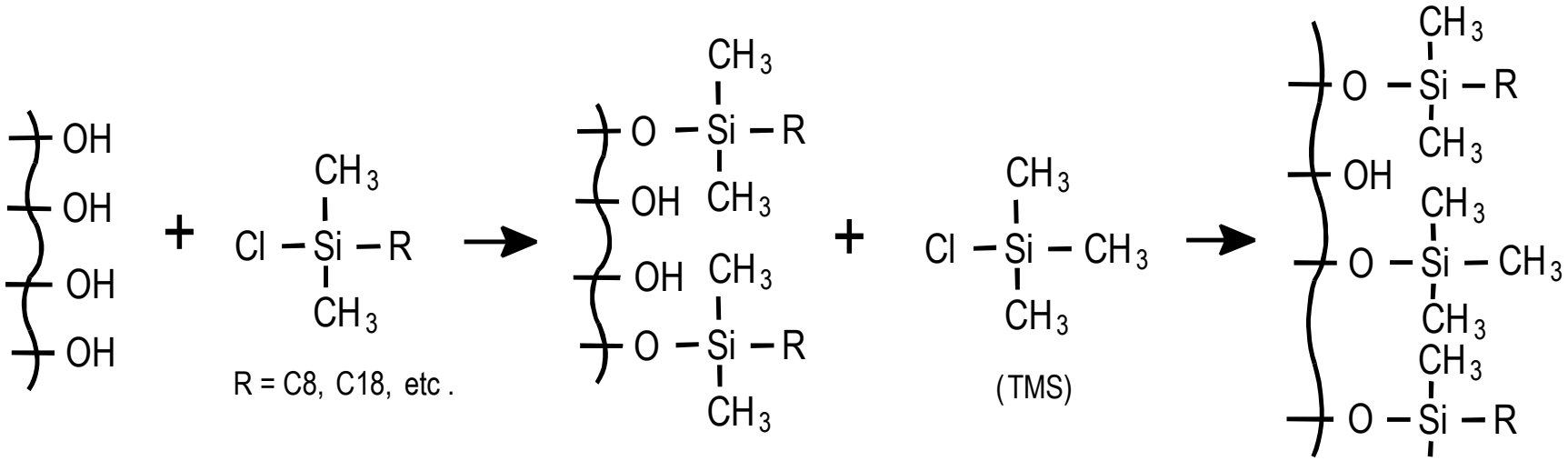
- Single coacervation step
- High yields
- Better reproducibility

Apply the bonded phase

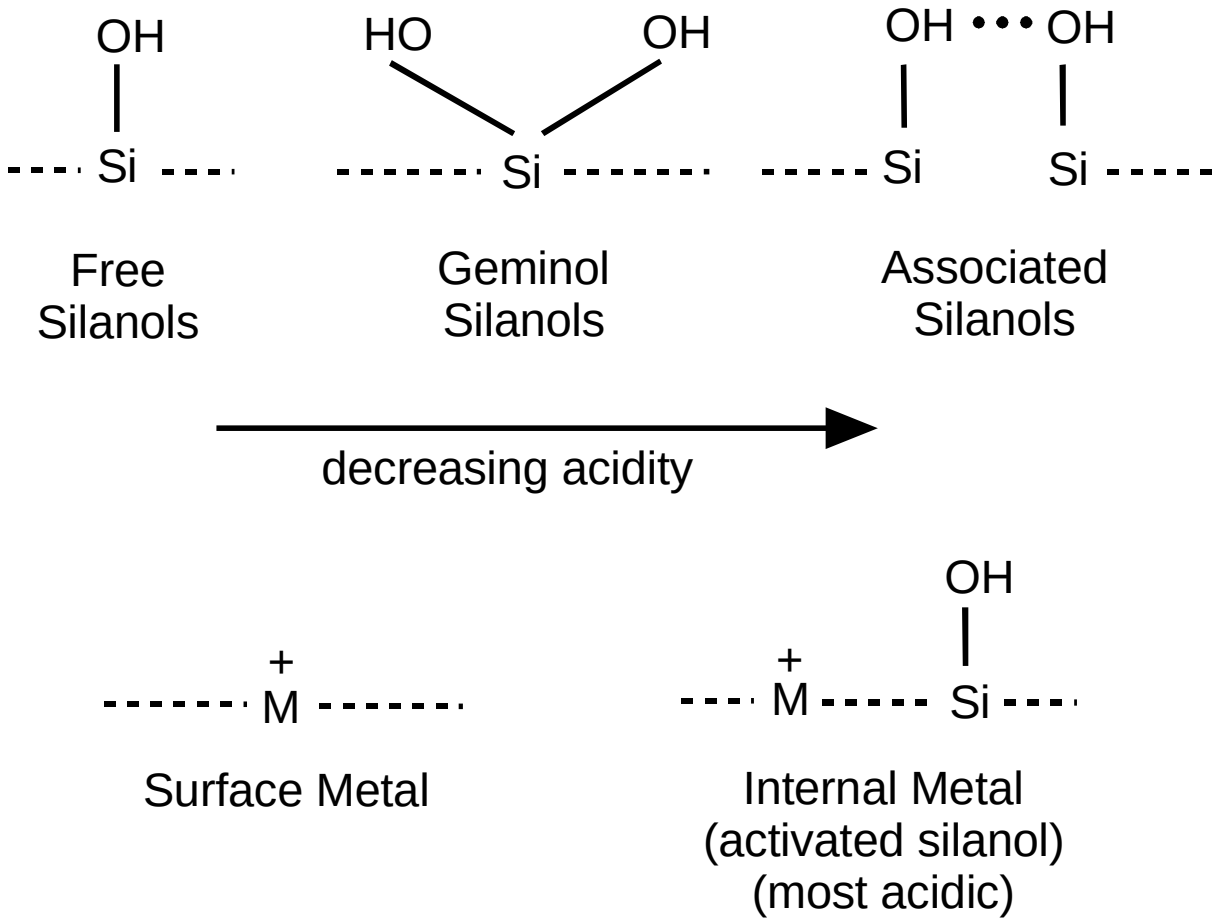
Silica Particle Surface



Typical Stationary Phase Bonding and Endcapping Reaction

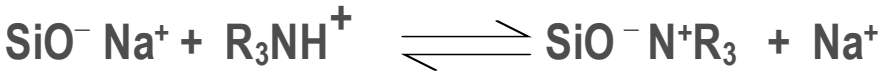


The Surface of Silica Supports



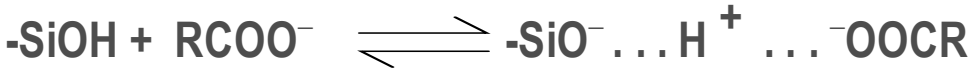
Potential Secondary Interactions

Ion-exchange



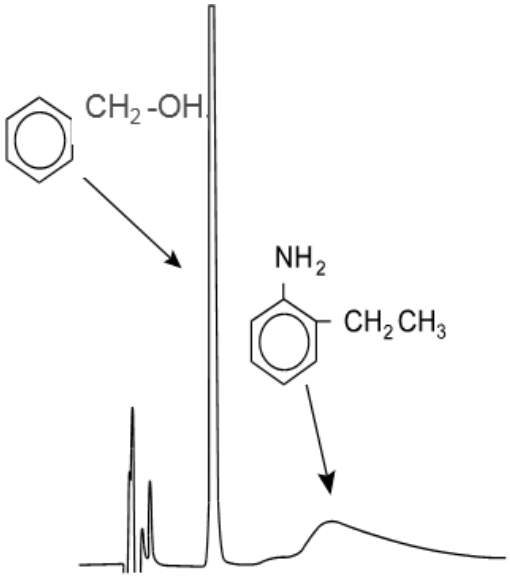
1. Ionized silanols (SiO^-) will ion-exchange with protonated bases (R_3NH^+) which can cause tailing and method variability. This occurs most often at mid pH where silanols are ionized.

Hydrogen bonding



2. Unprotonated acids can compete for H^+ with protonated silanols. This can occur at low pH.

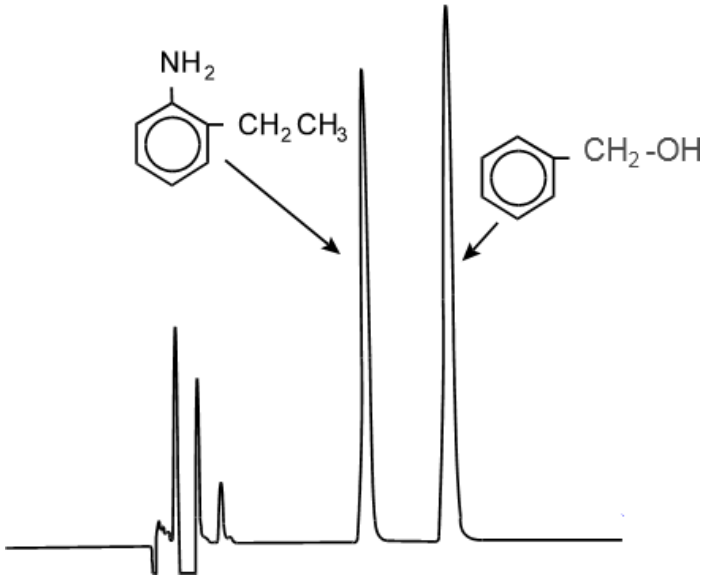
Highly Purified Zorbax Rx-Sil



Original ZORBAX, 1973 and other type A silicas

- basic compounds can tail

Conditions: Flow Rate: 2.0 mL / min.
Mobile Phase: 5% 2-Propanol in Heptane



ZORBAX Rx-Sil, 1987 and other Type B silicas

- basic compounds have less tailing
- lower effective silanol pKa

Poroshell 120 Column Chemistries

Multiple bonded phases for flexibility in method development

Poroshell 120 EC-C18 and C8

- Robust end-capped C18/C8 for best peak shape at pH 2-9

Poroshell 120 Stablebond C18 and C8

- Robust chemistries for pH<2
- Non-endcapped for alternate selectivity

Poroshell HPH-C18 and HPH-C8

- Long lifetime at high pH

Poroshell 120 Phenyl-Hexyl

- Excellent choice for pi-pi interactions
- Selectivity similar to phenyl, diphenyl, or other phenyl-hexyl columns

Poroshell 120 SB-Aq

- Proprietary bonded phase is an excellent choice for polar analytes

Poroshell 120 Bonus-RP

- Embedded polar group provides unique selectivity for polar compounds

Poroshell 120 EC-CN

- Flexible end-capped CN chemistry for normal and reversed-phase separations

Poroshell 120 HILIC

- Bare silica HILIC for use in hydrophilic interaction chromatography of polar molecules

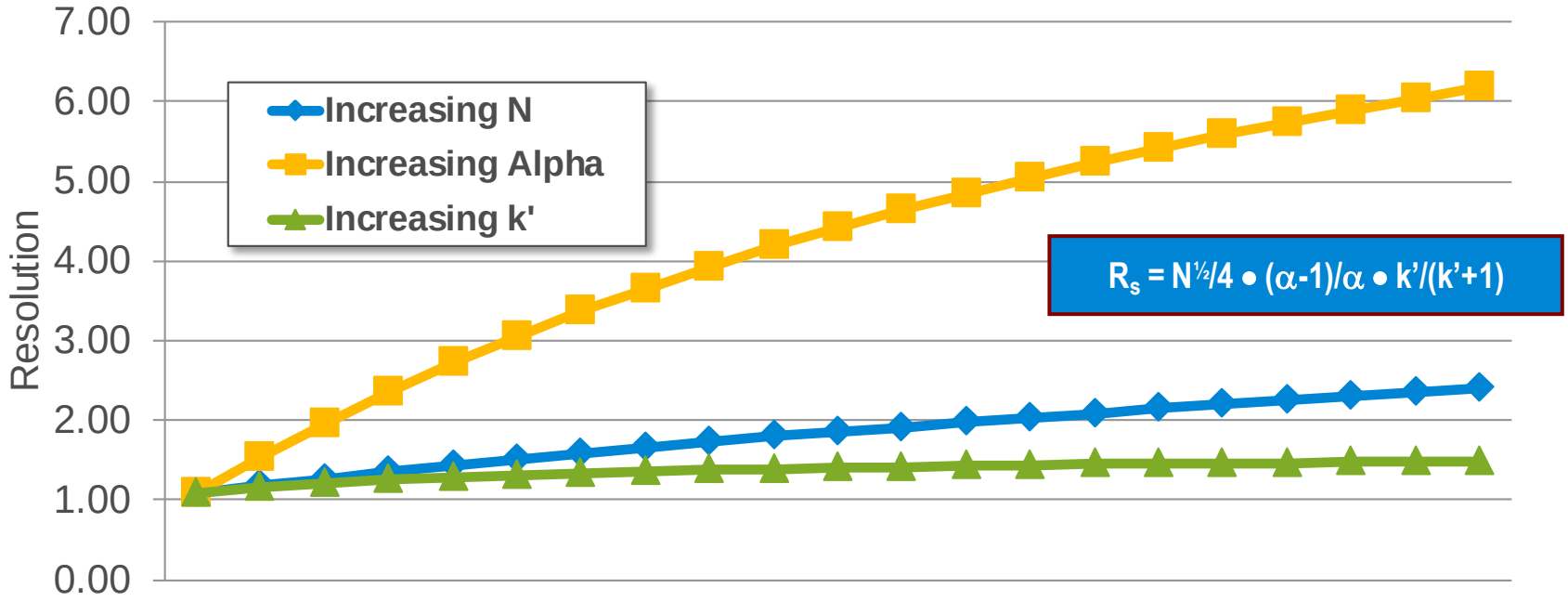
Poroshell 120 PFP

- Pentafluorophenyl chemistry for orthogonal selectivity relative to C18

Porshell 120 Column Chemistries

Bonded Phase	Pore Size (Å)	Temp. Limit (°C)	pH Range	Endcapped	Carbon Load (%)	Surface Area (m^2/g)
EC-C18	120	60	2 - 8	Double	10	130
EC-C8	120	60	2 - 8	Double	5	130
SB-C18	120	90	1 - 8	No	8	130
SB-C8	120	80	1 - 8	No	5.5	130
HPH-C18	100	60	3 - 11	Double	Proprietary	95
HPH-C8	100	60	3 - 11	Double	Proprietary	95
Phenyl-Hexyl	120	60	2 - 8	Double	9	130
SB-Aq	120	80	1 - 8	No	Proprietary	130
Bonus-RP	120	60	2 - 9	Triple	9.5	130
HILIC	120	60	0 - 8	No	N/A	130
EC-CN	120	60	2 - 8	Double	3.5	130
PFP	120	60	2 - 8	Yes	5.1	130

Why So Many Phase Chemistries?



