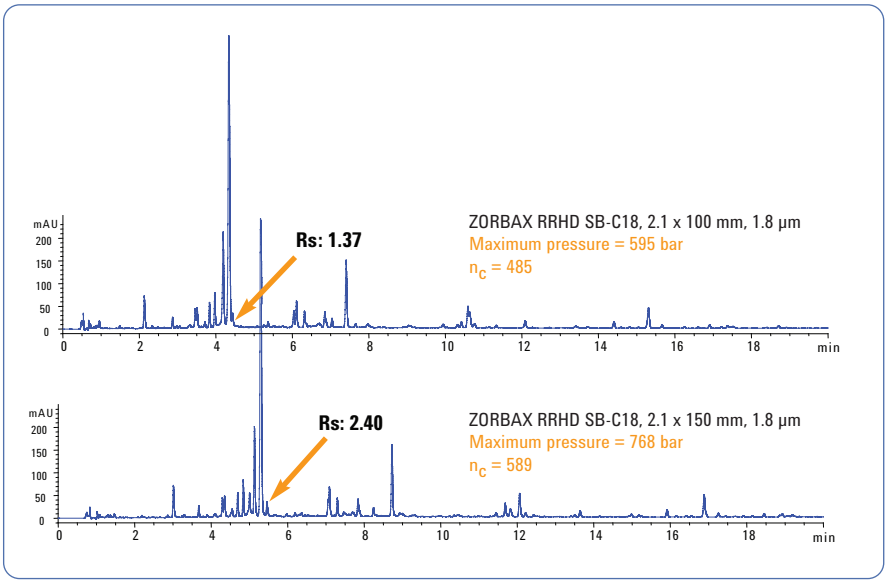
Increasing Resolution and Speed by Operating UHPLC Columns up to 1200 Bar

Technical Note



Abstract

LC columns with sub-2-µm particles have gained popularity because they deliver high productivity and resolving power. In the past, some of the efficiency and throughput benefits of these columns could not be realized because LCs and columns were limited to backpressures of 400 to 600 bar. That changed when Agilent ZORBAX Rapid Resolution High Definition (RRHD) columns with 1.8 µm particles were designed to take advantage of the unique 1200-bar pressure limit of the Agilent 1290 Infinity LC System. Operation in this high-pressure domain allows chromatographers to achieve even more speed and resolution for complex separations.

Introduction

The productivity and resolution benefits of ultra high performance liquid chromatography (UHPLC) and columns with sub-2-µm particles are well recognized in the scientific community. Shorter LC columns with 1.8 µm particles allow analysts to achieve faster runtimes while maintaining column efficiency and resolution. Longer columns with these small particles enable additional resolution for complex mixtures. However, smaller particles produce higher backpressure. When the backpressure limit of the column or the LC is reached, it is impossible to further increase the flow rate for a faster analysis, or to use a longer column to achieve greater resolution.

The Agilent 1290 Infinity LC System uniquely addresses the need for a more flexible LC that operates under higher backpressures up to 1200 bar. (See Figure 1.) Agilent ZORBAX Rapid Resolution High Definition (RRHD) columns with 1.8 µm particles are designed for optimal performance over the entire operating range of this LC, and are the only commercially available columns that can be used up to the 1200-bar limit. This combination of the 1290 Infinity and ZORBAX RRHD (1.8 µm) columns forms a total solution that provides new levels of LC performance and flexibility.

The ZORBAX RRHD (1.8 µm) columns exceed the capabilities of the Agilent ZORBAX Rapid Resolution High Throughput (RRHT) columns with 1.8 µm particles, which have a 600-bar limit. New hardware and an improved packing process extend the stability of the ZORBAX RRHD (1.8 µm) columns to higher pressures. The ZORBAX RRHD (1.8 µm) columns are available in short and long lengths, and can be used up to 1200 bar to achieve the chromatographic definition needed to separate complex mixtures completely and reliably.

