A Look at
Agilent HPLC Columns Choices

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Product Specialist Chemistries and Consumable

Agilent
Trusted Answers

Considerating Column Properties for Column Selection

- Silica Particle Chemistry
  - Silica surface
  - End-capping
- Particle size options
  - Totally porous
  - Superficially porous
- Column Chemistry
  - Bonded phases

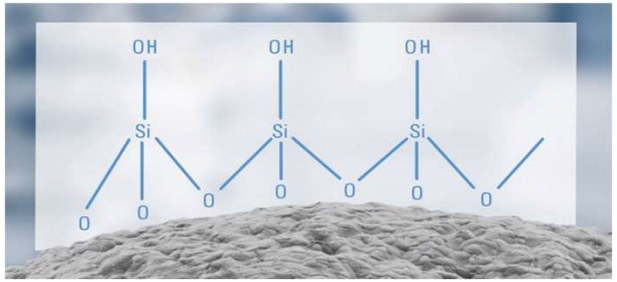
Silica column characteristics

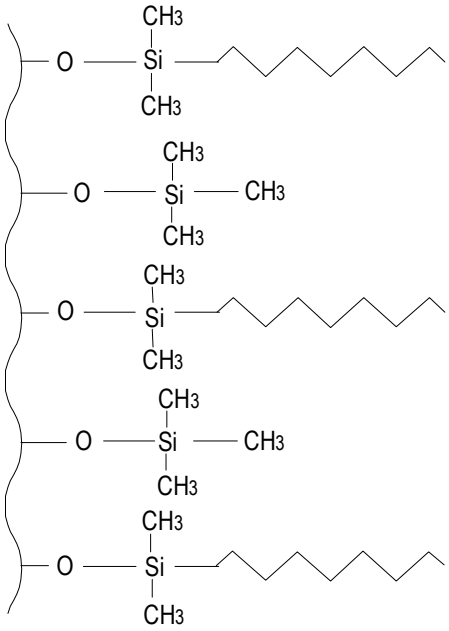
Surface area

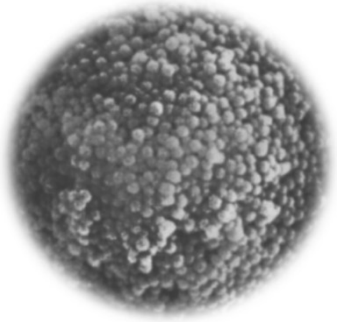
- Pore size
- Particle size

Silica Particle Chemistry

- Silanols
- Bonding density
- Endcapping



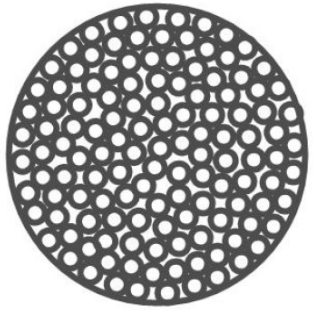




Typical Pore Size of Silica Particles

Small molecules

- 80 – 120 Å
- Maximizes loading and retention

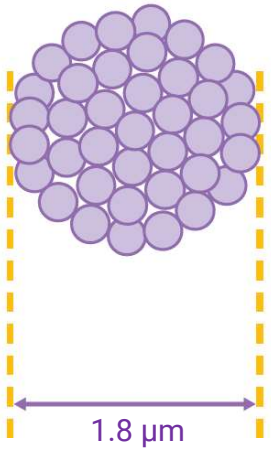


Peptides, proteins, other large biomolecules

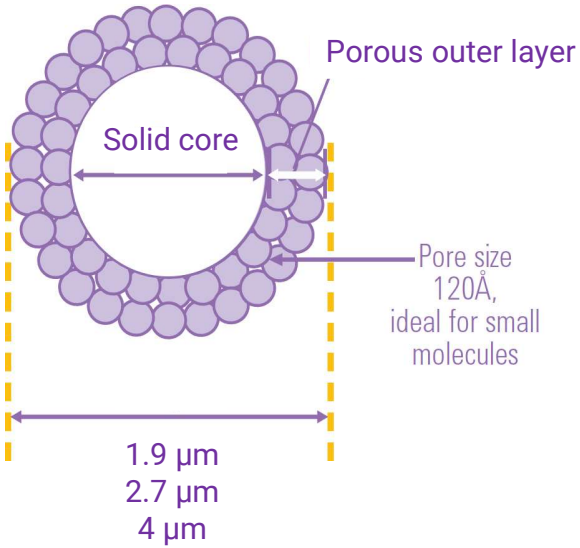
- 120 Å (Peptides)
- 300 Å to 450 Å (or higher)
- Maintain high efficiency

Silica Particle Technologies

Totally porous



Superficially porous



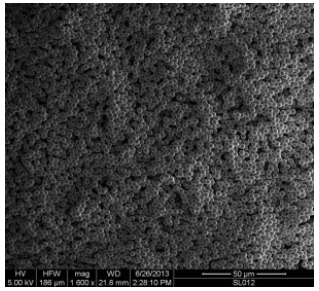
Example: ZORBAX InfinityLab Poroshell

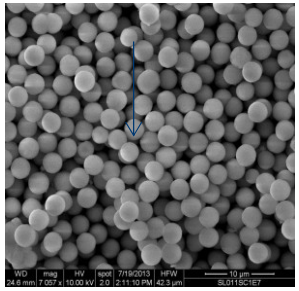
Agilent's small molecules LC column portfolio

- ZORBAX
- Pursuit
- Pursuit XRs
- Polaris

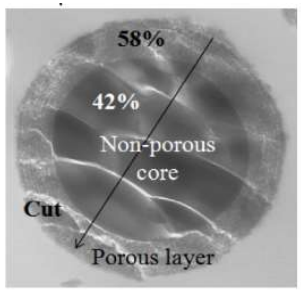
- InfinityLab Poroshell 120
- Poroshell
- AdvanceBio

