Available in more than TWELVE bonded phases and in 300Å configurations

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**

The Measure of Confidence

Agilent Technologies

More phases give you **more flexibility** to refine your analysis

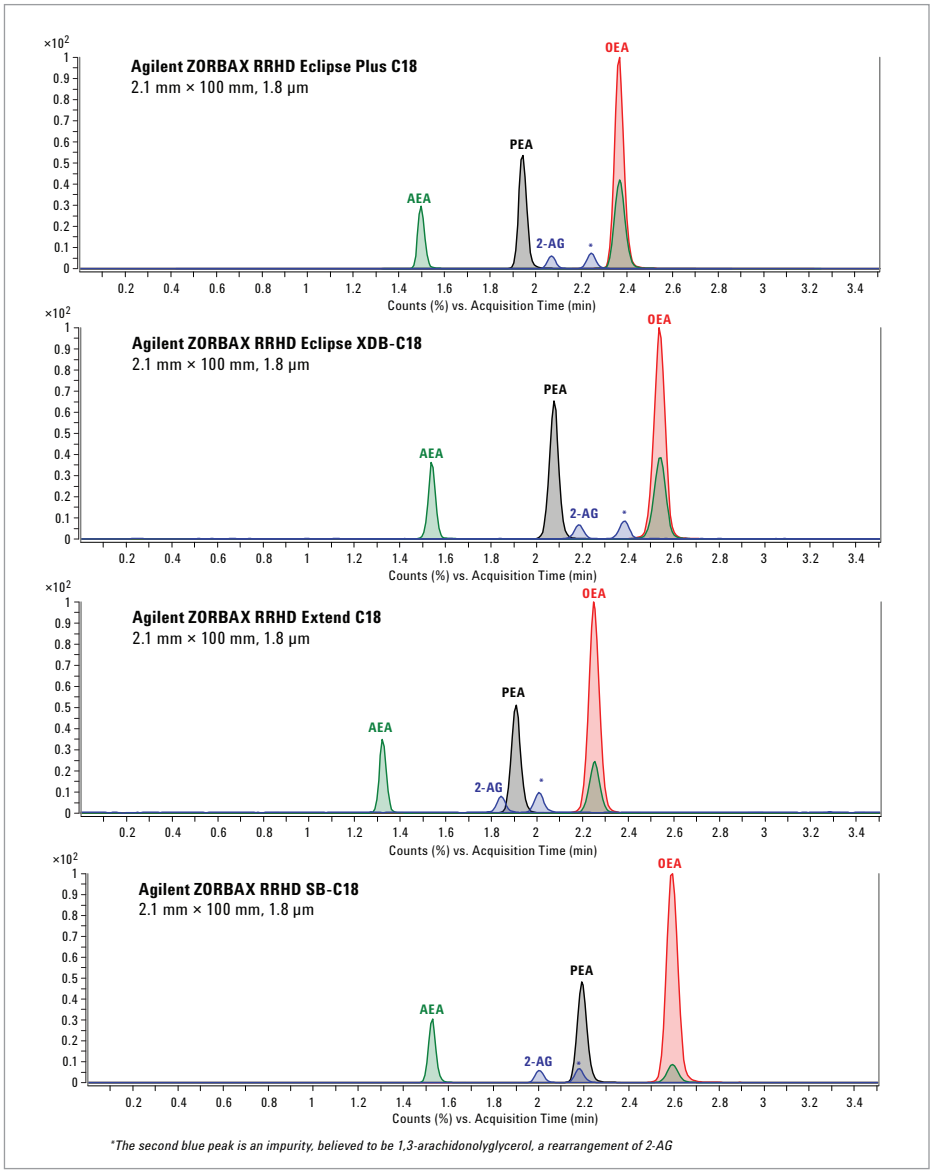
ZORBAX RRHD columns are available in more than twelve bonded phases, plus HILIC, allowing you to fine-tune your selectivity to meet your analysis needs.