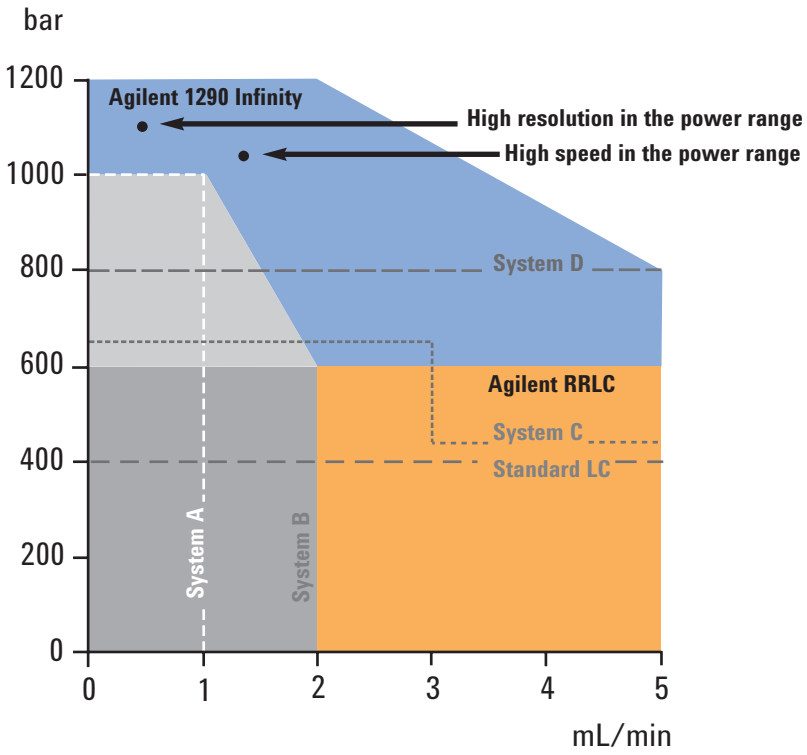


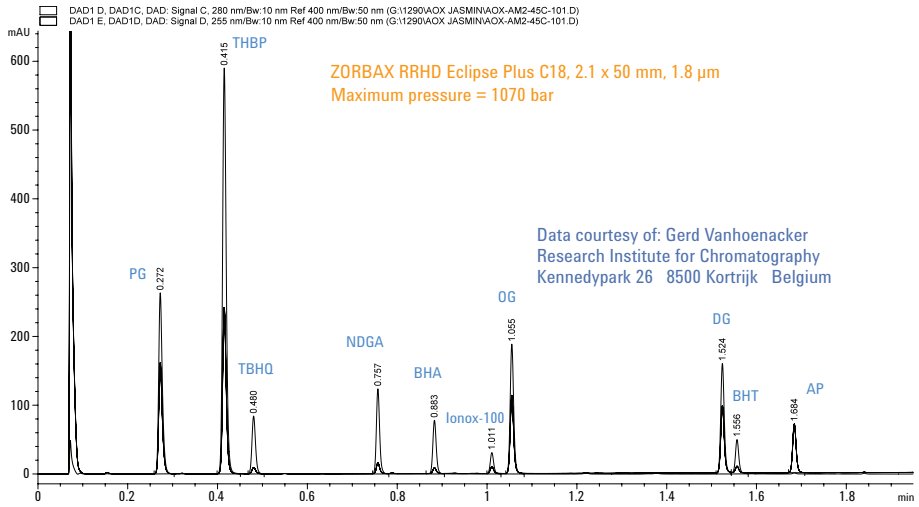
Figure 1: ZORBAX RRHD (1.8 µm) columns use the full pressure and flow range of the 1290 Infinity LC, for separations with higher speed and definition.

Results and discussion

As the following examples show, the combination of these columns with the 1290 Infinity LC System makes it possible to increase analysis speed for complex samples, or to achieve greater resolution in a limited amount of time.

Example 1: maximize speed with higher flow rates

Many labs need to decrease LC runtimes to increase sample throughput. In the example shown in Figure 2, the goal was to achieve maximum speed in a gradient separation of a 10-component mixture of antioxidants. This was a complex separation, but an analysis time of less than two minutes was achieved by using a fast flow rate of 1.6 mL/min with an Agilent ZORBAX RRHD Eclipse Plus C18 column, 2.1 x 50 mm, 1.8 µm. Note that a gradient can produce a pressure change of more than 300 bar during an analysis, especially if it covers a very wide range of organic mobile phase. The gradient program at this 1.6 mL/min flow rate generated a maximum pressure of 1070 bar – exceeding the limit on other UHPLC systems and columns. The extended pressure range of the 1290 Infinity LC with the ZORBAX RRHD (1.8 µm) columns enabled this fast separation.



