Can I Really Enjoy the Benefits of the Latest LC Column Technology?

Stephen Luke

LC Columns Product Manager

Agilent Technologies

Organization and Laboratory Needs

Agilent CrossLab

Organization needs

- Increased capacity
- Shorter time to market
- Increased profitability

Laboratory needs

- Better use of resources
- Increase productivity
- Reduce costs

Laboratory Needs

Translated to liquid chromatography

Agilent CrossLab

Better use of resources

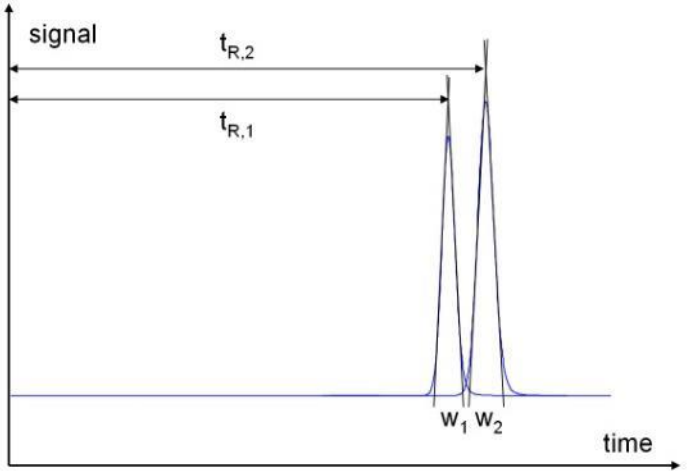
Increase productivity

Reduce costs

Use all the instruments in the lab

Run fast with high resolution

Get long column lifetimes



Laboratory Needs

What's stopping you achieving them?

Agilent CrossLab

Better use of resources
Increase productivity
Reduce costs

→

Use all the instruments in the lab
Run fast with high resolution
Get long column lifetimes

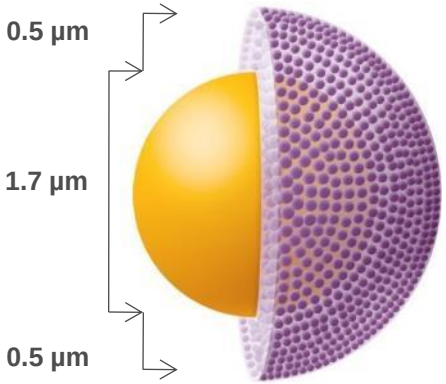
Yes, but…

- I don't all have UHPLC instruments
- I can't / don't want to change my methods much
- My "dirty" samples clog my columns
- My columns fail in the mobile phases we use

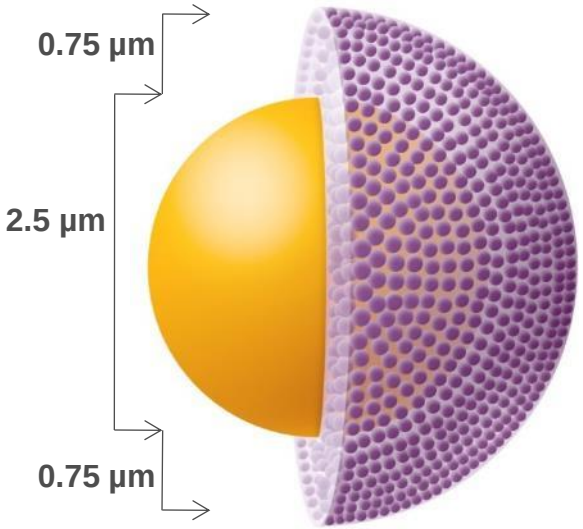
Superficially Porous Particles for LC

Make every LC and LC/MS in your lab work harder

Agilent CrossLab



Poroshell 120 2.7 µm



Poroshell 120 4 µm

www.agilent.com/chem/discoverporoshell

Laboratory Needs

Translated to liquid chromatography

Agilent CrossLab

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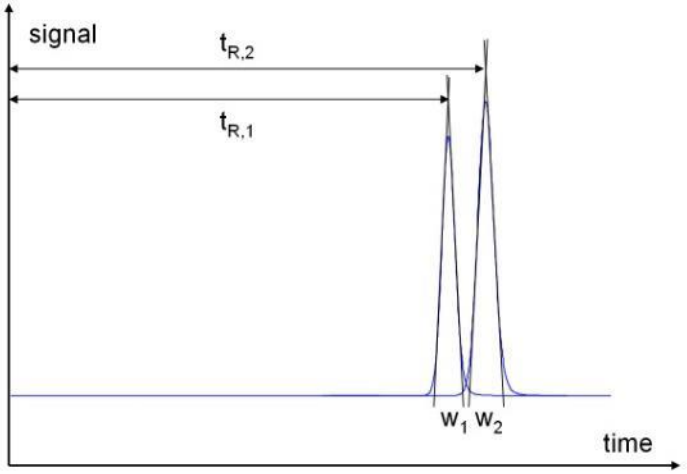

Use All the Instruments in the Lab

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Equation for Pressure Drop Across an HPLC Column

$$\Delta P = \frac{\eta \cdot L \cdot v}{\theta \cdot d_p^2}$$

ΔP = Pressure Drop

η = Fluid Viscosity

L = Column Length

v = Flow Velocity

d_p = Particle Diameter

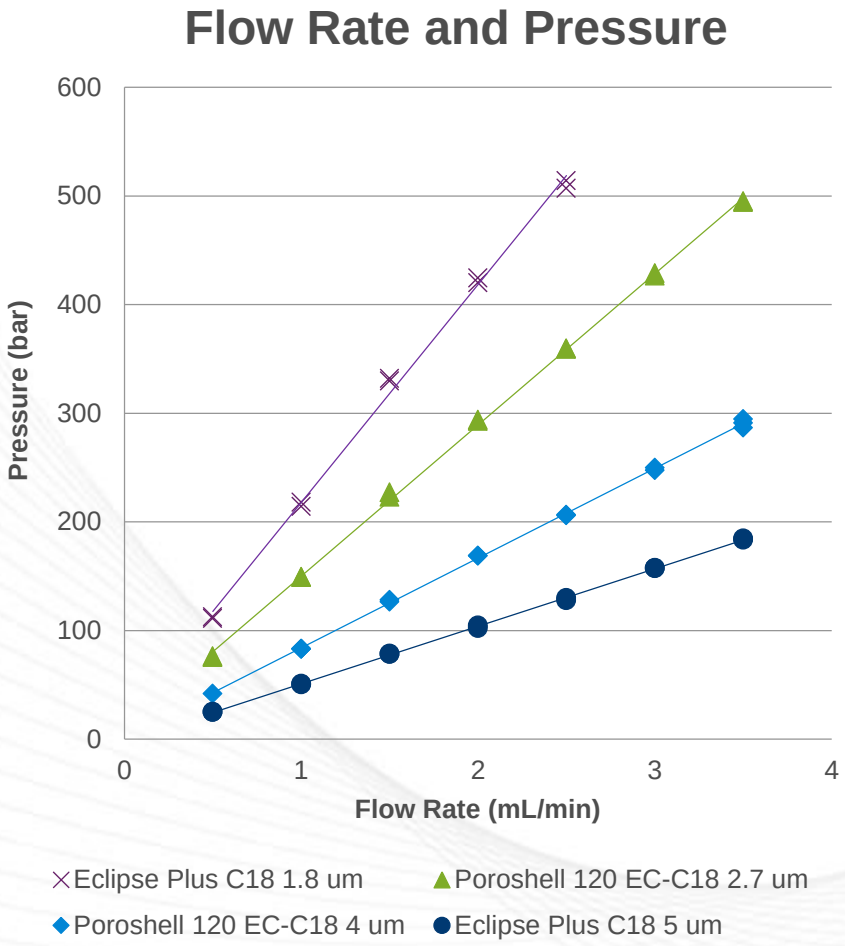
θ = Dimensionless Structural Constant
~ 600 For Packed Beds in LC

- Pressure and delay/dispersion volume are key components of instrument compatibility
- Shorter column lengths and larger particle diameters reduce column pressure

Use All the Instruments in the Lab

4 µm pressures suitable for any instrument

Agilent CrossLab



Column Dimensions: 4.6 x 100 mm

Mobile phase A: 0.1 % formic acid in water

Mobile phase B: 0.1 % formic acid in acetonitrile

Temperature: 35°C

Pressure reading at 5% B

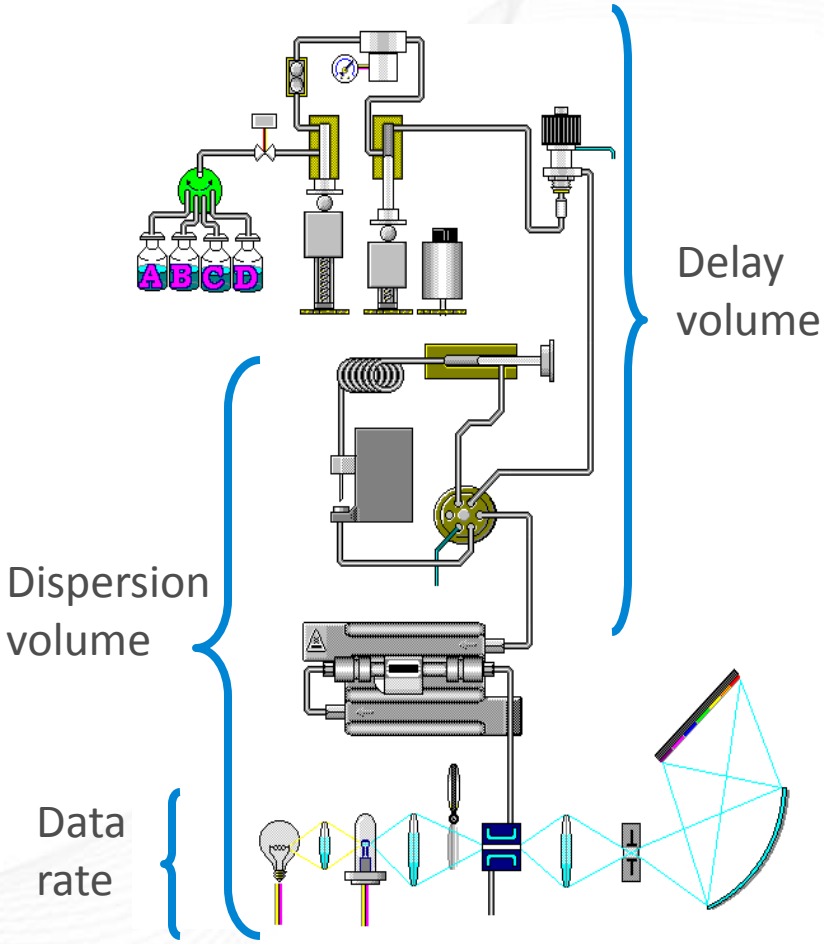
Application note 5991-5510EN

Use All the Instruments in the Lab

Agilent CrossLab

Volume Considerations for LC Systems

- The time taken for solvent to fill the delay volume represents an isocratic hold at the start of a run. For fast methods low delay volumes are required
- Sharp (efficient) peaks have a low peak volume. Low dispersion volumes are needed to avoid broadening of low volume peaks
- Both effects are most pronounced with narrow (2.1 mm) ID columns



Use All the Instruments in the Lab

Agilent CrossLab

Instrument / Preference		Column	Details
	UHPLC Low delay/dispersion Up to 1300 bar	Poroshell 120 2.7 µm	Pressure 50% of sub-2 µm TPP
	HPLC/UHPLC Mid delay/dispersion Up to 600 bar		
	HPLC High delay/dispersion Up to 400 bar	Poroshell 120 4 µm	Typical pressure < 200 bar

