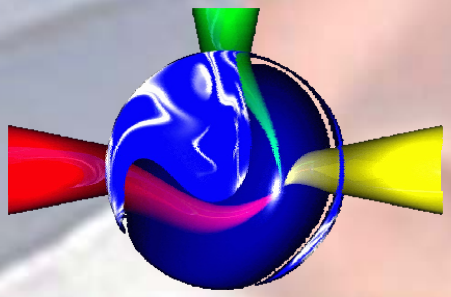
Zorbax Rapid Resolution High Throughput LC Columns (RRHT)



At the core of the
1200 Series HPLC,
it's all about
the chromatography.

What is Rapid Resolution HT (RRHT)?

A group of ZORBAX HPLC columns that provide *fast separations with more resolving power*

- ZORBAX Rapid Resolution HT columns are a *New Technology*:
  - The first sub 2μm (1.8μm), totally porous packing for ultra-fast separations.
- The small particles provide the high resolving power while very short column lengths (15-50mm) enable high-speed analyses.
- The most extensive separation portfolio for fast, ultra-fast and high resolution chromatography.
- The only full scaleable solution, sub two micron to prep portfolio, based on the same family of particle technology.

What Can You Do with RRHT 600 Bar Columns? And the Rapid Resolution System

Fast and very fast applications with short columns – ***50mm long and less***

High resolution separations with 100 and 150mm long 1.8um columns- ***high peak capacity with lower back pressures***

High temperature separations – up to 80-100°C with StableBond at low pH

A full solution approach to conventional chromatography and fast/ultra-fast, high resolution separations all on the same unit and within the same family of column choices.

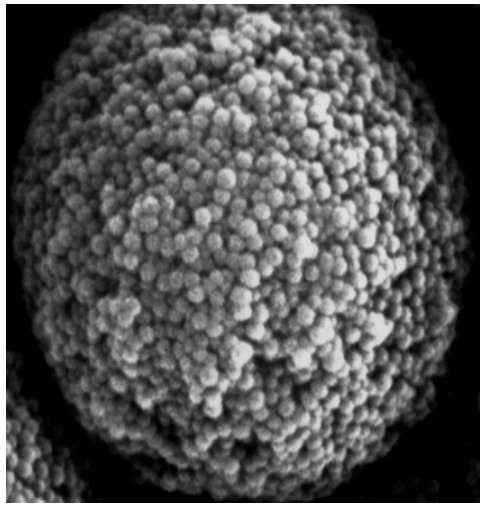
Fast separations with the lowest volume columns – 2.1 mm ID, 1.0mm ID

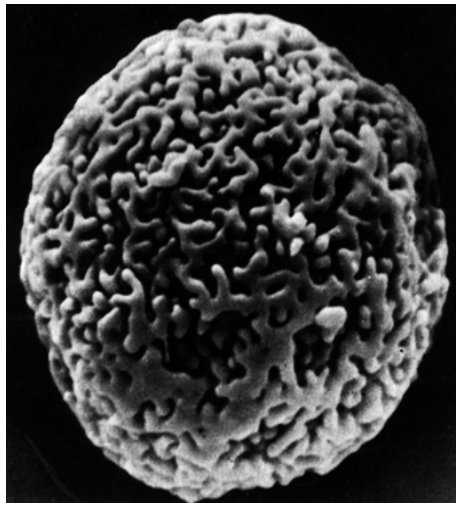
How Do We Achieve High Performance with 1.8µm (Rapid Resolution High Throughput) Columns

1. Pick a strong particle to withstand higher pressures for routine and high pressure operation
2. **Bond it with multiple familiar bonded phases – select the right one for the separation and all particle sizes 1.8, 3.5 and 5um give the same performance**
3. Pack it into columns specially designed for high performance with small particles at higher pressures
   a) Short columns for fast and ultra-fast LC, long columns for high resolution
   b) ID's from 1.0 – 4.6 mm for the widest range of applications
4. **Verify performance – use performance measures outlined (efficiency, resolution, scalability, lifetime)**
5. Use it routinely
   a) In new 1200 Series RRLC at pressures up to 600 bar and temperatures up to 100°C

Silica Comparisons

Rx-SIL (Sol type)



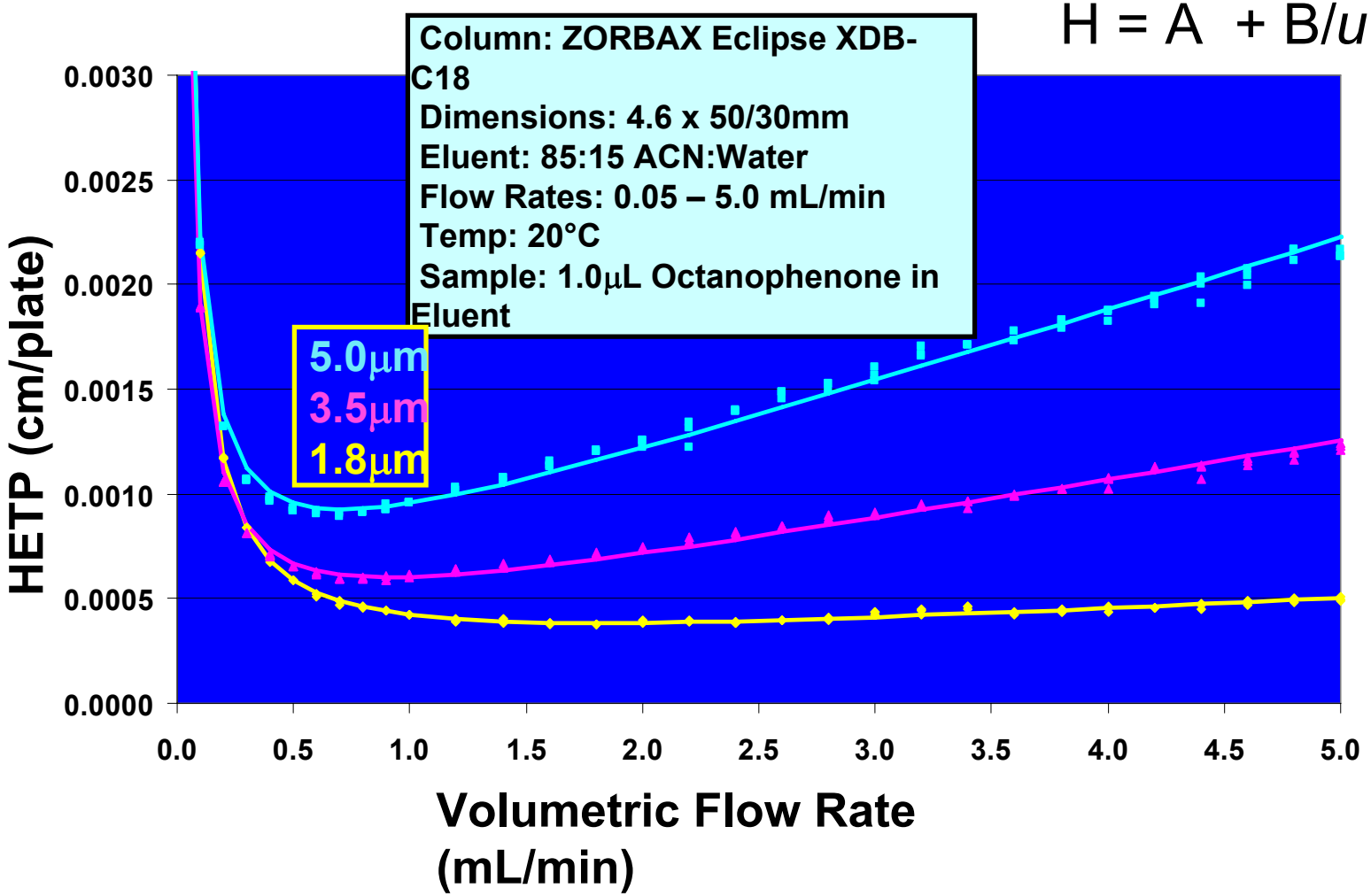


Xerogel (sil-type)

STRUCTURE:	UNIFORM SUB PARTICLES	"SPONGE-LIKE," POLYMERIC NETWORK
POROSITY (%):	50	70
PARTICLE SIZE/ SURFACE AREA (M^2/G)	80A/180	100A/300
STRENGTH:	HIGH	MODERATE
HIGH pH RESISTANCE:	GOOD	POOR
PURITY:	HIGH	LOW - HIGH
PORE SIZE, PARTICLE SIZE DISTRIBUTION	NARROW	BROAD