Plates:	5000	10000	15000	20000	25000
Alpha:	1.10	1.35	1.60	1.85	2.1
k	2.0	4.5	7.0	9.5	12.0

Selectivity Impacts Resolution Most

- Change bonded phase
- Change mobile phase

} Typical Method Development Parameters

- Plates are easiest to increase

Method Development Scheme

Changing selectivity to improve resolution:

Mobile phase (1st choice to change because it's easy)

- Mobile phase – organic modifier (ACN, MeOH etc.)
- Mobile phase – pH – over a wide pH range – pH 1-12 if needed

Bonded phase (optimization for robust methods)

- Phases other than C18/C8
- Phenyl-Hexyl, Polar-embedded, CN, PFP

Why Is Changing the Bonded Phase Effective?

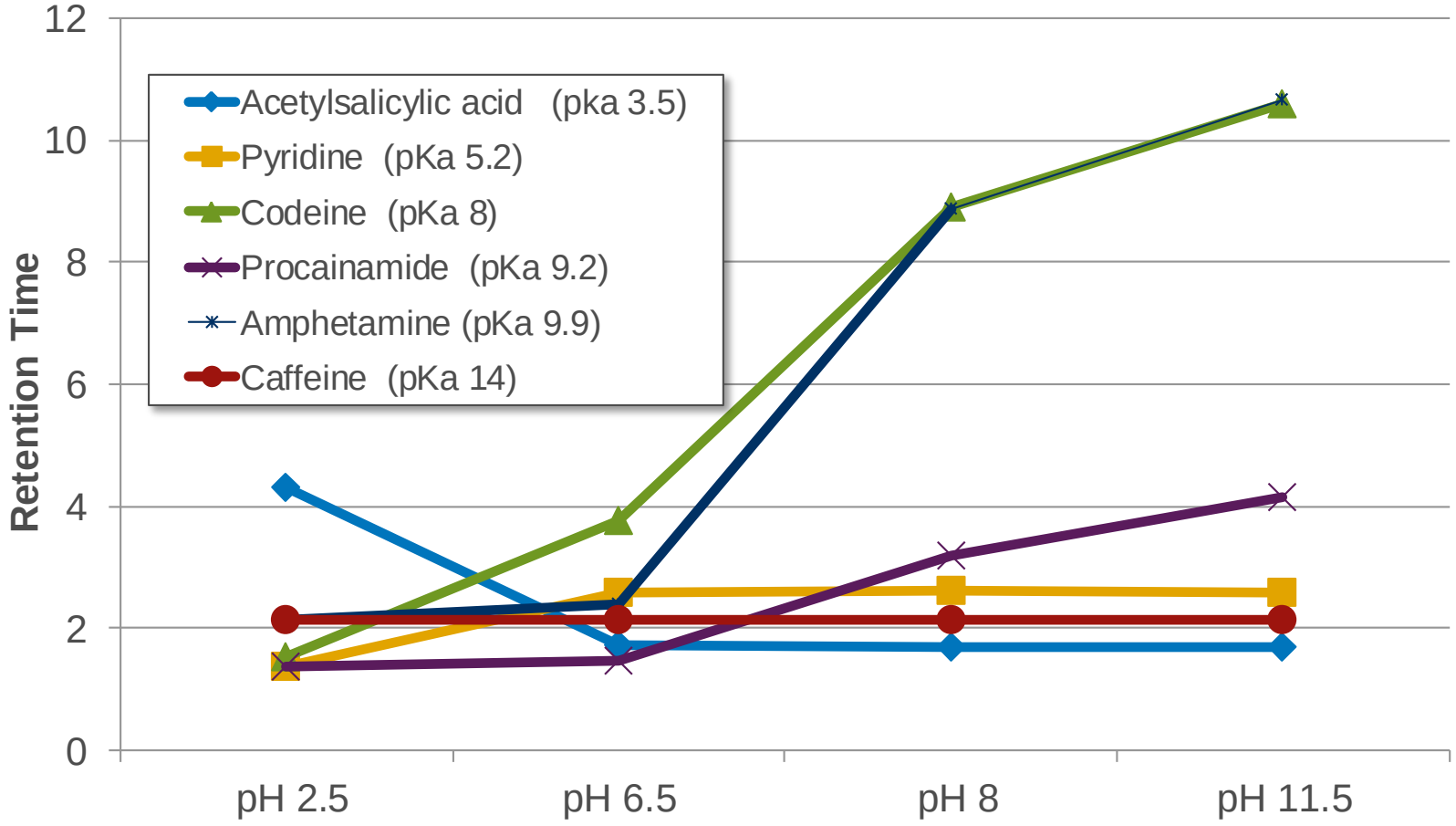
- Differences in interactions between polar and non-polar compounds.
- Other types of interactions with a bonded phase can be exploited (pi-pi interactions etc.)
- These all change with bonded phase!
- Changing the bonded phase can improve selectivity/resolution, reduce analysis time

When you use Poroshell 120 columns the comparison of bonded phases can be done quickly!!

- Multiple column choices available with different high speed technologies make this easy

Change in Retention with pH for Ionizable Compounds is Compound-Dependent

More retention for non-charged analytes (i.e. acids at low pH and bases at high pH)

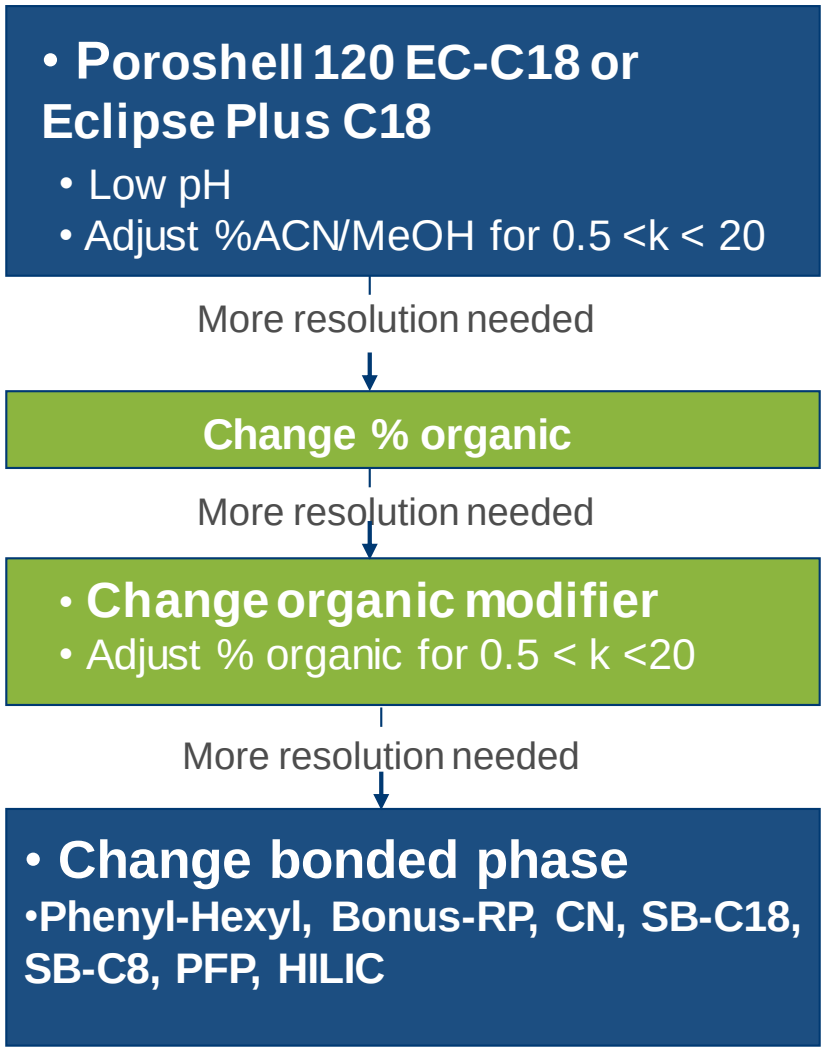


Mobile Phase: 45% MeOH, 55% 20 mM Phosphate Buffer

Change in Retention with pH for Ionizable Compounds is Key to Method Development

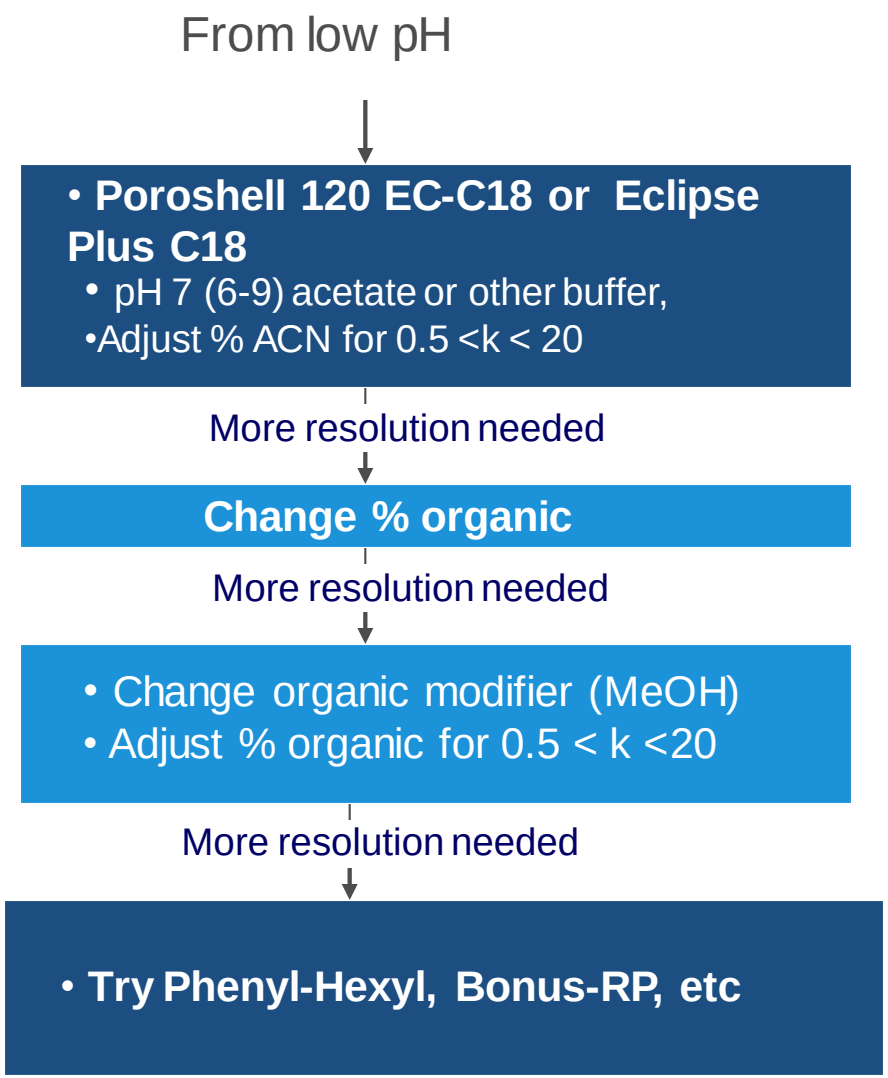
- Non-charged analytes have better retention (i.e. acids at low pH and bases at high pH)
- Silanols on silica ionize at mid-pH, increasing retention of basic analytes (i.e possible ion-exchange interactions)
- Choose mobile phase pH to optimize retention and selectivity during method development
- Poroshell 120 EC-C18 and C8 can be used over a wide pH range
- Other choices exist for high pH

First Steps in a Method Development Scheme



- Select a high quality (Poroshell 120 EC or Eclipse Plus) C18 bonded phase first for good retention and resolution with typical acidic, basic and neutral samples.
- Optimize the organic component of the mobile phase to change selectivity
- Choose alternate bonded phases to completely optimize method if needed
- Evaluate other bonded phases and conditions for most robust method
- **With superficially porous or sub-2um column choices, steps can be done quickly**
  - **Makes it possible to find a robust method**

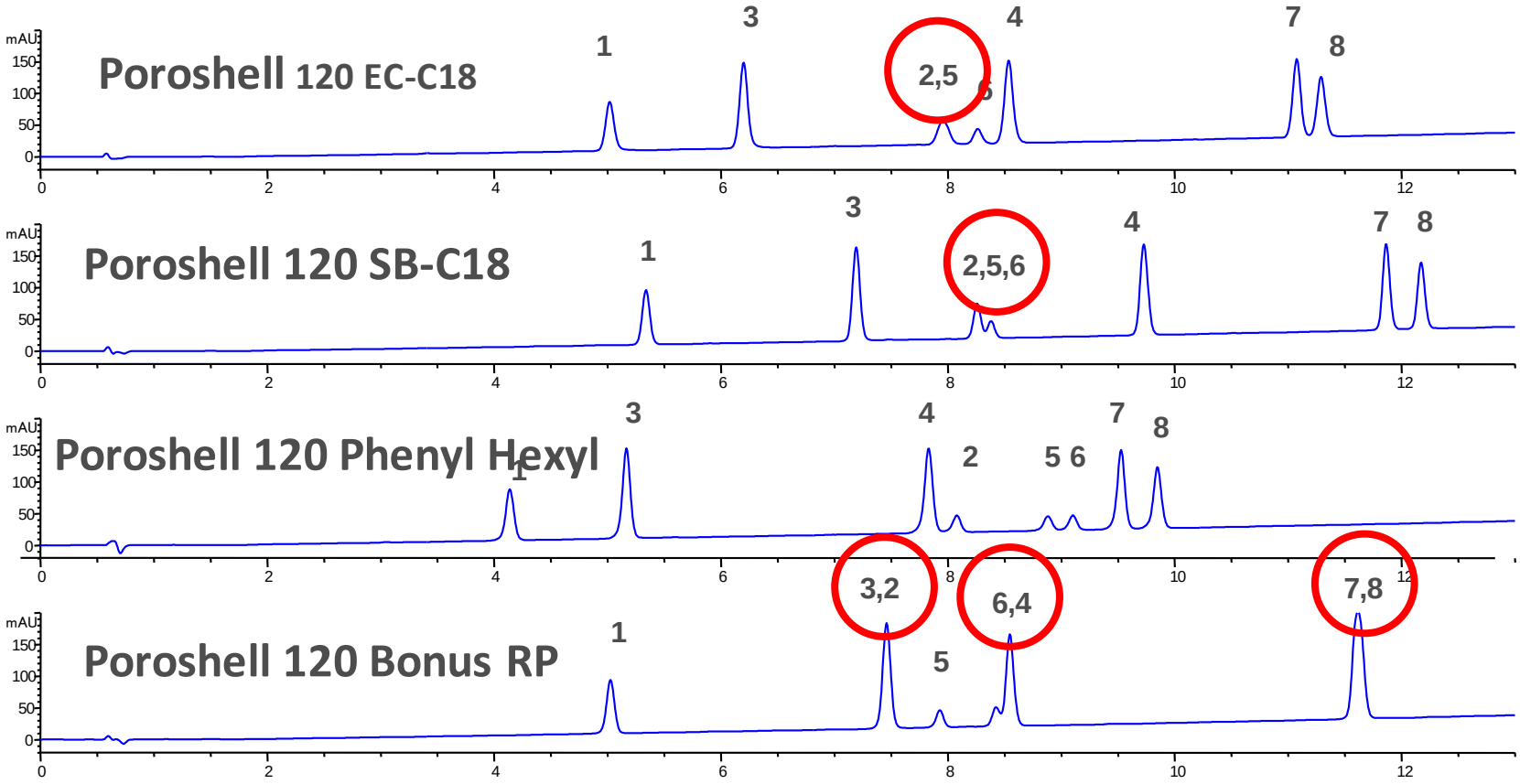
Method Development Scheme – Evaluating Mid pH