Laboratory Needs

Translated to liquid chromatography

Agilent CrossLab

Better use of resources

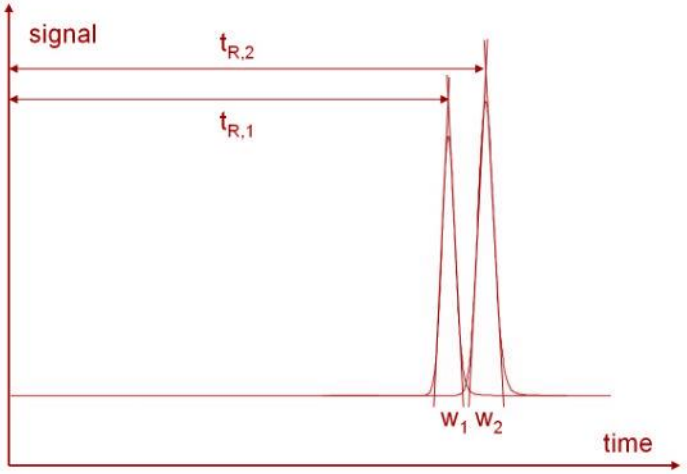
Increase productivity

Reduce costs

Use all the instruments in the lab

Run fast with high resolution

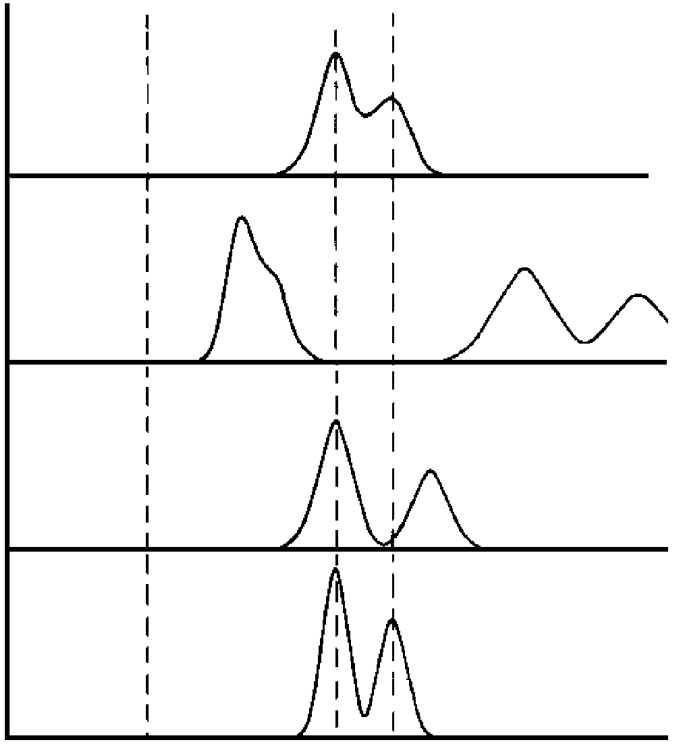
Get long column lifetimes



Increase Resolution

Fundamental Resolution Equation

$$R_s = \frac{\sqrt{N}}{4}\frac{(\propto -1)}{\propto}\frac{k}{(k+1)}$$



To increase resolution:

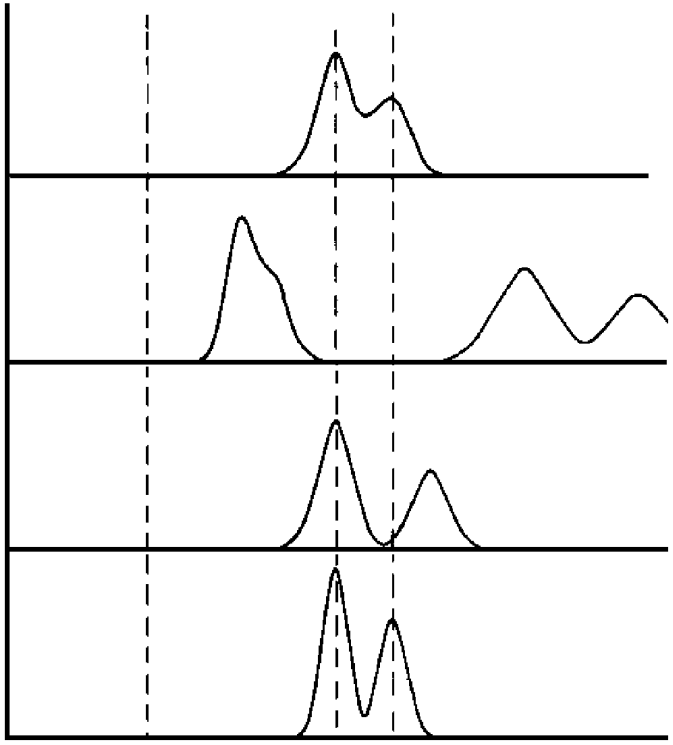
- Increase retention (*k*)
- Change selectivity (*a*)
- Increase efficiency (*N*)

Run Fast with High Resolution

Agilent CrossLab

Fundamental Resolution Equation

$$R_s = \frac{\sqrt{N}}{4}\frac{(\propto -1)}{\propto}\frac{k}{(k+1)}$$



- Retention and selectivity are impacted by changes to chemistry (mobile phase and stationary phase)
- Using a shorter column with high efficiency will maintain resolution in a shorter run time

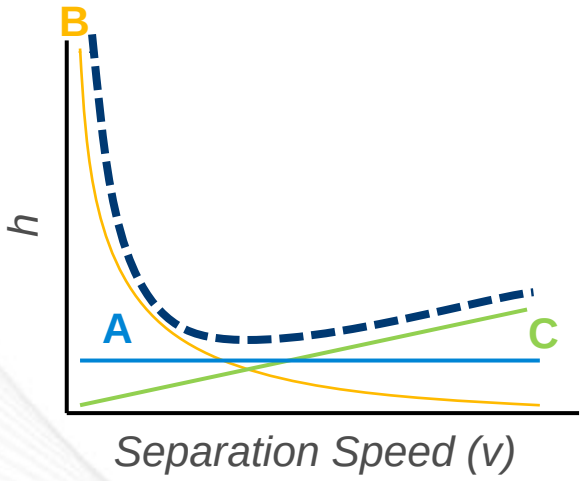
Run Fast with High Resolution

All 3 van Deemter terms are reduced with SPP

Agilent CrossLab

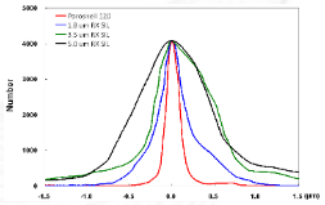
van Deemter equation:

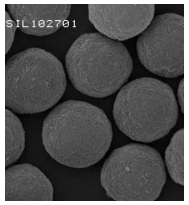
$$h = A + B/\nu + C \cdot \nu$$

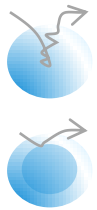


Lower *h* = higher efficiency!

- **A term – eddy diffusion**
  - Particle size & packing quality
  - Narrow particle size distribution
- **B term – longitudinal diffusion**
  - Less mobile phase in the column
  - Reduced diffusion
- **C term – mass transfer**
  - Shorter diffusion paths
  - More effect on large molecules

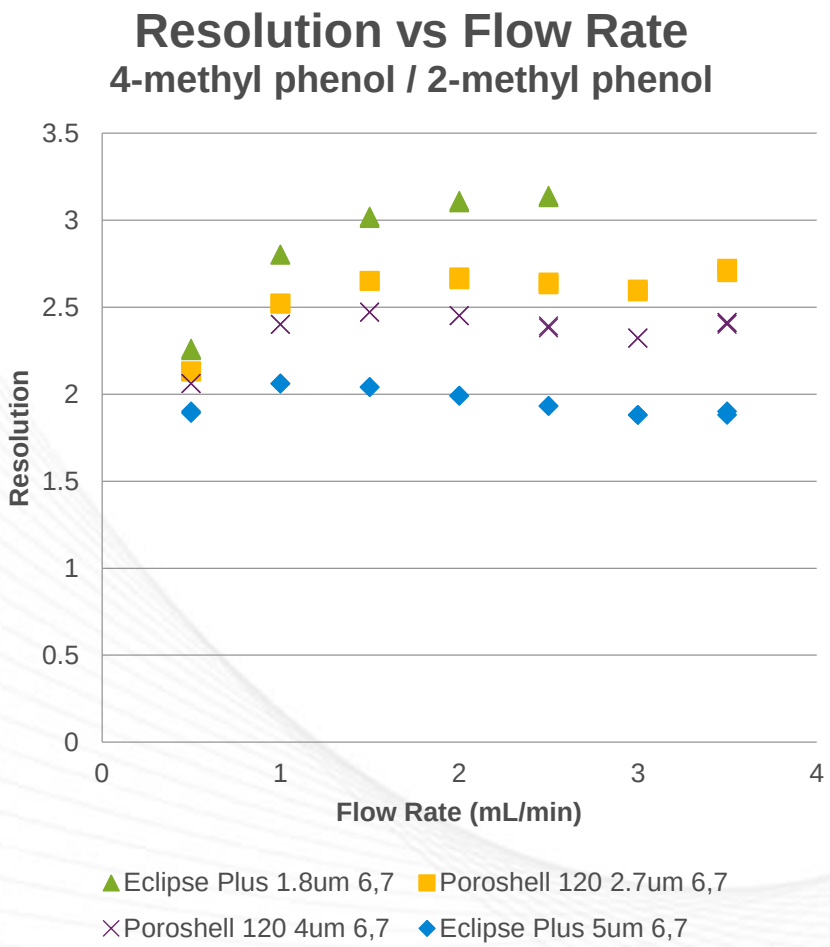






Increase Resolution

4 µm gives good resolution at low pressure



Column Dimensions: 4.6 x 100 mm

Mobile phase A: 0.1 % formic acid in water

Mobile phase B: 0.1 % formic acid in acetonitrile

Temperature: 35°C

% B	Time (min)						
5	4	2	1.33	1	0.8	0.67	0.34
40	34	17	11.33	8.5	6.8	5.67	2.84
40	40	20	13.33	10	8	6.67	3.34
5	42	21	14	10.5	8.4	7	3.5
5	50	25	16.67	12.5	10	8.34	4.17
Flow rate (mL/min)	0.5	1	1.5	2	2.5	3	3.5

Results at 2mL/min	R_s	P (bar)
Eclipse Plus 1.8 um	3.1	422
Poroshell 120 2.7 um	2.7	293
Poroshell 120 4 um	2.5	169
Eclipse Plus 5 um	2.0	103