Chromatographic conditions for Figure 2

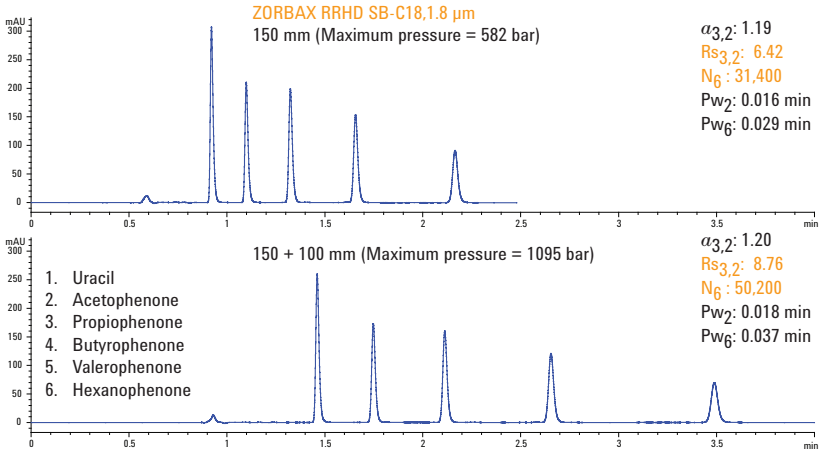
Column	ZORBAX RRHD Eclipse Plus C18, 2.1 x 50 mm, 1.8 µm
Mobile phase	A = 0.02% H_3PO_4, B = acetonitrile:methanol 3:1
Flow rate	1.6 mL/min
Gradient	0 – 1.85 min 30 – 100% B
Injection volume	1 µL
Detector	Sig = 280/10 nm, Ref = 400/50, 80 Hz Sig = 255/10 nm, Ref = 400/50, 80 Hz
Temperature	45 °C
Sample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

Figure 2. By operating at greater than 1000 bar, the ZORBAX RRHD (1.8 µm) column produced this gradient separation of 10 antioxidants in less than two minutes.

Agilent 1290 Infinity LC – *technology for more resolution, speed and sensitivity*

Example 2: maximize resolution with tandem columns

Longer LC columns deliver more resolving power for mixtures with a large number of components. While columns are available in standard lengths of 50, 100, and 150 mm, analysts may need additional options to increase resolution. As this example shows, they can combine columns in series and achieve even better separations. Figure 3 compares the separation of a group of alkylphenones on a 150 mm ZORBAX RRHD (1.8 µm) column versus two RRHD columns in series. In this example, tandem columns with a total length of 250 mm increased resolution by 36% and efficiency by the expected 60%. These columns in series generated very high resolution, but required close to 1100 bar pressure for this gradient separation. This high pressure was well within the limit for the Agilent LC and column.



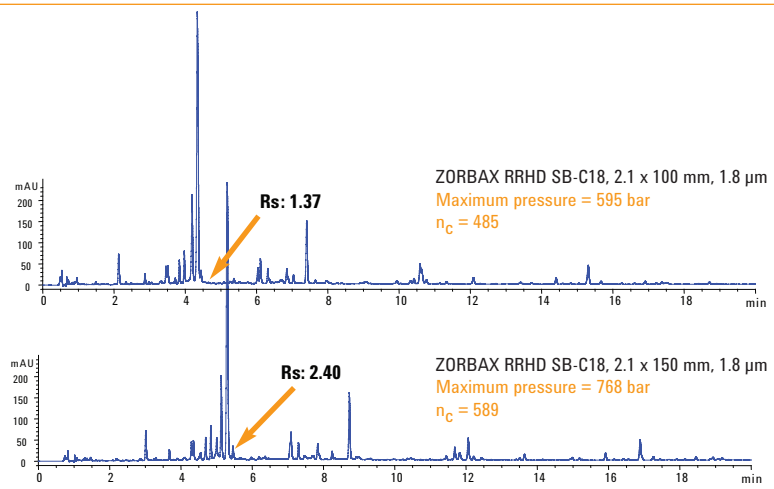
Chromatographic conditions for Figure 3

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

Figure 3. Because they operate at pressures up to 1200 bar, ZORBAX RRHD (1.8 µm) columns can be used in tandem to maximize efficiency and resolution.

Example 3: increase peak capacity with longer columns

A third example (Figure 4) shows a separation of a complex licorice root extract. As the column length increased, the peak capacity increased from 486 on the 100 mm column to 589 on the 150 mm column, and some minor components were completely resolved. This gradient separation used a maximum pressure of almost 800 bar, representing a more typical UHPLC separation, and the sample was well-resolved with the ZORBAX RRHD (1.8 µm) column.



Chromatographic conditions for Figure 4

Method

Columns	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
Abbreviation	Rs = resolution
	n_c = peak capacity

Figure 4. A longer 150 mm ZORBAX RRHD (1.8 µm) column enabled greater peak capacity and a better separation for this sample of licorice root.

Conclusion

Agilent ZORBAX Rapid Resolution High Definition (RRHD) columns with 1.8 µm particles are designed and manufactured for reliable operation at 1200 bar – the highest pressure in the industry. High-pressure operation enables use of longer columns, tandem columns, and/or higher flow rates. Chromatographers can use these columns over the full operating range of the Agilent 1290 Infinity LC System, providing greater flexibility to achieve maximum speed and resolution for complex samples.

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