- Mid pH can provide better selectivity
- It may be more compatible with your sample
- The process for investigating mid pH is the same as for low pH
- Poroshell 120 EC- and Eclipse Plus deliver outstanding peak shape and lifetime performance at mid pH
- Alternate bonded phases should also be considered if improved selectivity is desired

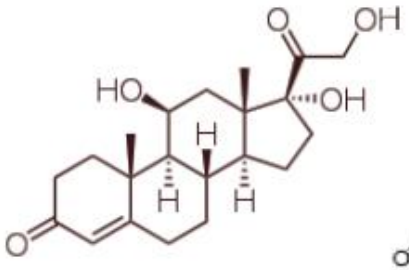
Separation of 8 Steroids with Methanol Gradient

Best Resolution of all analytes with Poroshell 120 Phenyl-Hexyl

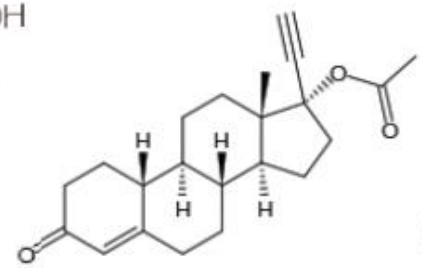


1. Hydrocortisone, 2. β-Estradiol, 3. Androstadiene 3,17 dione, 4. Testosterone, 5. Ethinylestradione, 6. Estrone, 7. Norethindone acetate, 8. Progesterone

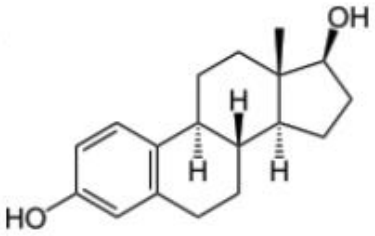
Separation of 8 Steroids with Methanol Gradient



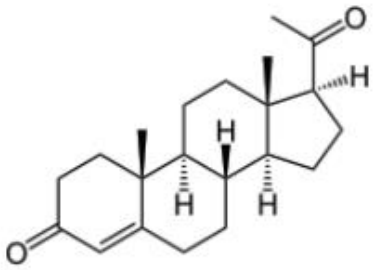
Hydrocortisone



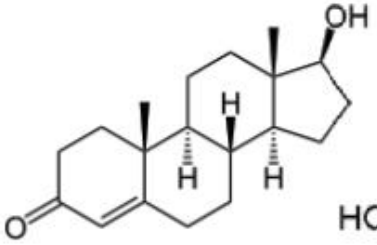
Norethindrone acetate



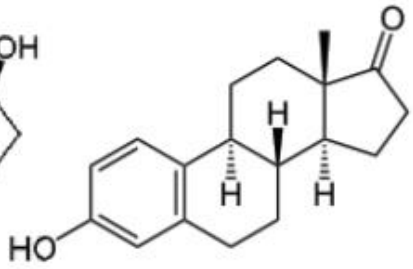
β Estradiol



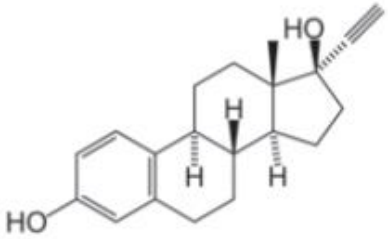
Progesterone



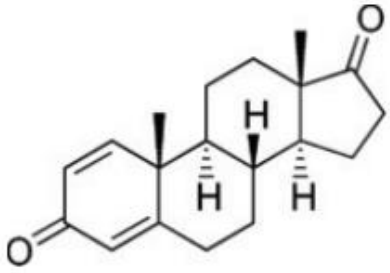
Testosterone



Estrone



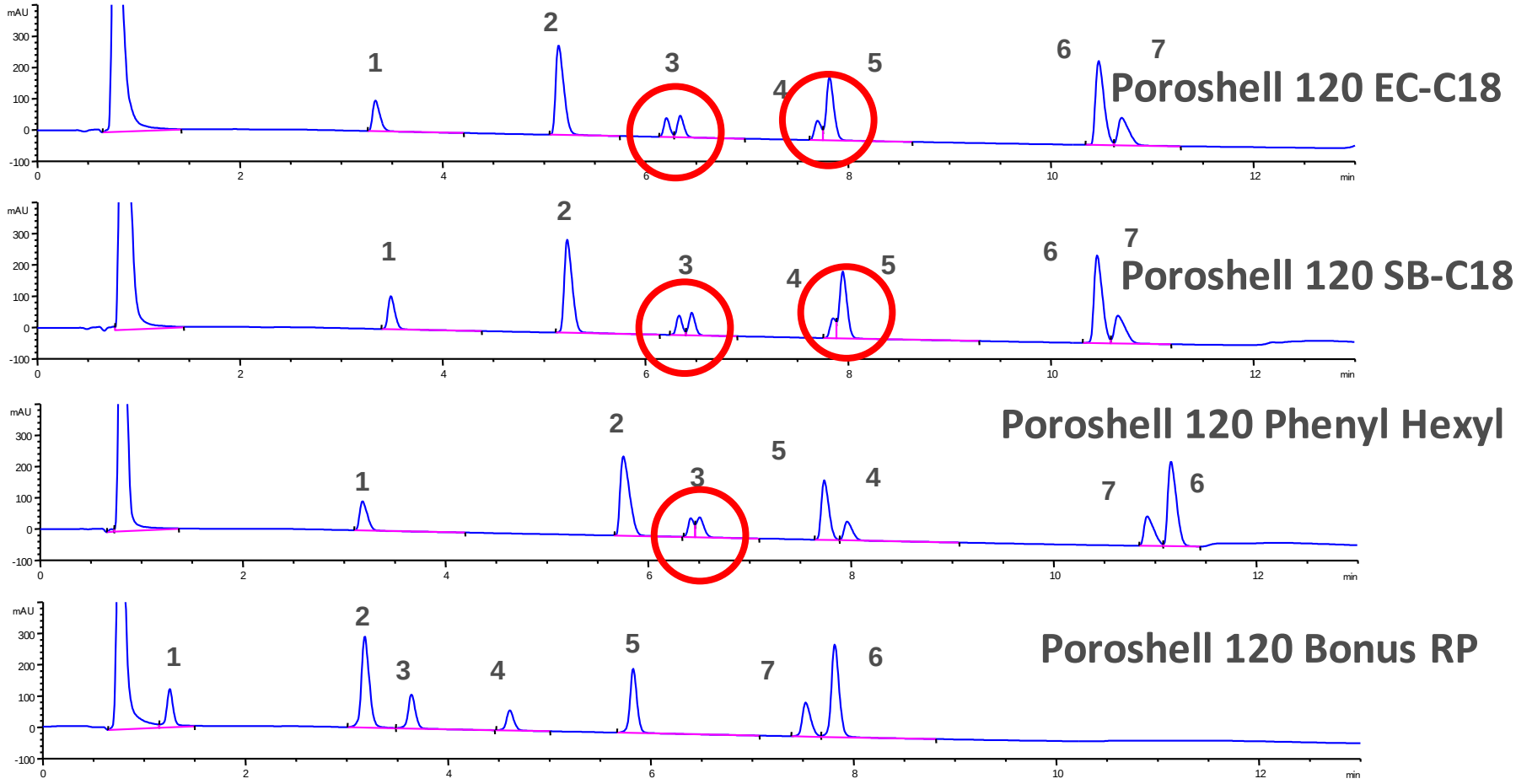
Ethinylestradiol



Androstadiene 3,17 dione

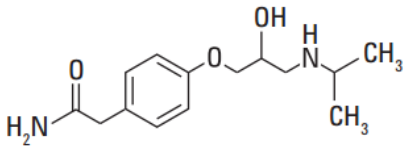
Beta Blockers with Methanol Gradient

Best Resolution of all analytes with Poroshell Bonus-RP

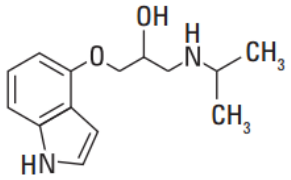


1.Atenolol, 2. Pindolol, 3. Naldolol, 4. Metoprolol, 5. Acebutolol, 6. Propranolol, 7. Alprenolol

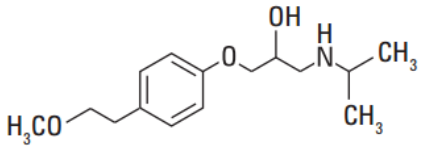
Beta Blockers with Methanol Gradient



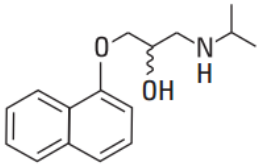
Atenolol



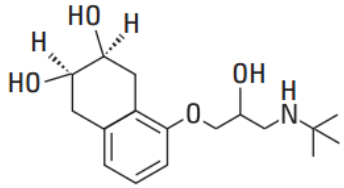
Pindolol



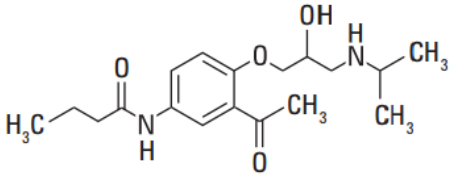
Metoprolol



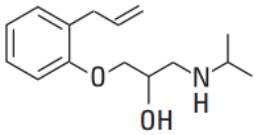
Propranolol



Nadolol



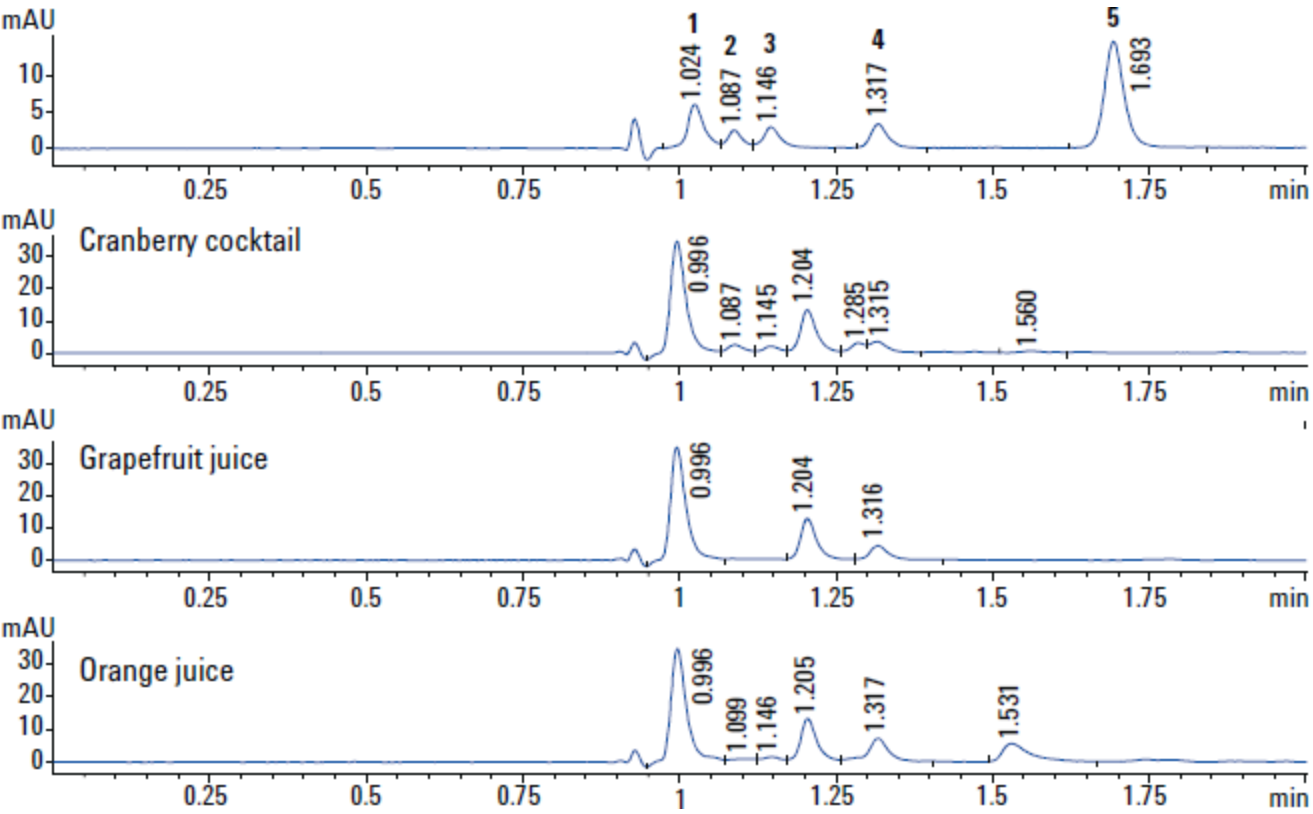
Acebutolol



Alprenolol

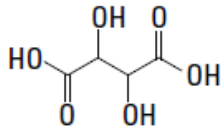
Samples prepared at 10 mg/mL in DMSO diluted in water to a final concentration of 0.1 mg/mL