Application note 5991-5510EN

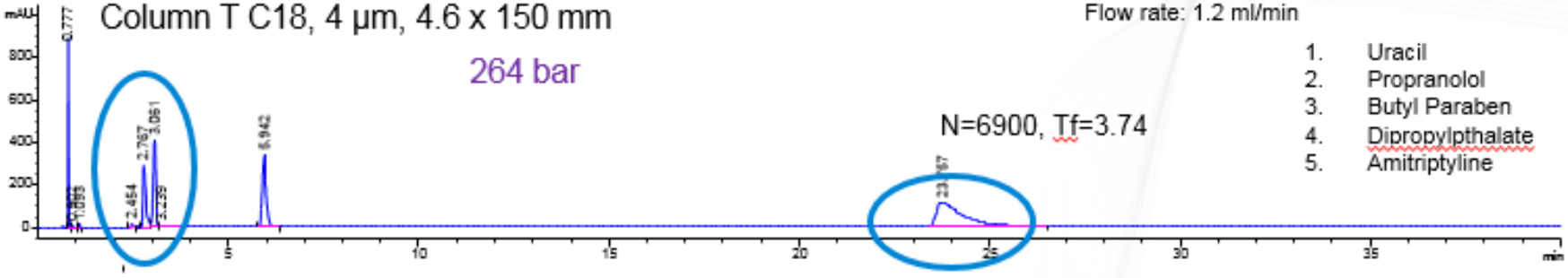
Other SPP Column Performance

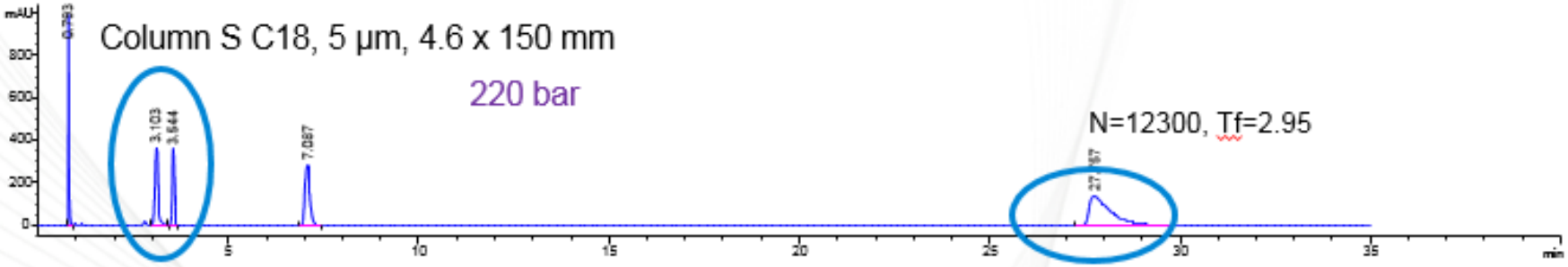
Agilent CrossLab

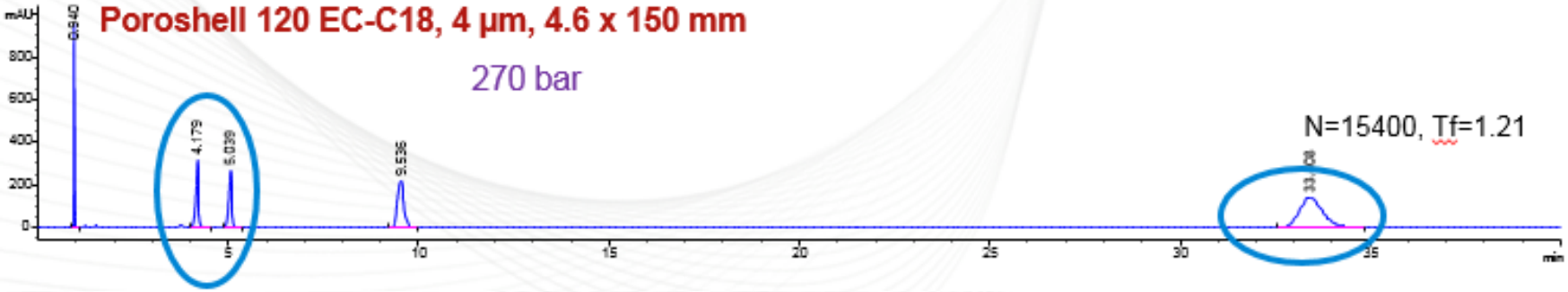
Poor retention and peak shape from non-Agilent columns

Mobile phase A: 20 mM K_2HPO_4/KH_2PO_4 pH 7
Mobile phase B: Methanol
Flow rate: 1.2 ml/min

1. Uracil
2. Propranolol
3. Butyl Paraben
4. Dipropylpthalate
5. Amitriptyline

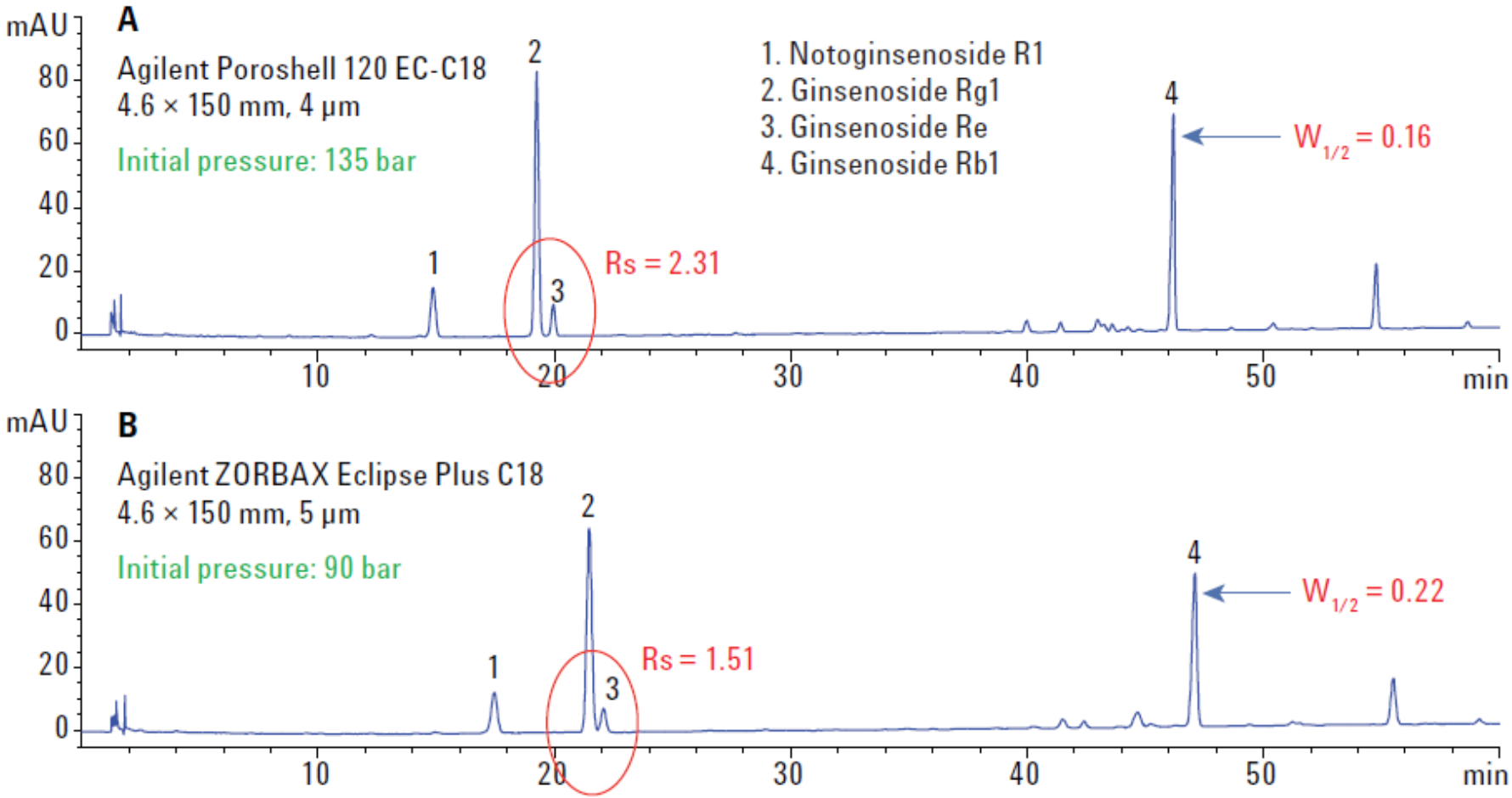






Increase Resolution

4 µm column increases resolution

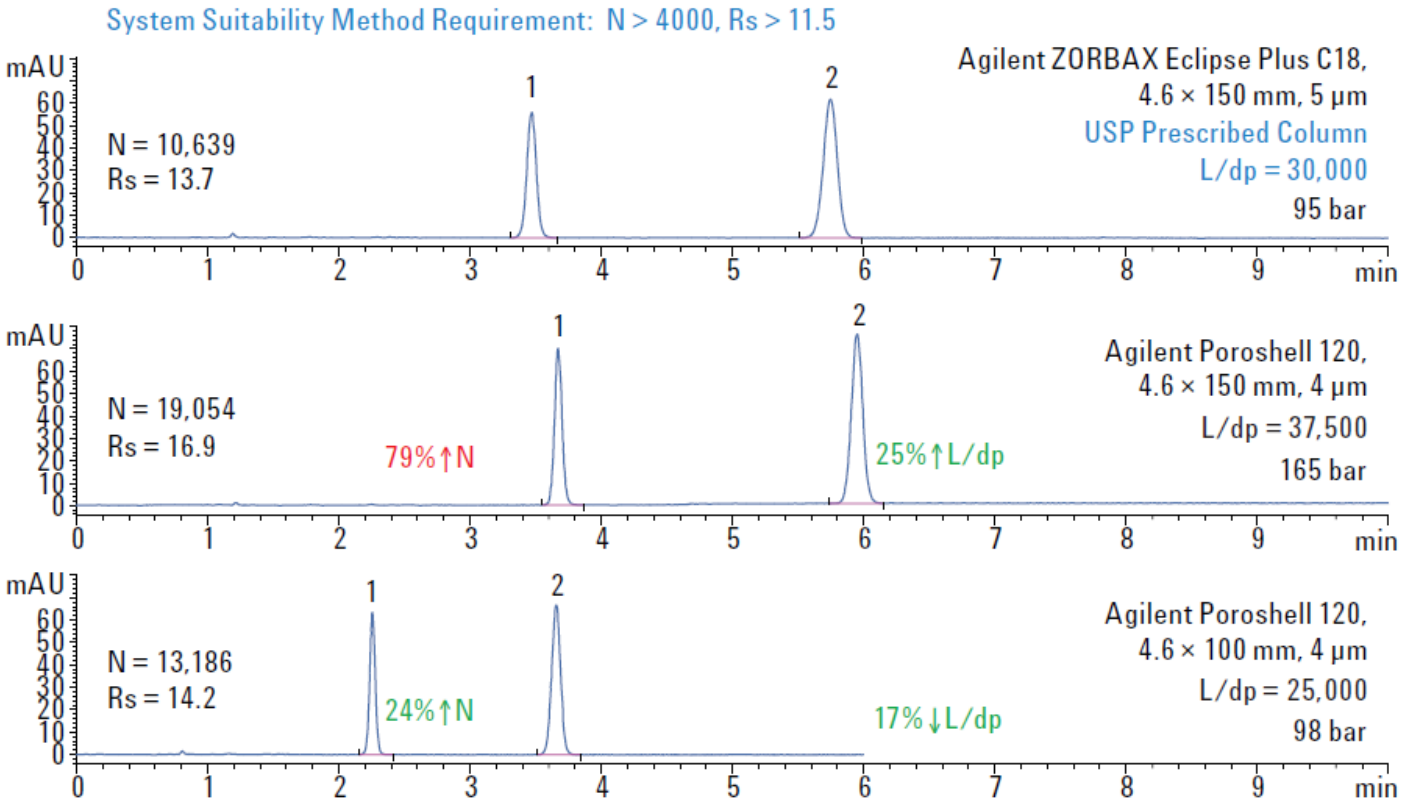


Notoginseng, China Pharmacopoeia, edition 2015

Application note 5991-5554EN

Run Fast with High Resolution

Shorter 4 µm column gives fast, high resolution runs



Mobile phase: 50:49:1 MeCN:H_2O:Acetic acid
Flow rate: 1.2 mL/min

Peak ID

1. Naproxen
2. Butyrophenone

Application note [5991-5408EN]

Return on Investment

$43K savings annually with 160% ROI / year

Scope	Number of instruments	1
	Number of years	5

		Conventional	**Fast**
Method	Analysese per year (1 instrument)	5,000	5,000
	Run time (minutes)	10	6
	Flow rate (mL/min)	1.2	1.2
	Column lifetime (injections)	1,000	1,000
	Development time (hours)	0	80

		Cost	**Conventional** *Amount/analysis*	**Fast** *Amount/analysis*	**Conventional** *Cost/year*	**Fast** *Cost/year*	**Saving** *Cost/year*
Value	Overhead per instrument ($/hour)	$125.00	$20.83	$12.50	$104,167	$62,500	$41,667
	Cost of mobile phase purchase ($/L)	$62.50	$0.75	$0.45	$3,750	$2,250	$1,500
	Cost of mobile phase disposal ($/L)	$5.00	$0.06	$0.04	$300	$180	$120
	Columns ($)	$550.00	$0.55	$0.55	$2,750	$2,750	$0
	Total		$22.19	$13.54	$110,967	$67,680	
	Saving			$8.66		$43,287	

		Cost	**Conventional** *Cost/method*	**Fast** *Cost/method*	**Cost** *Cost/method*
Costs	Overhead per instrument ($/hour)	$125.00	$0	$10,000	$10,000
	Method development ($/hour)	$175.00	$0	$14,000	$14,000
	Instrument	$0	$0	$0	$0
	Total		$0	$24,000	

		Conventional	**Fast**
ROI%	Value	$0.00	$216,433
	Cost	$0.00	$24,000
	ROI% / year		160%