Van Deemter Curve 1: HETP vs. Volumetric Flow Rate

$$H = A + B/u + Cu$$



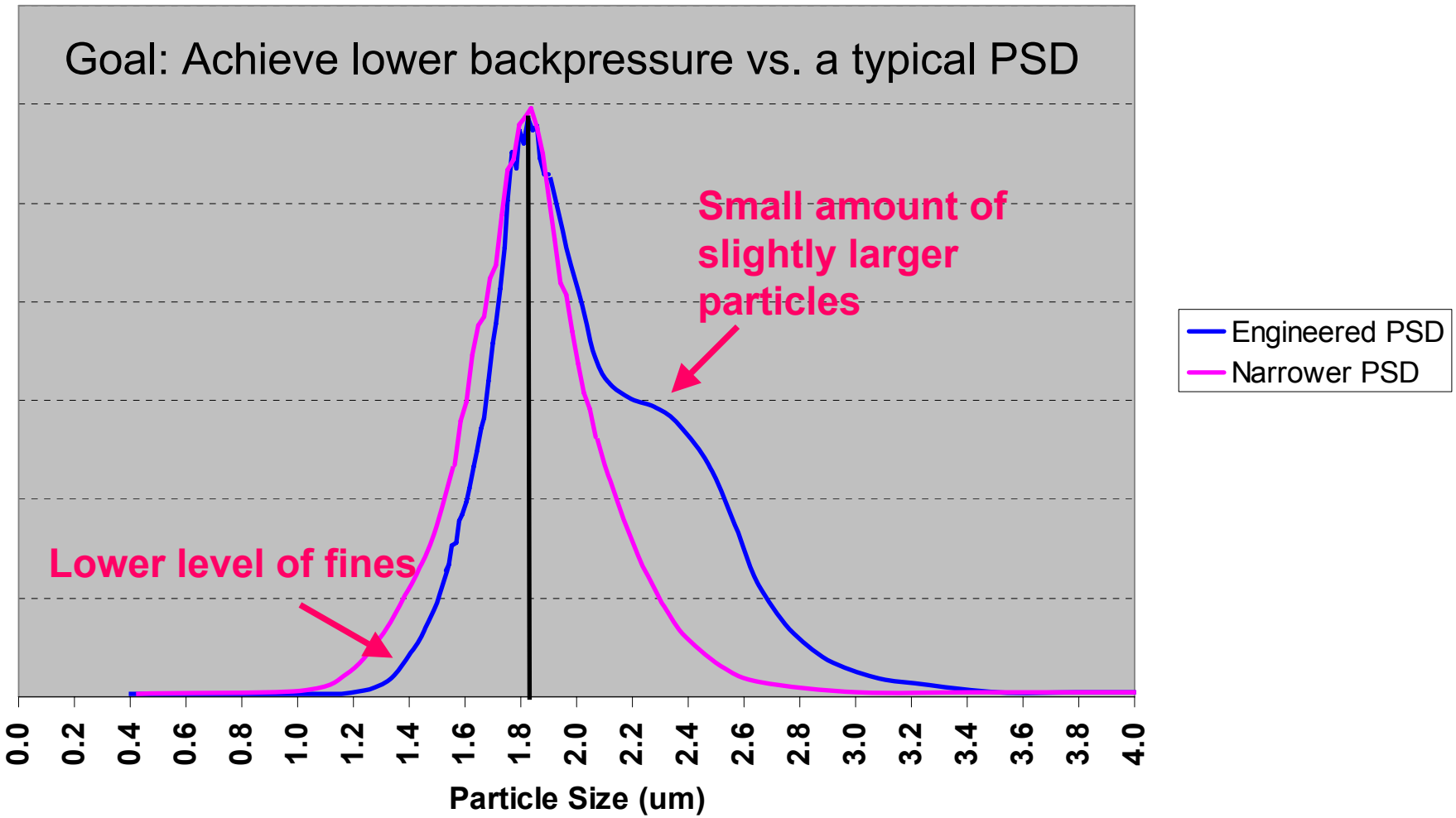
- Smaller particle sizes should have flatter curves, minimal shift out slightly

Why Lower Pressures on Rapid Resolution Systems with RRHT Columns

1. RRHT 1.8um columns generate on average 25% lower backpressure than other sub 2 um columns – optimized particle manufacturing technology permits high flow rates but with reduced backpressure on a comparable particle size

2. Rapid Resolution system generates lower pressure – shorter capillaries and advantageous flow paths

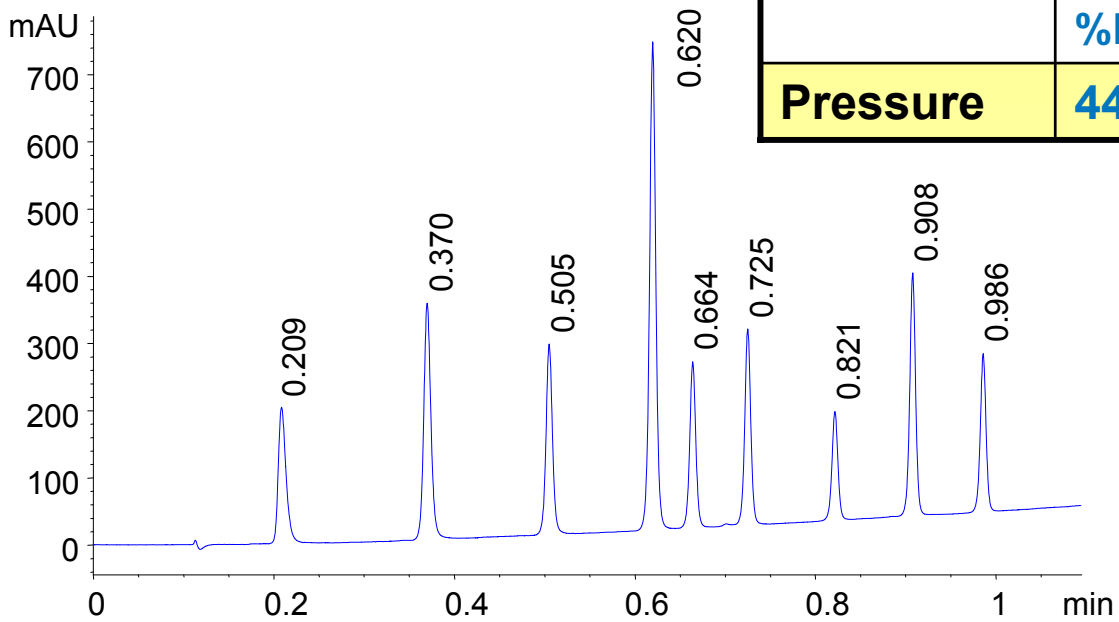
Comparison of Typical Particle Size Distribution and Engineered PSD of RRHT Columns



NEW Rapid Resolution System with RRHT –

Better Performance at Dramatically Lower Pressure THAN Acquity

	Rapid Resolution	Competitor
Column	**RRHT, SB-C18, 2.1 x 50mm, 1.8um**	**Competitor column 2.1x50mm,1.7um**
Anal. Time	0.949 min	0.914 min
Resolution	3.69	3.50
Precision	**0.025 – 0.094 %RSD**	**0.18 – 0.30 %RSD**
Pressure	**440 bar**	**700 bar (+60%)**

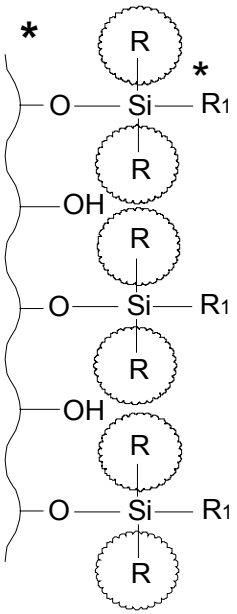


Sample: Phenones Test Mix
Flow Rate: 1ml
Gradient: 35-95% ACN in 0.9min
Temperature: 50°
Injection volume: 1µl
Injection Technique: ADVR, OI, MCO
WL: 245nm
Data Rate: 80Hz

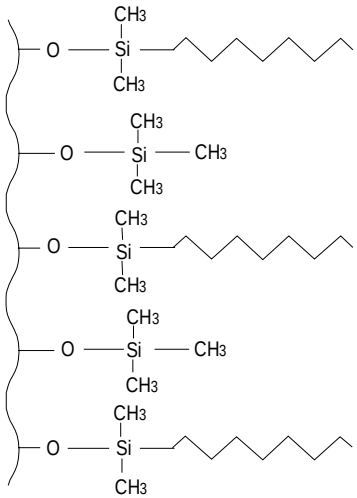
Match Method pH and Zorbax Column Choice

Choose the Best Bonded-Phase for Each pH Range

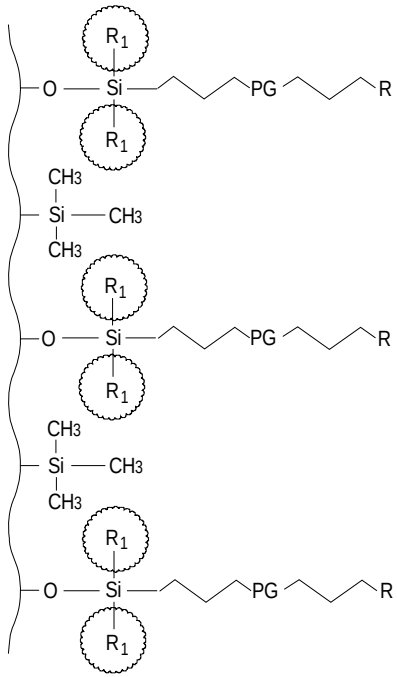
StableBond, pH 1-4
1. Uses bulky silanes
2. Non-endcapped



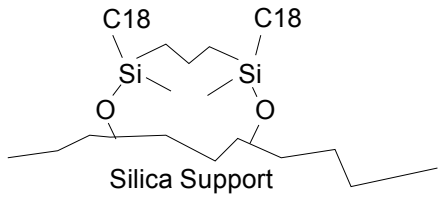
Eclipse XDB, pH 2-9
1. eXtra Densely Bonded dimethylalkylsilanes
2. proprietary double-endcapping