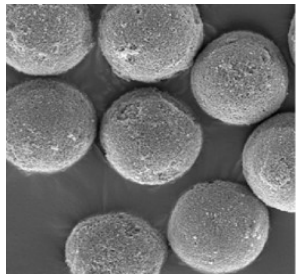
TPP



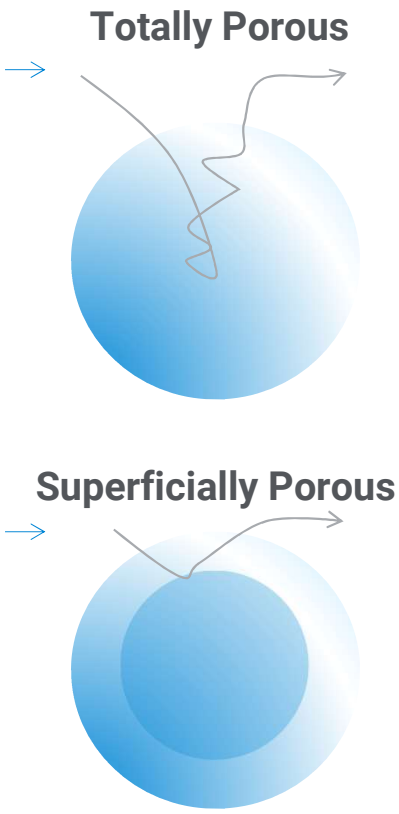


SPP





Analyte mass transfer improvements through lower diffusion



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

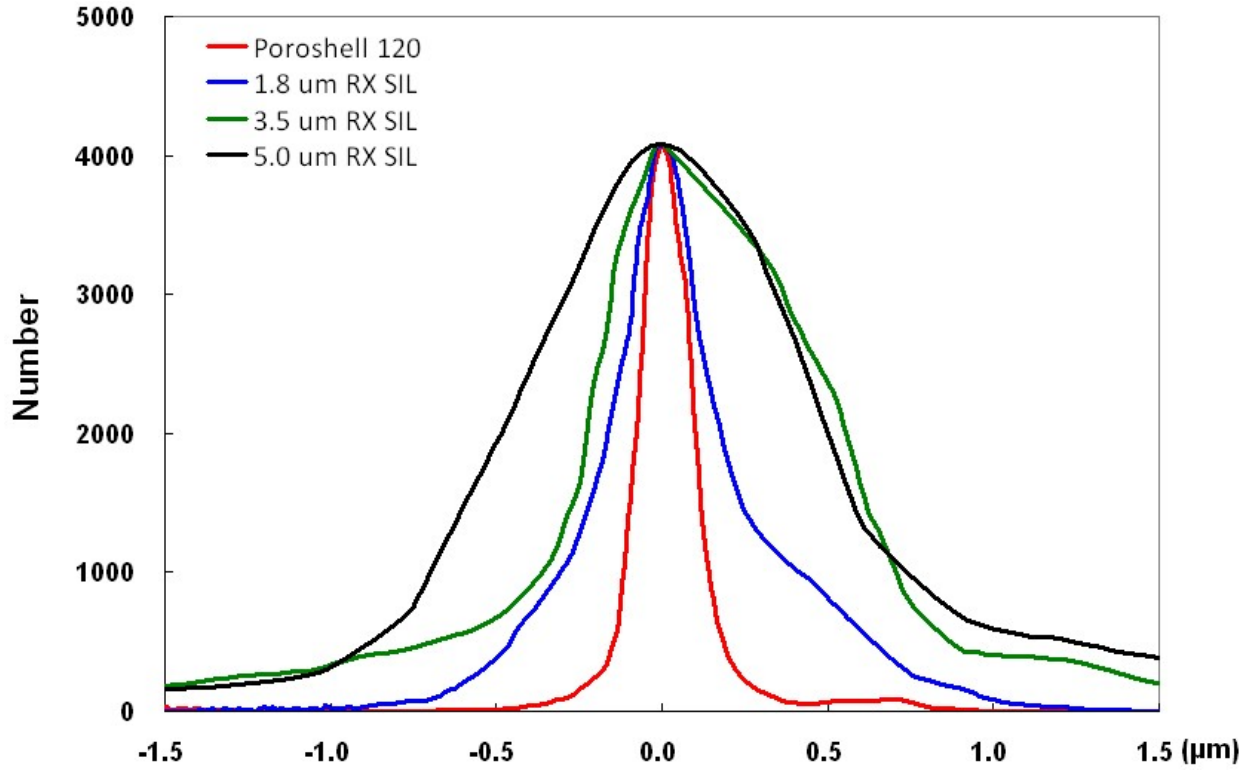
van Deemter equation:

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

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

Comparison of particle size distributions

Totally porous and InfinityLab Poroshell 120 particles



When to use Superficially Porous Particles vs Totally Porous Particles

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

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

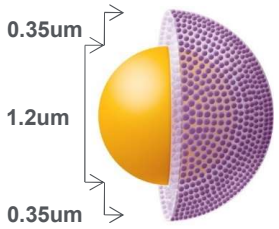
InfinityLab Poroshell alignment with ZORBAX chemistries

- 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 Chemistry	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 Bonus-RP	ZORBAX Bonus-RP
InfinityLab Poroshell 120 SB-Aq	ZORBAX StableBond SB-Aq
InfinityLab Poroshell 120 EC-CN	ZORBAX Eclipse XDB-CN
InfinityLab Poroshell 120 HILIC	ZORBAX HILIC-Plus

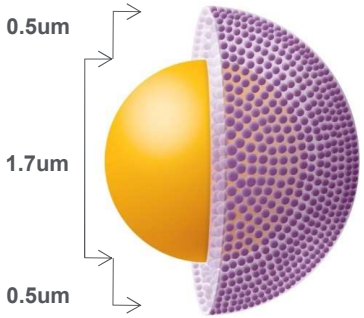
Scalability of SPP Particles

Scalability of InfinityLab Poroshell 120 Particles

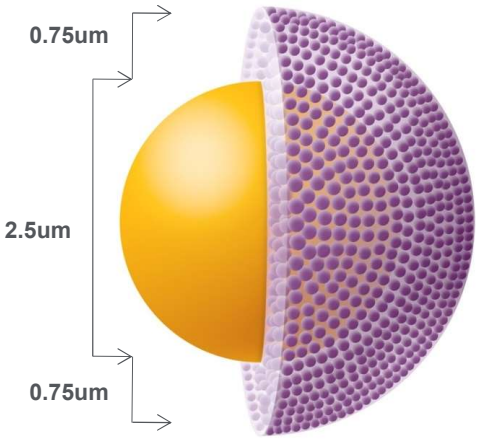


InfinityLab Poroshell 120
1.9 µm

Highest UHPLC performance



InfinityLab Poroshell 120
2.7 µm

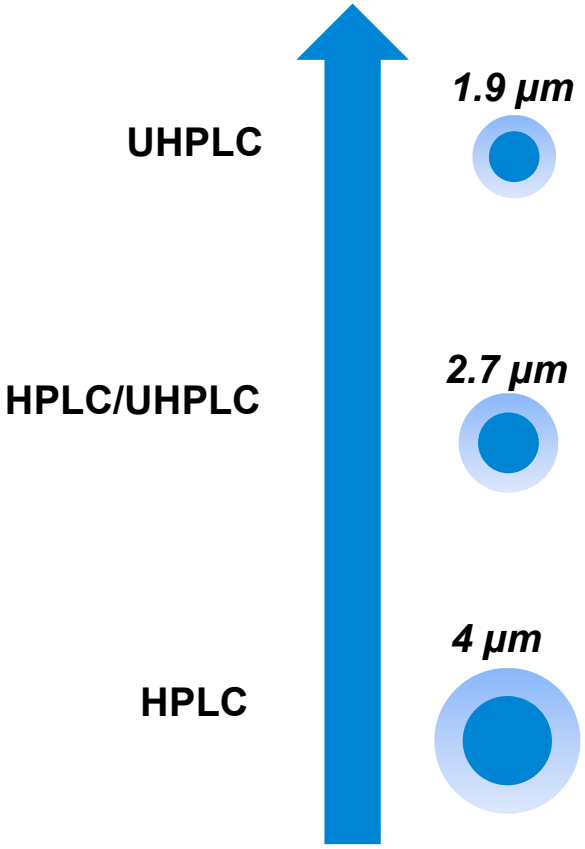


InfinityLab Poroshell 120
4 µm

Improved HPLC performance

www.agilent.com/chem/discoverporoshell

Which Particle works best on which LC system?