Increase Resolution

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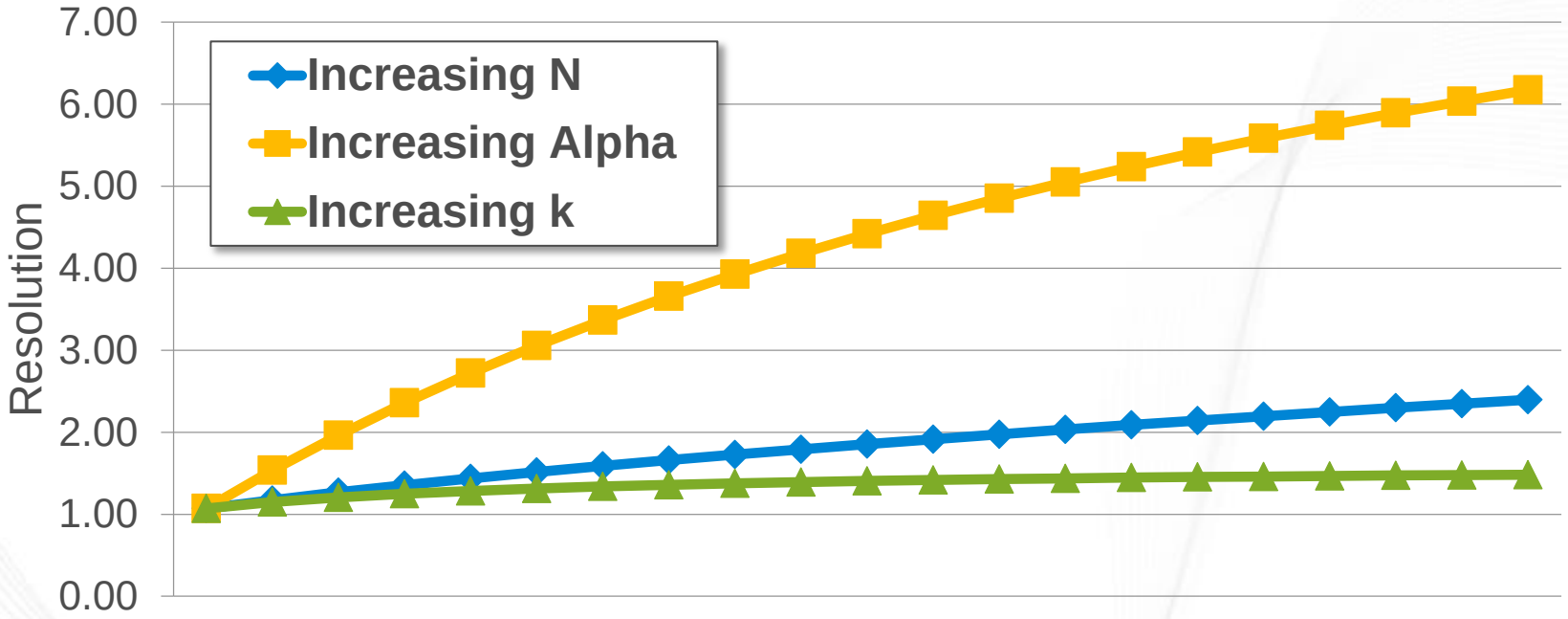

Plates:	5000	10000	15000	20000	25000
Alpha:	1.10	1.35	1.60	1.85	2.1
k	2.0	4.5	7.0	9.5	12.0

Selectivity Impacts Resolution Most

- Change bonded phase
- Change mobile phase

$$R_s = \frac{\sqrt{N}}{4}\frac{(\propto -1)}{\propto}\frac{k}{(k+1)}$$

Increase Resolution

Agilent CrossLab

Choice of bonded phase selectivity - 12 chemistries

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

Wall Chart 5991-6240EN

Increase Resolution

The right selectivity

Agilent CrossLab

Column	H	S*	A	B	$C_{(2.8)}$	$C_{(7.0)}$	$F_{s\ (pH\ 2.8)}$
Poroshell 120 EC-C18	1.02	0.01	-0.13	0.00	0.16	0.12	0.0
Poroshell 120 EC-C8	0.88	0.01	-0.23	0.02	0.13	0.09	6.0
Poroshell 120 Phenyl-Hexyl	0.75	-0.08	-0.39	0.02	0.14	0.14	13.1
Poroshell 120 PFP	0.63	-0.52	-0.52	0.43	-0.11	N/A	85.5

Hydrophobic Subtraction Model (HSM) Data provided by Dwight Stoll

Hydrophobic Subtraction Model	
Hydrophobicity	H
Steric interaction	S*
Hydrogen-bond acidity	A
Hydrogen-bond basicity	B
Ion-exchange capacity	C

F_s factor describes the similarity of two column selectivities. A small F_s indicates that two columns are very similar, while a large factor indicates that two columns are very different. Calculated according to the following equation:

$$F_s = \{[12.5(H_2 - H_1)]^2 + [100(S_2^* - S_1^*)]^2 + [30(A_2 - A_1)]^2 + [143(B_2 - B_1)]^2 + [83(C_2 - C_1)]^2\}^{\frac{1}{2}}$$

Further details at:
http://www.usp.org/app/USPNF/columnsDB.html

Reversed-phase only so Poroshell 120 HILIC not included

Increase Resolution

The right selectivity

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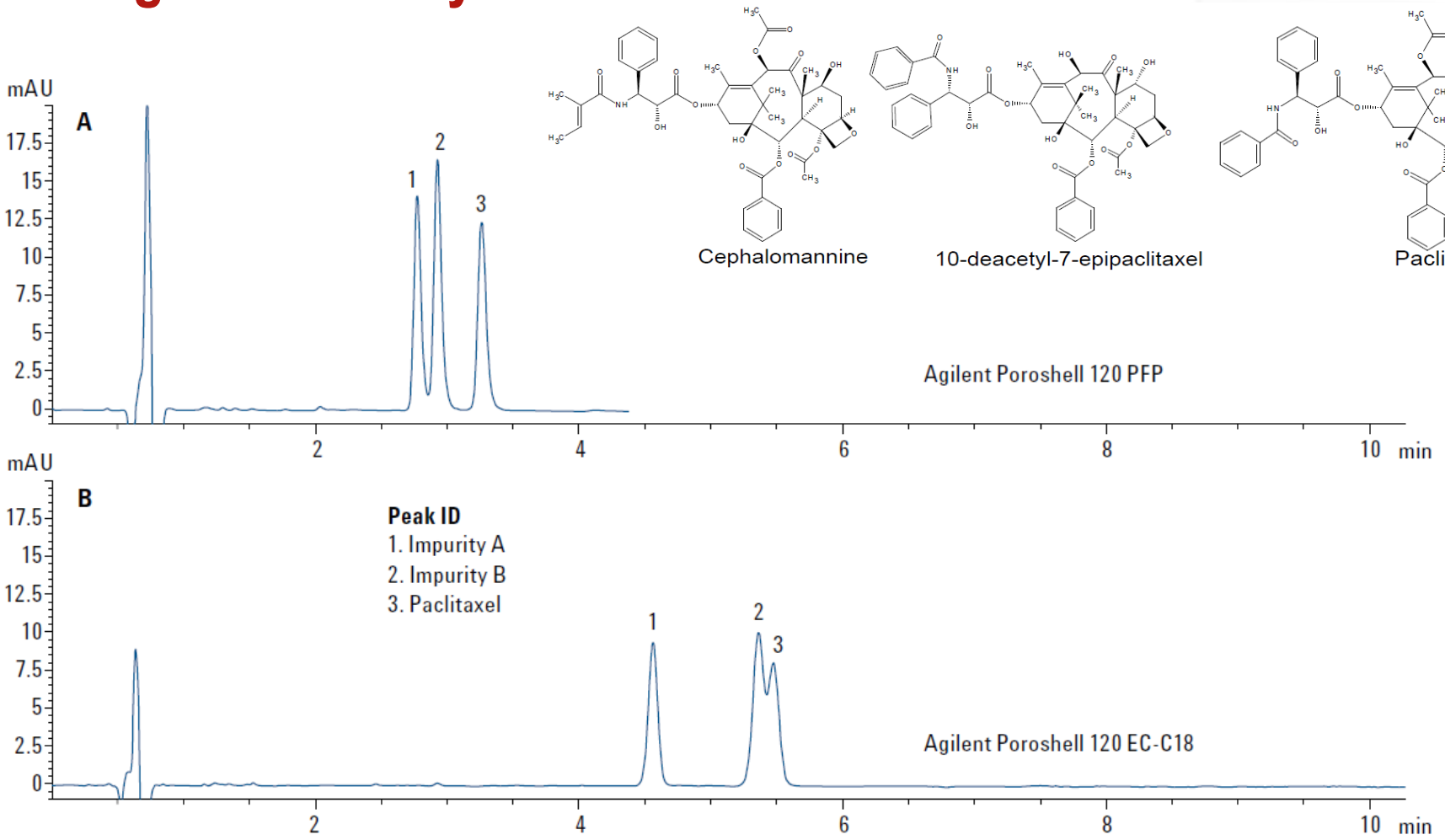
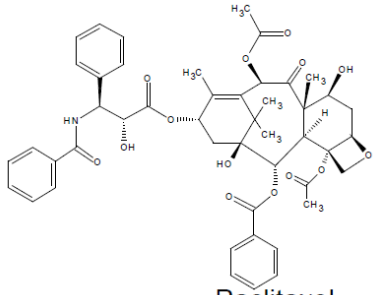


Cephalomannine

10-deacetyl-7-epipaclitaxel

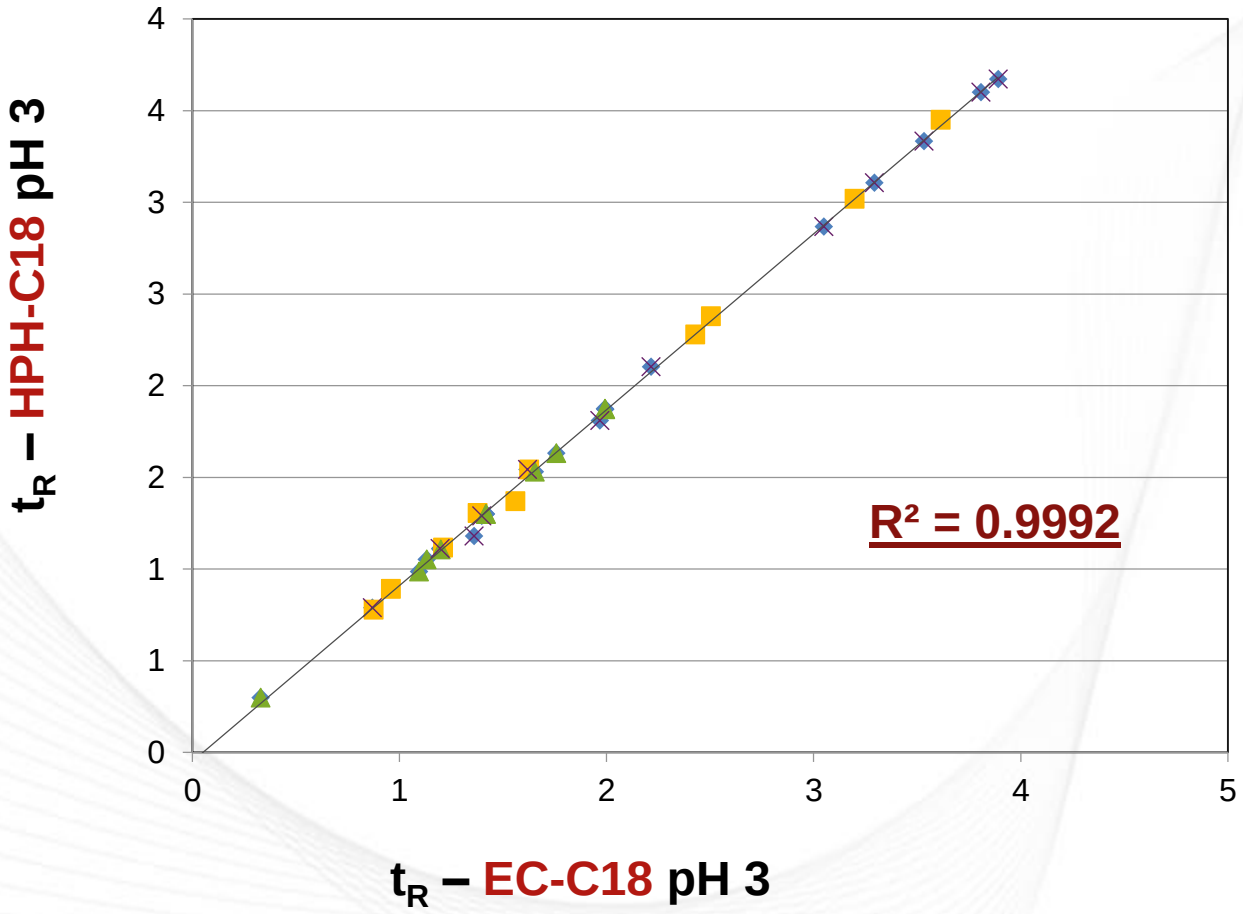


Paclitaxel

Application note [5991-6283EN]