Bonus-RP, pH 2-8
1. polar alkyl phase
2. triple endcapped
3. uses bulky silanes



Extend-C18, pH 2-11.5
1. unique bidentate structure
2. double endcapped



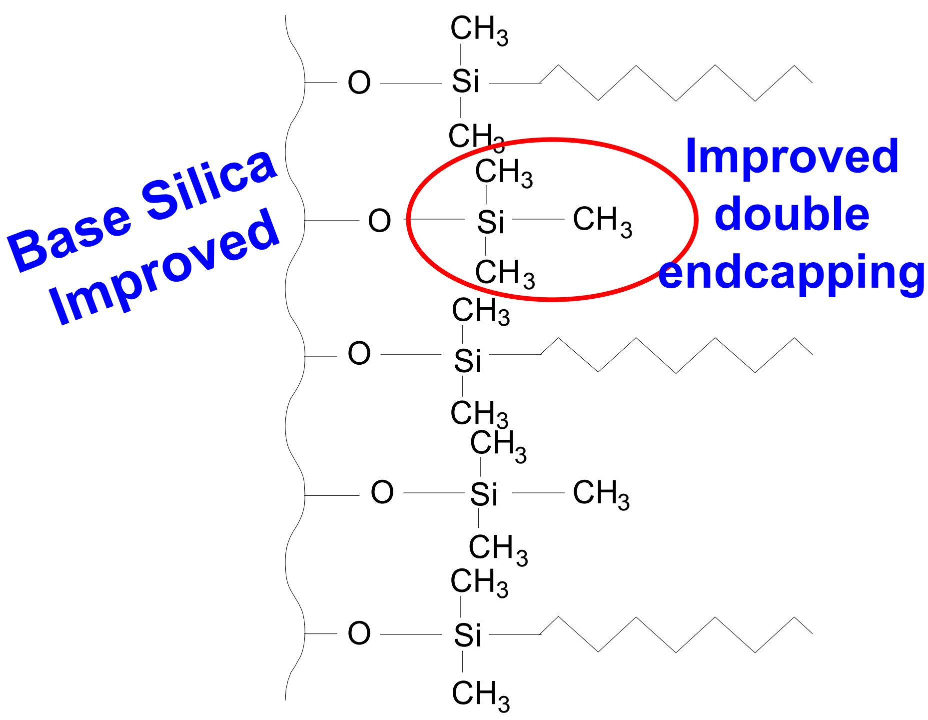
ZORBAX Eclipse Plus Columns

ZORBAX Eclipse Plus columns:

Use ZORBAX silica – improved with patented, proprietary treatments for better peak shape

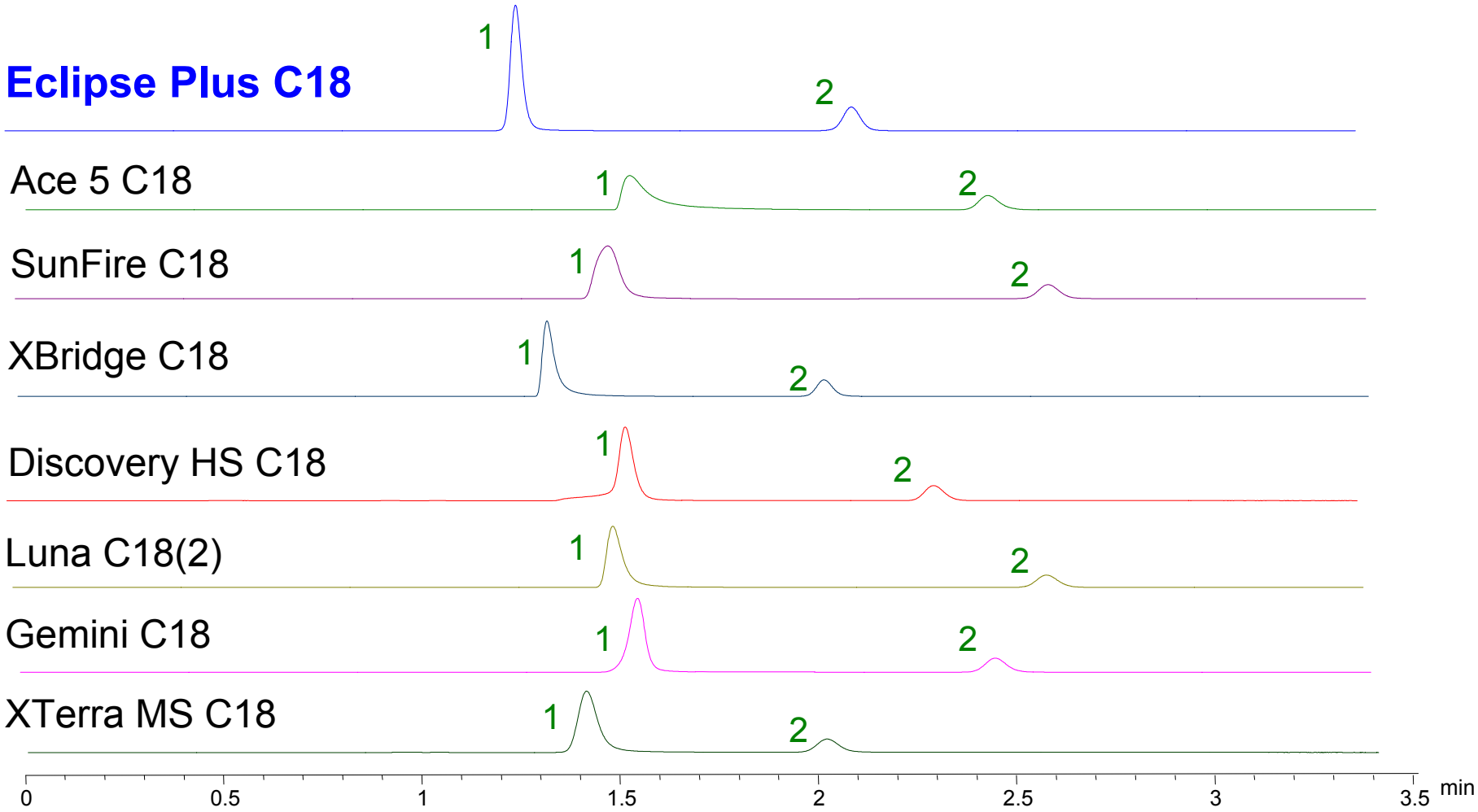
Use improved bonding reagents and processes for better peak shape

Have tighter specifications for superior lot-to-lot reproducibility and more reliable performance



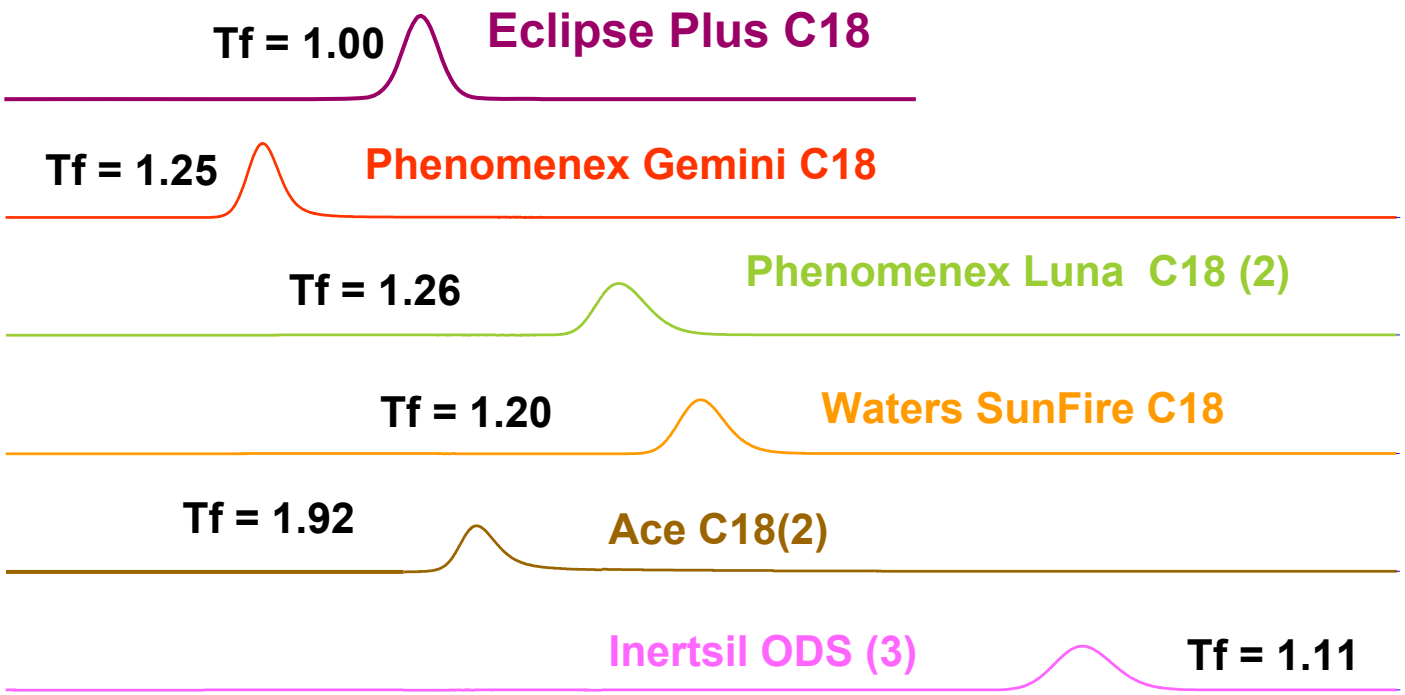
Eclipse Plus C18 – Best Peak Shape for Bases without Tailing – Standard Test Mix 1

1. Pyridine (Base) 2. Phenol (Acid) Mobile Phase: 60/40 Water/ACN



ZORBAX Eclipse Plus is the Best with Amitriptyline in Methanol Mobile Phases

Amitriptyline ~ 0.1µg 80% Methanol 8mM total Potassium Phosphate Buffer pH 7.0,
Detection: UV 215 nm, Flow Rate: 1.0 mL/min Columns: 4.6x100mm 5µ columns



Peak Shape is normally better in Methanol mobile phases.
Eclipse Plus is superior under these conditions.

