Achieve true **high-definition** separations without sacrificing stability

**Agilent ZORBAX Rapid Resolution High Definition (RRHD) Columns**

ZORBAX RRHD columns take your fast and high-resolution separations to the next level with:

- **Infinitely greater flexibility:** push flow rates to the limit without compromising the efficiency or quality of your separations
- **Stability up to 1200 bar** for ultimate compatibility with UHPLC instruments – including Agilent’s 1290 Infinity LC
- **Maximum resolution:** 1.8 μm particles ensure defined separations and scalability – 3.5 μm and 5 μm particles simplify method transfer
- **More reliable compound identification:** enhance your MS separations with high-definition chromatograms and 2.1 mm id columns
- **A wide selection of bonded phases**, aligned with Agilent’s popular ZORBAX and Poroshell 120 families for easy scalability and method transfer
  - **ZORBAX SB-C18** for different selectivity and enhanced stability at pH 1-2
  - **ZORBAX RRHD 300Å**, part of the AdvanceBio family of columns, provides options for intact proteins and peptides
  - **ZORBAX Eclipse Plus** for exceptionally symmetrical peaks with acids, bases, and neutrals

Learn how to analyze more complex separations, faster. Go to [www.agilent.com/chem/RRHD](http://www.agilent.com/chem/RRHD)
More phases give you more flexibility to refine your analysis

ZORBAX RRHD columns are available in more than twelve bonded phases, plus HILIC, allowing you to fine-tune your selectivity to meet your analysis needs. For most applications, Eclipse Plus C18 columns are a good first choice, because they deliver high performance and excellent peak shapes over pH 2 through 9. Other bonded phases include:

- Phenyl, PAH, and Cyano columns for optimizing separations that do not use the C18 bonded phase
- HILIC columns for analyzing small, polar analytes by LC/MS
- Bonus-RP for analyzing polar compounds

Selectivity comparison: C18 columns

Selectivity differences are due to subtle, yet important variations, such as bonding type, endcapping, or the amount and type of silanols on the silica. Other factors that influence selectivity include mobile phase composition, temperature, and pH. (Note that these factors are identical in the following example.)

Here we compared the selectivity of four Agilent ZORBAX RRHD C18 columns using an endocannabinoid analysis method. For full details, see Agilent pub #5990-7166EN

Sample
1. Anandamine (AEA)
2. Palmitoylethanolamide (PEA)
3. 2-arachinoylglycerol (2-AG)
4. Oleoylethanolamide (OEA)

Conditions
A: 0.1% HCOOH in H₂O (30%)
B: 0.1% HCOOH in CH₃CN (70%)
Flow rate: 1 mL/min, isocratic
Sample: 1 μL
Temperature: 30 ºC
MS2 Scan: 290-390, ESI positive mode, scan time: 500, MS1 positive mode, scan time: 500, fragmentor: 135 V, drying gas: 12 L/min, 325 ºC, nebulizer pressure: 35 psig, capillary voltage: 3000

AEA, 348 m/z
PEA, 300 m/z
2-AG, 379 m/z
OEA, 326 m/z

*A fifth peak with a mass of 379 was also detected.
This impurity is believed to be 1,3 arachidonoylglycerol, a rearrangement of 2-AG.

Here we compared the selectivity of four Agilent ZORBAX RRHD C18 columns using an endocannabinoid analysis method. For full details, see Agilent pub #5990-7166EN
**Selectivity comparison: Phenyl columns**

Two ZORBAX RRHD phenyl columns are currently available: Eclipse Plus Phenyl-Hexyl and SB-Phenyl. Both are excellent for analyzing conjugated anthocyanins, because π electrons present in the double bonds of anthocyanins interact with the π electrons in the phenyl stationary phase. This provides a unique selectivity mechanism over traditional alkyl phases, such as C18.

While the π-π interactions are responsible for retention with alkyl phases, they may provide slight selectivity advantages for phenyl columns when analyzing closely related conjugated compounds. The EIC’s displayed below clearly show the distinct glycosides and acylglycosides of five different anthocyanins, each marked with a unique color. The Eclipse Plus Phenyl-Hexyl column resolves a few more anthocyanin peaks with this methanol/formic acid gradient than the other three phases.