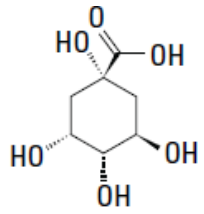
ZORBAX SB-Aq Phase

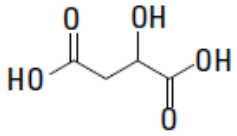


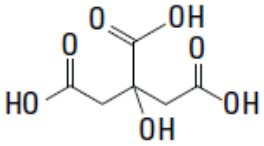
Peak ID

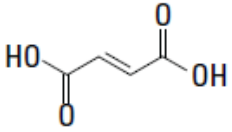
1. Tartaric acid
2. Quinic acid
3. Malic acid
4. Citric acid
5. Fumaric acid


Tartaric acid


Quinic acid


Malic acid


Citric acid


Fumaric 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

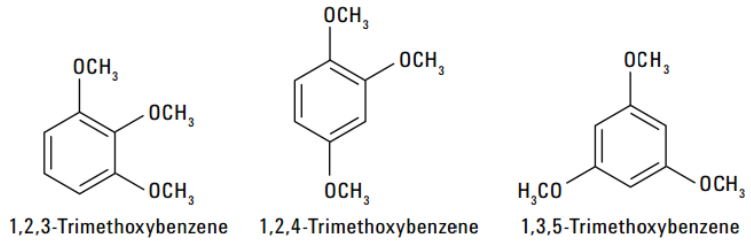
5991-1992EN

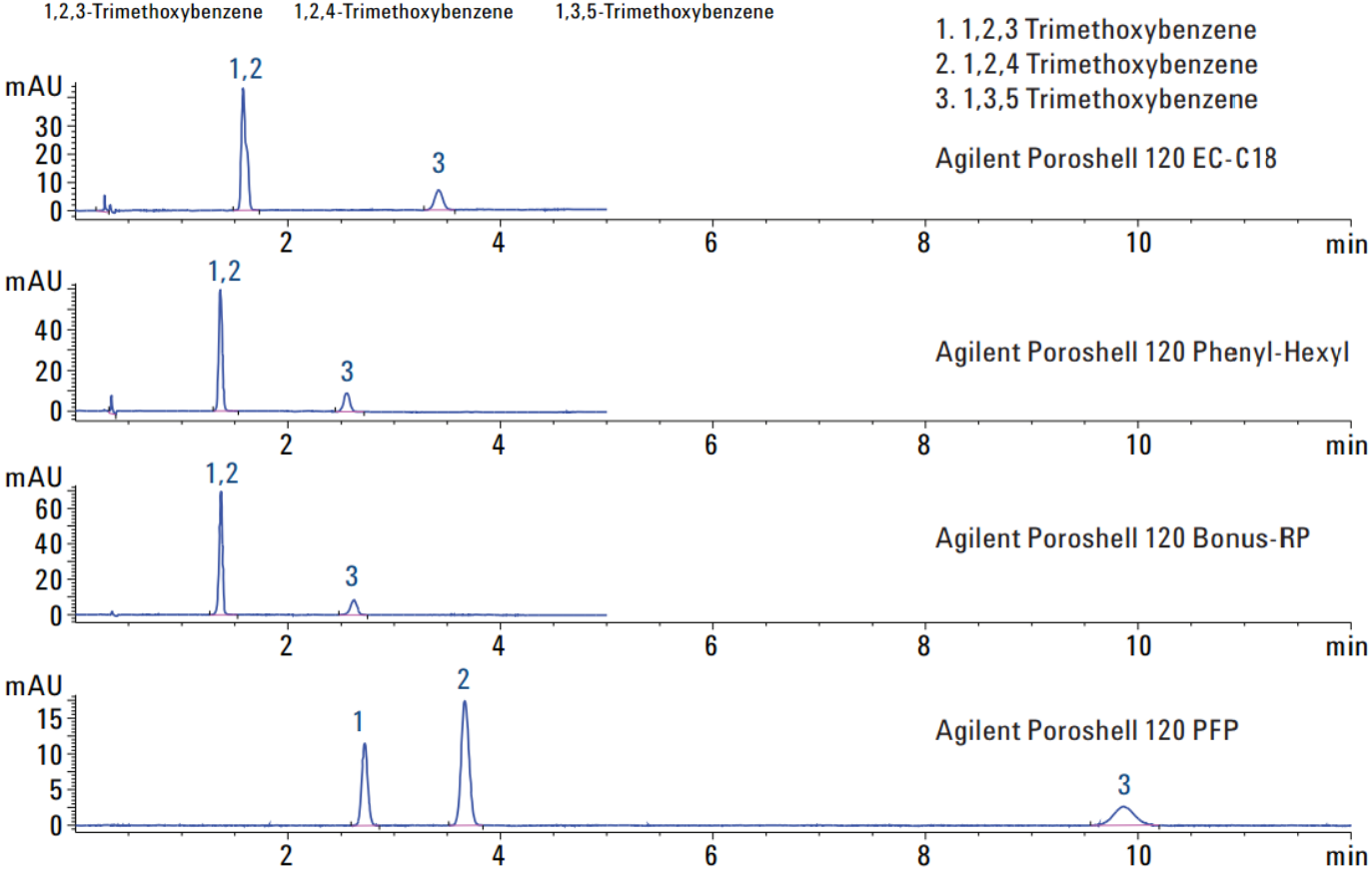
New Phase on Poroshell 120

Poroshell 120 PFP

- USP L43
- pentafluorophenyl bonded phase
- Excellent choice for polar analytes
- Unique pi-pi interactions
- The ring system electron deficient, making it a Lewis acid
- Allows for electronic interactions with electron-donating Lewis bases
- Alternative phase chemistry orthogonal to C18 chemistries
- **Recommended operating range**
  - **pH 2-8**
  - **Maximum temp: 60°C**

Positional isomers on Poroshell 120 phases

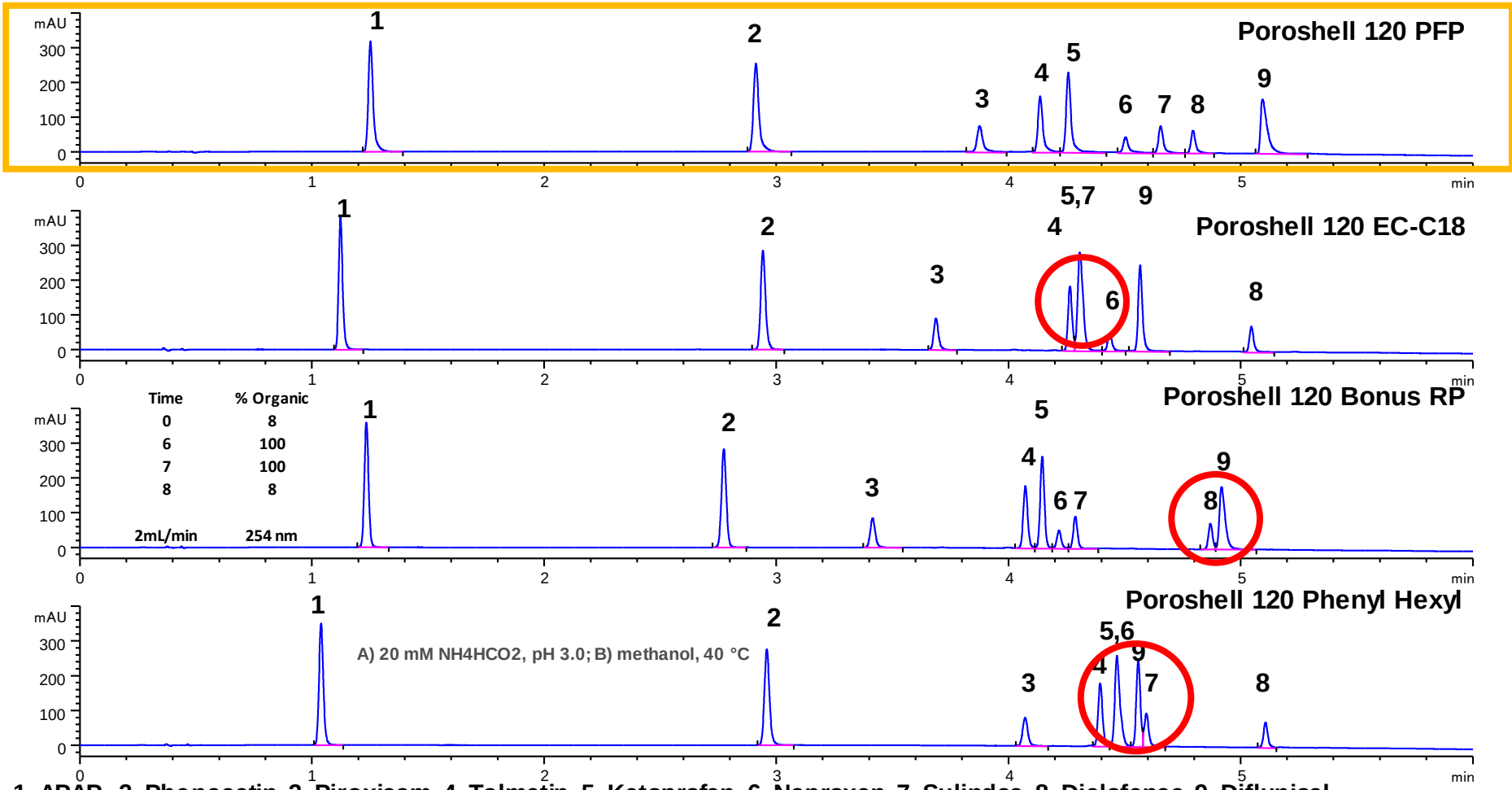




Poroshell 120,
4.6 × 50 mm,
70:30 water:MeOH,
1.5 mL/min, 40 °C,
254 nm

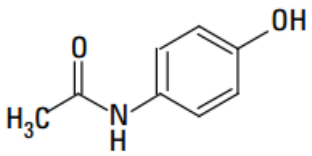
NSAID Separation Poroshell 120 with a Methanol Gradient

Best Resolution of all analytes with Poroshell 120 PFP

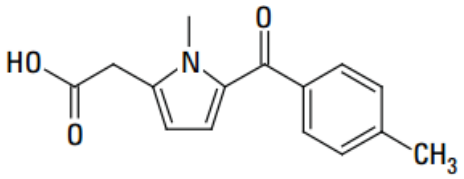


1. APAP, 2. Phenacetin, 3. Piroxicam, 4. Tolmetin, 5. Ketoprofen, 6. Naproxen, 7. Sulindac, 8. Diclofenac, 9. Diflunisal

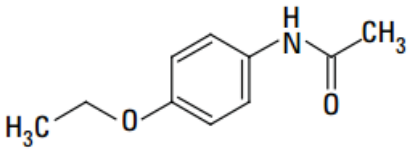
NSAID Separation Poroshell 120 with a Methanol Gradient



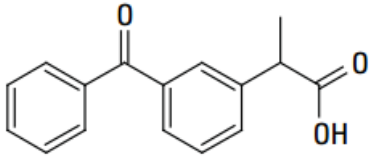
Acetaminophen (APAP)