ZORBAX RRHT Bonded Phase Choices

ZORBAX Eclipse Plus – (C18 and C8) first choice for method development, industry leading peak shape performance, good for most applications, use up to 60°C

ZORBAX Eclipse XDB – (C18 and C8) good choice for method development, but older than Eclipse Plus, general purpose bonded phase, for most applications, use up to 60°C

ZORBAX StableBond – good for low pH and high temperature applications, for those who want to raise temperature for faster analysis

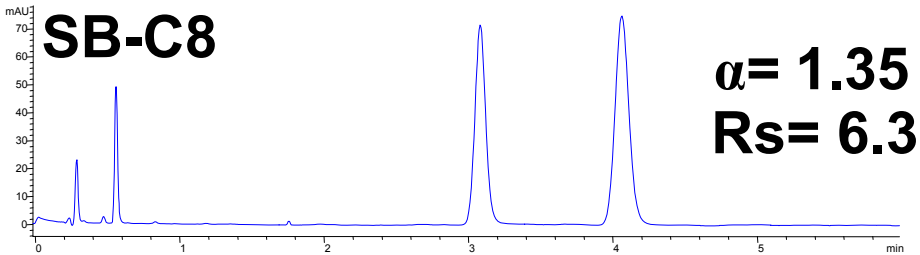
- **SB-C18** – use up to 90°C and down to pH <1 (high temp only at low pH)
- **SB-C8** – use up to 80°C and down to pH 1
- **SB-Phenyl** – use up to 80°C at pH 2
- **SB-CN** – use up to 80°C at pH 2
- **SB-AQ** – use in up to 100% aqueous mobile phases for good retention – up to 60°C at pH 2

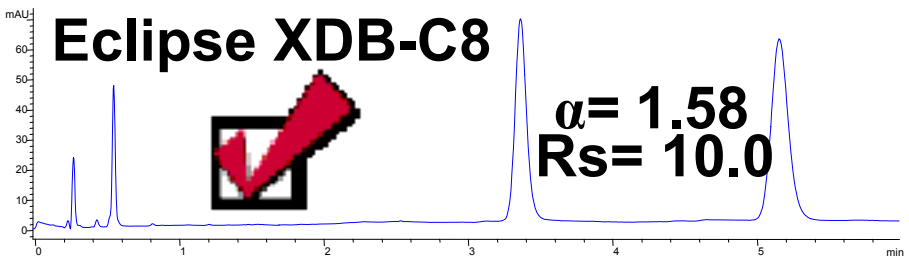
ZORBAX Extend-C18 – good at high pH – up to pH 11.5 at ambient temperature

ZORBAX Rx-Sil – for normal phase applications and "HILIC" type applications where an aqueous containing mobile phase is used on bare silica.

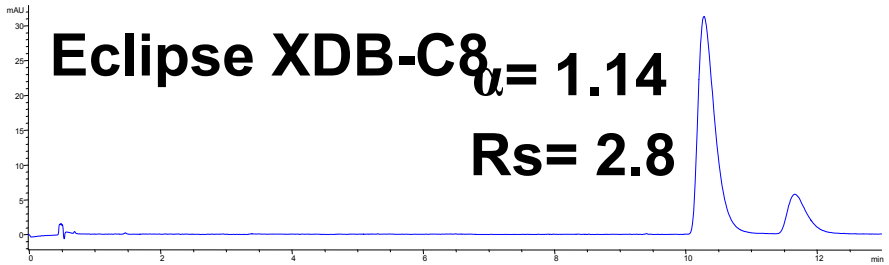
Multiple Bonded Phase Choices are Important for Best Separations

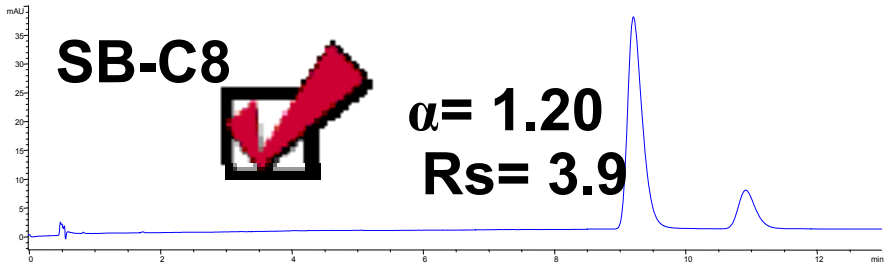
Improve selectivity by changing the stationary phase.





Mobile phase: (63:37) water :acetonitrile + 1.8 mL H_3PO_4
Flow = 2.0 mL/min. isocratic
Temp. : ambient
Sample: children's ibuprofen oral suspension,
with benzophenone as internal std.
prepared as described in USP





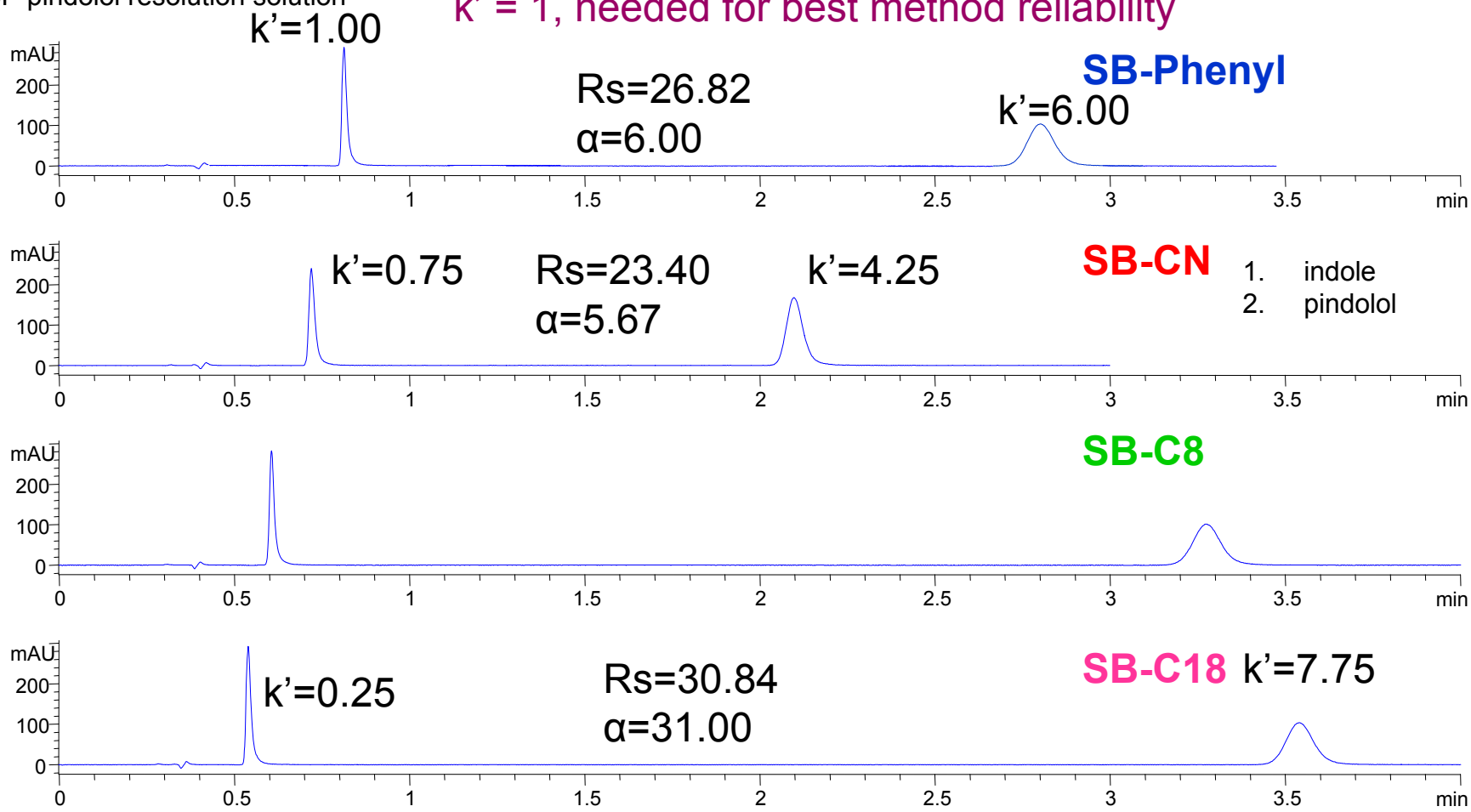
Mobile phase: (70:30) 200mM NaH_3PO_4 pH 2.5 :MeOH
Flow =1 mL/min. isocratic
Temp. : 50 °C
LC: Agilent 1100 w. thermally controlled column compartment
Sample: doxepin HCl in mp 0.1 mg/mL (82% E-isomer)

Columns: ZORBAX RRHT, 4.6 x 50 mm 1.8 µm

Optimum selectivity- Resolution, k' and analysis time should be considered (SB Family)