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

Conditions
A: 0.1% HCOOH in H_2O (30%)
B: 0.1% HCOOH in CH_3CN (70%)
Flow rate: 1 mL/min, isocratic
Sample: 1 µL
Temperature: 30 °C
MS2 Scan: 290-390, ESI positive mode, scan time: 500, fragmentor: 135 V; drying gas: 12 L/min, 325 °C; 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 arachidonolyglycerol, a rearrangement of 2-AG.*

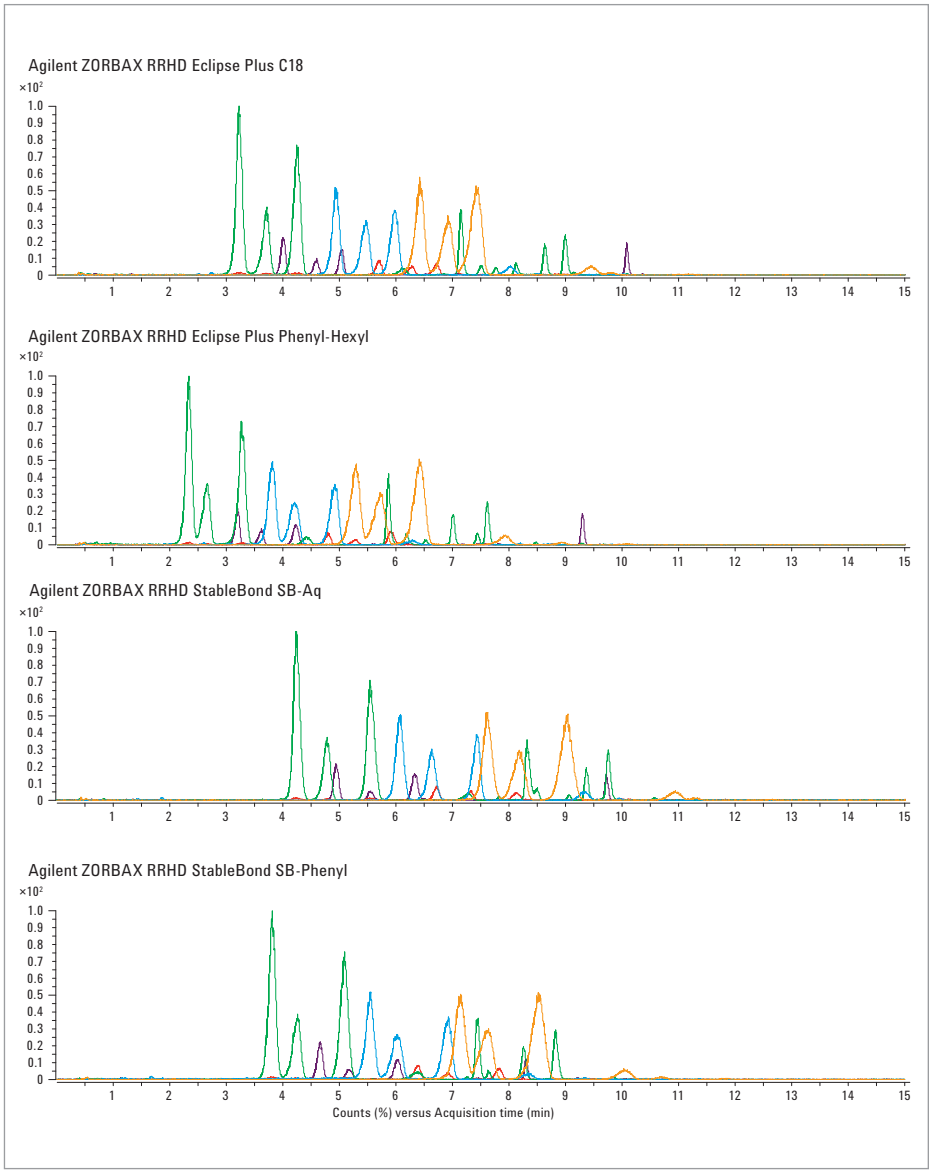
Sample
1. Anandamine (AEA)
2. Palmitoylethanolamide (PEA)
3. 2-arachinoylglycerol (2-AG)
4. Oleoylethanolamide (OEA)

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.