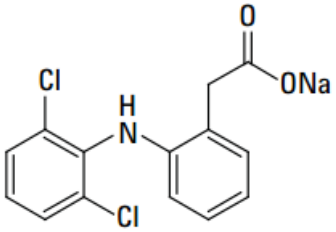
Tolmetin



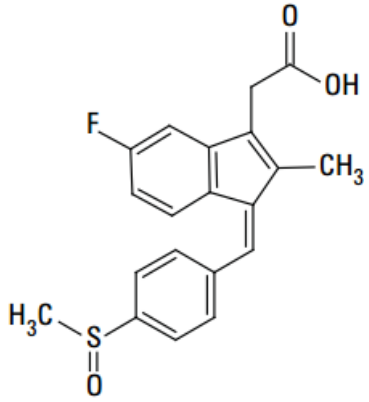
Phenacetin



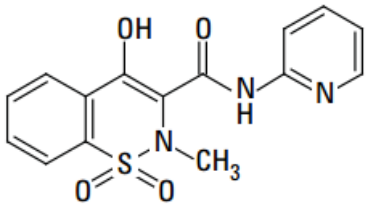
Ketoprophen



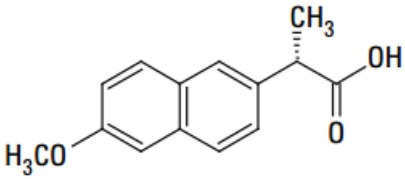
Diclofenac



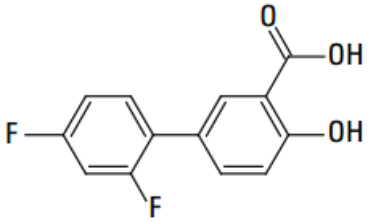
Sulindac



Piroxicam

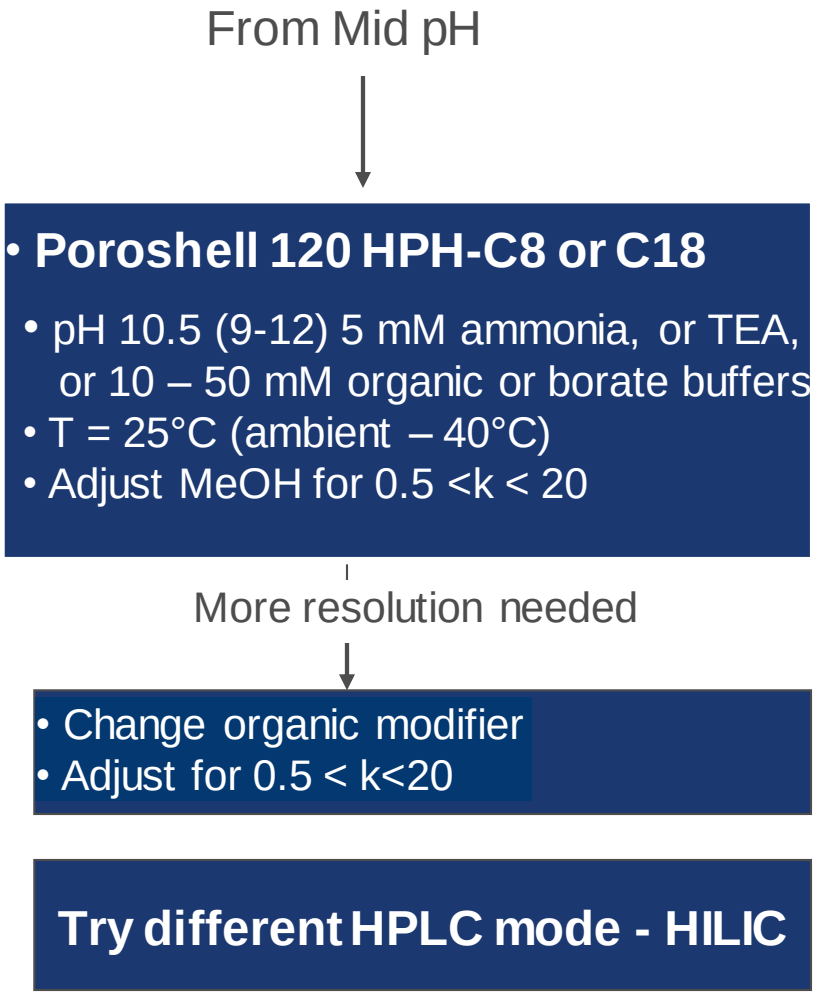


Naproxen



Diflunisal

Method Development at High pH

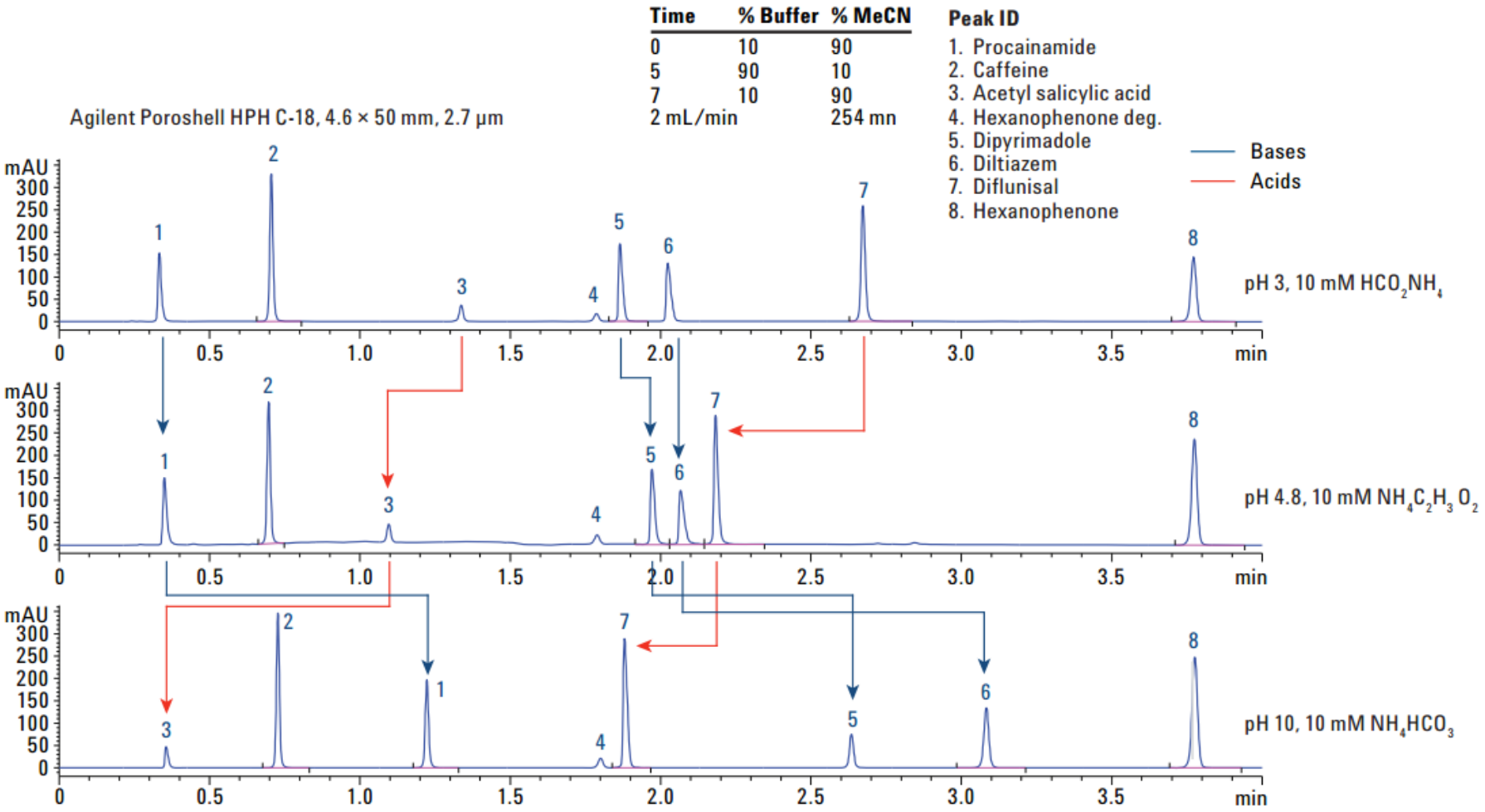


Reasons to Consider High pH

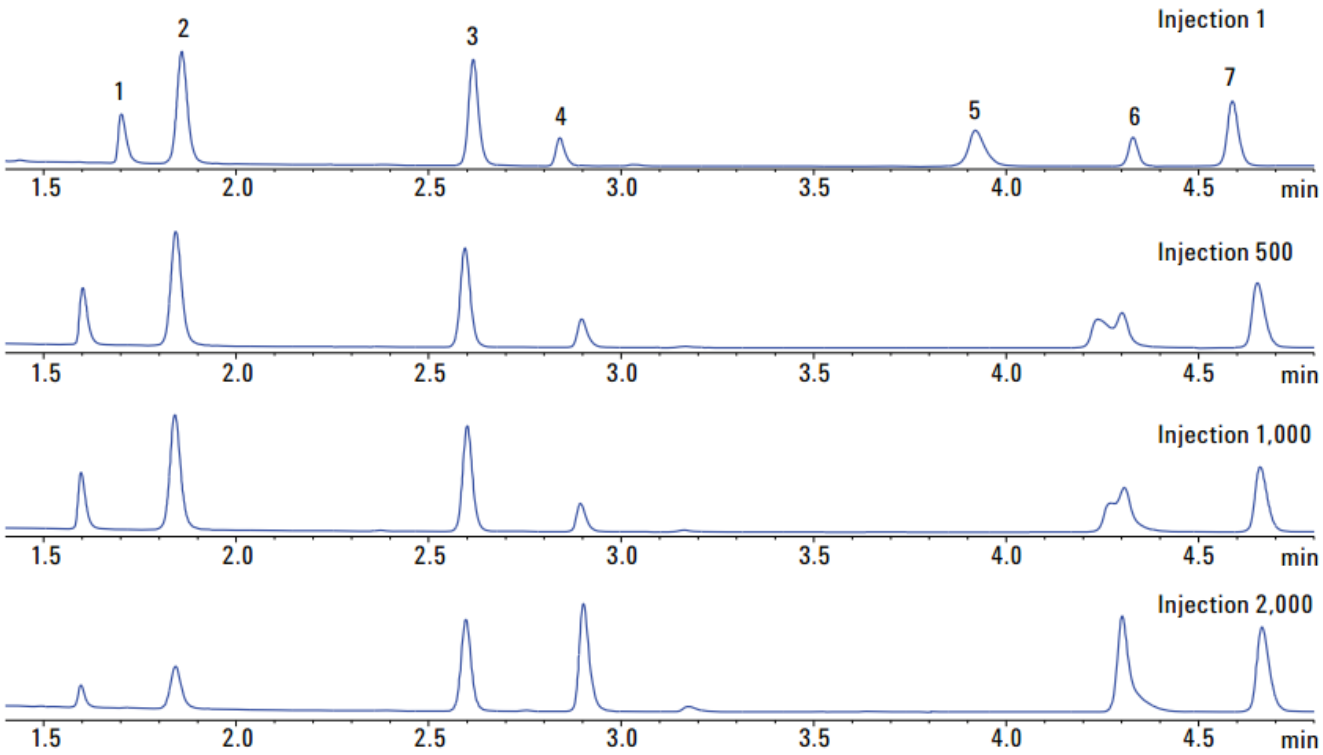
- **Increase retention of basic compounds by analyzing them in non-charged form**
- **Improve selectivity**

Poroshell HPH-C18 Low, Mid, & High pH

Why use multiple pH's?



Column Lifetime with High pH Methods



Other brand column on an ammonium bicarbonate gradient at pH 10.

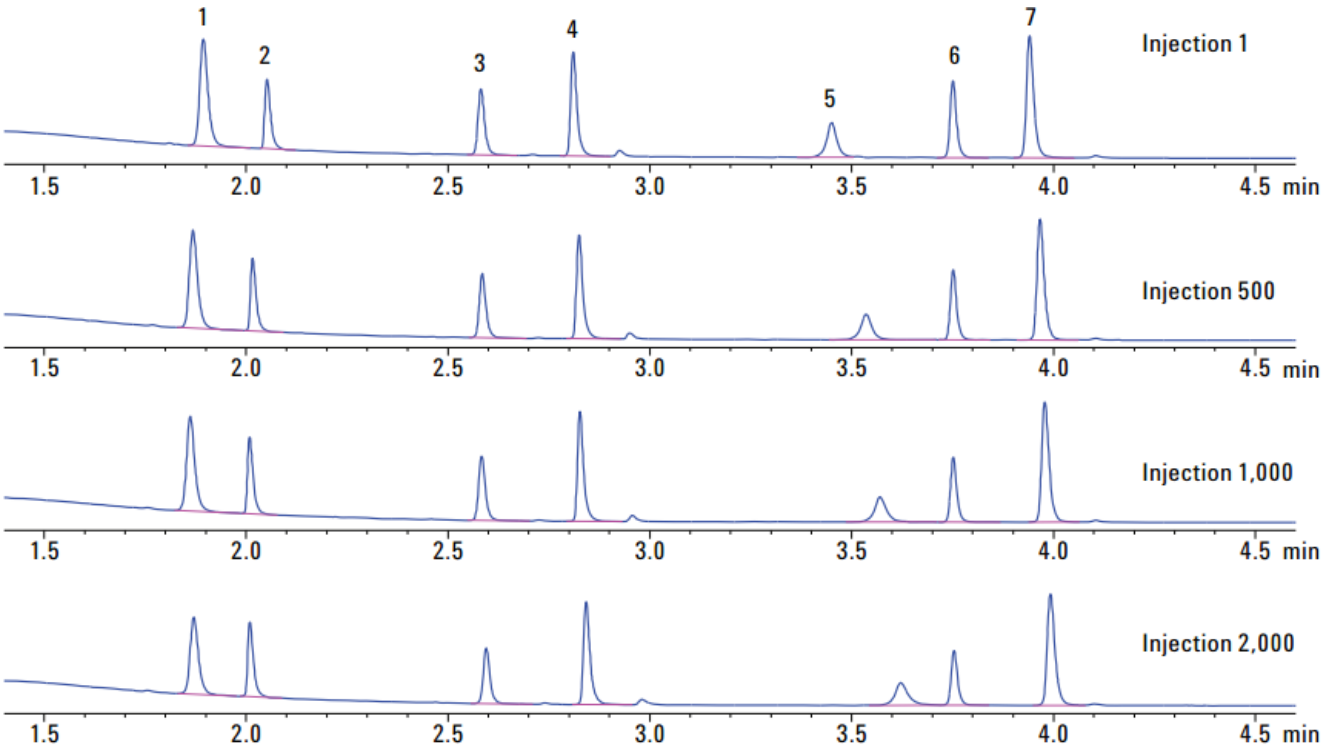
Peak ID

1. Methyl salicylate
2. 4-Chlorocinnamic acid
3. Acetophenone
4. Quinine
5. Nortriptyline
6. Heptanophenone
7. Amitriptyline

Conditions

Column:	Other brand, 2.1 x 50 mm, 3 µm	
Eluent:	A) 10 mM ammonium bicarbonate adjusted to pH 10.0 in water B) acetonitrile	
Flow rate:	0.4 mL/min	
Gradient:	Time (min)	%B
	0	5
	5	95
	5.1	5
Total run time:	7 min	

Column Lifetime with High pH Methods



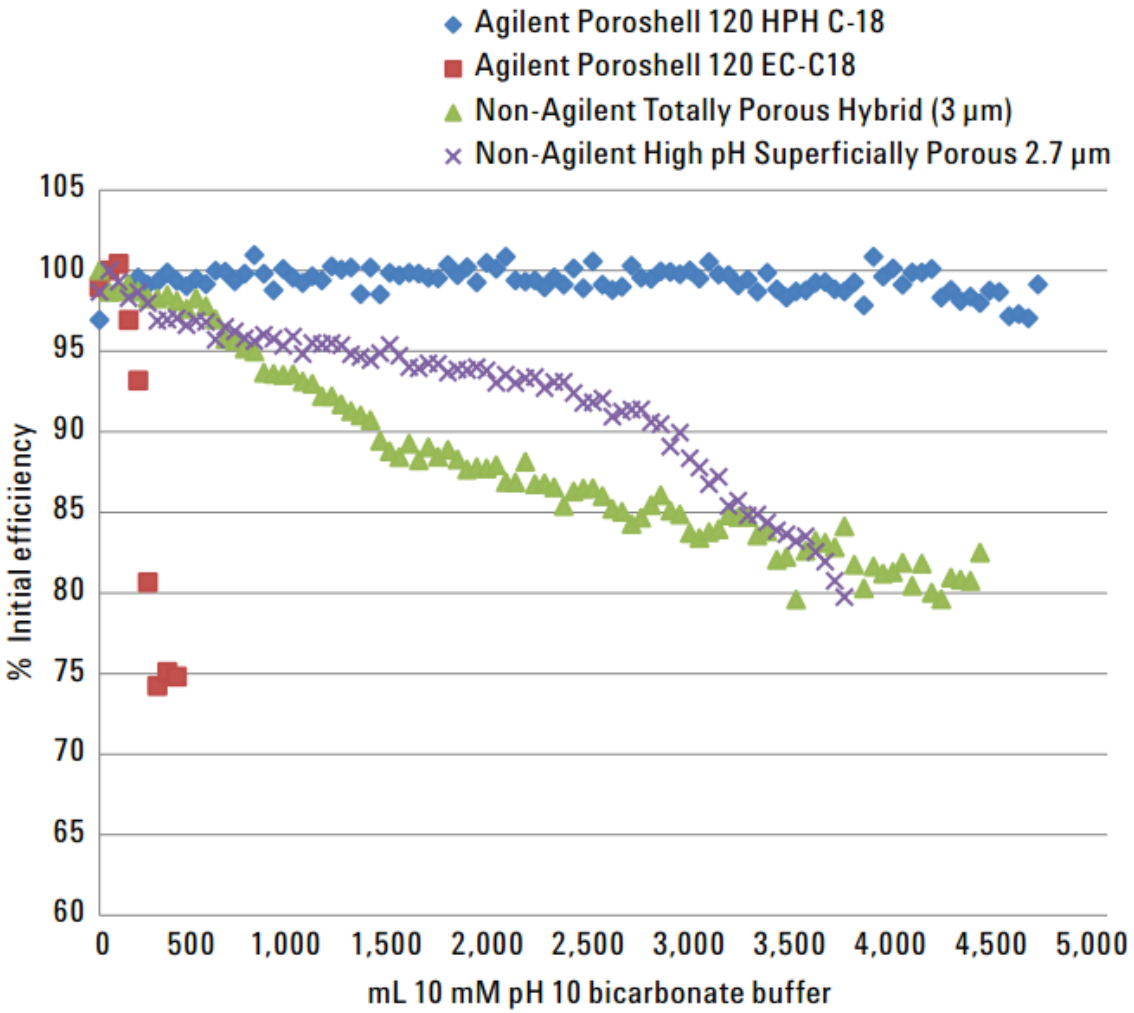
Peak ID

1. Methyl salicylate
2. 4-Chlorocinnamic acid
3. Acetophenone
4. Quinine
5. Nortriptyline
6. Heptanophenone
7. Amitriptyline