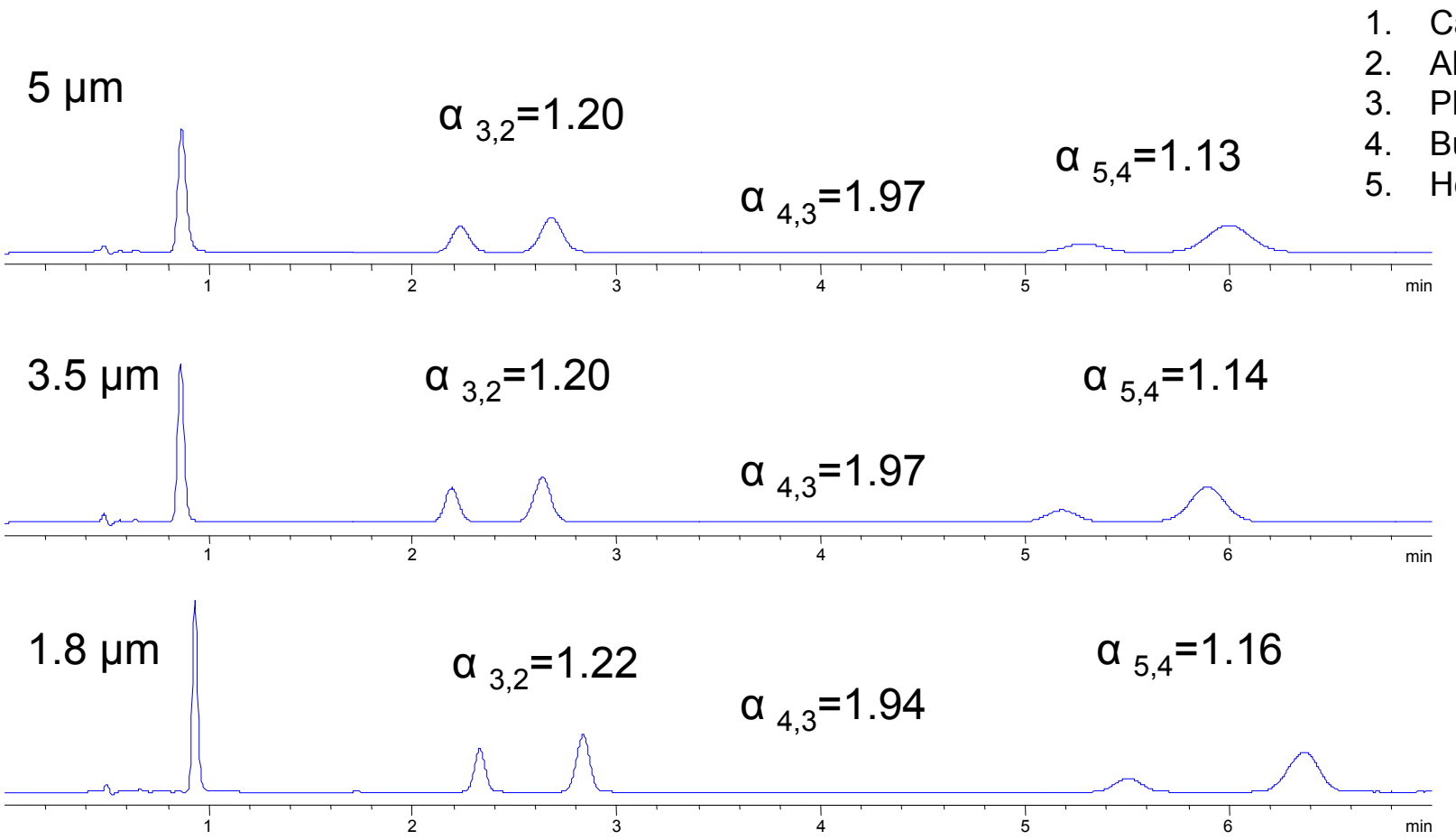
Columns: StableBond 4.6 x 50 mm 1.8 um, as described below. Mobile Phase: A: pH 5 Na Acetate (USP method), B: MeCN, (70:30) Temperature: 25°C Flow: 1.5 mL/min. Detection: 219, 4 nm, 0.2 s response time, semi-micro flow cell, Sample: USP pindolol resolution solution

k' = 1, needed for best method reliability



No difference in column selectivity as a function of particle size

Eclipse XDB-C18, USP Analysis of Phenobarbital



Column: 4.6 x 50mm Eclipse XDB-C18
A= pH 4.5 Na Acetate, B=MeOH (60:40)
F=1 Inj. Vol: 2 ul
Flowcell: 3uL, 2 mm flow path

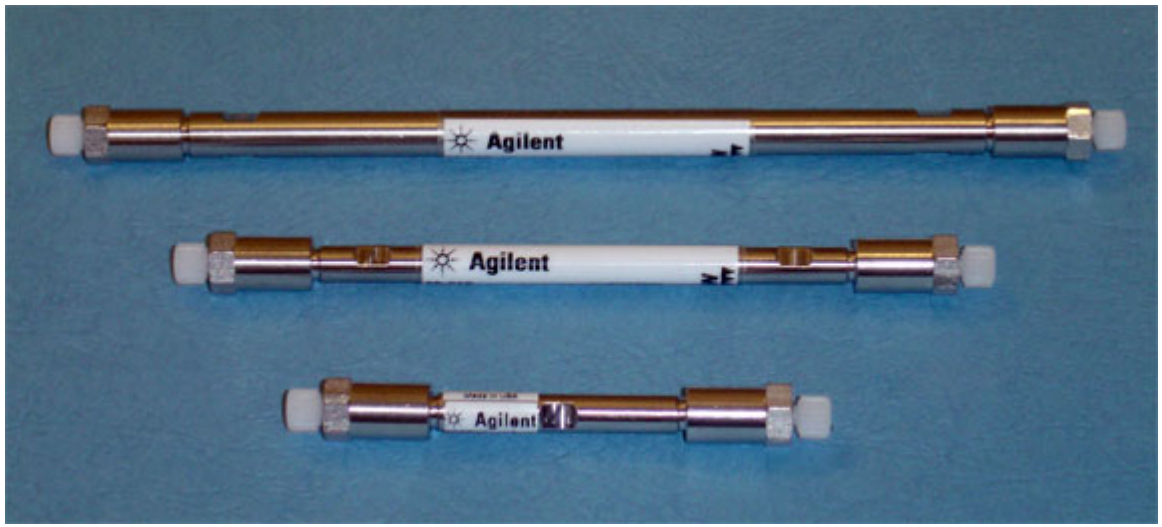
3. New RRHT Columns with Fixed, High-Pressure End-fittings for 600 Bar Use

High-pressure RRHT 1.8um columns

150mm

100mm

50mm



Fittings look different on 600 bar columns than those on 400 bar columns.

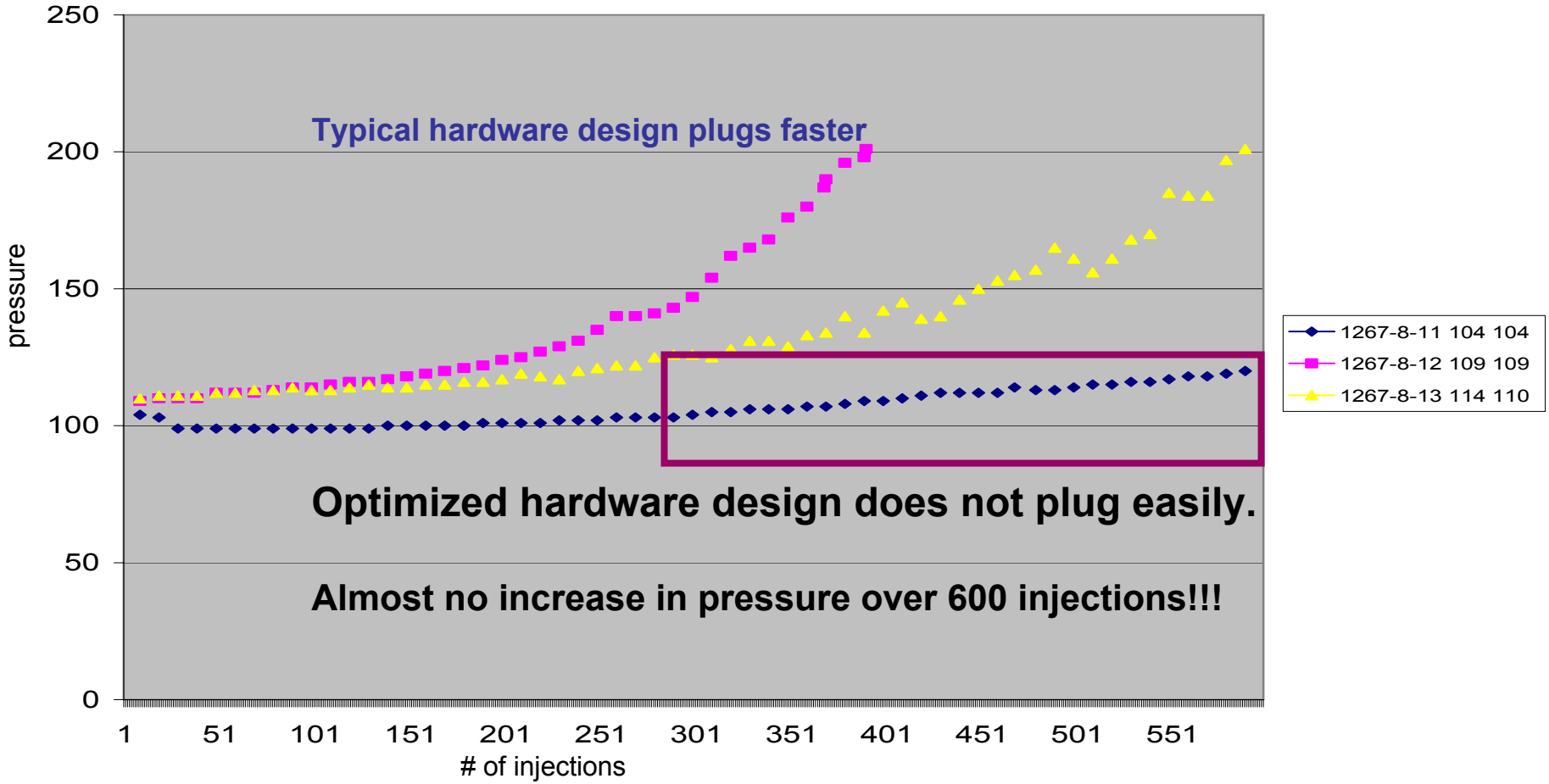
Metal-to-metal internal sealing for high pressure use

400 bar RRHT 1.8um column

Standard fittings, support typical pressures

Optimized Hardware Design Makes it Possible to Inject “Dirty” Samples

Sample: plasma sample, proteins precipitated, filtered 0.2um filter, only
Minimum sample prep means faster analysis

