Scalability of Agilent Columns Across HPLC and UHPLC Instruments

Application Note

Pharmaceutical

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Abstract

LC method transfer across several instrument types, using Agilent and non-Agilent systems, demonstrates the scalability of Agilent columns with different configurations and stationary phases. This ability to easily scale a method from one system to another is particularly useful in the pharmaceutical industry where samples may have to be analyzed by R&D, QC, or other laboratories where identical instrument setups may not be possible or where analytical needs may vary.

Introduction

Instrumentation and column technology for liquid chromatography continually improve, to deliver higher throughput, higher resolution, and higher sensitivity. Transferring an LC method from one instrument to another should be straightforward. However, columns specifically designed for one instrument are often not recommended, or necessary for another instrument. Newer, sub-2-µm columns are designed to withstand higher pressures than traditional HPLC systems, as a result, the smaller particles cannot be fully used within 400 bar. In addition, differences in system delay volume and extra-column volume could affect column performance from one instrument to another, most notably with small internal diameter columns. Therefore, scalability between column dimensions, especially with respect to particle size, is paramount to ensure straightforward method transfer. The flexibility of Agilent ZORBAX columns in several configurations and stationary phases is demonstrated by transferring an LC method across several instrument types, using Agilent and non-Agilent systems.

Agilent Technologies

Experimental

LC Method Parameters

An Agilent 1200 Series RRLC, a 1290 Infinity LC, a 1290 Infinity LC/6410 Triple Quadrupole MS system, and a non-Agilent UHPLC are used in this experiment.

Mobile phase	A: 0.2% formic acid in water; B: acetonitrile
Gradient	15% to 95% B, gradient time (t_g) varies according to column dimensions and flow rate, see Equations 1 and 2
DAD	Sig = 260, 4 nm; Ref = Off
MS Source	350 °C, 12 L/min, 50 psi, 3500 V
MS Scan	Positive ESI, Delta EMV 200, Fragmentor 135 V, Scan 100–400, 5 ms scan time, 0.2 amu step, 28.36 cycles/s, 35.3 ms/cycle
Analytes in elution order with identifying mass	acetaminophen (109), caffeine (194), 2-acetamidophenol (109), acetanilide (135), acetylsalicylic acid (120), phenacetin (179), salicylic acid (120), sulindac (356), piroxicam (332), tolmetin (257), ketoprofen (254), diflunisal (332), diclofenac (295), celecoxib (381), ibuprofen (160)
Sample	0.01 mg/mL (UV) and 1 µg/mL (MS) each in water

The following Agilent LC columns were used:

Description		P/N
Agilent ZORBAX Eclipse Plus C18	4.6 mm × 250 mm, 5-µm	959990-902
Agilent ZORBAX Eclipse Plus C18	4.6 mm × 150 mm, 5-µm	959993-902
Agilent ZORBAX Eclipse Plus C18	3.0 mm × 100 mm, 3.5-µm	959961-302
Agilent ZORBAX RRHD Eclipse Plus C18	3.0 mm × 100 mm, 1.8-µm	959758-302
Agilent ZORBAX RRHD Eclipse Plus C18	3.0 mm × 50 mm, 1.8-µm	959757-302
Agilent Poroshell 120 EC-C18	3.0 mm × 100 mm, 2.7-µm	695975-302
Agilent Poroshell 120 EC-C18	3.0 mm × 50 mm, 2.7-µm	699975-302

Gradient Scaling

Once a gradient separation has been optimized (selectivity and retention index), it is possible to further improve the chromatography by varying column length, particle size and flow rate. However, the k* value (Equation 1) must be maintained while varying these column conditions, so as not to lose selectivity while scaling the gradient.