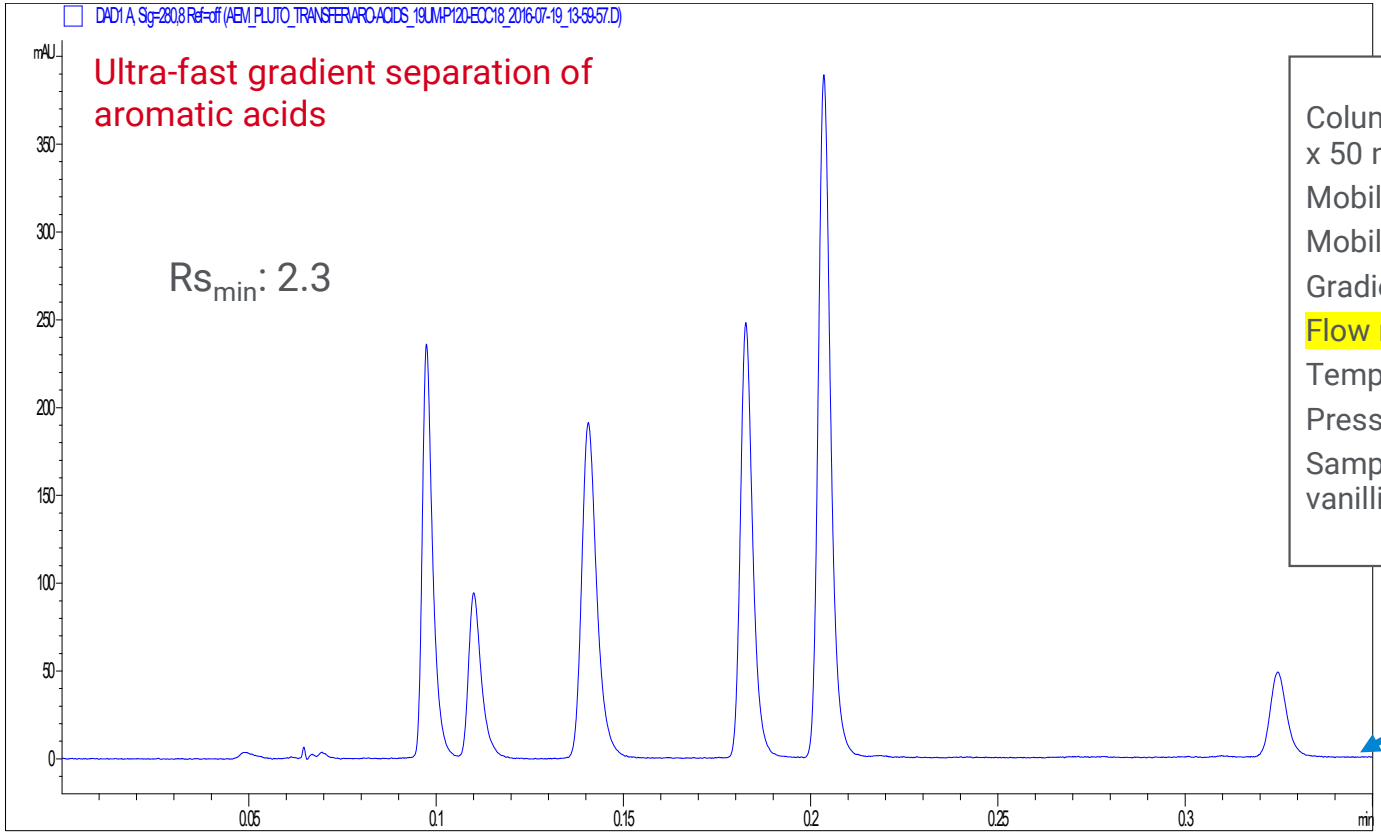


Highest UHPLC performance
- Pressure rating: 1300 bar
- Typical pressure: Similar to sub-2 µm totally porous
- Efficiency: ~120% of sub-2 µm totally porous
- Example: Agilent 1290 Infinity II LC system

UHPLC performance at lower pressure
- Pressure rating: 600 bar
- Typical pressure: 50% of sub-2 µm totally porous
- Efficiency: ~90% of sub-2 µm totally porous
- Example: Agilent 1260 Infinity II or Agilent 1260 Infinity II Prime LC

Improved HPLC performance
- Pressure rating: 600 bar
- Typical pressure: Often < 200 bar
- Efficiency: ~200% of 5 µm totally porous
- Agilent 1220 Infinity II LC system

InfinityLab Poroshell 1.9um:
Use high flow rates and low dispersion UHPLCs for fast separations



Column: InfinityLab Poroshell 120 EC-C18, 2.1 x 50 mm, 1.9 µm

Mobile phase A: 0.2% formic acid

Mobile phase B: acetonitrile

Gradient: 8-26% B in 0.3 min

Flow rate: 2.2 mL/min

Temperature: 60 °C

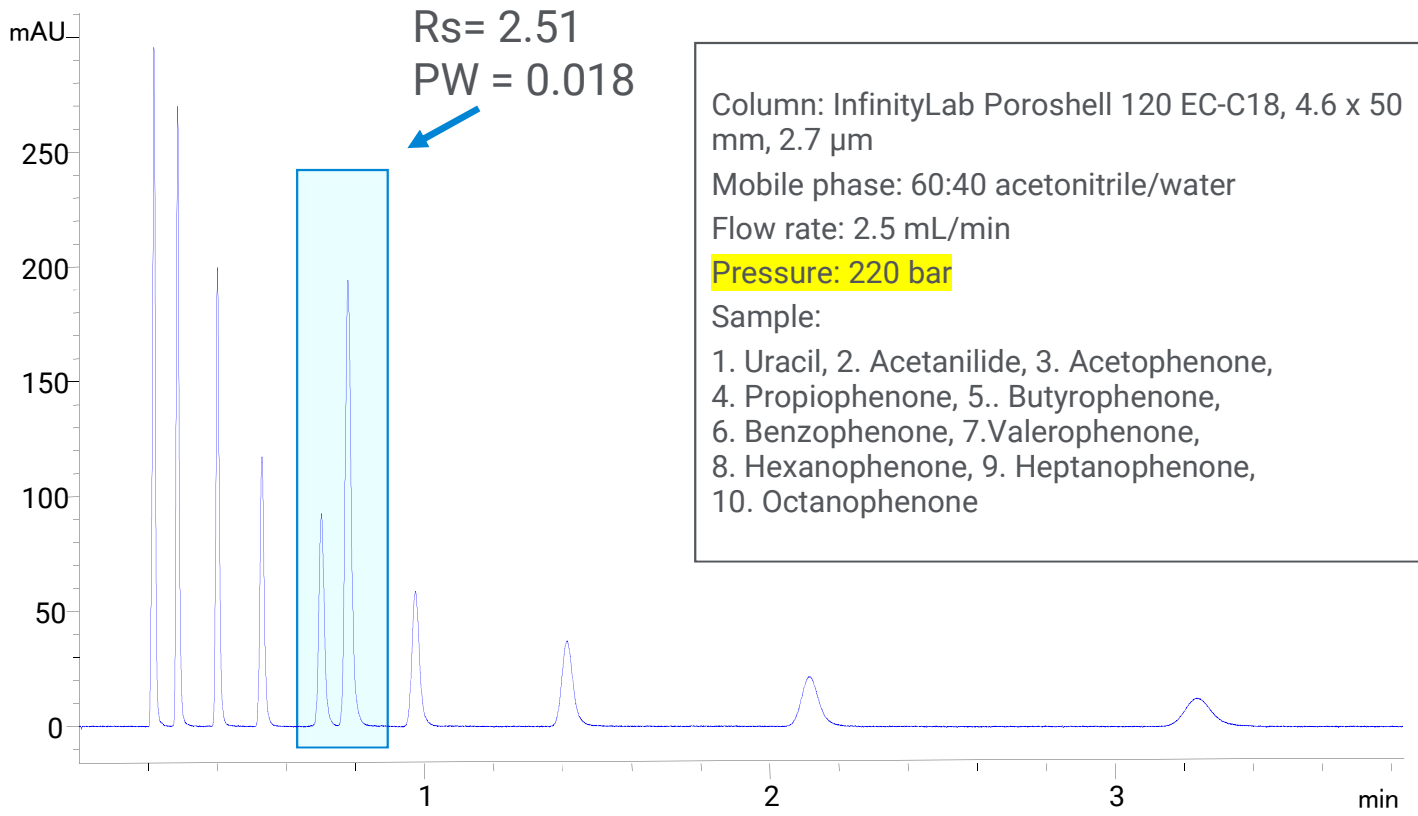
Pressure: 1150 bar

Sample: Protocatechuic acid, DOPAC, PABA, vanillic acid, syringic acid, salicylic acid