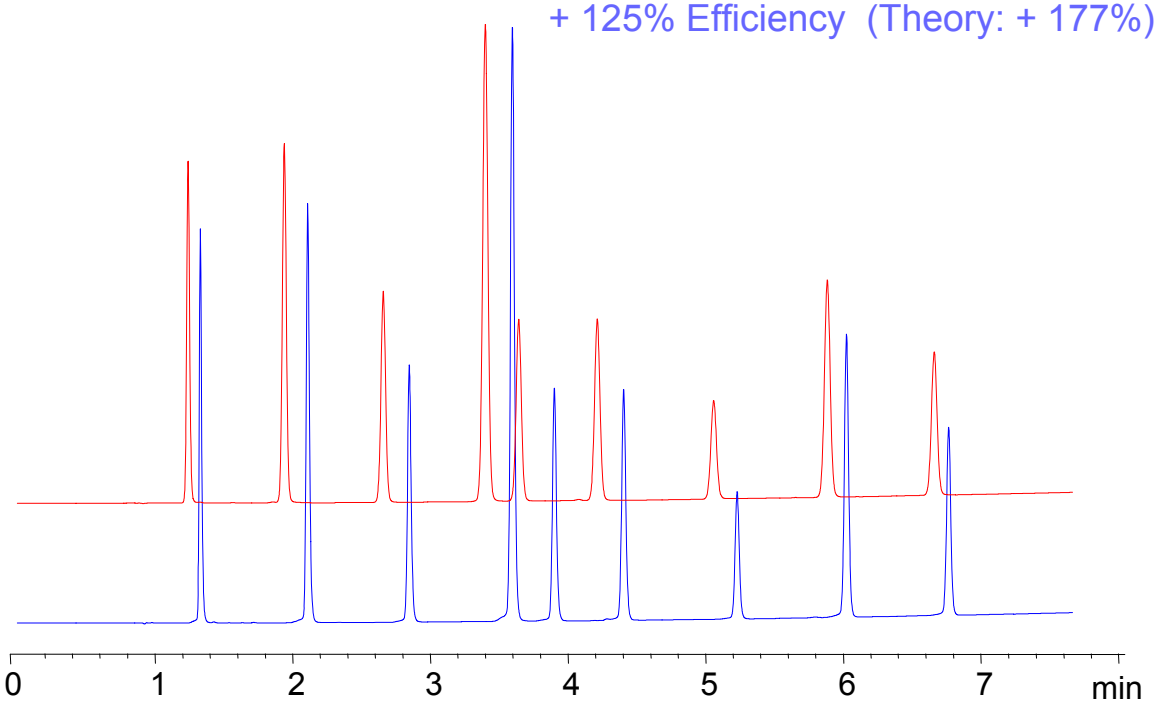


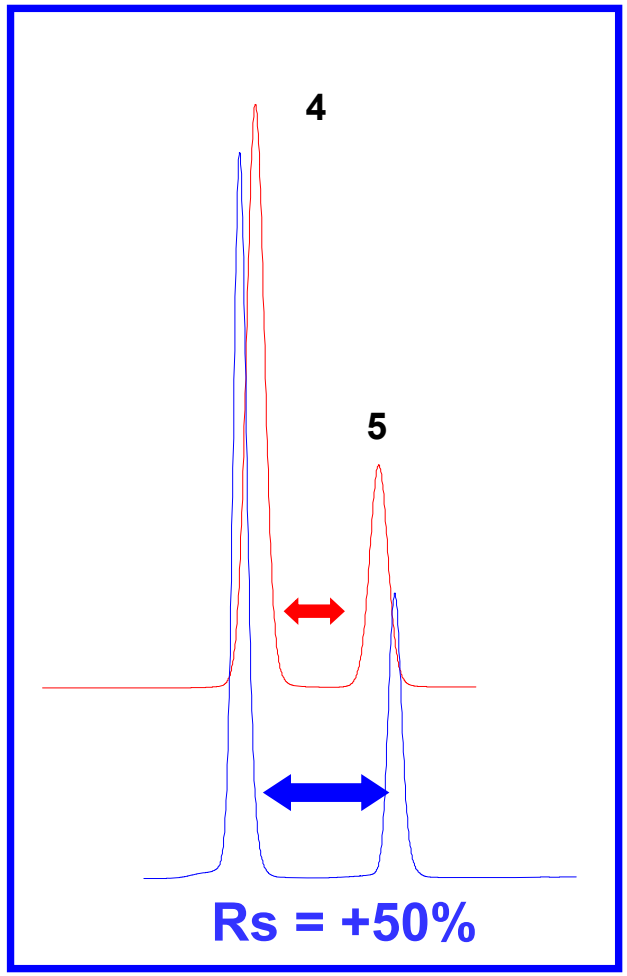
Resolution Improvements on Long 1.8 µm Columns

4.6 x150mm SB-C18 RRHT - 5µm vs. 1.8µm

5µm: **Rs(4,5) = 3.63**
Peak Width, 5σ (9) = 0.103

1.8µm: **Rs(4,5) = 5.45**
Peak Width 5s(9) = 0.075
+ 50% Resolution (Theory: + 67%)
+ 125% Efficiency (Theory: + 177%)

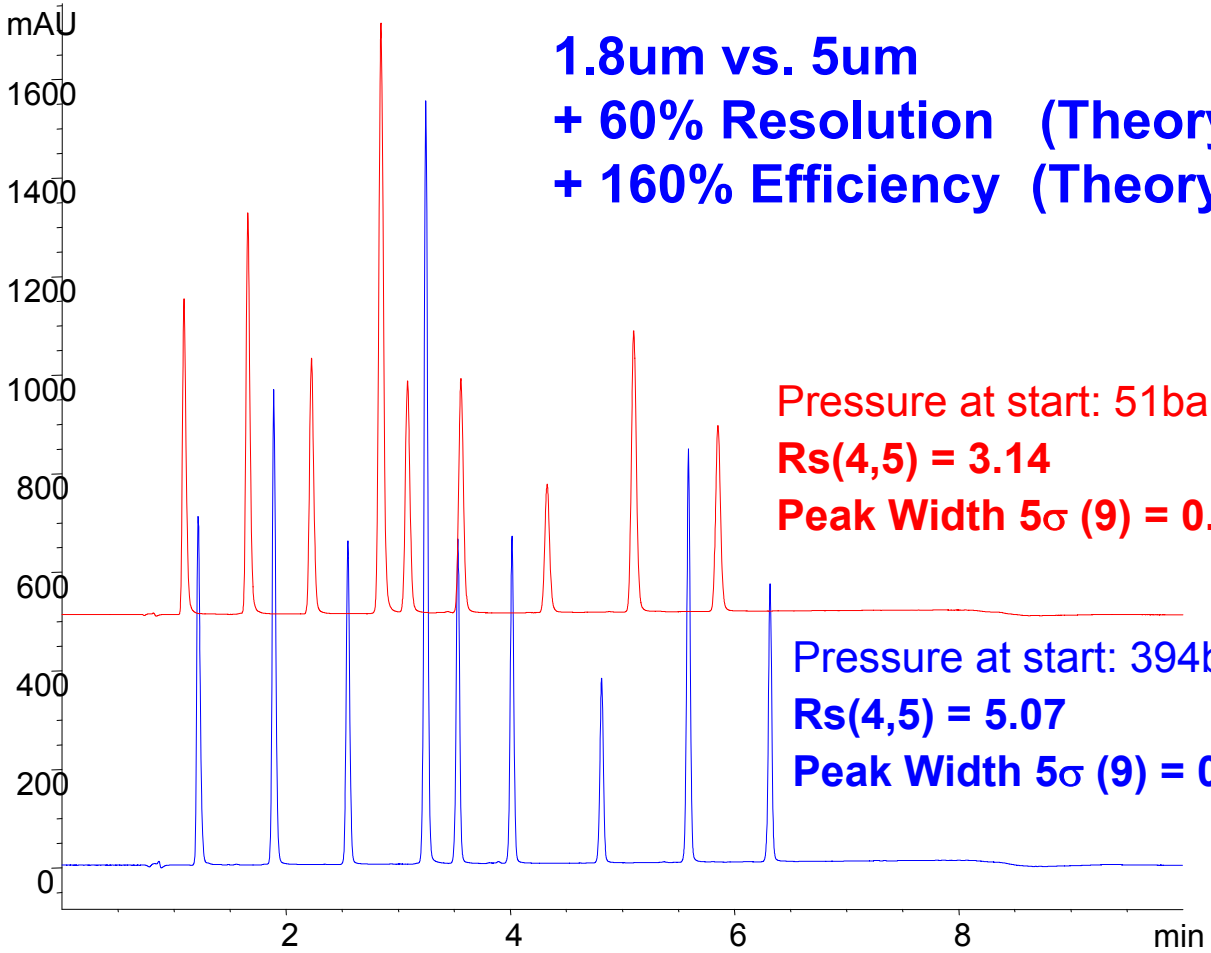




Resolution improvements on long columns are just as good as on short columns

Resolution Improvements on Long 1.8 µm Columns

2.1 x150mm SB-C18 RRHT - 5µm vs. 1.8µm



1.8um vs. 5um
+ 60% Resolution (Theory: 67%)
+ 160% Efficiency (Theory: 177%)