Increase Resolution

Very similar selectivity - Poroshell 120 HPH-C18 vs EC-C18

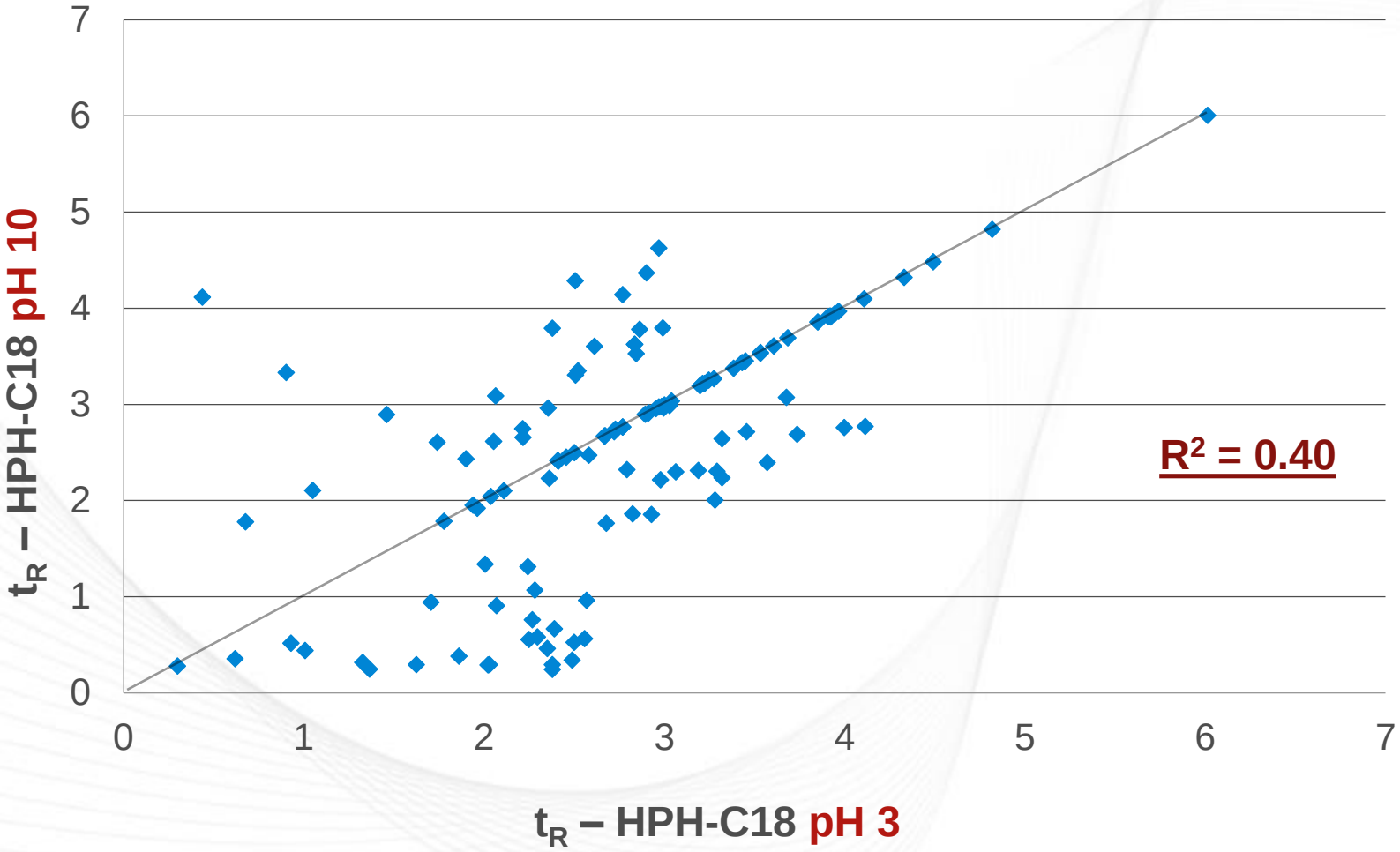


MP-A 10mM Ammonium formate/water adj. to pH3 using Formic Acid
MP-B ACN
Flow – 0.42 mL/min
Column Temp. Ambient
1 uL injection
Detection 254 nm
Gradient:

time, min	%B
0	5
4	95
5	95
6	5
7	stop run

Increase Resolution

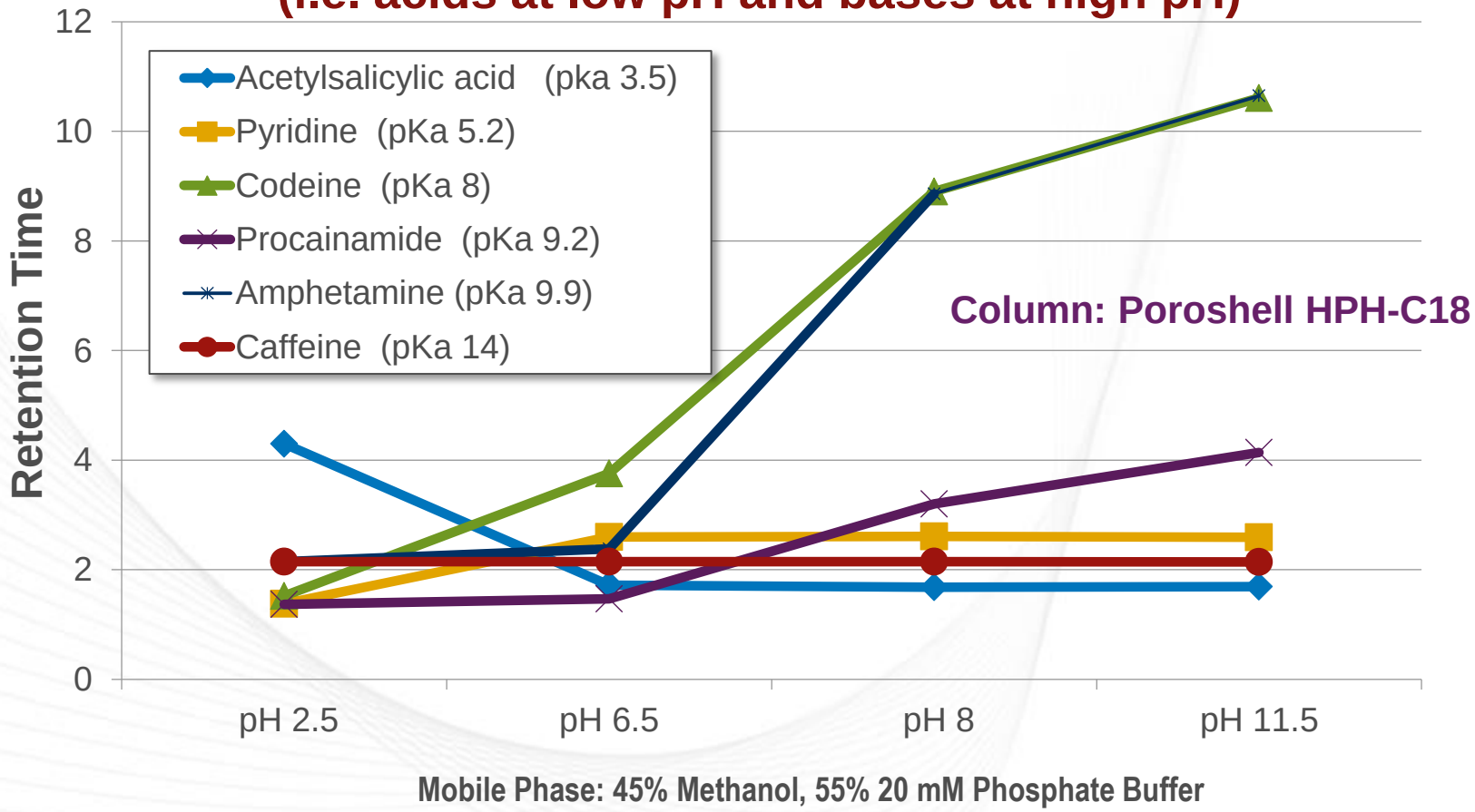
Very different selectivity – pH10 vs pH3



Increase Resolution

Change in retention with mobile phase pH

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

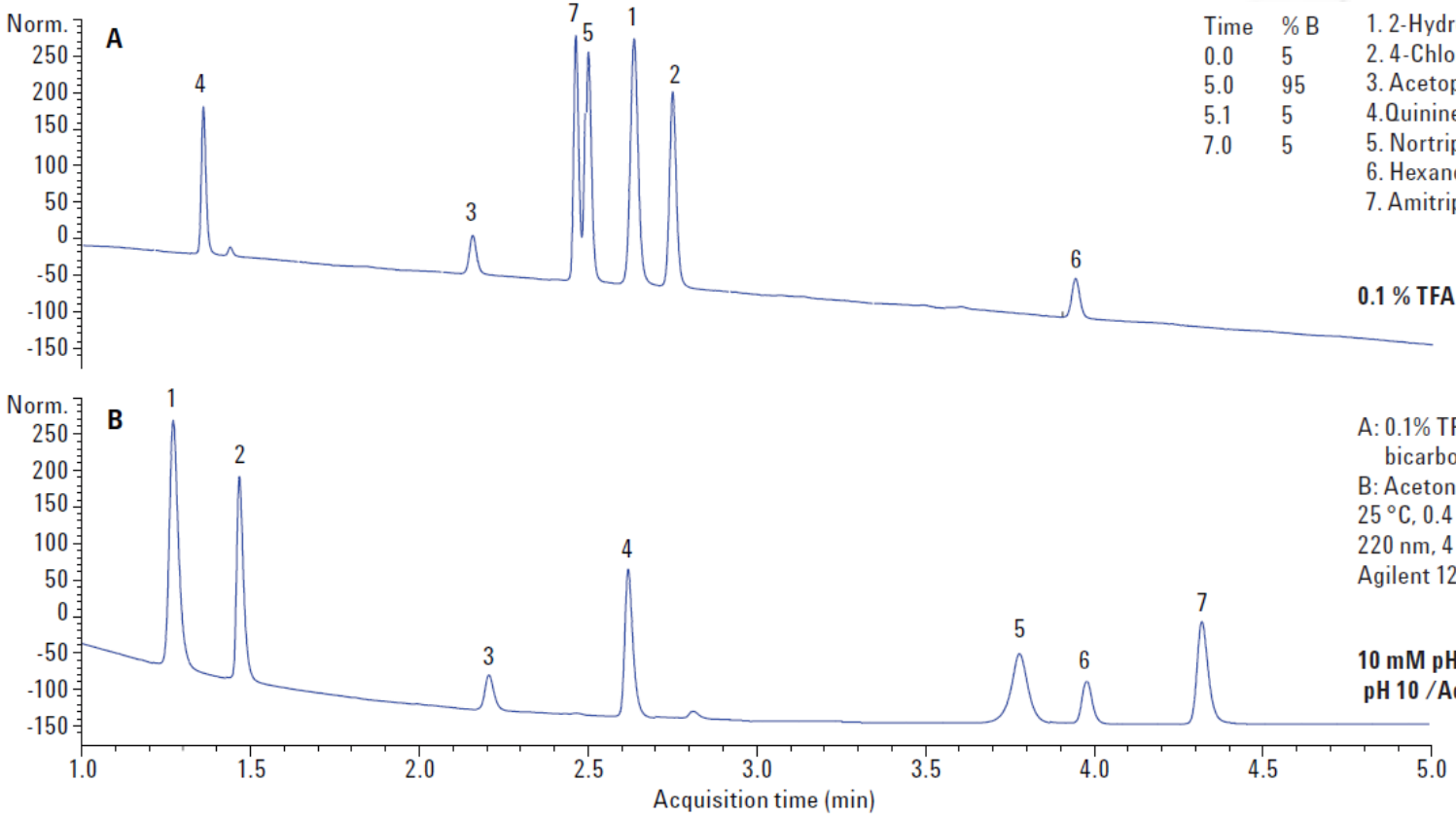


Mobile Phase: 45% Methanol, 55% 20 mM Phosphate Buffer

Increase Resolution

Very different selectivity at high and low pH

Mixture of acidic and basic analytes



Time	% B
0.0	5
5.0	95
5.1	5
7.0	5

1. 2-Hydroxy-5-methyl-benzaldehyde
2. 4-Chloro-cinnamic acid
3. Acetophenone
4. Quinine sulfate
5. Nortriptyline
6. Hexanophenone
7. Amitriptyline