**Extracted ion chromatograms from LC/MS scan data of blueberry anthocyanins. For full details, see Agilent pub #5990-8470EN**

To place your order now, go to www.agilent.com/chem/RRHD
**Push the boundaries of protein and peptide analysis**

Wide pore ZORBAX RRHD 300SB-C18, -C8, -C3, 300-Diphenyl, and 300-HILIC 1.8 µm columns deliver UHPLC performance for reversed-phase separations of intact proteins and peptide digests. Together with UHPLC instruments, such as Agilent’s 1290 Infinity LC, these versatile columns enable higher order characterization and shorter analysis times. ZORBAX StableBond technology (C18, C8, and C3) gives you the advantages of:

- **Low pH stability**, which lets you confidently perform protein and peptide separations down to pH 1 using trifluoroacetic acid (TFA) and formic acid eluents
- **Temperature stability, up to 80 °C**, allowing you to run separations at higher temperatures without compromising column lifetime. So you can improve efficiency and reduce eluent viscosity

The diphenyl phase is a unique phase previously only available on the 100Å Pursuit XRs and 200Å Pursuit columns. By applying this proven bonding chemistry to the ZORBAX 300Å 1.8 µm particle, this unique selectivity can now be exploited for protein separations using TFA and formic acid mobile phases. Also available in HILIC, for fast, high resolution separation of polar glycopeptides.