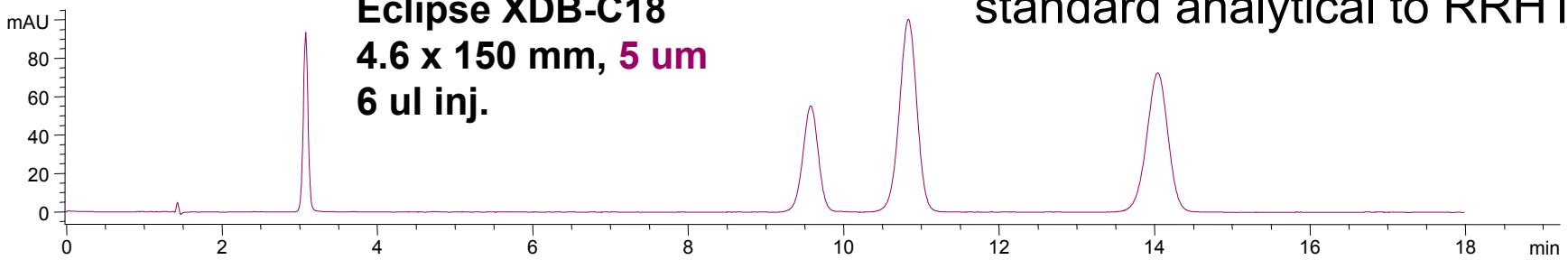
Pressure at start: 51bar, 5µm
Rs(4,5) = 3.14
Peak Width 5σ (9) = 0.107

Pressure at start: 394bar, 1.8µm
Rs(4,5) = 5.07
Peak Width 5σ (9) = 0.074

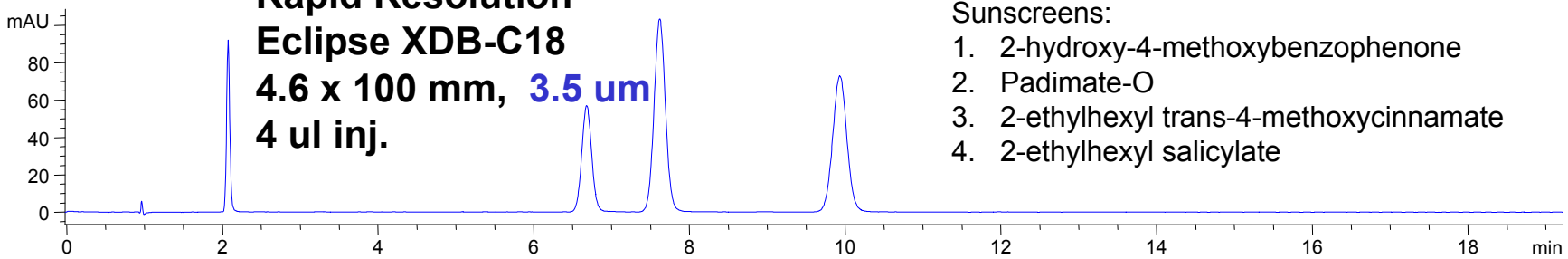
➢**Resolution gains are independent of column ID on a low volume LC**

Choose a Simple-to-Scale Bonded-Phase – Compare 5, 3.5 and 1.8 µm Columns

Easy conversion from standard analytical to RRHT

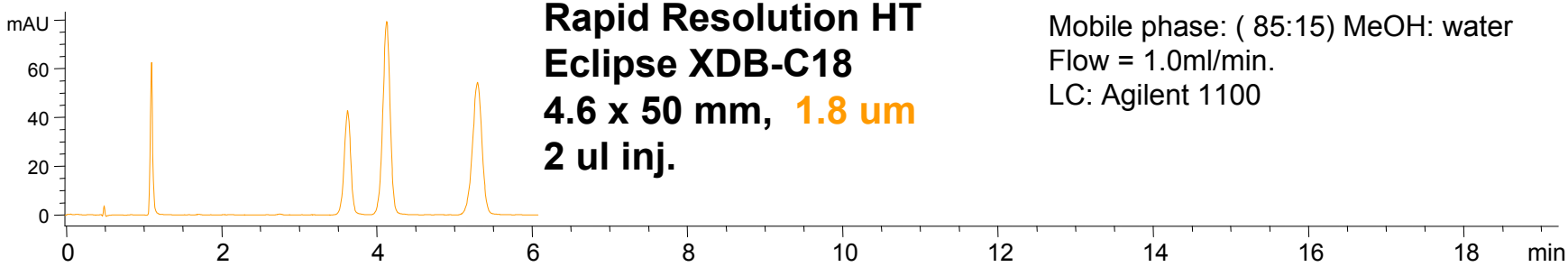


Eclipse XDB-C18
4.6 x 150 mm, 5 um
6 ul inj.



Rapid Resolution
Eclipse XDB-C18
4.6 x 100 mm, 3.5 um
4 ul inj.

Sunscreens:
1. 2-hydroxy-4-methoxybenzophenone
2. Padimate-O
3. 2-ethylhexyl trans-4-methoxycinnamate
4. 2-ethylhexyl salicylate



Rapid Resolution HT
Eclipse XDB-C18
4.6 x 50 mm, 1.8 um
2 ul inj.

Mobile phase: (85:15) MeOH: water
Flow = 1.0ml/min.
LC: Agilent 1100

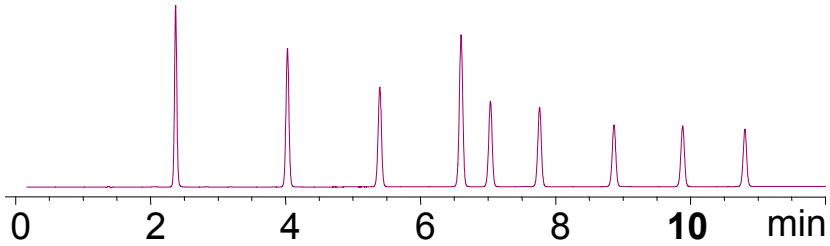
Rapid Resolution HT Columns –

Up to 20X Faster than Traditional 5um Columns on std LC systems

Up to 20x faster than conventional HPLC

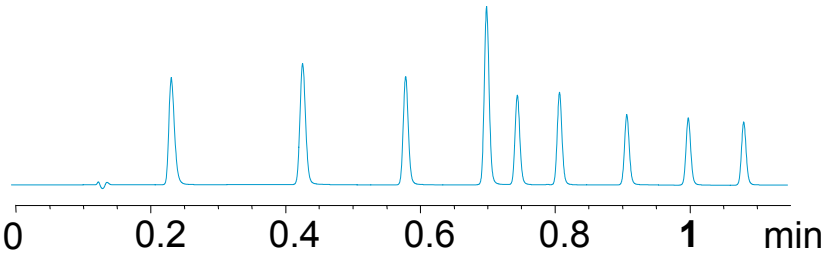
- With same or better performance (**resolution**, precision, sensitivity, carry over)
- Compliant with strictest regulatory performance requirements

SB-C18
4.6 x 150mm, 5µm
1.20ml/min, 40°C
Analysis Time = 11min



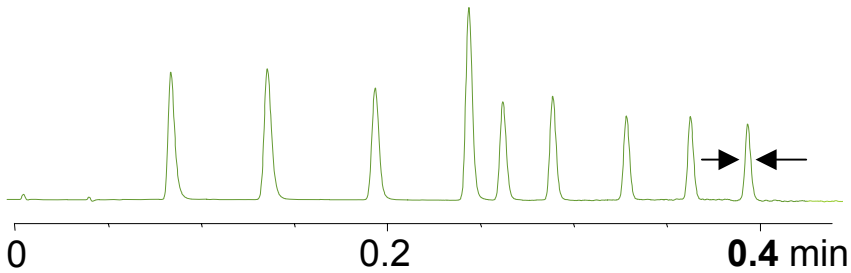
HPLC, 40°C

RRHT SB-C18
2.1mm x 50mm 1.8µm
1.00ml/min, 40°C
Analysis Time= 1.1min



RRLC, 40°C
10x faster

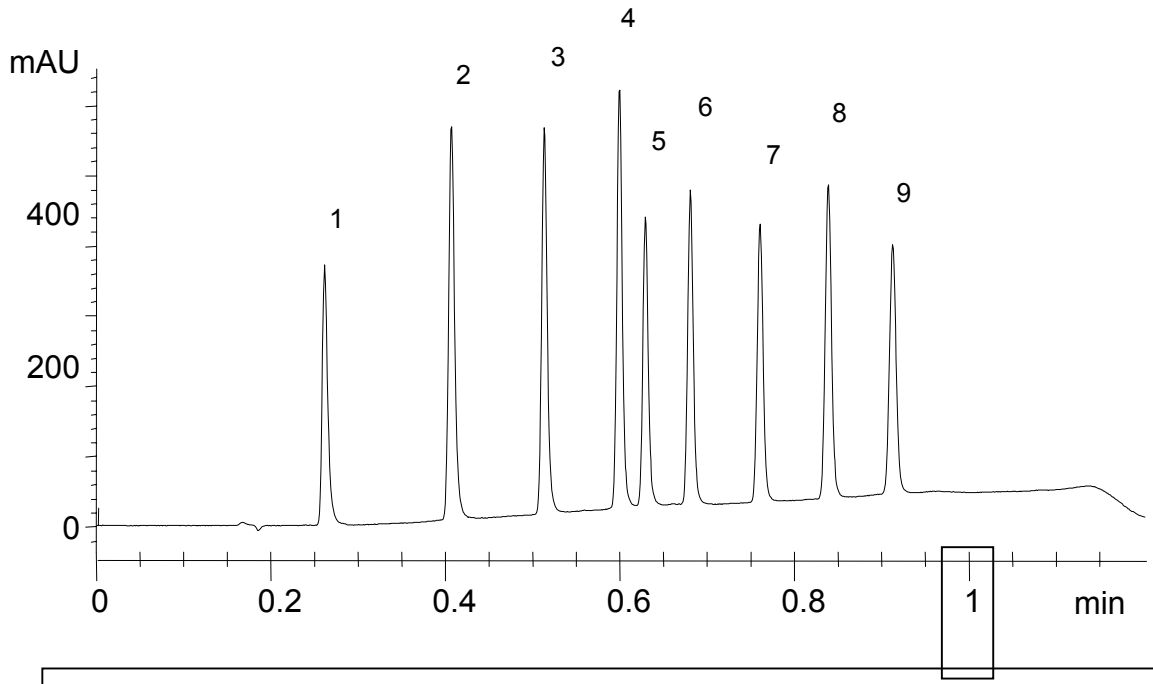
RRHT SB-C18
2.1mm x 50mm 1.8µm
2.40ml/min, 95°C
Analysis Time: 0.4min



