

New high definition UHPLC columns increase resolution and speed by operating up to 1200 bar

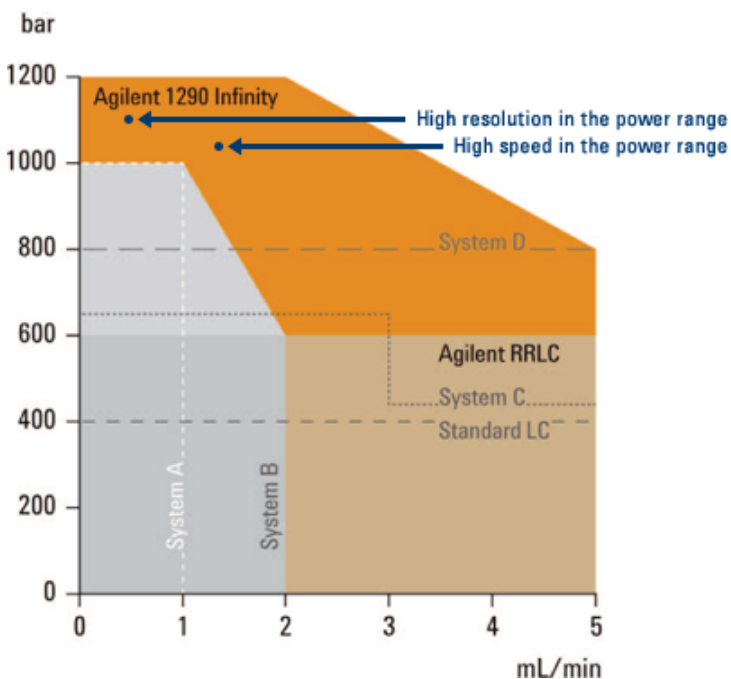


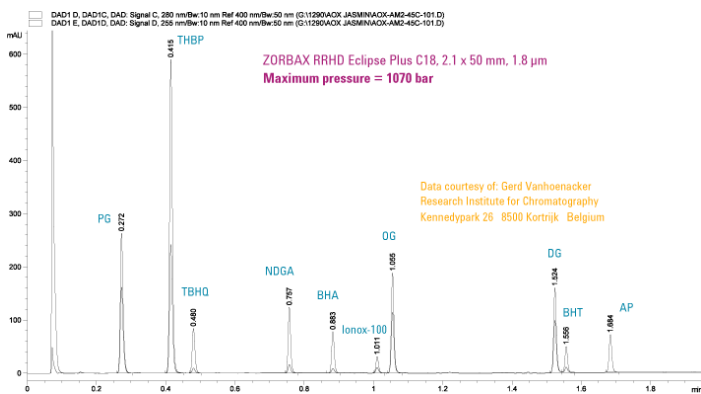
Figure 1. ZORBAX RRHD columns use the full power range of the 1290 Infinity LC, for separations with higher speed and definition.

Many labs have adopted short LC columns with sub-2- μm particle sizes to achieve greater productivity with fast separations and high resolution. But questions have remained about the ability to separate complex mixtures completely and reliably on longer sub-2- μm columns. These questions have now been answered with innovative columns that you can use in a higher pressure range to achieve the chromatographic definition you need.

New **Agilent ZORBAX Rapid Resolution High Definition (RRHD) columns** with 1.8 μm particles are available in short and long lengths, and are designed for optimal performance over the entire operating range of the new Agilent 1290 Infinity LC System (**Figure 1**). The ZORBAX RRHD columns exceed the capabilities of the Agilent ZORBAX Rapid Resolution High Throughput columns with 1.8 μm particles. The new RRHD columns allow you to achieve high-definition separations by taking advantage of the 1200 bar pressure limit of the 1290 Infinity LC

Even faster separations, even greater resolution

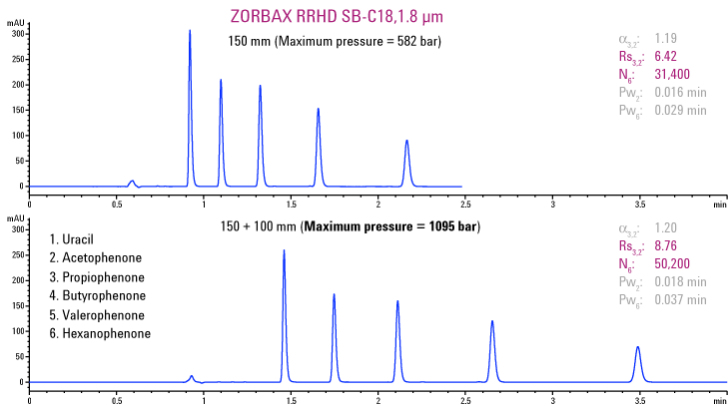
A new packing process extends the stability of the ZORBAX RRHD columns, to allow you to consider more options for fast and high-resolution separations. Two examples highlight these capabilities. In the first example, shown in **Figure 2**, the goal was to achieve maximum speed in a gradient separation of a 10-component mixture of antioxidants. This is a complex separation, but a sub-two-minute analysis time was achieved by using a flow rate of 1.6 mL/min with the Agilent ZORBAX RRHD Eclipse Plus C18 column, 2.1 x 50 mm. The gradient program at this flow rate generated a maximum pressure of 1070 bar – higher than the limit on other ultra high performance liquid chromatography (UHPLC) systems. The extended pressure range of the 1290 Infinity LC with the RRHD columns enabled this fast separation.