0.1 % TFA /Acetonitrile

A: 0.1% TFA or 10 mM, pH 10 Ammonium bicarbonate
B: Acetonitrile
25 °C, 0.4 mL/min, 2.1 × 50 mm column
220 nm, 4 nm ref off
Agilent 1290 Infinity, PEEK rotor seals

10 mM pH Ammonium bicarbonate, pH 10 /Acetonitrile

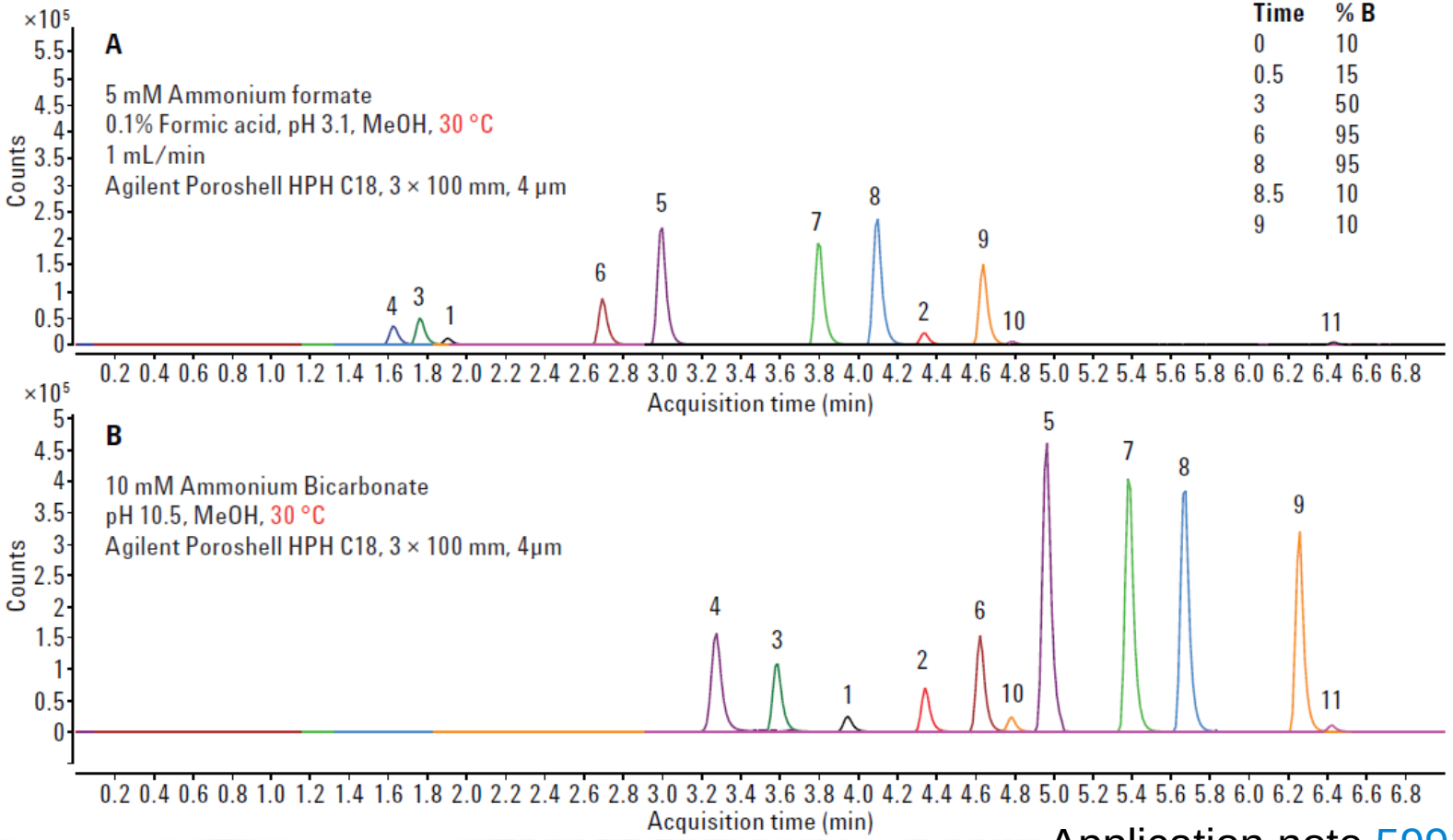
Application note 5991-6525EN

Advantages of High pH with LC-MS

Improved retention, resolution and response at high pH

Agilent CrossLab

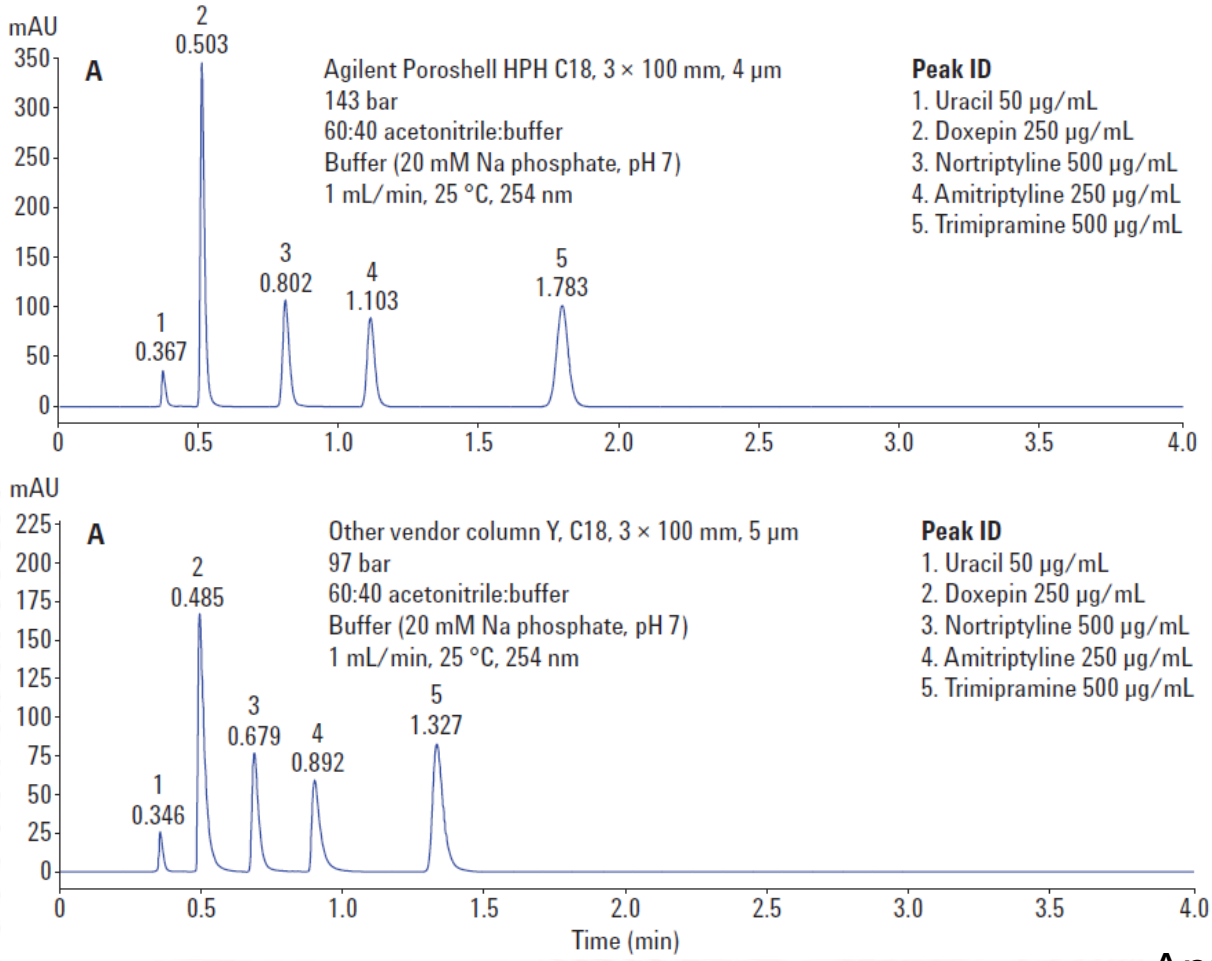
Drugs of abuse analysis by LC-MS



Application note [5991-6523EN]

Other High-pH SPP Column Performance

Poor retention and peak shape from non-Agilent column



Compound	HPH Column T_f	Column Y T_f
Doxepin	1.68	2.97
Nortriptyline	1.36	1.96
Amitriptyline	1.25	2.41
Trimipramine	1.07	1.60

Application note 5991-6512EN

Instrument Considerations at High pH

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- In general, Agilent Infinity modules operate over a pH range of 1 to 12.5
- pH < 2.3
  - Solvents must not contain acids that attach stainless steel
- pH > 9.5
  - Replace standard (Vespel) rotor seals in all valves with either Tefzel or PEEK seals*
  - Replace standard glass solvent inlet filters with stainless steel filters
  - Be aware that quartz flow cells in detectors will etch slowly. Do not leave high pH solvents stationary in the cells for extended periods

** Examples:*
5068-0171 Rotor Seal PEEK FL for 1290 Infinity Binary Pump
5068-0170 Rotor Seal PEEK FL for 1290 Infinity High Performance Autosampler
5068-0172 Rotor Seal PEEK FL for 1290 Infinity Quaternary Pump
5068-0223 Rotor Seal PEEK for 6-Column Selector Valve