Equation 1

$$k^* = (t_g F)/(d/2)^2 L(\Delta\%B)$$

where: t_g = gradient time
F = flow rate
d = column internal diameter
L = column length
$\Delta\%B$ = change in organic content across gradient segment

Assuming a constant k*, Equation 1 can be simplified to Equation 2 below:

Equation 2

$$t_{g2} = (t_{g1} d_2^2 L_2 F_1)/(d_1^2 L_1 F_2)$$

where: t_{g1} and t_{g2} = original and new gradient times
F_1 and F_2 = original and new flow rates
d_1 and d_2 = original and new column internal diameters
L_1 and L_2 = original and new column lengths

Additionally, in Equation 2, v_1 and v_2 can be substituted for t_{g1} and t_{g2} respectively to accurately scale a method's injection volume according to a new column's dimensions.

Instrument ⇔ Column Compatibility Considerations

While almost any column can be installed and run on any instrument, pressure limitations by the LC and pressure generated by the column can lead to the inability to optimally utilize any column on any LC. For example, small particles generate substantial back pressure, especially when packed into long columns, this particular column configuration is not best suited for a conventional 400 bar HPLC, as that pressure limit will easily be exceeded as flow rates increase. Table 1 lists the specifications of several Agilent LC systems that are critical to good performance, while Table 2 shows compatibility between instruments and columns for UHPLC and HPLC.

Table 1. Critical Parameters of Agilent LC Systems

	Agilent 1100/1200 Series Binary HPLC	Agilent 1200 Series RRLC (std)	Agilent 1260 Infinity Binary LC	Agilent 1290 Infinity Binary LC
Pressure limit (bar)	400	600	600	1200
Max flow rate (mL/min)	5	5	5	5
Pump Delay volume (µL)	600–900	600–800	600–800 (120*)	45/75
Capillary id (mm)	0.17	0.17	0.17 (0.12*)	0.12
Dispersion volume w/o cell (µL)	15	15	15 (7.5*)	7.5
Injection principle	Variable loop	Variable loop	Variable loop	Variable loop
Injection volume (std/ext) (µL)	100/1500	100/1500	100/1500	20/40 (100 up to 600 bar)
Area RSD (%)	< 0.25	< 0.25	< 0.25	< 0.25

*optimized for 2.1 mm id

Table 2. Instrument and Column Compatibility in Agilent UHPLC and HPLC Systems (Green [Compatible] to Red [Incompatible])

	UHPLC 1.8 µm particles						UHPLC superficially porous particles		HPLC 3.5–5 µm particles
Column length (mm)	**Short, 30–50**			**Long, 100–150**			**30–150**		**50–300**
Column id (mm)	2.1	3	4.6	2.1	3	4.6	2.1	3–4.6	3–4.6
Max pressure (bar)	1200	600	600	1200	600	600	600	600	400
Agilent column	RRHD*	RRHT^	RRHT	RRHD	RRHT	RRHT	Poroshell	Poroshell	Various
Agilent 1290 Infinity (1200 bar)									
Agilent 1260 Infinity/1200 RRLC (600 bar)									
Agilent 1100/1200 Series (400 bar)									

*rapid resolution high definition
^rapid resolution high throughput

Results and Discussion

Maintaining Selectivity with Different Column Dimensions

As shown in Figure 1, selectivity is maintained when a 5-µm column is shortened from 250 mm to 150 mm, and when transferred to a 3 mm × 100 mm, 3.5-µm column. Some resolution, however, is lost with the shorter columns, most notably with ibuprofen, the last peak to elute.