Conditions

Column:	Agilent Poroshell 120 HPH-C18, 2.1 × 50 mm, 2.7 µm	
Eluent:	A) 10 mM ammonium bicarbonate adjusted to pH 10.0 in water	
	B) acetonitrile	
Flow rate:	0.4 mL/min	
Gradient:	Time (min)	%B
	0	5
	5	95
	5.1	5
Total run time:	7 min	

Column Lifetime with High pH Methods



Conditions

Columns:	2.1 × 50 mm, 2.7 µm
Eluent:	A) 0.1% ammonium hydroxide in water B) acetonitrile
Flow rate:	0.4 mL/min
Gradient:	Time (min) % B 0 5 3 95 3.5 5
Total run time:	4 min

Excellent column life with HPH-C18 and C8

HILIC
Hydrophilic Interaction Chromatography

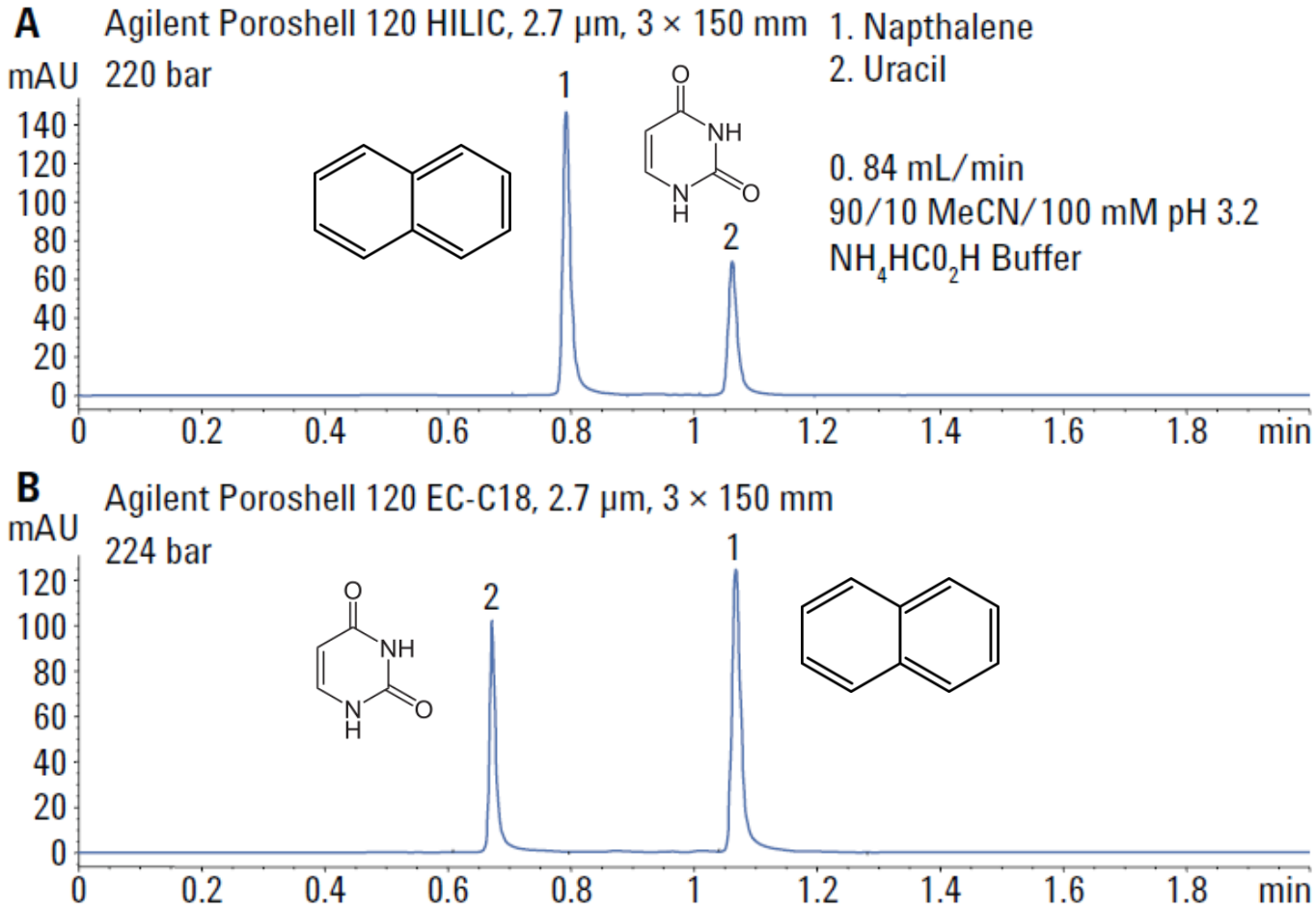
- HILIC offers more retention than reversed-phase for very polar bases

- Polar stationary phase:
  - Silica
  - Amine
  - Amide

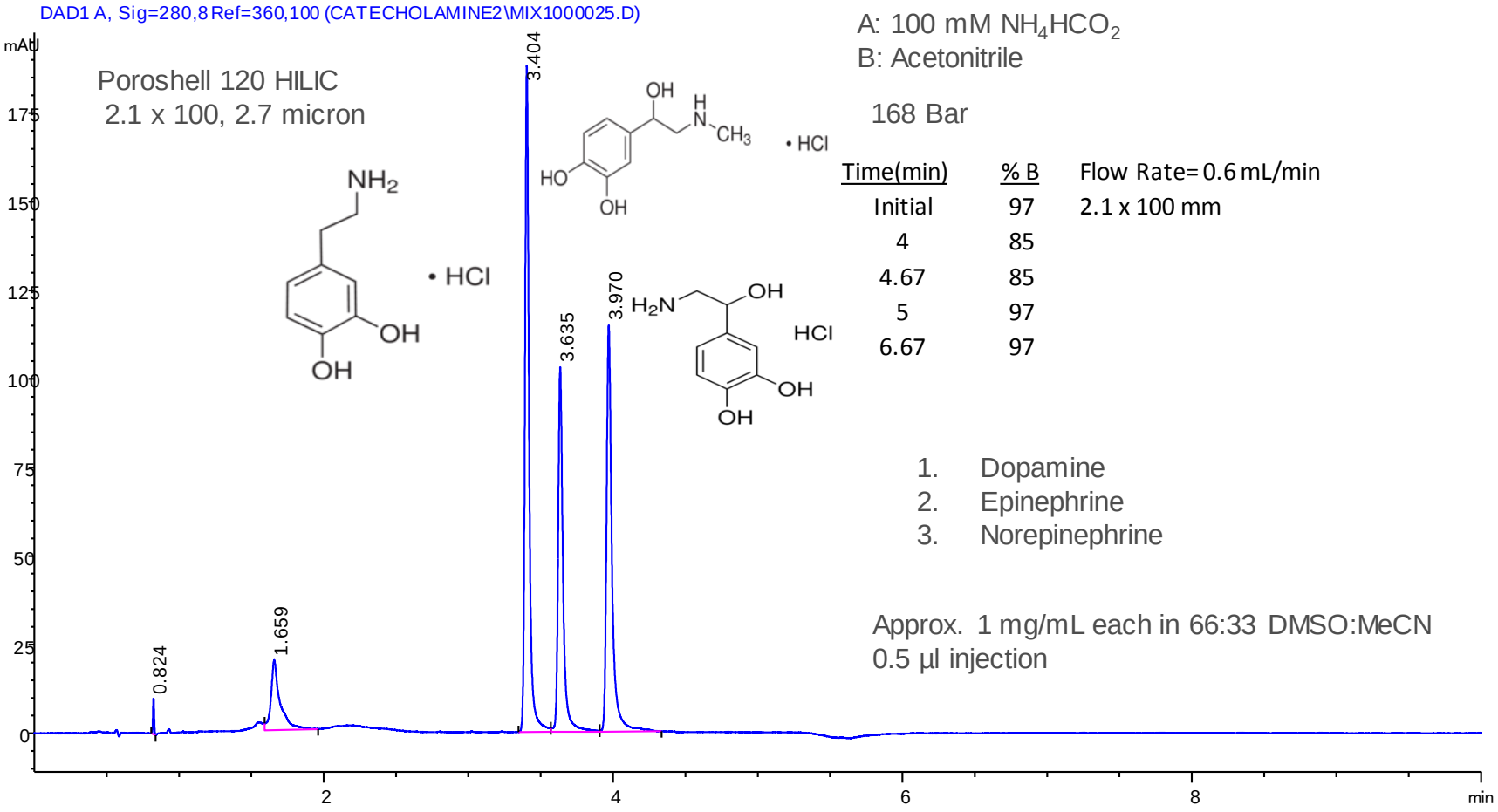
- Polar mobile phase:
  - Water is the strong solvent
  - Typically ACN/water
  - Buffer controls ionization of analyte and stationary phase
  - Typically ammonium acetate or ammonium formate

- Retention/elution is from least to most polar

HILIC – comparison with C18



HILIC Separation of Catecholamines Poroshell 120 2.1 x 100, 2.7 micron



Poroshell 120 Options

Poroshell 120 (2.7 µm)

Size (mm)	EC-C18	EC-C8	SB-C18	SB-C8	NEW HPH-C18	NEW HPH-C8
4.6 x 150	693975-902	693975-906	683975-902	683975-906	693975-702	693975-706
4.6 x 100	695975-902	695975-906	685975-902	685975-906	695975-702	695975-706
4.6 x 75	697975-902	697975-906	687975-902			
4.6 x 50	699975-902	699975-906	689975-902	689975-906	699975-702	699975-706
4.6 x 30	691975-902	691975-906	681975-902			
3.0 x 150	693975-302	693975-306	683975-302	683975-306	693975-502	693975-506
3.0 x 100	695975-302	695975-306	685975-302	685975-306	695975-502	695975-506
3.0 x 75	697975-302	697975-306	687975-302			
3.0 x 50	699975-302	699975-306	689975-302	689975-306	699975-502	699975-506
3.0 x 30	691975-302	691975-306	681975-302			
2.1 x 150	693775-902	693775-906	683775-902	683775-906	693775-702	693775-706
2.1 x 100	695775-902	695775-906	685775-902	685775-906	695775-702	695775-706
2.1 x 75	697775-902	697775-906	687775-902			
2.1 x 50	699775-902	699775-906	689775-902	689775-906	699775-702	699775-706
2.1 x 30	691775-902	691775-906	681775-902			

Size (mm)	Phenyl-Hexyl	SB-Aq	Bonus-RP	HILIC	EC-CN	NEW PFP
4.6 x 150	693975-912	683975-914	693968-901	693975-901	693975-905	693975-408
4.6 x 100	695975-912	685975-914	695968-901	695975-901	695975-905	695975-408
4.6 x 50	699975-912	689975-914	699968-901	699975-901	699975-905	699975-408
3.0 x 150	693975-312	683975-314	693968-301	693975-301	693975-305	693975-308
3.0 x 100	695975-312	685975-314	695968-301	695975-301	695975-305	695975-308
3.0 x 50	699975-312	689975-314	699968-301	699975-301	699975-305	699975-308
2.1 x 150	693775-912	683775-914	693768-901	693775-901	693775-905	693775-408
2.1 x 100	695775-912	685775-914	695768-901	695775-901	695775-905	695775-408
2.1 x 50	699775-912	689775-914	699768-901	699775-901	699775-905	699775-408

Note: Poroshell 120 columns have a 600 bar/9000 psi pressure limit.