Conditions
A: 5% HCOOH in H_2O
B: CH_3CN
0.65 mL/min
10-50% B in 15 minutes
5 µL injection of blueberry
TCC: 30 °C
MS2 Scan, ESI +, 200-1000
Cyanidin, m/z 286
Peonidin, m/z 300
Delphinidin, m/z 302
Petunidin, m/z 316
Malvidin, m/z 330

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

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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.

Flow rate (mL)	Run number	Pressure (bar)	Retention time (min)	Tailing factor (5%)	Plate count
1	1	680 to 520	1.789	1.08	9258
1	50	680 to 520	1.790	1.06	9241
1	100	680 to 520	1.788	1.07	9252
1	200	680 to 520	1.789	1.10	9305

***Agilent ZORBAX 300SB-C18** performance characteristics at intervals during the 200 run reproducibility test*

Faster separations

ZORBAX RRHD 300SB-C18 columns are packed with 1.8 µm particles, allowing them to maintain their performance at higher flow rates (as with small-molecule UHPLC).

Flow rate (mL)	Pressure (bar)	Retention time (min)	Tailing factor (5%)	Plate count
0.3	230 to 150	2.39	1.47	8855
0.5	350 to 250	2.04	1.27	9226
1.0	680 to 520	1.78	1.09	8980
1.5	890 to 670	1.72	1.13	8912

***Agilent ZORBAX 300SB-C18** performance as a function of flow rate. Peak symmetry improves with minimal reduction in efficiency, as the flow rate increases*

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.

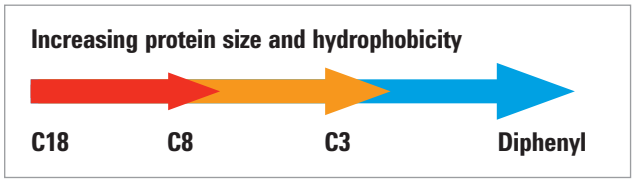
Increased resolution of peptide fragments

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

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

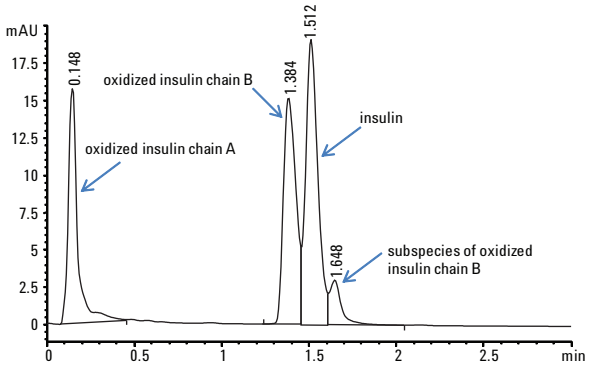


Figure 1. *In less than two minutes, the oxidized insulin chains were resolved*

Column:	ZORBAX RRHD 300SB-C18 2.1 x 100 mm, 1.8 µm
Sample:	Enzymatic protein digest (MAb)
Injection:	5 µL
Flow rate:	0.5 mL/min
Temperature:	50 °C
Mobile phase A:	0.1% TFA
Mobile phase B:	0.01% TFA + 80% ACN
Gradient:	2% B for 1 min, 2 to 45% B for 8.8 min, 45 to 95% B for 0.2 min, 95% B for 2 min, 95 to 2% B for 0.2 min
Detection:	1290 Infinity LC at 280 nm

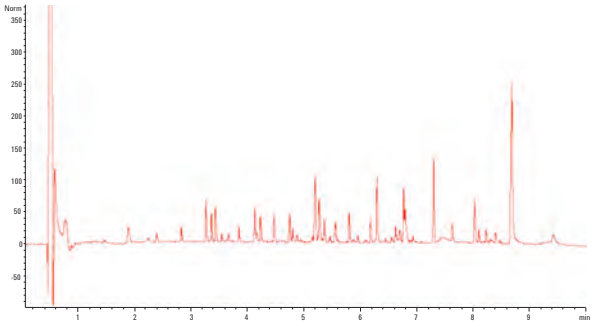


Figure 2. *The longer 100 mm Agilent ZORBAX 300SB-C18 column provides maximum resolution for protein digests – in this sample the total run time, including washing and equilibration, is* ***under fifteen minutes***