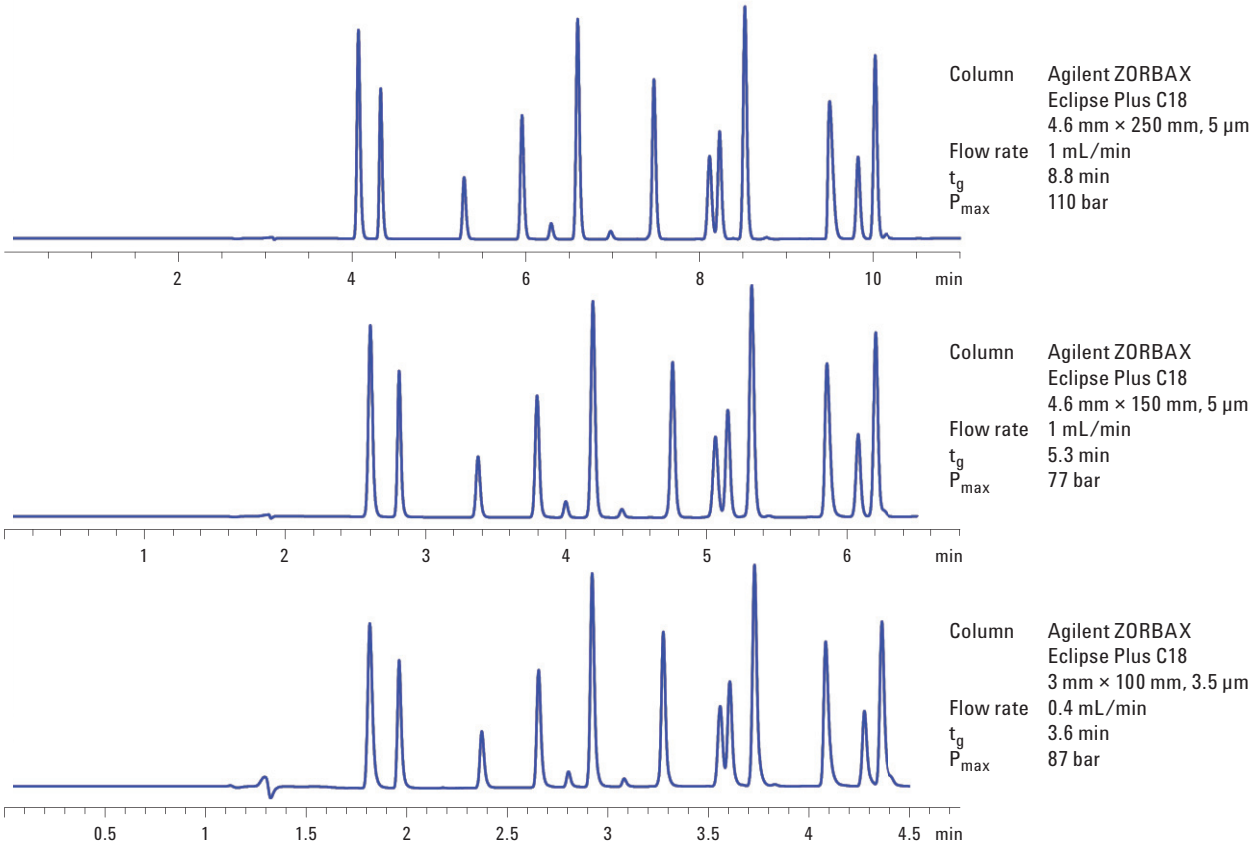

Figure 1. Maintaining selectivity on Agilent ZORBAX Eclipse Plus C18 columns with different dimensions and particle sizes using an Agilent 1200 Series RRLC.

Effect of System Delay Volume

A manual change in system delay volume is the cause of a pronounced difference in the early eluting peaks (Figure 2). The relatively large delay volume of an Agilent 1200 Series RRLC system if set-up in a standard delay volume and not in low delay volume configuration with a smaller dimension column (in this case 3 mm × 100 mm, 3.5 µm) causes delayed elution of all peaks. In order to make the chromatography more similar to the larger dimension columns shown in Figure 1, the automatic system delay volume reduction feature of the Agilent 1200 Series autosampler can be used, as shown in the bottom chromatogram in Figure 2.

Method Transfer across Agilent Instruments

Transferring the method from a 1200 Series RRLC to an Agilent 1290 Infinity LC yields very similar results with the 3.5-µm column, as shown in Figure 3, when the automatic delay volume reduction feature of the 1200 Series autosampler is used. As shown in Table 1, the delay volume of a 1290 Infinity LC system is substantially lower than the 1100/1200 Series or 1260 Infinity LC systems. The same is true for other vendors UHPLC systems optimized for lowest delay volumes. Also, typically the mixing behavior, that is, the slope of a step gradient, is much steeper for such instruments.