0.35 min runtime

UHPLC separations at HPLC pressures with 2.7um InfinityLab Particles

Fast isocratic separation of alkylphenones



Column: InfinityLab Poroshell 120 EC-C18, 4.6 x 50 mm, 2.7 µm

Mobile phase: 60:40 acetonitrile/water

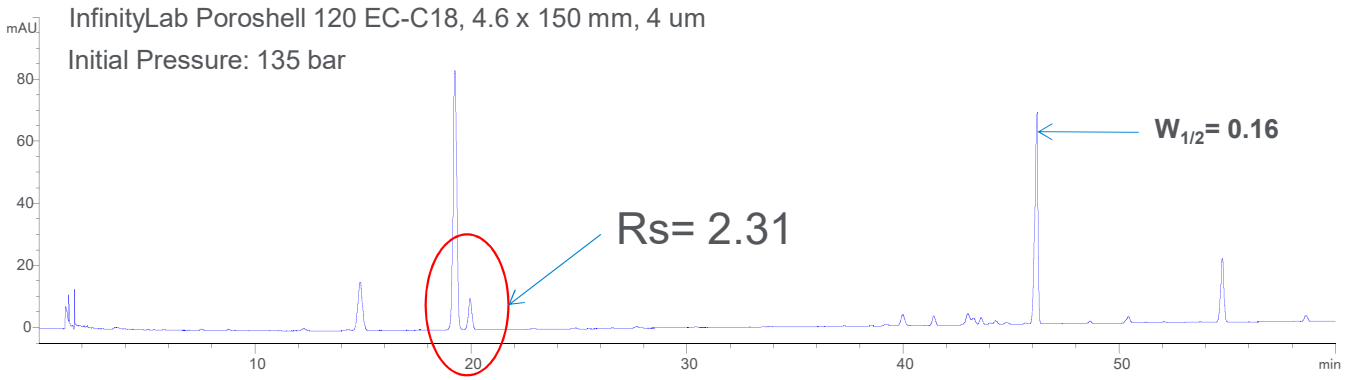
Flow rate: 2.5 mL/min

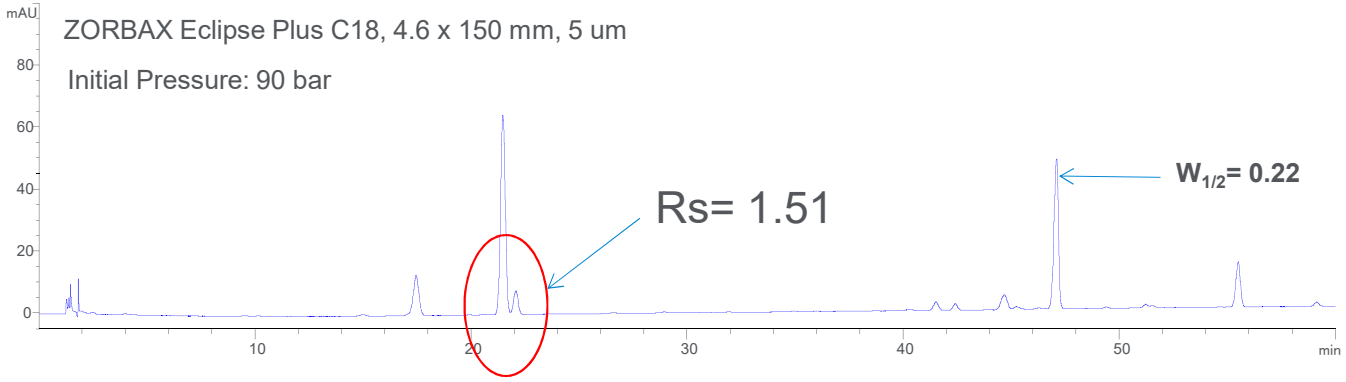
Pressure: 220 bar

Sample:

1. Uracil, 2. Acetanilide, 3. Acetophenone,
4. Propiophenone, 5.. Butyrophenone,
6. Benzophenone, 7.Valerophenone,
8. Hexanophenone, 9. Heptanophenone,
10. Octanophenone

Improving HPLC Performance with 4um particles





Mobile phase A: Water,
Mobile phase B: Acetonitrile
Flow rate: 1.0 mL/min
Temperature: 30 °C
Sample:

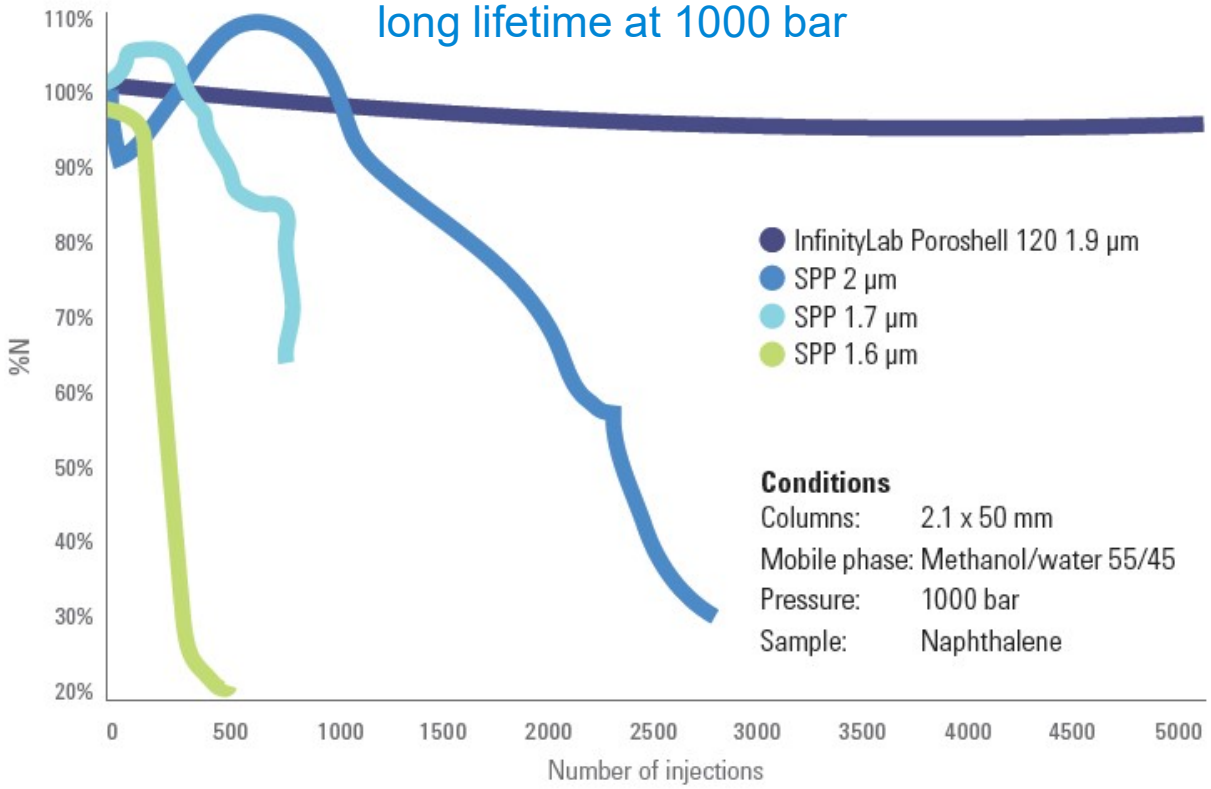
- Notoginsenoside R1,
- Ginsenoside Rg1
- Ginsenoside Re
- Ginsenoside Rb1