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**

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:	H_2O:IPA (98:2), 0.1% TFA
Mobile phase B:	IPA:ACN:H_2O (70:20:10), 0.1% TFA
Detection:	1290 Infinity LC at 225 nm

Gradient timescale

Time (min)	% Solvent B
0.00	25
3.00	35
4.00	90
5.00	25

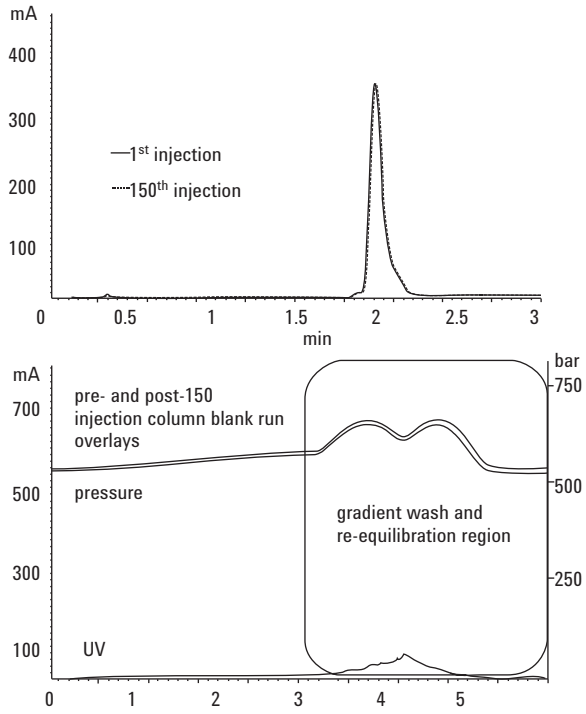


Figure 3. *Excellent column reproducibility and protein recovery using Agilent ZORBAX RRHD 300SB-C8*

Columns:	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, 10% 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

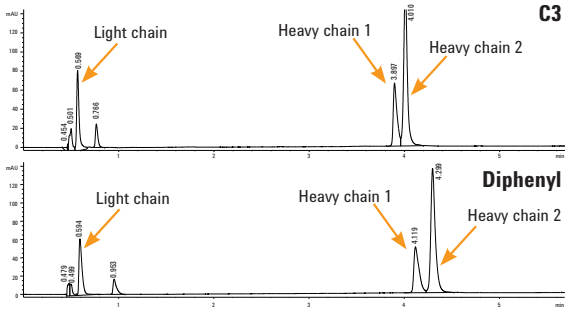


Figure 4. *Separation of the light and heavy chains of a monoclonal antibody after reduction and alkylation*

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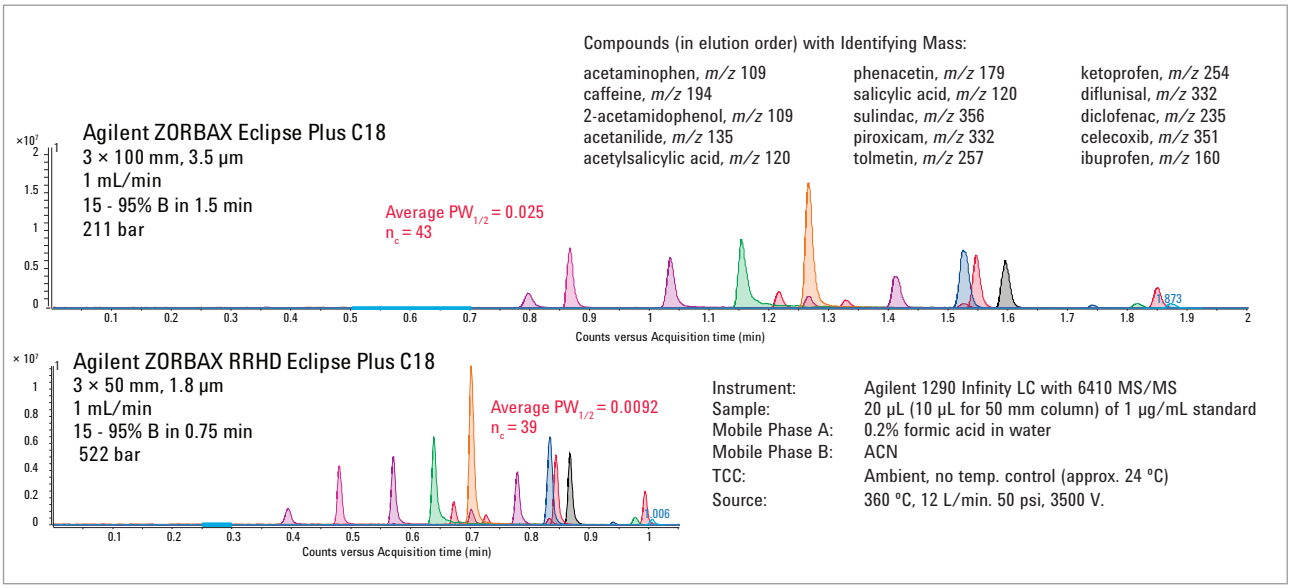
Harness the **full potential** of your UHPLC system