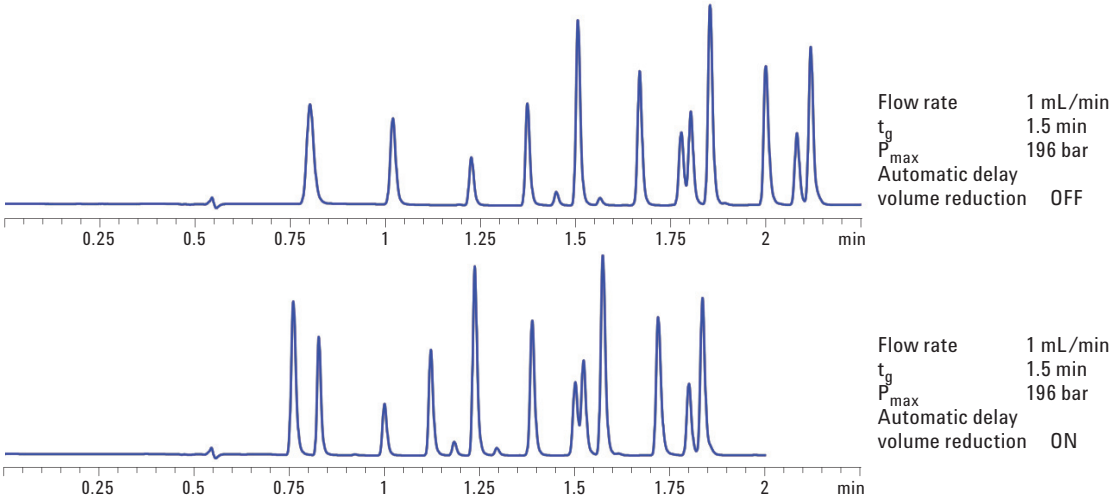

Figure 2. Effect of system delay volume on an Agilent ZORBAX Eclipse Plus C18 3.0 mm × 100 mm, 3.5-µm column using an Agilent 1200 Series RRLC without (top) and with (bottom) the delay volume reduction function on the autosampler.

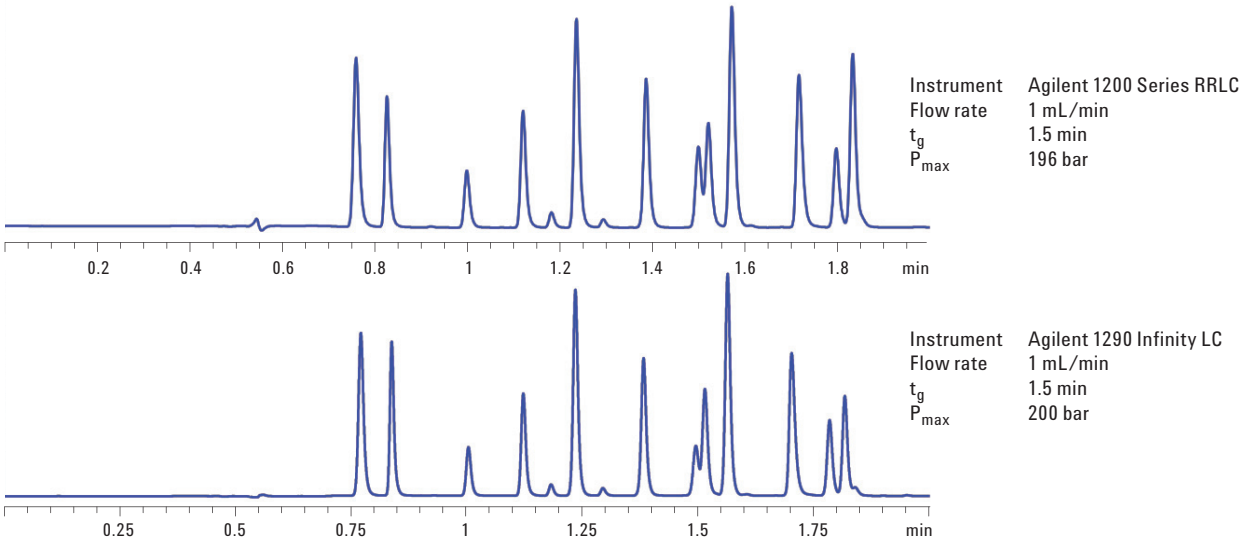

Figure 3. Comparing separations on Agilent 1200 Series RRLC and Agilent 1290 Infinity LC instruments, using an Agilent ZORBAX Eclipse Plus C18 3.0 mm × 100 mm, 3.5-µm column.