Poroshell 120 Fast Guards for UHPLC

Size (mm)	EC-C18	EC-C8	SB-C18	Phenyl-Hexyl	NEW PFP
4.6 x 5	820750-911	820750-913	820750-912	820750-914	
3.0 x 5	823750-911	823750-913	823750-912	823750-914	
2.1 x 5	821725-911	821725-913	821725-912	821725-914	821725-915

Method Development Kits

Method Development Kits	Description (One of each)	Dimension	Part No.
Poroshell 120 Selectivity	EC-C18, Phenyl-Hexyl, Bonus-RP	2.1 x 50 mm	5190-6155
Poroshell 120 Selectivity	EC-C18, Phenyl-Hexyl, Bonus-RP	4.6 x 50 mm	5190-6156
Poroshell 120 Aqueous	SB-Aq, Phenyl-Hexyl, Bonus-RP	2.1 x 50 mm	5190-6157
Poroshell 120 Aqueous	SB-Aq, Phenyl-Hexyl, Bonus-RP	4.6 x 50 mm	5190-6158
Poroshell 120 USP L1, L7, and L10	EC-C18, EC-C8, EC-CN	4.6 x 100 mm	5190-6159
Poroshell 120 USP L1, L7, and L10	EC-C18, EC-C8, EC-CN	3.0 x 100 mm	5190-6160
ZORBAX RRHD pH	SB-C18, Eclipse Plus C18, and Extend-C18	2.1 x 50 mm	5190-6152
ZORBAX Eclipse Plus	C18, C8, Phenyl-Hexyl	2.1 x 50 mm	5190-6153
ZORBAX RRHD Aqueous	SB-Aq, Bonus-RP, Eclipse Plus Phenyl-Hexyl	2.1 x 50 mm	5190-6154

Poroshell 120 Options

Agilent Poroshell 120 4 µm

NEW

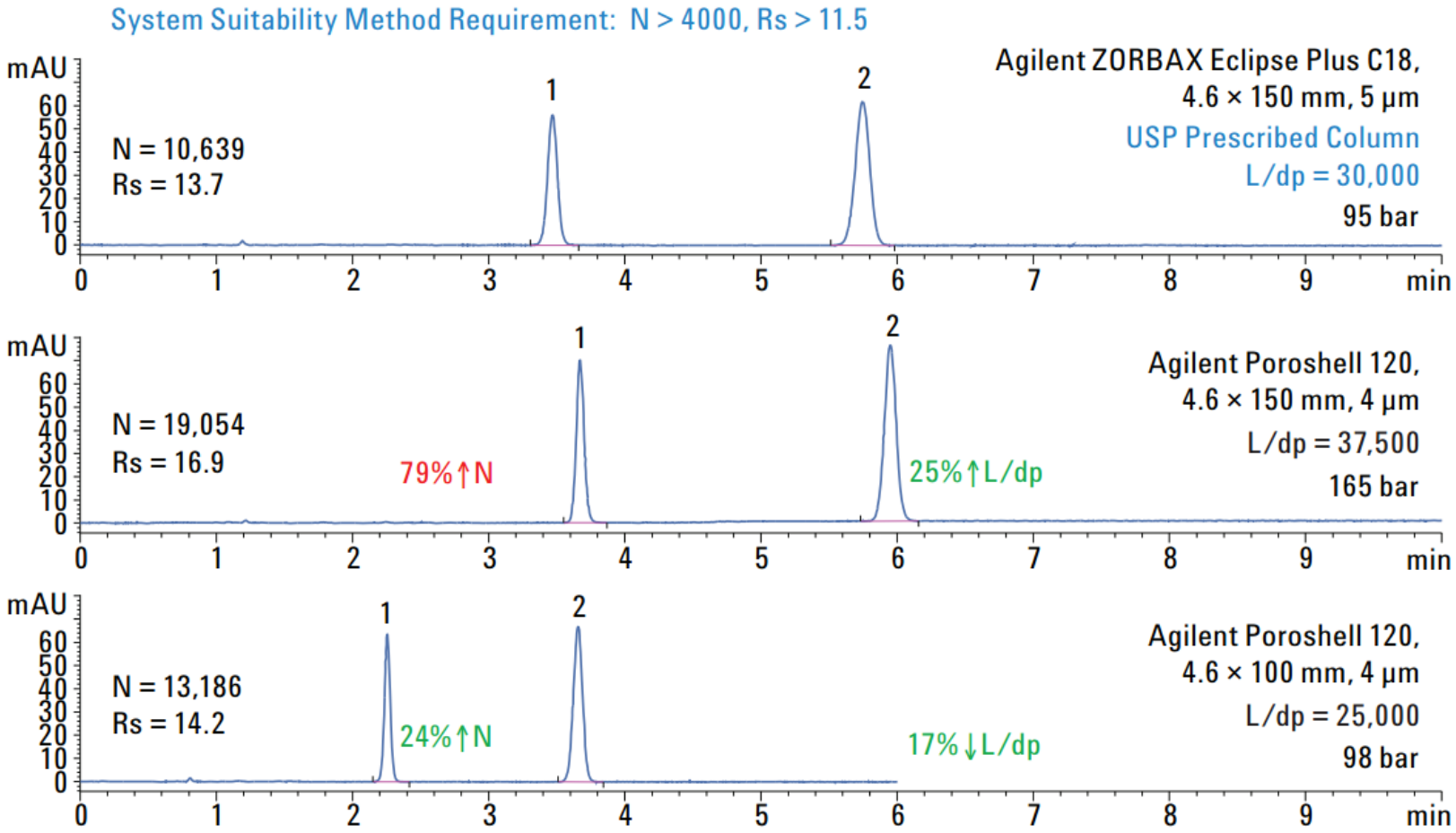
Size (mm)	EC-C18	EC-C8	PFP	Phenyl-Hexyl	HILIC
4.6 x 250	690970-902	690970-906	690970-408	690970-912	690970-901
4.6 x 150	693970-902	693970-906	693970-408	693970-912	693970-901
4.6 x 100	695970-902	695970-906	695970-408	695970-912	695970-901
4.6 x 50	699970-902	699970-906	699970-408	699970-912	699970-901
3.0 x 250	690970-302	690970-306	690970-308	690970-312	690970-301
3.0 x 150	693970-302	693970-306	693970-308	693970-312	693970-301
3.0 x 100	695970-302	695970-306	695970-308	695970-312	695970-301
3.0 x 50	699970-302	699970-306	699970-308	699970-312	699970-301
2.1 x 250	650750-902	650750-906	650750-408	650750-912	650750-901
2.1 x 150	693770-902	693770-906	693770-408	693770-912	693770-901
2.1 x 100	695770-902	695770-906	695770-408	695770-912	695770-901
2.1 x 50	699770-902	699770-906	699770-408	699770-912	699770-901

Guard columns for 4 µm

NEW

Size (mm)	EC-C18
4.6 x 5	820750-916
3.0 x 5	823750-916
2.1 x 5	821725-916

Benefits of 4 µm Poroshell 120



1. Naproxen
2. Butyrophenone

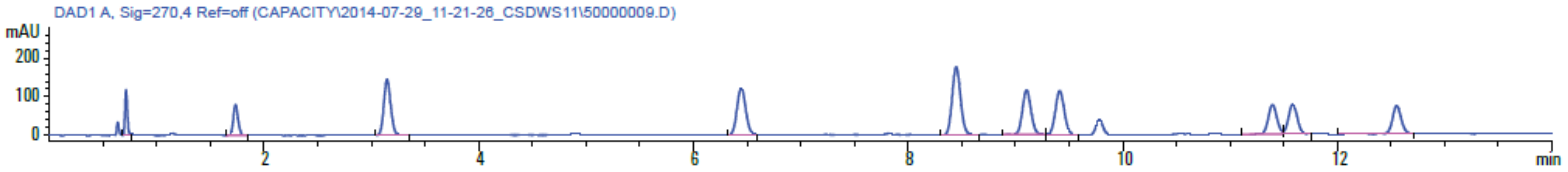
50:49:1 MeCN:water:acetic acid
Flow rate: 1.2 mL/min

5991-5408EN

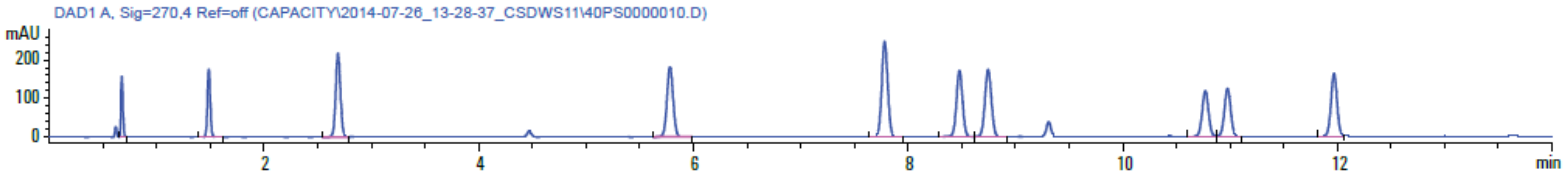
Consistent Selectivity Across Particle Sizes