**Extended column lifetime**

Throughout a 200-run reproducibility test, column performance remained consistent, no increases in column pressure or loss of performance.

```
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<th>Flow rate (mL)</th>
<th>Run number</th>
<th>Pressure (bar)</th>
<th>Retention time (min)</th>
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*Agilent ZORBAX 300SB-C18 performance characteristics at intervals during the 200 run reproducibility test*

**Higher recovery of intact proteins**

A shorter column generally results in greater recovery of intact proteins, because the protein has less distance to migrate and elute through the column. Because the C18 ligand is the most hydrophobic alkyl chain used in peptide and protein separations, it is well suited for analyzing small globular intact proteins. The separation in **Figure 1** illustrates an analysis of insulin, a small protein of 5,800 Daltons.

**Increased resolution of peptide fragments**

For peptide mapping applications, a longer column is best, because it provides increased resolution of peptide fragments from the protein enzymatic digest.

In the separation seen in **Figure 2**, the higher efficiency of the 1.8 µm particles increases the resolution of the individual peptide fragments for rapid identification of post translational amino acid modifications.

**Improved resolution and recovery of monoclonal antibodies**

For larger proteins, such as monoclonal antibodies, a shorter, less hydrophobic C8, C3, and diphenyl functionality provides improved resolution and high recovery.

In **Figure 3**, we demonstrate the outstanding reproducibility and lifetime of ZORBAX RRHD columns over 150 injections, **with no retention time or peak abnormalities**. The bottom chromatogram shows the blank runs and gradient pressure curves before and after the 150 injections, confirming that there is no ghosting or pressure increase.

In **Figure 4**, the larger heavy chains are retained longer than the light chains and both the C3 and the diphenyl resolve two heavy chains. However, for this particular monoclonal antibody the more retentive diphenyl phase provides baseline resolution of the two heavy chains for improved reproducibility of the quantitation.

For additional information on the RRHD Biocolumns, download Agilent pub #5990-8124EN
**Figure 1.** In less than two minutes, the oxidized insulin chains were resolved.

Column: ZORBAX RRHD 300SB-C18 2.1 x 50 mm, 1.8 µm  
Sample: Insulin, insulin chain A and chain B, oxidized (bovine-sigma, 1 mg/mL)  
Injection: 2 µL  
Flow rate: 1.0 mL/min  
Mobile phase A: 0.1% TFA  
Mobile phase B: 0.01% TFA + 80% ACN  
Run time: 8 min  
Gradient: 33 to 50% mobile phase B, 0 to 4 min  
Detection: 1290 Infinity LC at 280 nm

**Figure 2.** The longer 100 mm Agilent ZORBAX 300SB-C18 column provides maximum resolution for protein digests – in this sample the total run time, including washing and equilibration, is under fifteen minutes.

Column: ZORBAX RRHD 300SB-C8 2.1 x 50 mm, 1.8 µm  
Sample: MAb  
Flow rate: 1.0 mL/min  
Temperature: 80 °C  
Mobile phase A: H₂O:IPA (88:2), 0.1% TFA  
Mobile phase B: IPA:ACN:H₂O (70:20:10), 0.1% TFA  
Detection: 1290 Infinity LC at 225 nm  
Gradient timescale:

<table>
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<th>Time (min)</th>
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<td>4.00</td>
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<tr>
<td>5.00</td>
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**Figure 3.** Excellent column reproducibility and protein recovery using Agilent ZORBAX RRHD 300SB-C8.

Column: ZORBAX RRHD 300SB-C3 and 300-Diphenyl, 2.1 x 100 mm, 1.8 µm  
Sample: Reduced monoclonal antibody (IgG1) (1.0 mg/mL)  
Sample injection: 2 µL  
Mobile phase A: 0.1% TFA in water  
Mobile phase B: 80% n-propyl alcohol, 18% ACN, 9.9% water and 0.1% TFA  
Gradient: 0 min-1% B, 2 min-20% B, 5 min-50% B  
Flow rate: 0.5 mL/min  
Temperature: 74 °C  
Detection: UV280

**Figure 4.** Separation of the light and heavy chains of a monoclonal antibody after reduction and alkylation.

Column: ZORBAX RRHD 300Å 1.8 µm columns are part of the AdvanceBio family of columns, designed for faster analysis and efficiency in your lab. To learn about this column family, visit [www.agilent.com/chem/AdvanceBio](http://www.agilent.com/chem/AdvanceBio).

To place your order now, go to [www.agilent.com/chem/RRHD](http://www.agilent.com/chem/RRHD).
Harness the **full potential** of your UHPLC system

**Easy scalability and method transfer**

ZORBAX RRHD columns allow you to achieve the **same selectivity** as you would with ZORBAX Rapid Resolution High Throughput (RRHT) 1.8 µm, and Rapid Resolution 3.5 µm and 5 µm columns with the same bonded phase.

**New levels of sensitivity and resolution**

By transferring your method to an Agilent RRHD column, you can enhance resolution for difficult analyses — allowing you to save time by using shorter columns without compromising performance.


**Separation of seven biocides in 0.7 min on a ZORBAX RRHD Eclipse Plus C18 2.1 x 50 mm, 1.8 µm column**

In this example, we separated seven biocides using Agilent ZORBAX RRHD Eclipse Plus C18 columns. High-resolution results were obtained in **less than one minute**. For a full description, along with two comparison methods, see Agilent pub #5990-4899EN
Infinitely more powerful: The 1290 Infinity LC

The advanced technology in the Agilent 1290 Infinity LC provides you with access to virtually limitless separation, detection, automation and throughput possibilities. Agilent’s new Intelligent System Emulation Technology (ISET) makes the 1290 Infinity LC the world’s first truly universal system – executing any legacy HPLC or latest UHPLC method while delivering the same chromatographic results – all through a single mouse click.

• **Infinitely more performance:** best-in-class performance in terms of resolution per time, sensitivity, accuracy and precision in LC/UV and LC/MS applications.

• **Infinitely more flexibility:** enables ultra-high pressures up to 1200 bar and high flow rates up to 5 mL/min for maximum chromatographic performance, compatibility, flexibility and investment protection.

• **Infinitely lower cost of ownership:** designed for highest reliability and for easy and fast maintenance.

For more information on ZORBAX RRHD 300-HILIC, see Agilent pub #5991-1435EN
Agilent Chemistries:
Keeping you in command of your analyses

Agilent designs and manufactures columns to suit most techniques for small molecule, large molecule and synthetic polymer analysis, allowing you to scale methods from conventional 5 µm... to “Fast LC” sub-2 µm... to prep.

You also have access to Agilent’s extensive applications library for faster method development – plus worldwide technical support, speedy problem resolution, and our global infrastructure and delivery network.

Remember, too, that Agilent’s meticulous production controls ensure column consistency and performance. With more than 40 years of experience in producing polymers and silica chemistries, our team is committed to continuously developing new column advances that make you more productive.

For more information

To order Agilent ZORBAX RRHD columns, go to www.agilent.com/chem/RRHD

In the U.S. and Canada, call toll free: 1-800-227-9770, option 3, then option 3 again

In other countries, please call your local Agilent Representative or Agilent Authorized Distributor – see www.agilent.com/chem/contactus

Use Agilent lamps and capillaries in your instrument for best performance. Request your Infinity Series supplies brochure (pub #5990-6511EN) at www.agilent.com/chem/getguides

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