Time (min)	%B
0	19
12	19
60	36
61	90
65	90
66	19
70	19

Long column lifetime under UHPLC conditions

Best UHPLC lifetime

Optimized 1.9 µm particles and column loading provide long lifetime at 1000 bar



Column ID

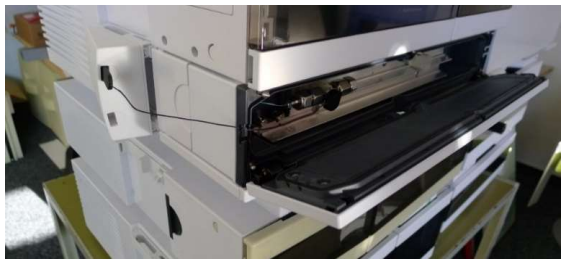
Usability, traceability and security

- All InfinityLab Poroshell 120 columns are available with pre-installed and pre-programmed Column ID

1.9 µm	A columns shipped with Column ID
2.7 µm & 4 µm	Add “T” suffix to part no to get Column ID

Column ID

Clip that holds column ID to column



Column Identification Tag

Understand key details and use of your column

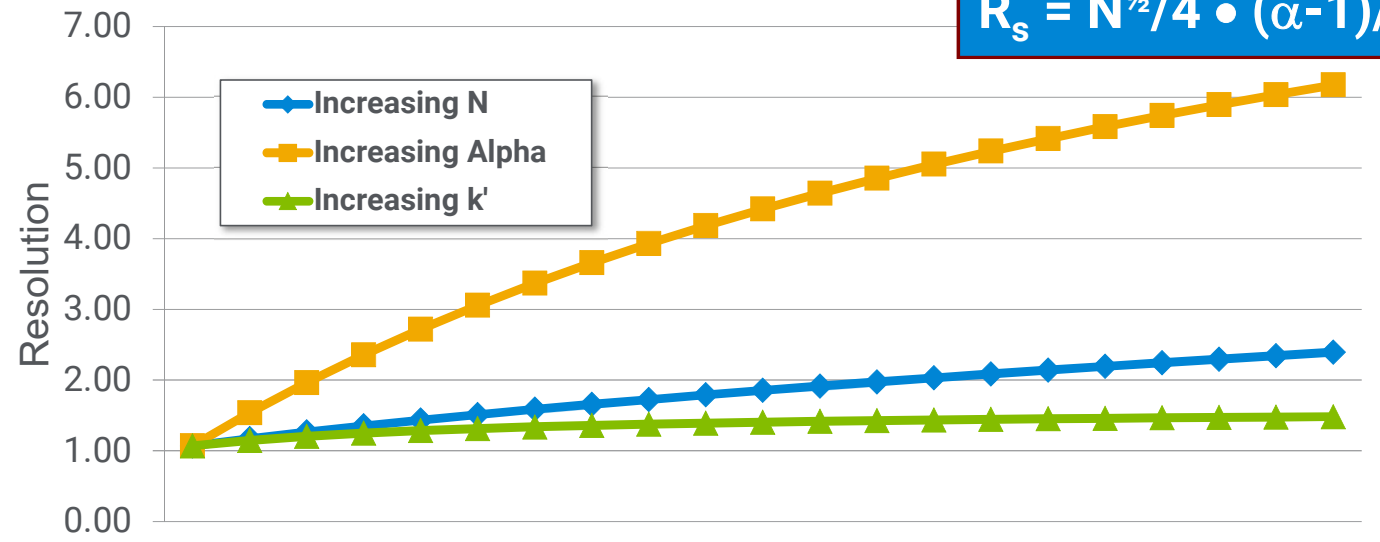
Field	Example
Description	Poroshell EC-C18
Length [mm]	100
Diameter [mm]	4.6
Particle size [µm]	2.7
Maximum pressure [bar]	600
Number of injections	[counter]
Product number	695975-902T
Serial number	USABC12345
Batch number	B12345
Maximum temperature [°C]	60
Maximum measured temperature [°C]	[updated from instrument]
Minimum pH	2.0
Maximum pH	8.0
Void volume [mL]	1.00
First injection date	[updated from instrument]
Recent injection date	[updated from instrument]

5067-5917 Column Identification Tag

- **Usability**
  - Easily find column details
- **Traceability**
  - Always know exactly which column is/was installed
- **Security**
  - Protect against the use of methods incompatible with the column

Resolution is a Common Goal for LC Method Development

$$R_s = N^{1/2}/4 \bullet (\alpha-1)/\alpha \bullet k'/(k'+1)$$



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 the most

- Change bonded phase
- Change mobile phase

} **Typical Analytical Method Development Parameters**

InfinityLab Poroshell 120 Selectivity
Choice of 18 chemistries

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

InfinityLab Poroshell 120
Phase specifications

Bonded Phase	Pore Size	Temp Limit	pH Range	Endcapped	Carbon Load	Surface Area
EC-C18	120Å	60 °C	2.0-8.0	Double	10%	130 m^2/g
EC-C8	120Å	60 °C	2.0-8.0	Double	5%	130 m^2/g
Phenyl-Hexyl	120Å	60 °C	2.0-8.0	Double	9%	130 m^2/g
SB-C18	120Å	90 °C	1.0-8.0	No	9%	130 m^2/g
SB-C8	120Å	80 °C	1.0-8.0	No	5.5%	130 m^2/g
HPH-C18	100Å	60 °C	3.0-11.0	Double	Proprietary	95 m^2/g
HPH-C8	100Å	60 °C	3.0-11.0	Double	Proprietary	95 m^2/g
Bonus-RP	120Å	60 °C	2.0-9.0	Triple	9.5%	130 m^2/g
PFP	120Å	60 °C	2.0-8.0	Yes	5.1%	130 m^2/g
SB-Aq	120Å	80 °C	1.0-8.0	No	Proprietary	130 m^2/g
EC-CN	120Å	60 °C	2.0-8.0	Double	3.5%	130 m^2/g
HILIC	120Å	60 °C	0.0-8.0	No	N/A	130 m^2/g

ZORBAX family

Eclipse Plus C18 is a good phase to start with

ZORBAX Family	Particles	Chemistries	Details
Eclipse Plus	1.8, 3.5, 5 um	C18, C8, Phenyl-Hexyl, PAH	Best all round - exceptional peak shape, efficiency, resolution and lifetime
StableBond	1.8, 3.5, 5, 7 um	SB-C18, SB-C8, SB-Phenyl, SB-C3, SB-AQ, SB-CN	Best for low pH mobile phases – great for method development
Eclipse XDB	1.8, 3.5, 5, 7 um	C18, C8, Phenyl, CN	High performance over a wide pH range
Extend-C18	1.8, 3.5, 5 um	Extend-C18	A good option for separations at high pH
Bonus-RP	1.8, 3.5, 5, 7 um	Bonus-RP	Alternative selectivity to alkyl, phenyl and cyano phases
HILIC Plus	1.8, 3.5 um	HILIC Plus	Retention of polar molecules, high sensitivity for LC/MS applications

Other columns families

Columns for more specialized applications

Column Family	Particles	Chemistries	Details
Pursuit	3, 5, 10 um	C18, C8, PFP, Diphenyl	**Higher surface area**
Pursuit XRs	3, 5, 10 um	C18, C8, Diphenyl, Si	**Highest surface area offers higher loadability**
Pursuit XRs ULTRA	2.8 um	C18, C8, Diphenyl	**Loaded for higher pressure stability**
Polaris	3, 5, 10 um	C18-A, C8-A, C18-Ether, C8-Ether, Amide-C18, NH2, Si-A	**More options for polar molecule separations**
Hi-Plex	8, 10 um	H, Ca, Ca (Duo), Pb, Na, Na (Octo), K	**Analysis of carbohydrates and organic acids**