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.

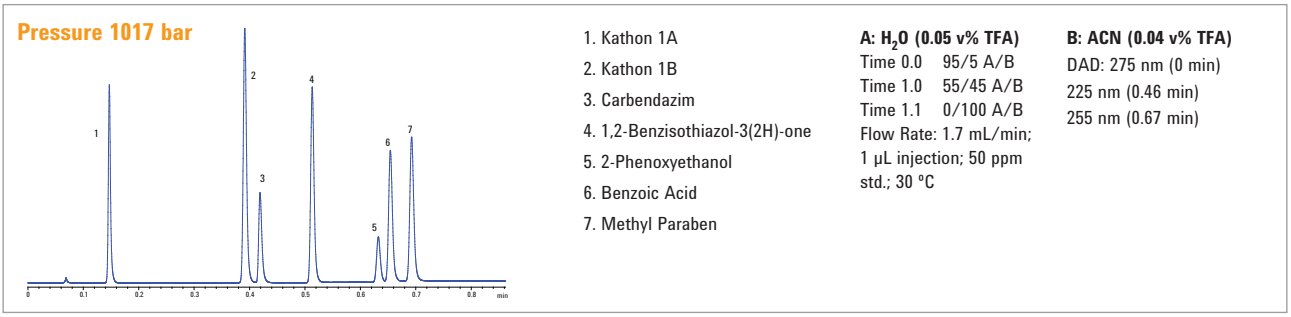
New levels of sensitivity and resolution

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.



A comparison of Agilent ZORBAX Eclipse Plus C18 columns with RRHD Eclipse Plus C18 columns. Scaling gradient methods according to column volume preserves selectivity during method transfer. The RRHD column saves analytical time without sacrificing 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*

ZORBAX Rapid Resolution High Definition (RRHD) Columns for High Pressure Use (Maximum Pressure: 1200 bar)

	Eclipse Plus C18 (USP L1)	Eclipse Plus C8 (USP L7)	Eclipse XDB-C18 (USP L1)	Extend-C18 (USP L1)	Eclipse PAH (USP L1)	Eclipse Plus Phenyl-Hexyl	Bonus RP
RRHD 2.1 x 150 mm, 1.8 µm	959759-902	959759-906	981759-902	759700-902	959759-918	959759-912	859768-901
RRHD 2.1 x 100 mm, 1.8 µm	959758-902	959758-906	981758-902	758700-902	959758-918	959758-912	858768-901
RRHD 2.1 x 50 mm, 1.8 µm	959757-902	959757-906	981757-902	757700-902	959757-918	959757-912	857768-901
RRHD 3.0 x 150 mm, 1.8 µm	959759-302	959759-306	981759-302				
RRHD 3.0 x 100 mm, 1.8 µm	959758-302	959758-306	981758-302	758700-302	959758-318	959758-312	
RRHD 3.0 x 50 mm, 1.8 µm	959757-302	959757-306	981757-302	757700-302	959757-318	959757-312	