Conversely, if a method originally developed on a 1100/1200 Series or 1260 Infinity LC needs to be transferred to a 1290 Infinity LC system, a larger delay volume would be needed and, ideally, also an adoption of the mixing curve. For this, an isocratic hold can be added manually to the method, or physically the volume can be added by additional capillary tubing. Both need up-front determination of the required volume. The use of the new Intelligent System Emulation Technology (ISET), available only for 1290 Infinity LC systems, is a new technology that makes this process easier. ISET emulates the different delay volumes and mixing behaviors of Agilent's or other LC systems on a 1290 Infinity LC and delivers the same results without manual changes of the method or hardware. For more information see Agilent Pub. No. 5990-8670EN.

Method Transfer across Agilent Columns

Totally porous 1.8-µm and 3.5-µm Agilent ZORBAX columns yield the same selectivity, while superficially porous 2.7-µm Poroshell 120 delivers very similar selectivity as a result of similar bonding chemistry. Both 1.8-µm Agilent ZORBAX RRHD and 2.7-µm Agilent Poroshell 120 provide better resolution than the 3.5-µm ZORBAX column of the same dimensions, as can be seen in Figure 4. Referring to Table 2, the scalability illustrated in Figure 4 indicates that there is an Agilent LC column to meet any instrument need. In this case, the same separation can be performed on a 400 bar instrument with a 3.5-µm column, on a 600 bar instrument with a superficially porous 2.7-µm column, or on a 1200 bar instrument with a 1.8-µm column.