Laboratory Needs

Translated to liquid chromatography

Agilent CrossLab

Better use of resources

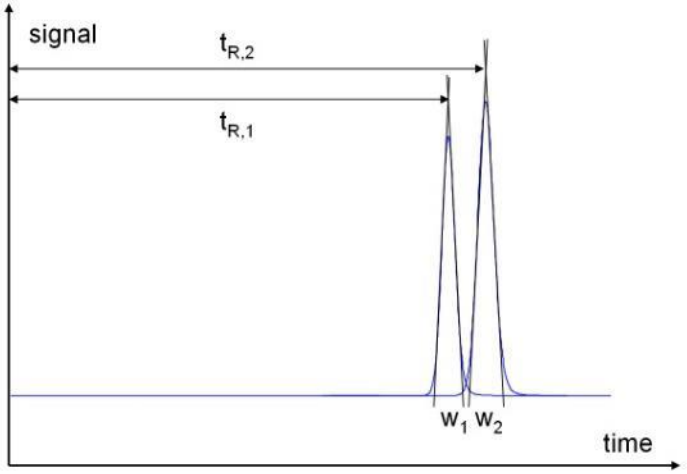
Increase productivity

Reduce costs

Use all the instruments in the lab

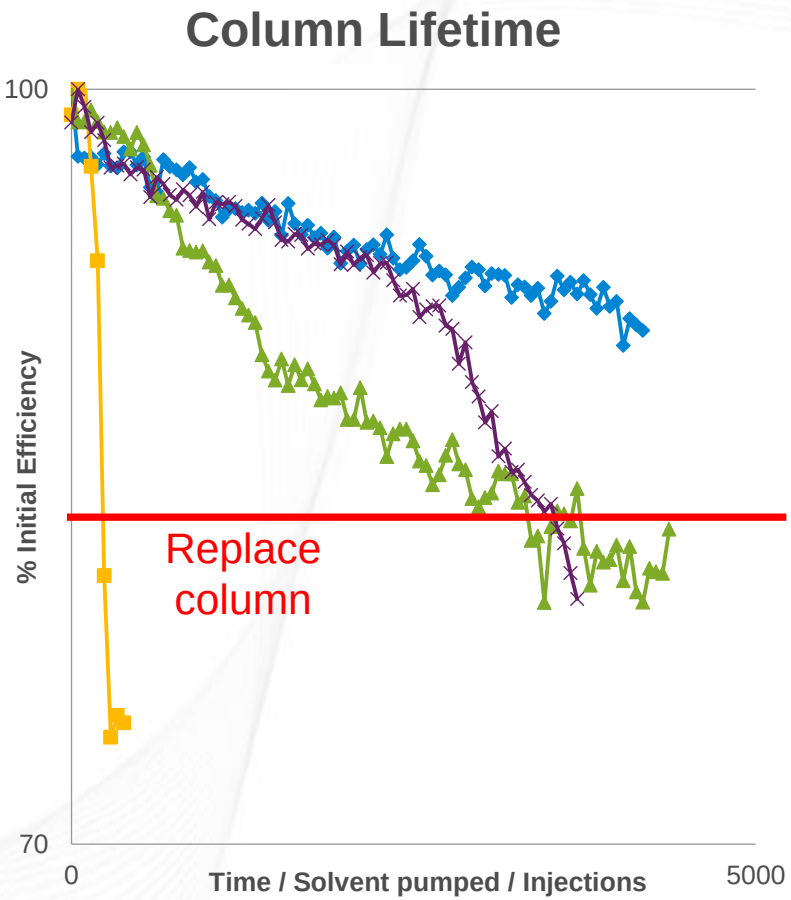
Run fast with high resolution

Get long column lifetimes



Costs of Short Column Lifetime

- More columns to purchase
- Disruption to workflow
- Re-work required



Get Long Column Lifetimes

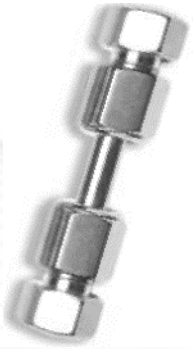
Agilent CrossLab

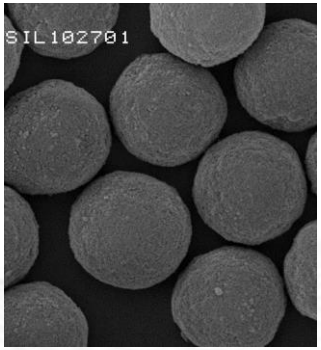
Causes of Column Failure

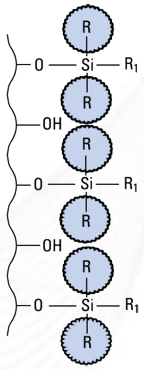
- Blocked frits
- Contamination/blockage
- Voiding of packed bed
- Dissolution of particles
- Loss of bonded phase

Can be Avoided Using

- Wide frit porosity
- Guard columns
- Robust packed bed
- High stability particles
- Robust bonding

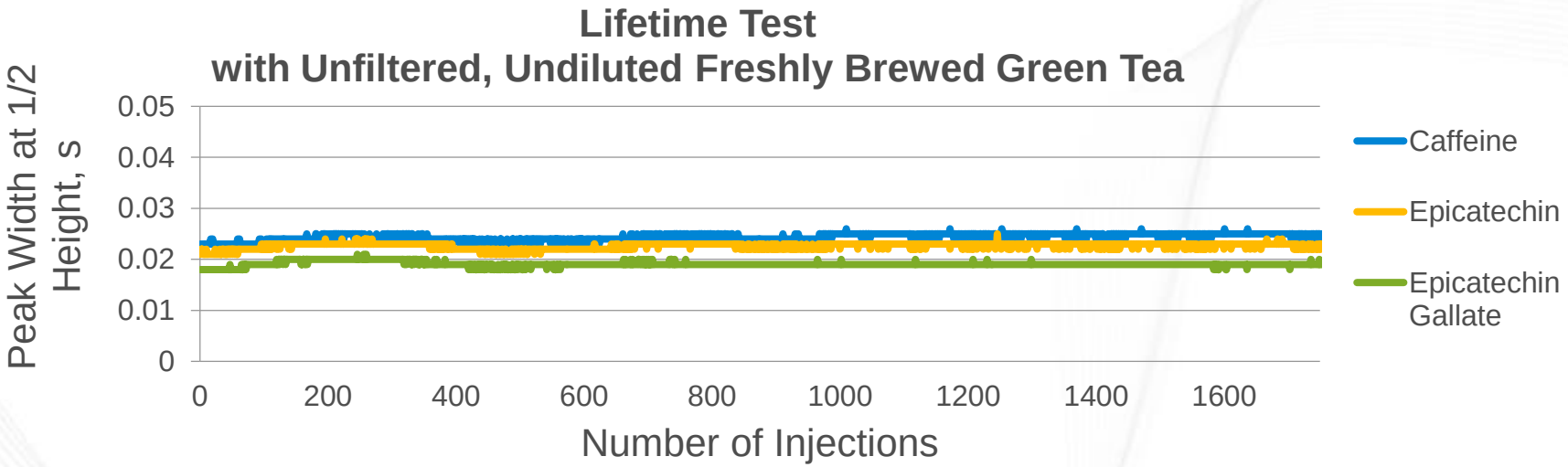


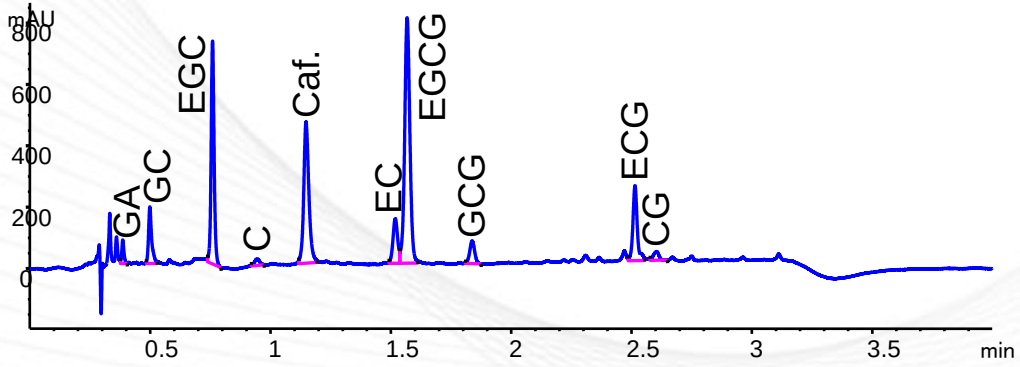




Get Long Column Lifetimes

>1800 Injections - no performance change





A: 0.2% HCOOH in H_2O, B: 0.2% HCOOH in CH_3CN

0.833 mL/min

Time	0.00	1.25	2.50
%B	10	15	27

40 °C P_{max} = 550 bar

Agilent Poroshell 120 SB-C18, 2.1 x 100 mm, 2.7 µm

Sig=210,4nm, Ref=Off

2-µL, 3-mm micro flow cell (PN G1315-60024)

Sample: 2 µL of freshly brewed green tea

(brewed from a commercial tea bag in 6 oz of initially boiling water for six minutes)

2 µm inlet frit prevents clogging and extends column lifetime

Get Long Column Lifetimes

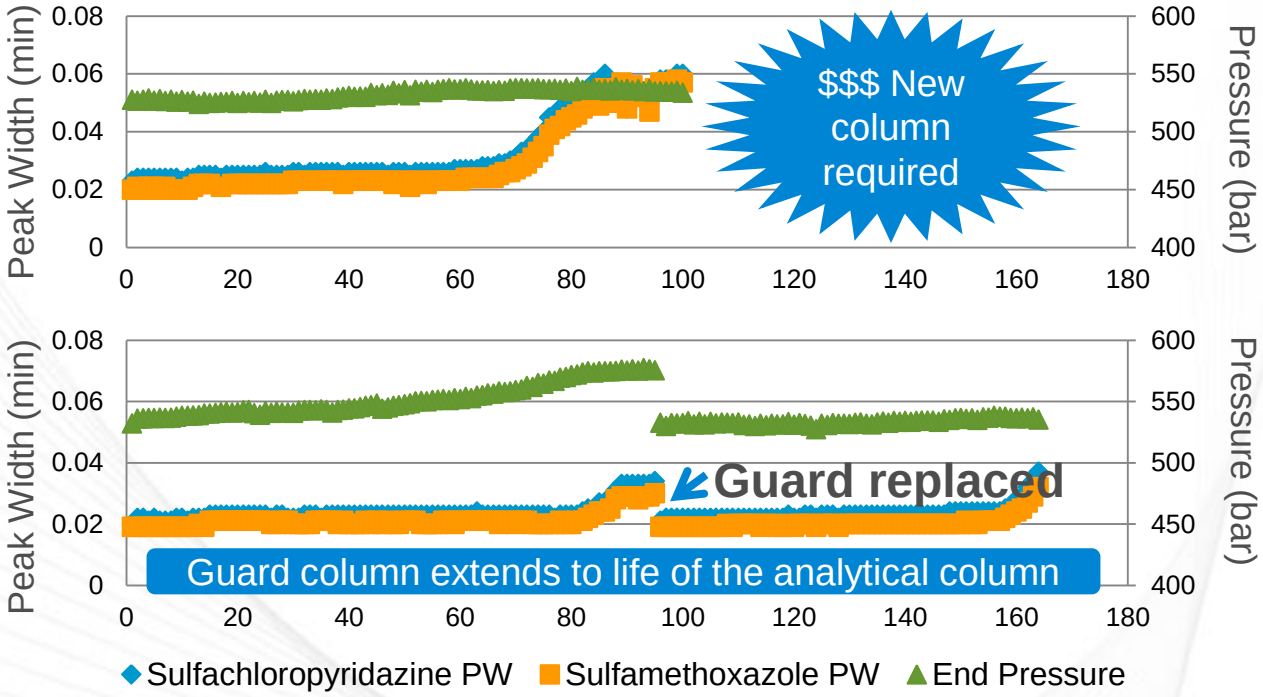
Advantages of guard columns

Agilent CrossLab

Accelerated Lifetime Test

Similac sample (milk substitute diluted 300:1) containing 2 sulfa drugs

Peak width change indicating column failure



No Guard

- Column failure @ inj. 70; new column required

With Guard

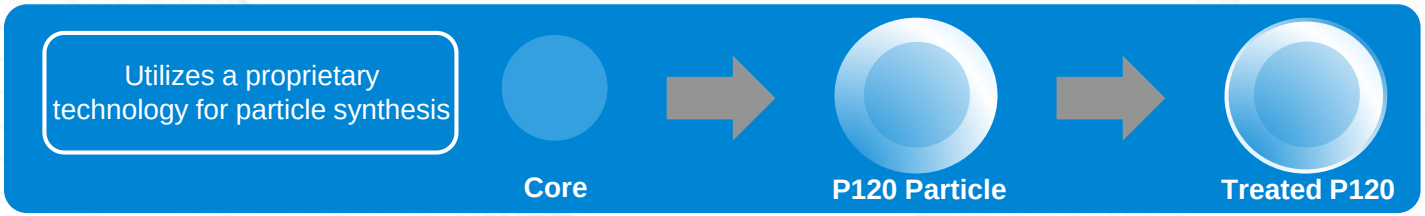
- Guard failure @ inj. 80; guard replaced
- Same column used throughout analysis