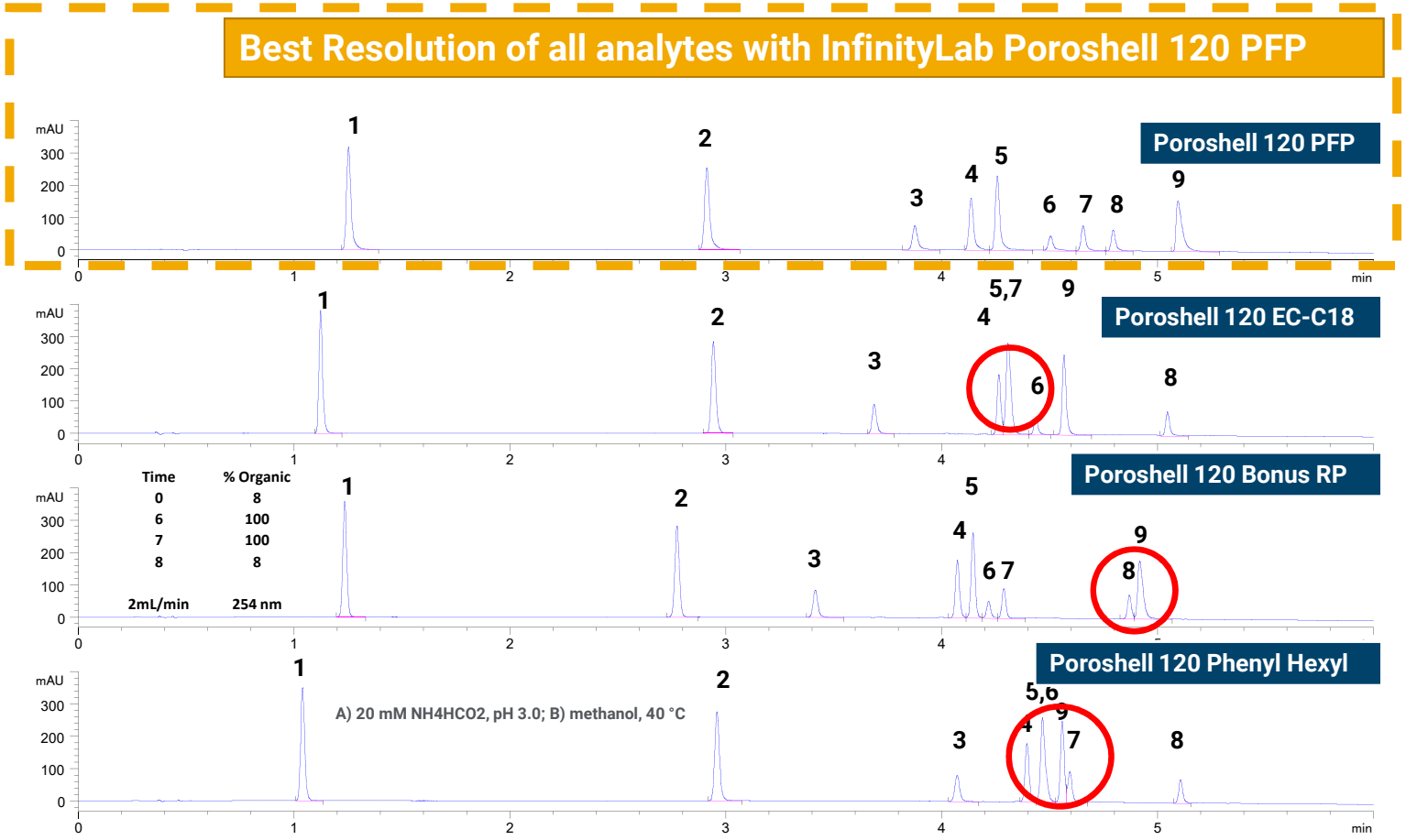
Column Family	Pressure Rating
Pursuit	400 bar
Pursuit XRs	400 bar
Pursuit XRs ULTRA	400 bar
Polaris	400 bar
Hi-Plex	25 – 50 bar

Why is changing the bonded phase effective?

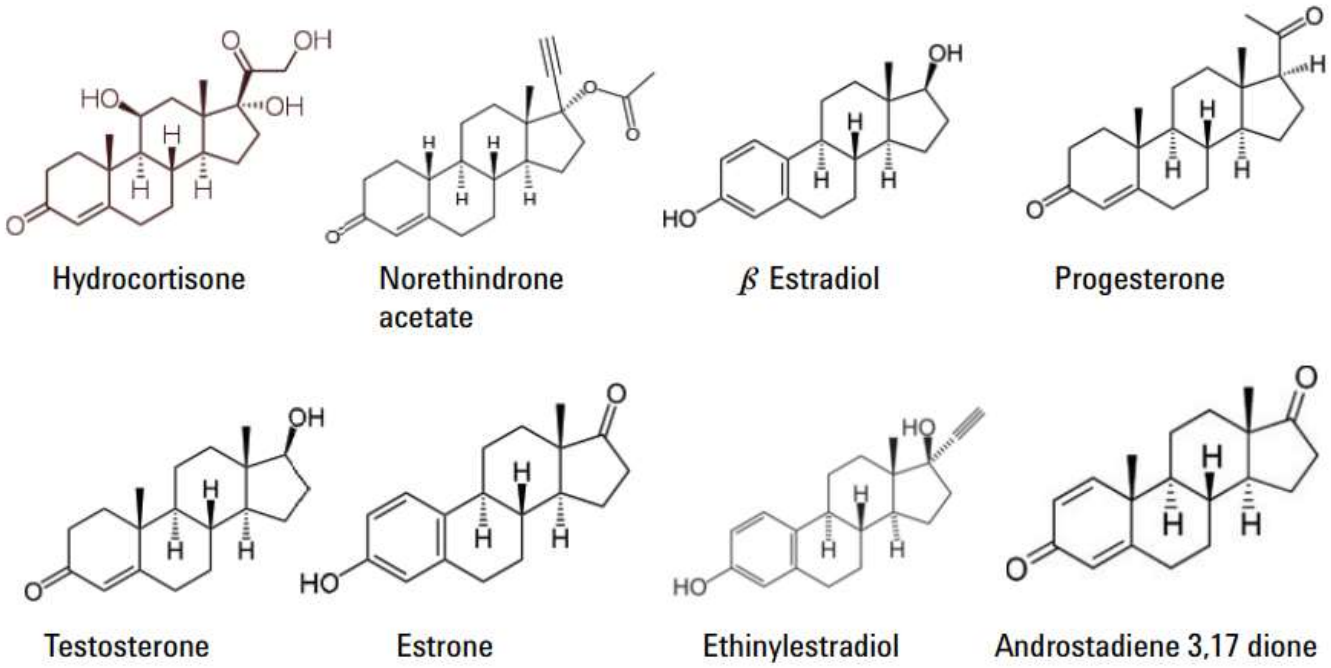
- **Differences in interactions with polar and non-polar compounds**.
- Other types of interactions with a bonded phase can be exploited (pi-pi interactions etc.)
- **These all change with bonded phase!**
- Changing the bonded phase can improve **selectivity/resolution**, reduce analysis time
- **When you use InfinityLab Poroshell 120 columns the comparison of bonded phases can be done quickly!!**
- **Multiple column choices** available make this easy

