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

<table>
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<tr>
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<th>Rx-SIL (Sol type)</th>
<th>Xerogel (sil-type)</th>
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<tbody>
<tr>
<td><strong>STRUCTURE:</strong></td>
<td>UNIFORM SUB PARTICLES</td>
<td>“SPONGE-LIKE,” POLYMERIC NETWORK</td>
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<tr>
<td><strong>POROSITY (%):</strong></td>
<td>50</td>
<td>70</td>
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<td><strong>PARTICLE SIZE/SURFACE AREA (M²/G):</strong></td>
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<td>100A/300</td>
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<td><strong>STRENGTH:</strong></td>
<td>HIGH</td>
<td>MODERATE</td>
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<td><strong>HIGH pH RESISTANCE:</strong></td>
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<td><strong>PURITY:</strong></td>
<td>HIGH</td>
<td>LOW - HIGH</td>
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<tr>
<td><strong>PORE SIZE, PARTICLE SIZE DISTRIBUTION:</strong></td>
<td>NARROW</td>
<td>BROAD</td>
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</table>
Van Deemter Curve 1: HETP vs. Volumetric Flow Rate

\[ H = A + \frac{B}{u} + Cu \]

Column: ZORBAX Eclipse XDB-C18
Dimensions: 4.6 x 50/30mm
Eluent: 85:15 ACN:Water
Flow Rates: 0.05 – 5.0 mL/min
Temp: 20°C
Sample: 1.0μL Octanophenone in Eluent

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

Goal: Achieve lower backpressure vs. a typical PSD

- Narrower PSD
- Lower level of fines
- Small amount of slightly larger particles
NEW Rapid Resolution System with RRHT – Better Performance at Dramatically Lower Pressure THAN Acquity

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

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

Sample
0.209 0.370 0.505 0.664 0.725 0.821 0.908 0.986

Agilent Technologies
**Match Method pH and Zorbax Column Choice**

**Choose the Best Bonded-Phase for Each pH Range**

<table>
<thead>
<tr>
<th>pH Range</th>
<th>Column Name</th>
<th>Features</th>
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</thead>
</table>
| 1-4      | StableBond | 1. Uses bulky silanes  
2. Non-endcapped |
| 2-9      | Eclipse XDB | 1. eXtra Densely Bonded dimethylalkylsilanes  
2. proprietary double-endcapping |
| 2-8      | Bonus-RP | 1. polar alkyl phase  
2. triple endcapped  
3. uses bulky silanes |
| 2-11.5   | Extend-C18 | 1. unique bidentate structure  
2. double endcapped |

![Diagram of StableBond, Eclipse XDB, Bonus-RP, Extend-C18 structures]
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

Eclipse Plus C18

Ace 5 C18

SunFire C18

XBridge C18

Discovery HS C18

Luna C18(2)

Gemini C18

XTerra MS C18

0  0.5  1  1.5  2  2.5  3  3.5  min
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.

SB-C8
\[ \alpha = 1.35 \]
\[ R_s = 6.3 \]

Eclipse XDB-C8
\[ \alpha = 1.14 \]
\[ R_s = 2.8 \]

Eclipse XDB-C8
\[ \alpha = 1.58 \]
\[ R_s = 10.0 \]

SB-C8
\[ \alpha = 1.20 \]
\[ R_s = 3.9 \]

Mobile phase: (63:37) water : acetonitrile + 1.8 mL H$_3$PO$_4$
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$_3$PO$_4$ 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

**SB-Phenyl**
- $k' = 1.00$
- Rs = 26.82
- $\alpha = 6.00$

**SB-CN**
- $k' = 0.75$
- Rs = 23.40
- $k' = 4.25$
- $\alpha = 5.67$

**SB-C8**
- $k' = 0.25$
- Rs = 30.84
- $\alpha = 31.00$

**SB-C18**
- $k' = 7.75$
No difference in column selectivity as a function of particle size

Eclipse XDB-C18, USP Analysis of Phenobarbital

1. Caffeine (I.S.)
2. Allobarbital
3. Phenobarbital
4. Butabarbital
5. Hexobarbital

Column: 4.6 x 50 mm Eclipse XDB-C18
A = pH 4.5 Na Acetate, B = MeOH (60:40)
F = 1 Inj. Vol: 2 ul Flowcell: 3 uL, 2 mm flow path
3. New RRHT Columns with Fixed, High-Pressure End-fittings for 600 Bar Use

High-pressure RRHT 1.8um columns

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

Metal-to-metal internal sealing for high pressure use

Standard fittings, support typical pressures
Optimized Hardware Design Makes it Possible to Inject “Dirty” Samples

Sample: plasma sample, proteins precipitated, filtered 0.2μm filter, only minimum sample prep means faster analysis.

Typical hardware design plugs faster.

Optimized hardware design does not plug easily.

Almost no increase in pressure over 600 injections!!!
Resolution Improvements on Long 1.8 μm Columns
4.6 x 150mm SB-C18 RRHT - 5μm vs. 1.8μm

5μm: \( R_s(4,5) = 3.63 \)
Peak Width, \( 5\sigma \) (9) = 0.103

1.8μm: \( R_s(4,5) = 5.45 \)
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

1.8μm vs. 5μm
+ 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

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

Rapid Resolution
Eclipse XDB-C18
4.6 x 100 mm, 3.5 μm
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 μm
2 ul inj.

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

Easy conversion from standard analytical to RRHT
Rapid Resolution HT Columns –
Up to 20X Faster than Traditional 5μm 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

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

**HPLC, 40°C**

**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**
>20X faster
27x faster
PW = 197msec

*Agilent Technologies*
Ultra-fast Gradient Analysis of 9 Alkylphenones

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

2.1x150mm, 5 μm
P/N 883700-922
70 min gradient
0.2 mL/min
120 peaks
60 mins

2.1x50mm, 1.8 μm
P/N 822700-902
10 min gradient
0.5mL/min
125 peaks
10 mins!

2.1x50mm, 1.8 μm
P/N 822700-902
30 min gradient
0.5mL/min
156 peaks!
25 mins

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

<table>
<thead>
<tr>
<th>Dimensions</th>
<th>Eclipse Plus C18</th>
<th>Eclipse Plus C8</th>
<th>Eclipse XDB-C18</th>
<th>Eclipse XDB-C8</th>
<th>Extend-C18</th>
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<tbody>
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<th>SB-C18</th>
<th>SB-C8</th>
<th>SB-Phenyl</th>
<th>SB-CN</th>
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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.