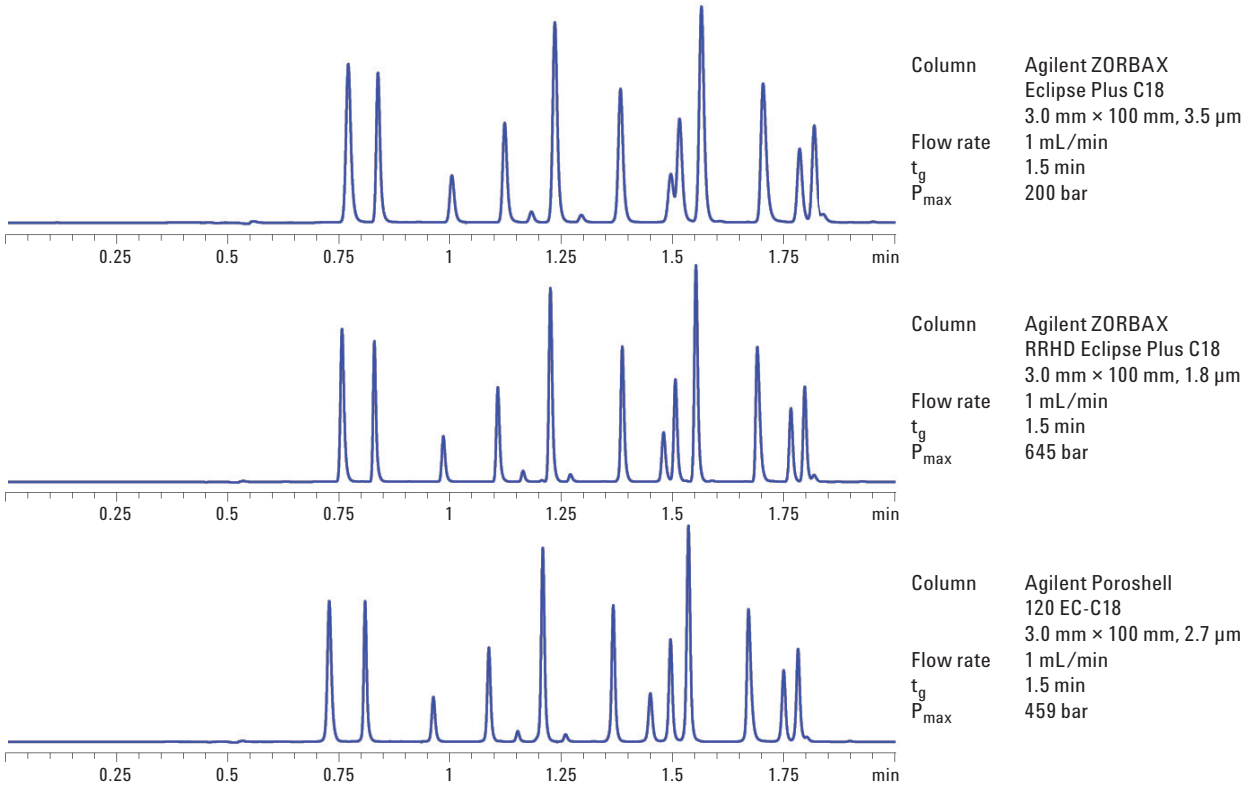

Figure 4. Comparing 1.8 and 3.5-µm Agilent ZORBAX Eclipse Plus C18 and 2.7-µm Agilent Poroshell 120 EC-C18 columns using an Agilent 1290 Infinity LC.

Method Transfer with Different Agilent Detectors

Selectivity is maintained when transferring this method from an Agilent 1290 Infinity LC with UV detection to a 1290 Infinity LC with MS detection, shown in Figure 5. Some peak broadening occurs with the MS due to more extra column volume in the detector, as compared to the DAD. Additionally, extra transfer tubing connecting the LC and MS accounts for the increase in system pressure and the slightly later elution time of all peaks.

Increasing Analysis Speed

Due to the highly selective nature of MS detection analysis speed can be increased to take full advantage of the high pressure limit of the RRHD column and the low back pressure generated by the Agilent Poroshell 120 column. With a 50 mm RRHD column, analysis time is increased significantly, resulting in a 0.4 minute run time for the 15 compounds, as seen in Figure 6.