Conditions

Column	ZORBAX RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 μm
Mobile phase	A = 0.02% H_3PO_4 , B = acetonitrile:methanol 3:1
Flow rate	1.6 mL/min
Gradient	0-1.85 min 30-100% B
Injection volume	1 μL
Detector	Sig = 280/10 nm, Ref = 400/50, 80 Hz Sig = 255/10 nm, Ref = 400/50, 80 Hz
Temperature	45°C
Sample	Antioxidant mixture, ~ 100 ppm

Figure 2. This ZORBAX RRHD column separated 10 antioxidants in less than two minutes – and withstood backpressures greater than 1000 bar.



Conditions

Column ZORBAX RRHD SB-C18, 1.8 μm
 Mobile phase 25:75 water:acetonitrile
 Flow rate 0.5 mL/min
 Temperature Ambient
 Sample Alkylphenones
 α = separation factor
 R_s = resolution
 N = number of theoretical plates
 Pw = peak width

Figure 3. You can maximize efficiency and resolution with longer ZORBAX RRHD columns, by operating at pressures up to 1200 bar.

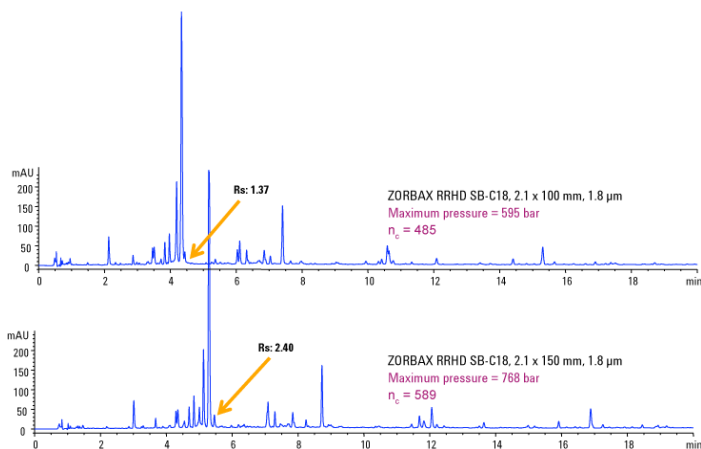


Figure 4. A longer 150 mm ZORBAX RRHD column enabled a high-resolution separation of this complex sample of licorice root.

Conditions

Column ZORBAX RRHD SB-C18, 2.1 mm, 1.8 μm
 Mobile phase A = 0.1% formic acid
 B = acetonitrile with 0.1% formic acid
 Gradient 10-100% B in 30 min
 Flow rate 0.4 mL/min
 Detector UV, 280 nm
 Temperature Ambient
 Sample Licorice root extract
 Abbreviations R_s = resolution
 n_c = peak capacity

The second example (**Figure 3**) demonstrates the power of the ZORBAX RRHD columns for high-resolution separations. The figure compares the separation of a group of alkylphenones on a 150 mm RRHD column versus two RRHD columns in series – a 100 mm and a 150 mm column. In this example, tandem columns with a total length of 250 mm increased resolution by 36% as it increased efficiency by the expected 60%. These columns in series generated close to 1100 bar pressure for this gradient separation.

Excellent chromatography in the conventional UHPLC pressure range

A third example (**Figure 4**) shows a complex separation of a licorice root extract. In this case, you can achieve the desired high resolution with the 150 mm RRHD column. By simply adjusting the gradient length, you can completely resolve some minor components and maximize peak capacity. This gradient separation used a maximum pressure of 800 bar, representing a more typical UHPLC separation, and the sample was well-resolved with the ZORBAX RRHD 1.8 μm column.

These three examples show the advantages of the new ZORBAX Rapid Resolution High Definition columns on the Agilent 1290 Infinity LC. The columns are used over the power range of the LC for maximum speed and separation. You can easily use them at any pressure up to 1200 bar for greater resolution – greater definition – in your most complex samples..

For more information about Agilent Technologies ZORBAX RRHD columns, contact the Chromatographic Specialties Inc. Technical Support team at 1-800-267-8103 or email tech@chromspec.com