NSAID separation with a methanol gradient

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



Separation of 8 steroids with methanol gradient

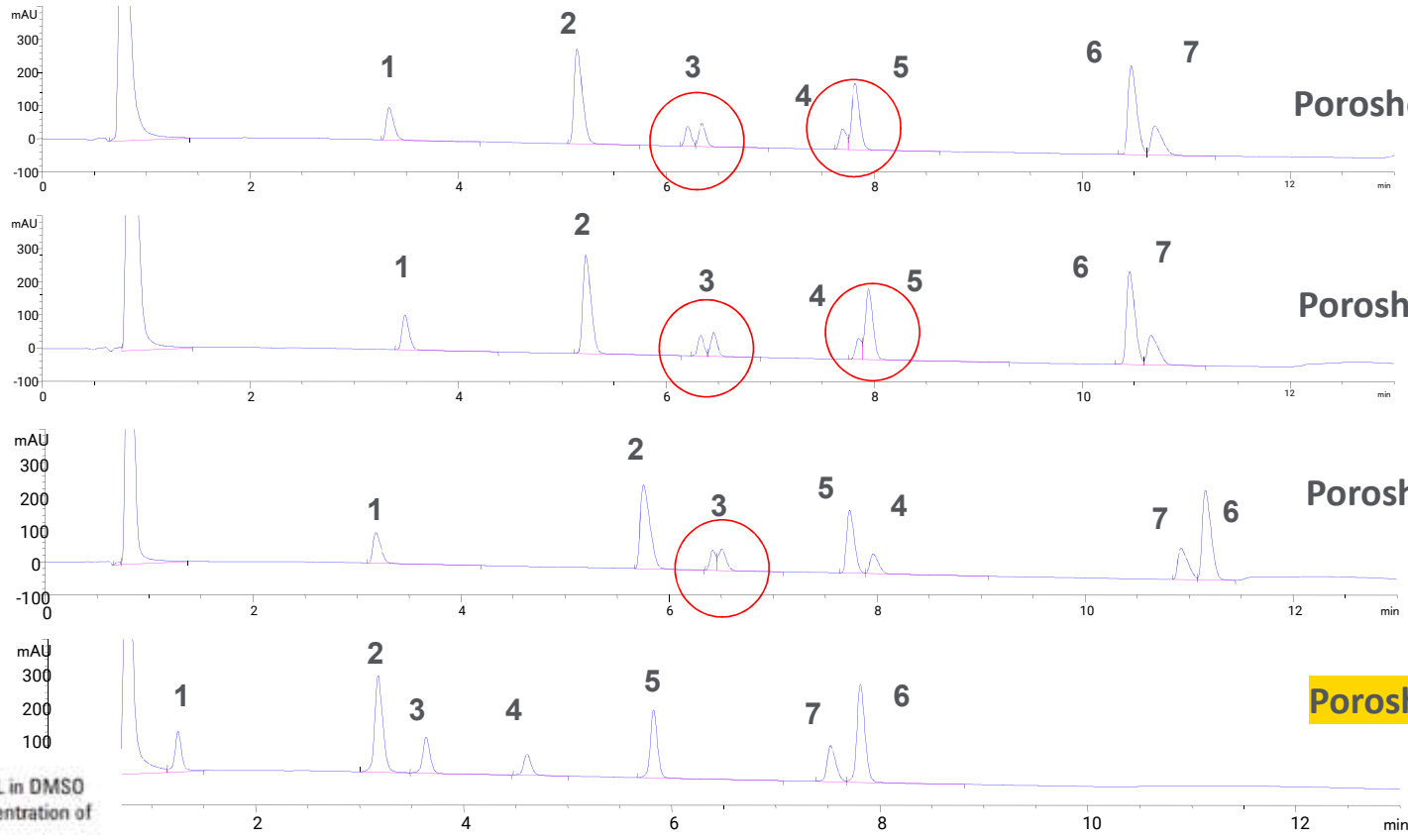


Poroshell Phenyl Hexyl: Alternate Selectivity

Best Resolution of all analytes with InfinityLab 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

Poroshell Bonus-RP: Alternate Selectivity

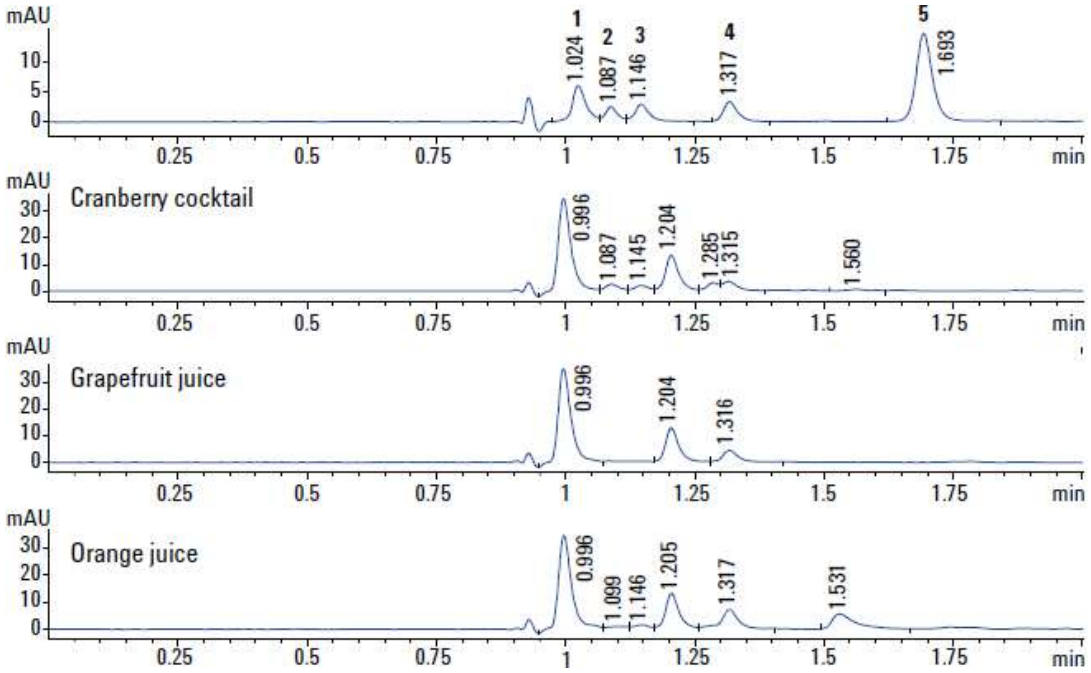


Beta Blockers
1. Atenolol
2. Pindolol
3. Naldolol
4. Metoprolol
5. Acebutolol
6. Propranolol
7. Alprenolol

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

Best Resolution of all analytes with Poroshell Bonus-RP

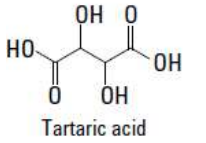
ZORBAX SB-Aq phase



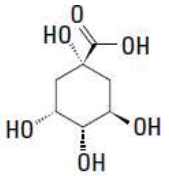
Column: Agilent Poroshell 120 SB-Aq, 3 × 100 mm, 2.7 µm (p/n 685975-314)
Eluent: 100 mM Potassium phosphate buffer, pH 2.5
Injection volume: 5 µL
Flow rate: 0.5 mL/min
Temperature: 50 °C
Detector: DAD, at 226 nm

Peak ID

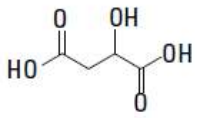
1. Tartaric acid
2. Quinic acid
3. Malic acid
4. Citric acid
5. Fumaric acid