	StableBond SB-C18 (USP L1)	StableBond SB-C8 (USP L7)	StableBond SB-Phenyl (USP L11)	StableBond SB-CN (USP L10)	StableBond SB-Aq	HILIC Plus
RRHD 2.1 x 150 mm, 1.8 µm	859700-902	859700-906	859700-912	859700-905	859700-914	959759-901
RRHD 2.1 x 100 mm, 1.8 µm	858700-902	858700-906	858700-912	858700-905	858700-914	959758-901
RRHD 2.1 x 50 mm, 1.8 µm	857700-902	857700-906	857700-912	857700-905	857700-914	959757-901
RRHD 3.0 x 150 mm, 1.8 µm	859700-302	859700-306				
RRHD 3.0 x 100 mm, 1.8 µm	858700-302	858700-306	858700-312	858700-305	858700-314	959758-301
RRHD 3.0 x 50 mm, 1.8 µm	857700-302	857700-306	857700-312	857700-305	857700-314	959757-301

Wide pore ZORBAX RRHD Columns for Protein and Peptide Separations

	StableBond 300SB-C18	StableBond 300SB-C8	StableBond 300SB-C3	300-Diphenyl	300-HILIC
RRHD 2.1 x 100 mm, 1.8 µm	858750-902	858750-906	858750-909	858750-944	858750-901
RRHD 2.1 x 50 mm, 1.8 µm	857750-902	857750-906	857750-909	857750-944	857750-901

Part of the AdvanceBio Family

For more information on ZORBAX RRHD 300-HILIC, see Agilent pub #5991-1435EN

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.

Agilent 1290 Infinity LC

For more details, visit
www.agilent.com/chem/infinity

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ZORBAX RRHD Column Specifications

Bonded Phase	Pore Size	Surface Area	pH Range	End-capped
ZORBAX Eclipse Plus C18	95Å	160 m_2/g	2.0-9.0	Double
ZORBAX Eclipse Plus C8	95Å	160 m_2/g	2.0-9.0	Double
ZORBAX Eclipse Plus Phenyl-Hexyl	95Å	160 m_2/g	2.0-9.0	Double
ZORBAX Eclipse XDB-C18	80Å	180 m_2/g	2.0-9.0	Double
ZORBAX Extend-C18	80Å	180 m_2/g	2.0-11.5†	Double
ZORBAX Bonus RP	80Å	180 m_2/g	2.0-9.0	Triple
ZORBAX StableBond SB-C18	80Å	180 m_2/g	1.0-8.0*	No
ZORBAX StableBond SB-C8	80Å	180 m_2/g	1.0-8.0*	No
ZORBAX StableBond SB-Phenyl	80Å	180 m_2/g	1.0-8.0*	No
ZORBAX StableBond SB-CN	80Å	180 m_2/g	1.0-8.0*	No
ZORBAX StableBond SB-Aq	80Å	180 m_2/g	1.0-8.0*	No
ZORBAX Eclipse PAH	95Å	160 m_2/g	2.0-8.0	No
ZORBAX HILIC Plus	95Å	160 m_2/g	0.0-8.0	No
BioHPLC columns for proteins and peptides				
ZORBAX StableBond 300SB-C8	300Å	45 m_2/g	1.0-8.0*	No
ZORBAX StableBond 300SB-C18	300Å	45 m_2/g	1.0-8.0*	No
ZORBAX StableBond 300SB-C3	300Å	45 m_2/g	1.0-8.0*	No
ZORBAX 300-Diphenyl	300Å	45 m_2/g	1.0-8.0	Yes
ZORBAX 300-HILIC	300Å	45 m_2/g	1.0-8.0	No

*StableBond columns are designed for optimal use at low pH. At pH >6, highest column stability for all silica based columns is obtained by operating at temperatures <40 °C and using lower buffer concentrations – 10-20 mM or organic buffers. 300SB-C18 may be used up to 90 °C. For pH 6-8, select the Eclipse Plus C18 column.

†Temperature limits are 60 °C up to pH 8, 40 °C from pH 8-11.5.

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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.

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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**

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

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