RRLC, 95°C
27x faster
PW = 197msec

>20X faster

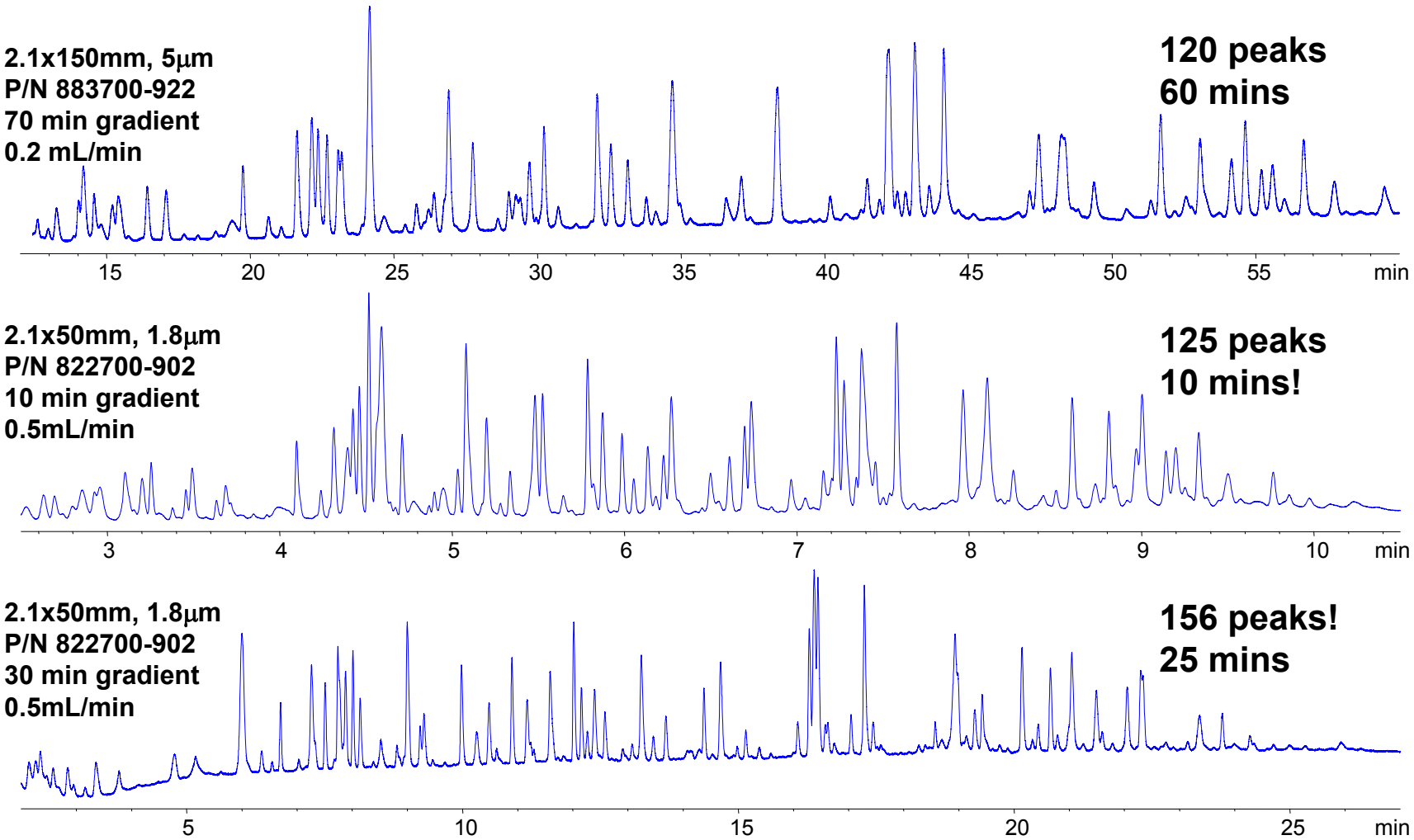
Ultra-fast Gradient Analysis of 9 Alkylphenones



	1100 HT
Flow Rate	**2.6ml/min**
Temp.	**32°C**
Pressure	**346 bar**
CycleTime	**1.5 min**
Run Time	**1.2 min**
Analysis T.	**0.912 min**
Rs (4,5)	**2.76**
RT% RSD	**<0.2%.**

Chromatographic conditions
Column: 4.6x50mm Zorbax StableBond-C18, 1.8µm
Injection: 1 µL
Separation: Mobile phase: A: water + 0.1% HCOOH; B: acetonitrile + 0.1% HCOOH.
Gradient: from 50% B to 100% B in 0.65 min, hold over 0.2 min.
Stop time = 1.2 min.
Sample: alkylphenones and acetanilide (100 ng/µL each) consisting of: 1 acetanilide, 2 acetophenone, 3 propiophenone, 4 butyrophenone, 5 benzophenone, 6 valerophenone, 7 hexanophenone, 8 heptanophenone and 9 octanophenone
Temperature: 32 °C
DAD detection: UV signal = 245 nm, 10 nm Reference = 360 nm, 80 nm
Slit: 8 nm
Peak width (response time): < 0.01 min (0.1 sec), i.e. 20 Hz data acquisition rate

Complex Samples Can be Transferred to RRHT Columns



Conditions: Mobile Phase A: Water w/ 0.1% TFA, B: ACN w/0.1% TFA, Gradient 2%B to 50%B, Temperature: 50°C
Detection: UV 214 nm Sample: HSA Tryptic Digest

What Can You Do with RRHT Columns?

1 Up to 20x faster* than conventional LC on 5um columns

Same or better performance (resolution, sensitivity) * with new Rapid Res systems

2. Easy & secure method transfer of conventional LC methods (from Zorbax 5um)

3. Up to 60% higher resolution compared to conventional LC

4. Highest Flexibility (narrow and std bore, large range of RRHT columns) –

5. Support of ultra-fast and conventional LC (Compatible with existing methods)

6. Highest Investment Protection (Backwards-compatible with 400bar, smooth transition to ultra-fast, high-resolution LC, minimum training & switching costs)

Key Messages and take aways.

1. **600 bar = 1000 bar** – *The performance of the Agilent Rapid Resolution System with Rapid Resolution HT (RRHT) columns allows users to achieve the same or better level of results* ***without the pressure.***
2. **Agilent has a competitive/superior column portfolio-** Greater number of column lengths, IDs and five bonded phases permit greater flexibility to meet any challenge -- over 100 configurations and 11 bonded phases to choose from.
3. **Agilent offers a complete solution and a migration path to fast LC-** move methods or method development to fast LC in a logical, proven path to ensure return on their investment in time and money.

The Complete List of RRHT 600 bar Columns – Jan 1

Dimensions	Eclipse Plus C18	Eclipse Plus C8	Eclipse XDB-C18	Eclipse XDB-C8	Extend-C18
4.6 x 150	959994-902				
4.6 x 100	959964-902	959964-906	928975-902	928975-906	728975-902
4.6 x 50	959941-902	959941-906	927975-902	927975-906	727975-902
4.6 x 30	959931-902	959931-906	924975-902	924975-906	724975-902
4.6 x 20			926975-902	926975-906	726975-902
3.0 x 150	959994-302				
3.0 x 100	959964-302	959964-306	928975-302	928975-306	728975-302
3.0 x 50	959941-302	959941-306	927975-302	927975-306	727975-302
3.0 x 30			924975-302	924975-306	724975-302
3.0 x 20			926975-302	926975-306	726975-302
2.1 x 150	959794-902				
2.1 x 100	959764-902	959764-906	928700-902	928700-906	728700-902
2.1 x 50	959741-902	959741-906	927700-902	927700-906	727700-902
2.1 x 30	959731-902	959731-906	924700-902	924700-906	724700-902
2.1 x 20			926700-902	926700-906	726700-902

Dimensions	SB-C18	SB-C8	SB-Phenyl	SB-CN	SB-AQ	Rx-Sil
4.6 x 150	829975-902	829975-906	829975-912	829975-905	829975-914	
4.6 x 100	828975-902	828975-906	828975-912	828975-905	828975-914	828975-901
4.6 x 50	827975-902	827975-906	827975-912	827975-905	827975-914	827975-901
4.6 x 30	824975-902	824975-906	824975-912	824975-905	824975-914	
4.6 x 20	826975-902	826975-906				
3.0 x 150	829975-302	829975-306	829975-312	829975-305		
3.0 x 100	828975-302	828975-306	828975-312	828975-305	828975-314	828975-301
3.0 x 50	827975-302	827975-306	827975-312	827975-305	827975-314	827975-301
3.0 x 30	824975-302	824975-306		824975-305		
3.0 x 20	826975-302	826975-306				
2.1 x 150	820700-902	820700-906	820700-912	820700-905		
2.1 x 100	828700-902	828700-906	828700-912	828700-905	828700-914	828700-901
2.1 x 50	827700-902	827700-906	827700-912	827700-905	827700-914	827700-901
2.1 x 30	824700-902	824700-906	824700-912	824700-905	824700-914	
2.1 x 20	826700-902	826700-906				

2 Recommended Starting Choices:

- **959941-902**
- **959741-902**

They are Eclipse Plus C18 Columns.

Conclusions

- ✓ High performance with RRHT, 1.8um columns is easily achieved.
- ✓ The columns can be used routinely for fast and high resolution analyses.
- ✓ A limited range of columns can be used on existing LC's.
- ✓ The new 1200 RRLC, with a higher pressure and temperature limit, expands the range of columns for achieving high performance.
- ✓ The widest range of applications can be explored with high temperature and high flow rate.