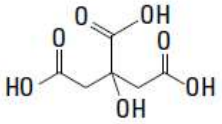
Tartaric acid



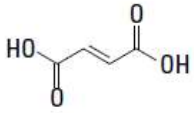
Quinic acid



Malic acid



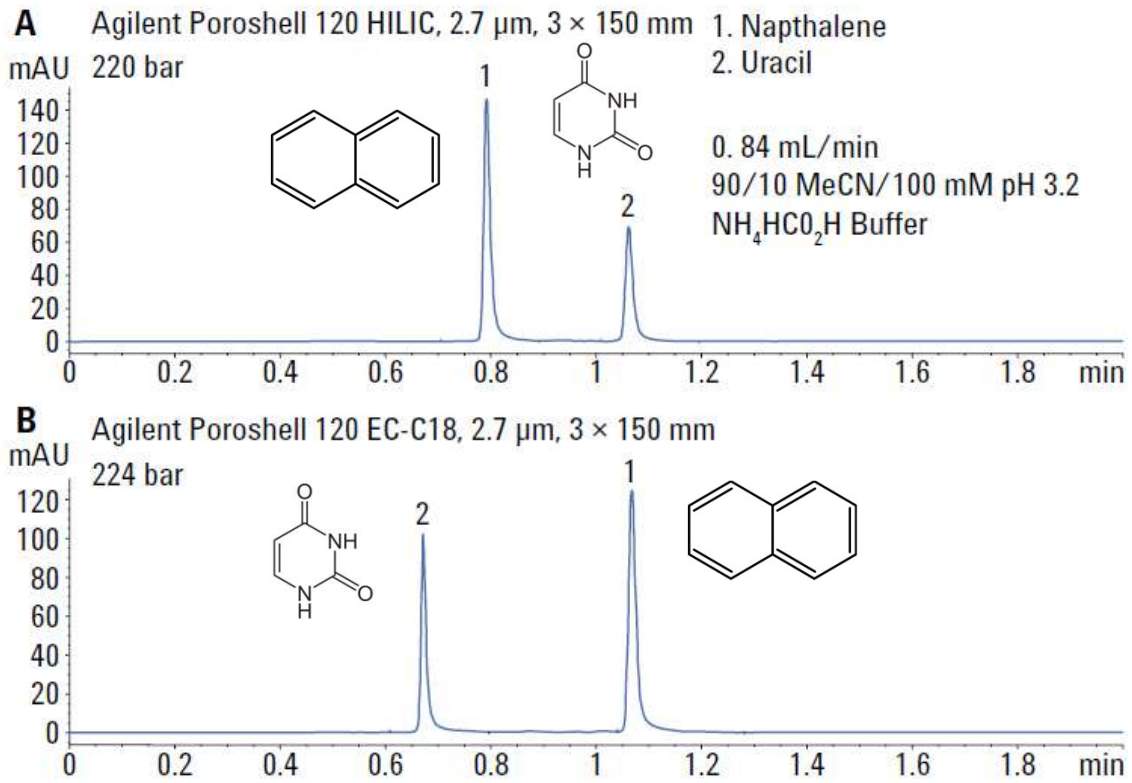
Citric acid



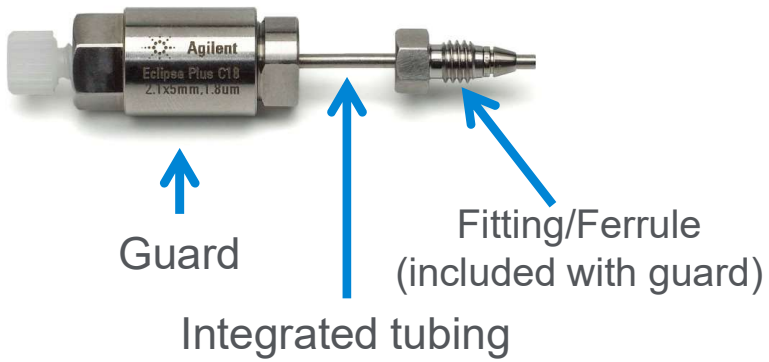
Fumaric acid

Application note: 5991-1992EN

HILIC Technique – Comparison with Reversed-Phase



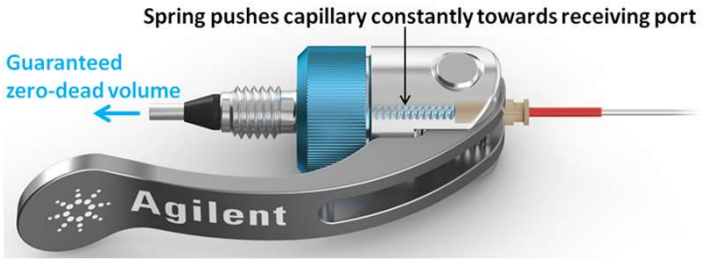
Fast Guard columns



- Agilent Fast Guards for UHPLC are a one-piece guard hardware solution designed for use with InfinityLab Poroshell 120 and ZORBAX RRHD 1.8 um columns, sold in 3-packs
- By installing a guard column when using dirtier samples, users can extend the life of their column, and utilize less expensive guard columns rather than column replacements

InfinityLab Quick Connect and Quick Turn Fittings

- Spring loaded design
- Easy! No tools needed
- Works for all column types
- Reusable
- Consistent ZDV connection



Quick Connect Fitting
- Finger tight up to 1300 bar
- Hand tighten the nut, then depress the lever

Spring load function for dead volume!

Quick Turn Fitting
- Finger tight up to 400 bar
- Up to 1300 bar with a wrench
- Compact design

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

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

InfinityLab Poroshell 120 columns Summary

Taking you to the next level of efficiency

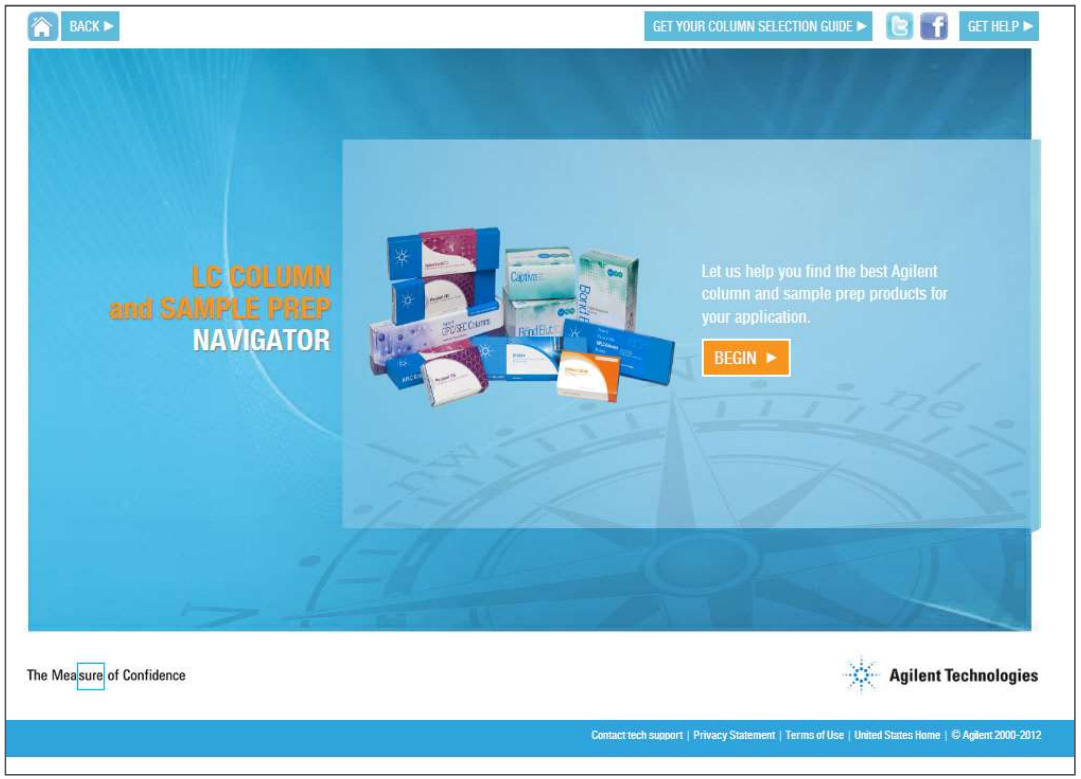
Analytical efficiency
Achieve the efficiency and selectivity to meet almost any application need. Enjoy high resolution and fast analysis for the best results

Instrument efficiency
Understand key details and use of a column that's a perfect fit for your LC with Column ID. Get the most from your instrument

Laboratory efficiency
Get high throughput and high quality data with consistent performance from batch to batch. Operate your lab at peak efficiency

On-Line tool “Navigator”

LC column and sample prep selection tool



http://navigator.chem.agilent.com

Thank you