Phenol Mix gradient at 1.5 mL/min

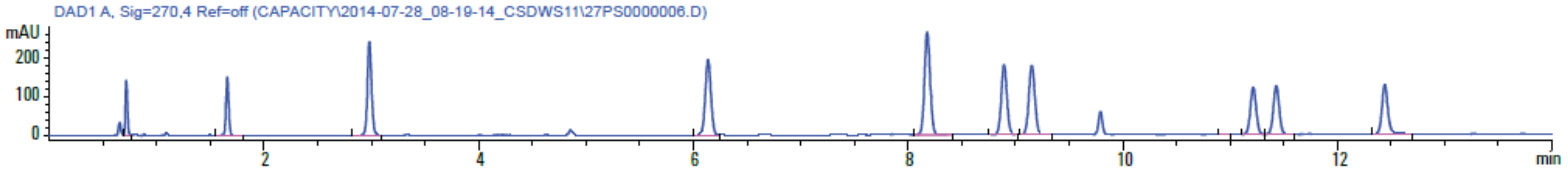
ZORBAX Eclipse Plus C18 5 µm



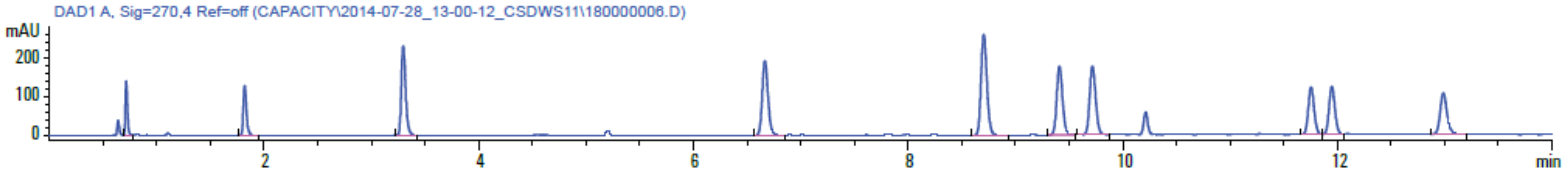
Poroshell 120 EC-C18 4 µm



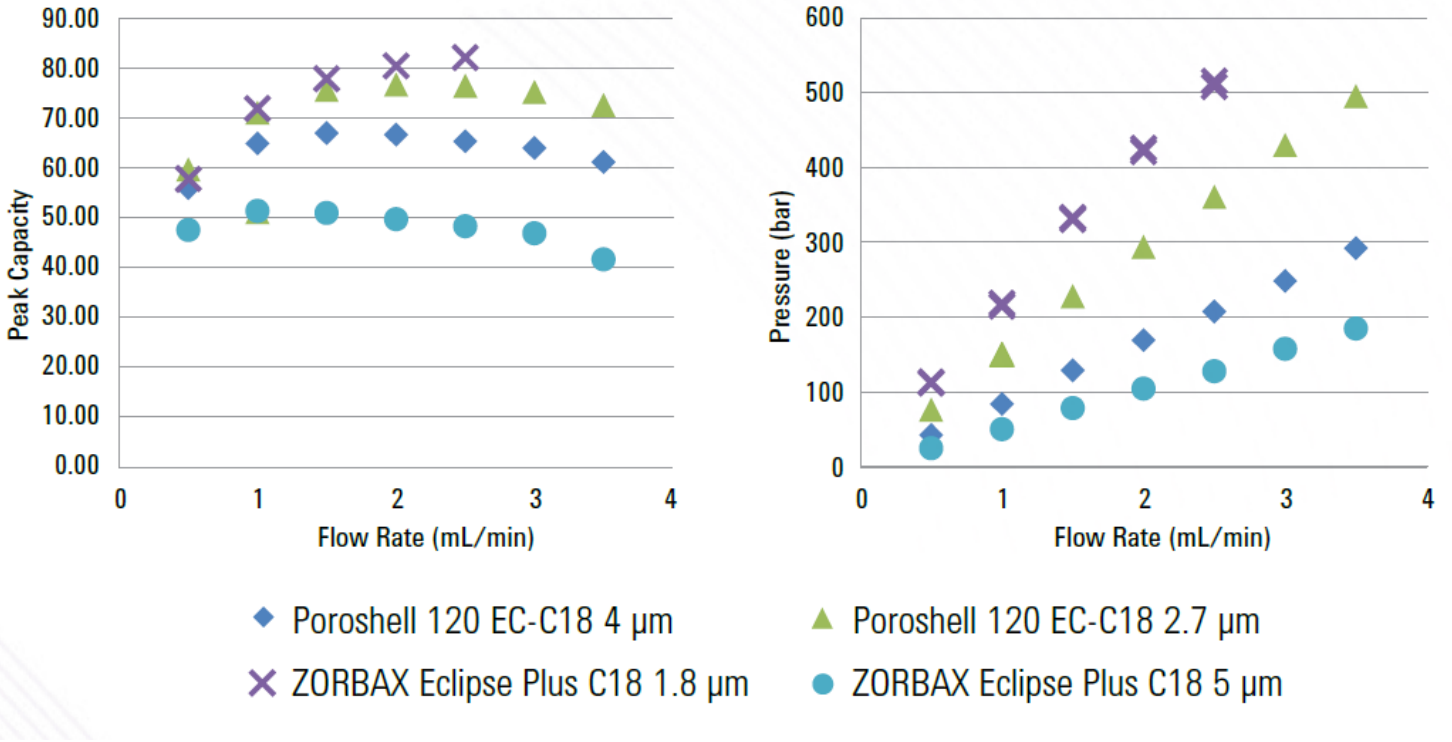
Poroshell 120 EC-C18 2.7 µm



ZORBAX Eclipse Plus C18 1.8 µm



Consistent Selectivity Across Particle Sizes

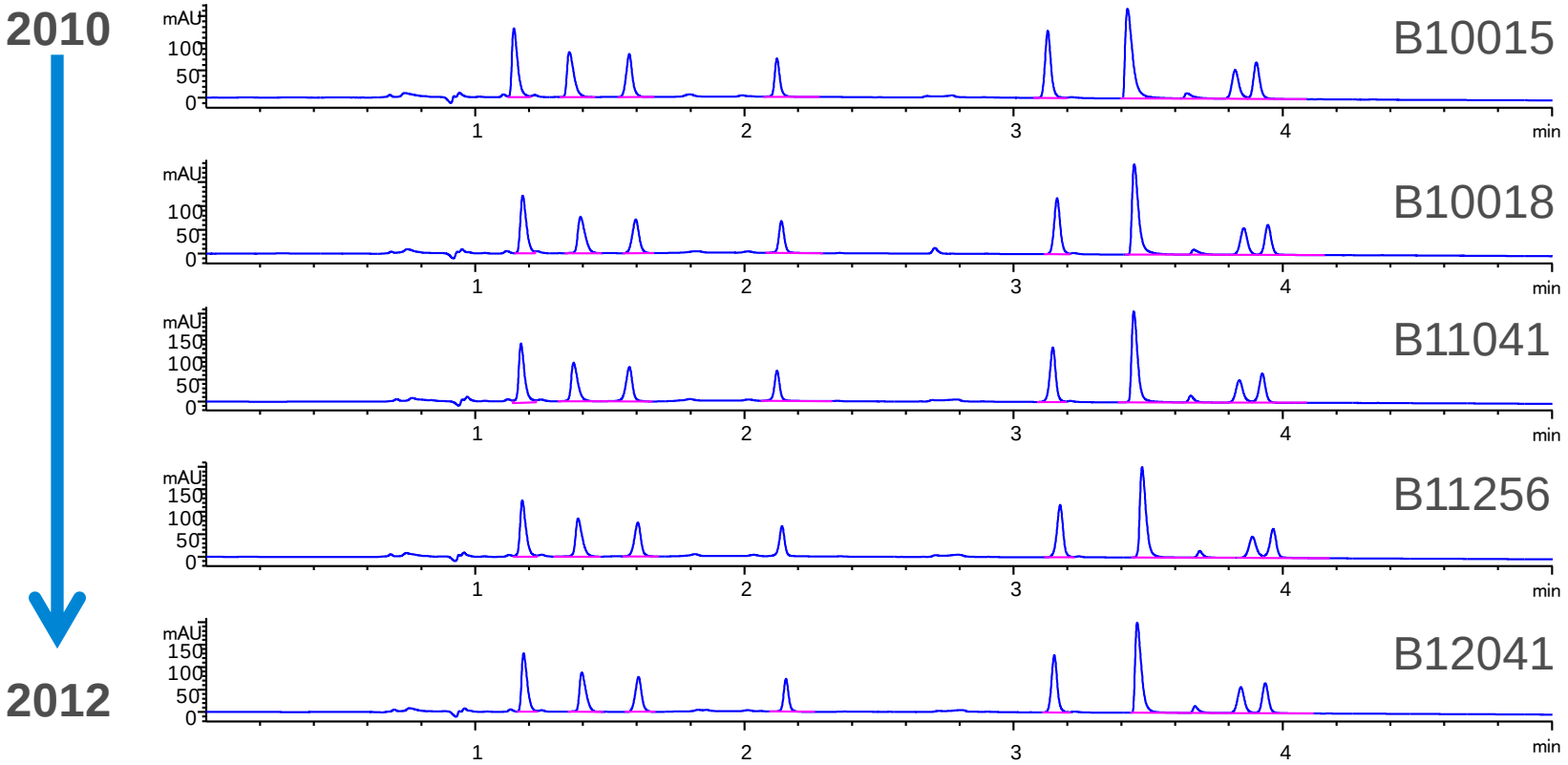


Columns:	All columns 4.6 x 100 mm
Instrument:	Agilent 1260, pulse damper and mixing column bypassed
Mobile phase:	A: 0.1% Formic acid B: MeOH + 0.1% formic acid

Flow rate:	0.4 mL/min
Temperature:	25 °C
Detection:	260 nm
Sample:	8 steroids
Gradient:	40-80% MeOH/14 min

Lot Reproducibility

Batch-to-batch reproducibility of Poroshell 120 columns



Beverage Additives

Method Validation Kits

Agilent ZORBAX Rapid Resolution High Definition (RRHD) Method Validation Kits

Size (mm)	Particle Size (µm)	Eclipse Plus C18	Eclipse Plus C8	Eclipse XDB-C18	Extend-C18	Eclipse Plus Phenyl-Hexyl	Bonus-RP	SB-C18	SB-C8	SB-Phenyl	SB-Aq
3.0 x 150	1.8	959759-302K	959759-306K	981759-302K				859700-302K	859700-306K		
3.0 x 100	1.8	959758-302K	959758-306K	981758-302K	758700-302K	959758-312K		858700-302K	858700-306K	858700-312K	858700-314K
3.0 x 50	1.8	959757-302K	959757-306K	981757-302K	757700-302K	959757-312K		857700-302K	857700-306K	857700-312K	857700-314K
2.1 x 150	1.8	959759-902K	959759-906K	981759-902K	759700-902K	959759-912K	859768-901K	859700-902K	859700-906K	859700-912K	859700-914K
2.1 x 100	1.8	959758-902K	959758-906K	981758-902K	758700-902K	959758-912K	858768-901K	858700-902K	858700-906K	858700-912K	858700-914K
2.1 x 50	1.8	959757-902K	959757-906K	981757-902K	757700-902K	959757-912K	857768-901K	857700-902K	857700-906K	857700-912K	857700-914K

Agilent ZORBAX Method Validation Kits

Size (mm)	Particle Size (µm)	Eclipse Plus C18	Eclipse Plus C8	Eclipse XDB-C18	Eclipse XDB-C8	Extend-C18	Eclipse Plus Phenyl-Hexyl	Bonus-RP	SB-Aq	SB-C18	SB-C8	SB-Phenyl
4.6 x 250	5	959990-902K	959990-906K	990967-902K	990967-906K	770450-902K	959990-912K	880668-901K	880975-914K	880975-902K	880975-906K	880975-912K
4.6 x 150	5	959993-902K	959993-906K	993967-902K	993967-906K	773450-902K		883668-901K	883975-914K	883975-902K	883975-906K	883975-912K
3.0 x 150	5	959993-302K										
4.6 x 250	3.5									884950-567K		
4.6 x 150	3.5	959963-902K	959963-906K	963967-902K	963967-906K	763953-902K	959963-912K	863668-901K	863953-914K	863953-902K	863953-906K	863953-912K
4.6 x 100	3.5	959961-902K	959961-906K	961967-902K	961967-906K	764953-902K	959961-912K	864668-901K	861953-914K	861953-902K	861953-906K	861953-912K
4.6 x 50	3.5	959943-902K	959943-906K	935967-902K	935967-906K	735953-902K	959943-912K	835668-901K	835975-914K	835975-902K	835975-906K	835975-912K
4.6 x 150	1.8	959994-902K							829975-914K	829975-902K	829975-906K	829975-912K
4.6 x 100	1.8	959964-902K	959964-906K	928975-902K	928975-906K	728975-902K	959964-912K	828668-901K	828975-914K	828975-902K	828975-906K	828975-912K
4.6 x 50	1.8	959941-902K	959941-906K	927975-902K	927975-906K	727975-902K	959941-912K	827668-901K	827975-914K	827975-902K	827975-906K	827975-912K
3.0 x 100	1.8				928975-306K			828668-301K				
3.0 x 50	1.8				927975-306K			827668-301K				
2.1 x 100	1.8				928700-906K							
2.1 x 50	1.8				927700-906K							

Agilent Poroshell 120 Method Validation Kits

Size (mm)	Particle Size (µm)	EC-C18	EC-C8	Phenyl-Hexyl	SB-C18	SB-C8	SB-Aq	Bonus-RP
4.6 x 150	2.7	693975-902K	693975-906K	693975-912K	683975-902K	683975-906K	683975-914K	693968-901K
4.6 x 100	2.7	695975-902K	695975-906K	695975-912K	685975-902K	685975-906K	685975-914K	695968-901K
4.6 x 50	2.7	699975-902K	699975-906K	699975-912K	689975-902K	689975-906K	689975-914K	699968-901K
3.0 x 150	2.7	693975-302K	693975-306K	693975-312K	683975-302K	683975-306K	683975-314K	693968-301K
3.0 x 100	2.7	695975-302K	695975-306K	695975-312K	685975-302K	685975-306K	685975-314K	695968-301K
3.0 x 50	2.7	699975-302K	699975-306K	699975-312K	689975-302K	689975-306K	689975-314K	699968-301K
2.1 x 150	2.7	693775-902K	693775-906K	693775-912K	683775-902K	683775-906K	683775-914K	693768-901K
2.1 x 100	2.7	695775-902K	695775-906K	695775-912K	685775-902K	685775-906K	685775-914K	695768-901K
2.1 x 50	2.7	699775-902K	699775-906K	699775-912K	689775-902K	689775-906K	689775-914K	699768-901K

Summary

What to consider when choosing a column?

- Make sure you choose the best pore size

- Consider newer particle technologies
  - Poroshell 120 2.7 µm
  - Poroshell 120 4.0 µm

- Take advantage of selectivity changes
  - Mobile phase
  - pH
  - Bonded phase

Thank you!

LC-column-support@agilent.com

AdvanceBio RP-mAb

New

Particle

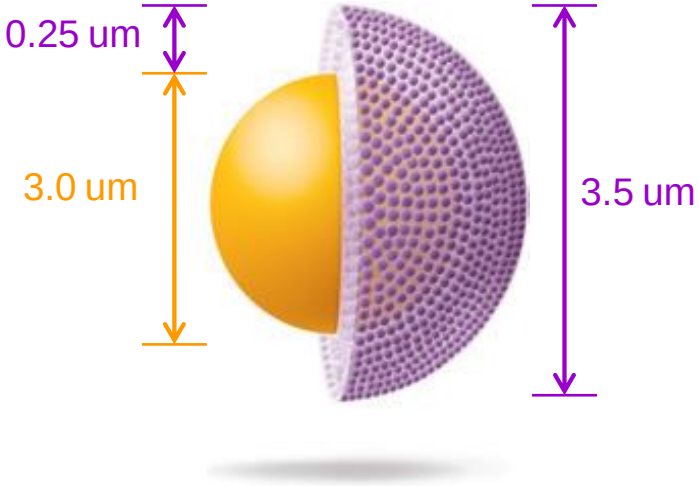
- 3.5 µm SP particle
- 0.25 µm porous layer depth
- 450Å pore diameter

Phases

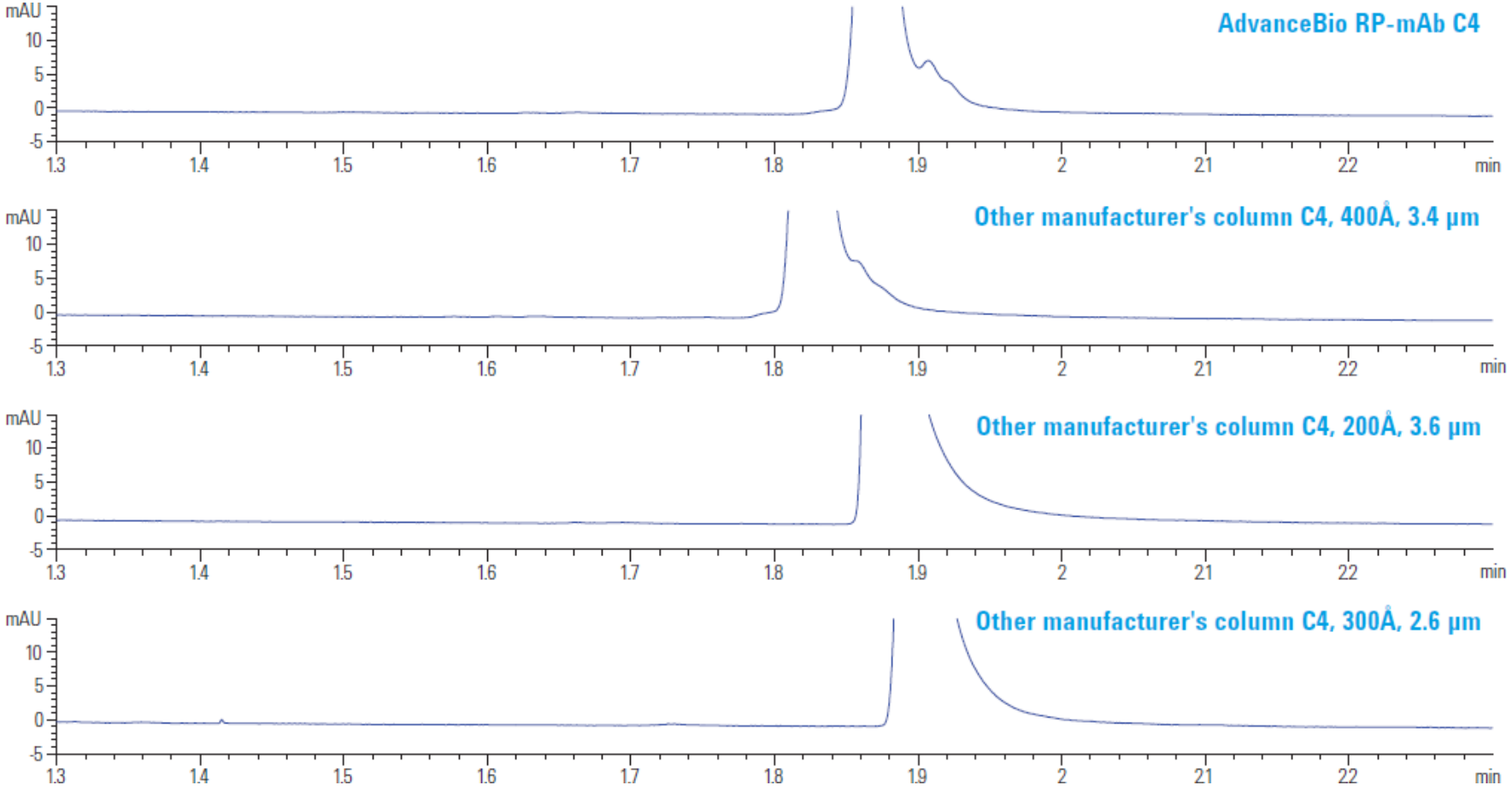
- C4
- SB-C8
- Diphenyl

The most popular phases for proteins, plus a choice for unique selectivity

The optimum large molecule resolution for use with both HPLC and UHPLC systems



AdvanceBio RP-mAb



Column dimensions: 2.1 x 100 mm, 3.5 µm
Mobile phase A: 0.1% TFA in water:IPA (98:2) Mobile phase B: IPA:acetonitrile:MPA (70:20:10)
Flow rate: 1.0 mL/min Gradient: 10-58% B in 4 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B
Temperature: 80 °C Detection: UV @ 254 nm
Sample: 5 µL injection of humanized recombinant Herceptin IgG1 intact from Creative Biolabs (1 mg/mL)