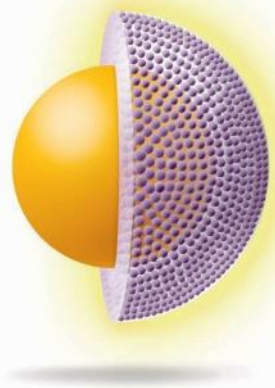
By installing a guard column when using dirtier samples, one can extend the life of their column, and utilize more inexpensive guard columns rather than analytical column replacements

Get Long Column Lifetimes

Approaches for longer lifetime at high pH

Approach	Comments
Totally porous silica-hybrid particles	Do not have the efficiency and moderate pressure of superficially porous particles
Bonding chemistry on superficially porous silica particles	Do not have the lifetime of silica-hybrid particles
Chemical modification of the outer layer of superficially porous silica particles	Combine the advantages of silica-hybrid and superficially porous particles

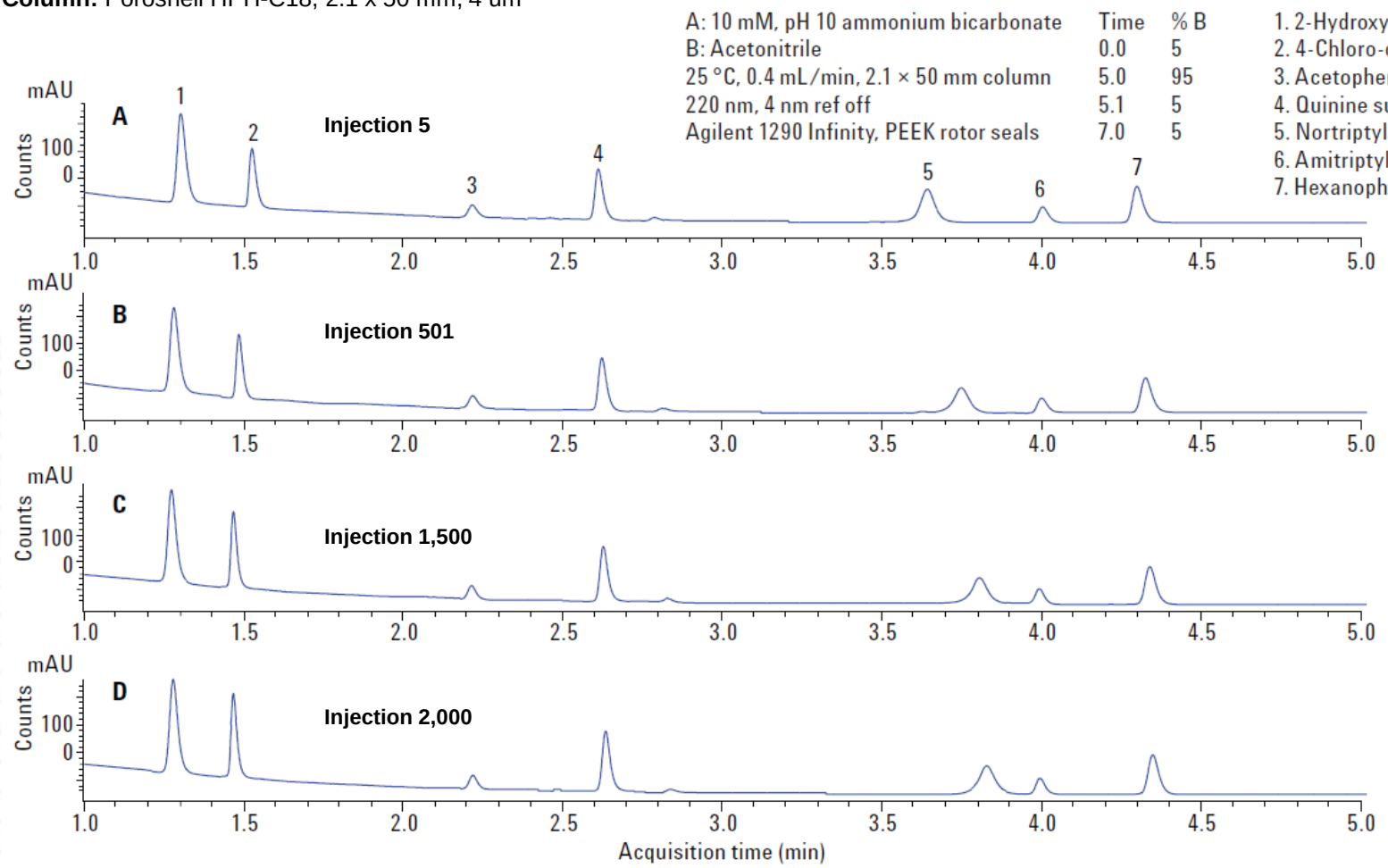




Get Long Column Lifetimes

Stability at high pH

Column: Poroshell HPH-C18, 2.1 x 50 mm, 4 um



Application note [5991-6525EN]

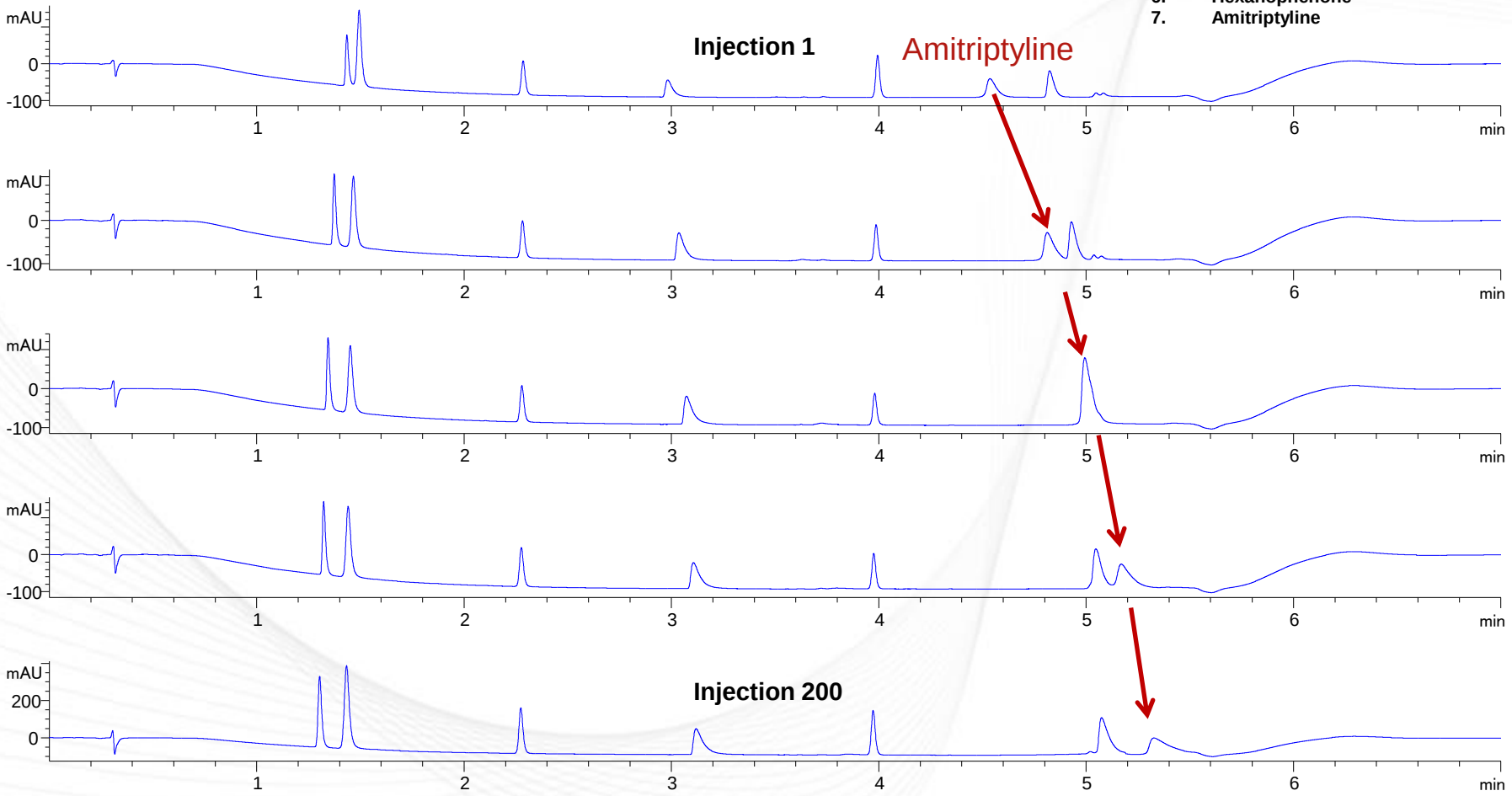
Other High-pH SPP Column Performance

Drifting retention times

Column: Column Y C18, 2.1 x 50 mm, 2.6 µm

Mobile phase A: 10 mM, pH 10 Ammonium Bicarbonate
Mobile phase B: Acetonitrile
Flow rate: 0.4 ml/min
Temperature: 25 C

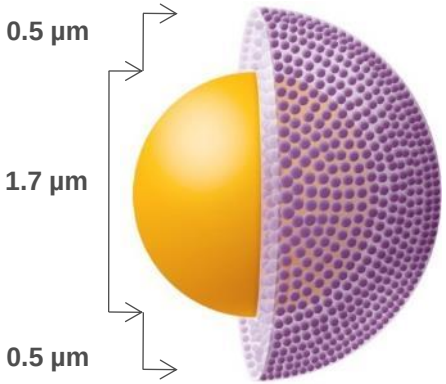
1. **2-Hydroxy-5 methyl-benzaldehyde**
2. **4-Chloro-cinnamic acid**
3. **Acetophenone**
4. **Quinine Sulfate**
5. **Nortriptyline**
6. **Hexanophenone**
7. **Amitriptyline**



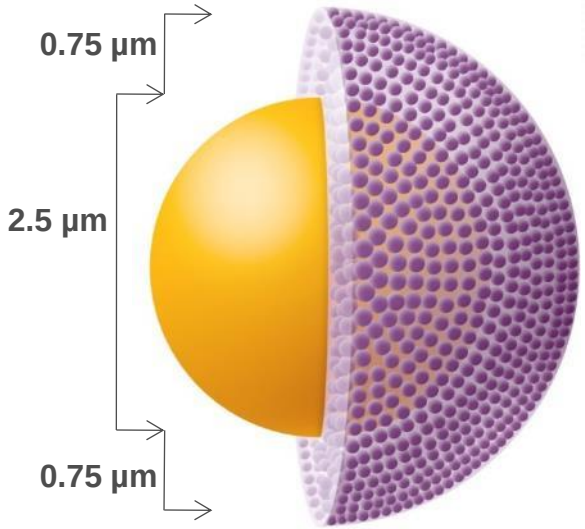
Poroshell 120

Make every LC and LC/MS in your lab work harder

Agilent CrossLab



Poroshell 120 2.7 µm



Poroshell 120 4 µm

Efficiency 90% of < 2 µm TPP
Pressure 50% of < 2 µm TPP
12 chemistries, including HPH
2 µm inlet frit

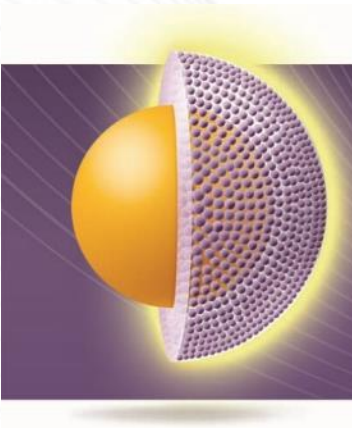
Efficiency 2x 5 µm TPP
Pressure often below 200 bar
7 chemistries, including HPH
2 µm inlet frit

www.agilent.com/chem/discoverporoshell

TPP = totally porous particle

Summary

- The pressure and peak widths of Poroshell 4 um particles allow you to use all the instruments in the lab
- High efficiency Poroshell particles and the right bonded phase selectivity enable fast runs with high resolution
- 2 µm inlet frits and innovative chemistries like HPH provide long column lifetimes – even at high pH
- You really can enjoy the benefits of the latest LC column technology



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