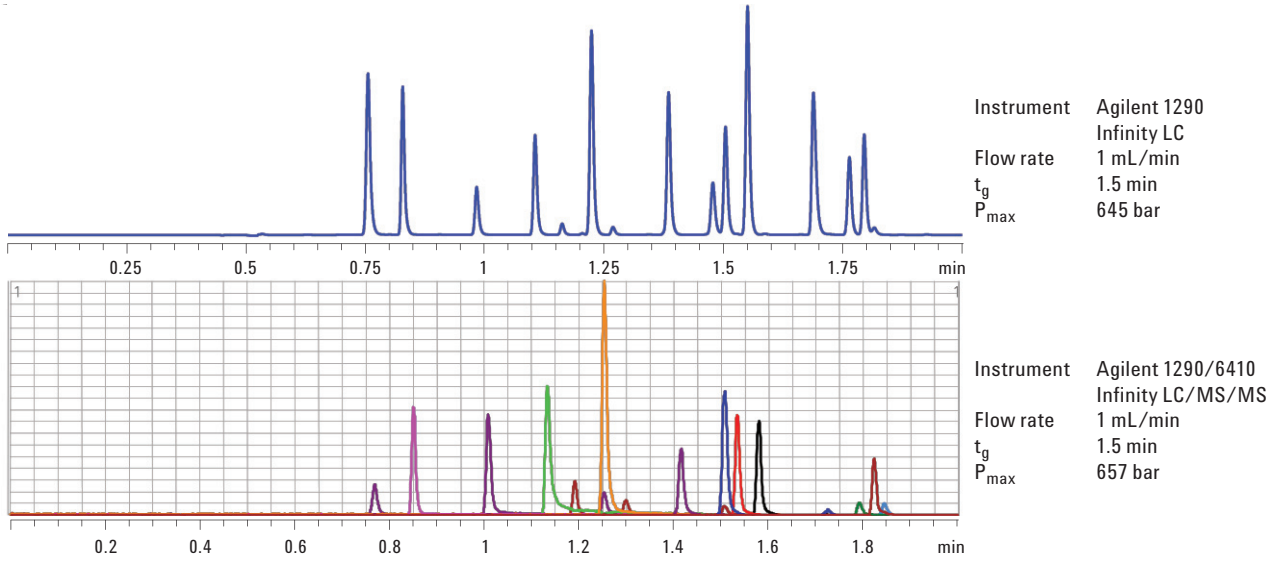

Figure 5. Comparing Agilent 1290 Infinity LC with UV detection with the Agilent 1290/6410 Infinity LC/MS/MS, using an Agilent ZORBAX RRHD Eclipse Plus C18 3.0 mm × 100 mm, 1.8-µm column.

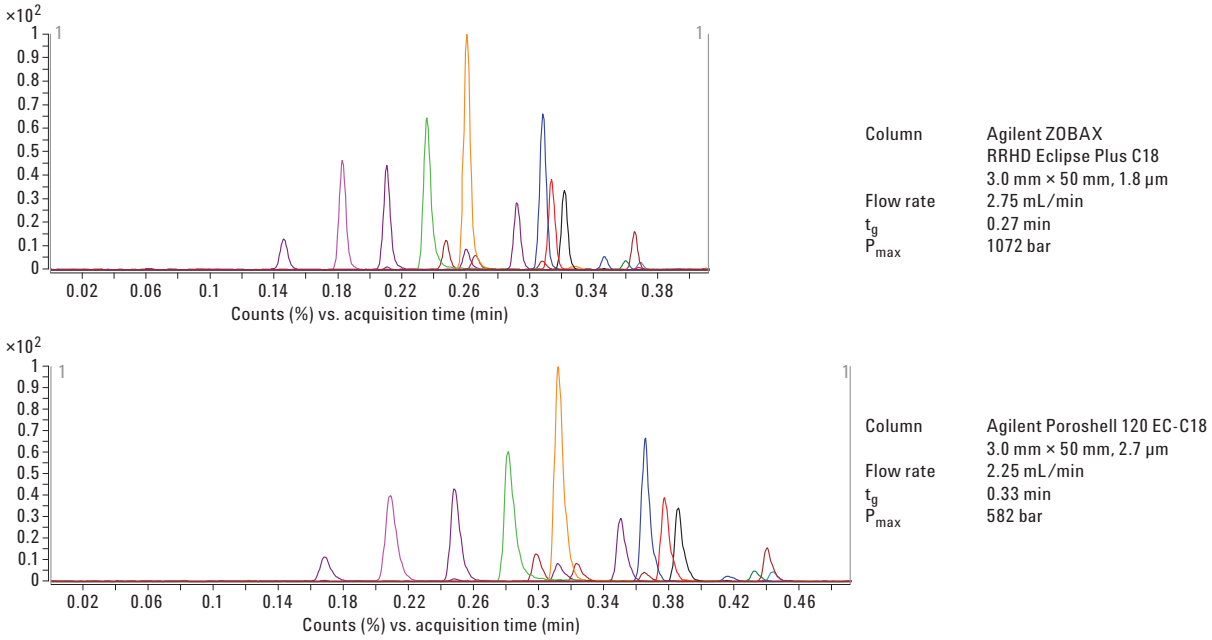

Figure 6. High-throughput methods with an Agilent 1290/6410 Infinity LC/MS/MS. (Note that these analyses were performed for demonstration purposes only, as it is not recommended to use such high flow rates with MS detection.)

Agilent Columns on a non-Agilent Instrument

Agilent's RRHD columns can be run not only with the Agilent 1290 Infinity LC, but also with a non-Agilent UHPLC system. The overall analysis is similar, but some slight modification would help resolve the last peak, ibuprofen. Smaller id capillary tubing in the non-Agilent UHPLC is probably the cause of increased system pressure and reduced delay volume, causing all peaks to elute earlier, as seen in Figure 7. The separation on the two instruments could be made more similar by adding either an isocratic hold to the beginning of the method on the non-Agilent LC or by adding additional capillary tubing to the non-Agilent LC to delay the gradient.

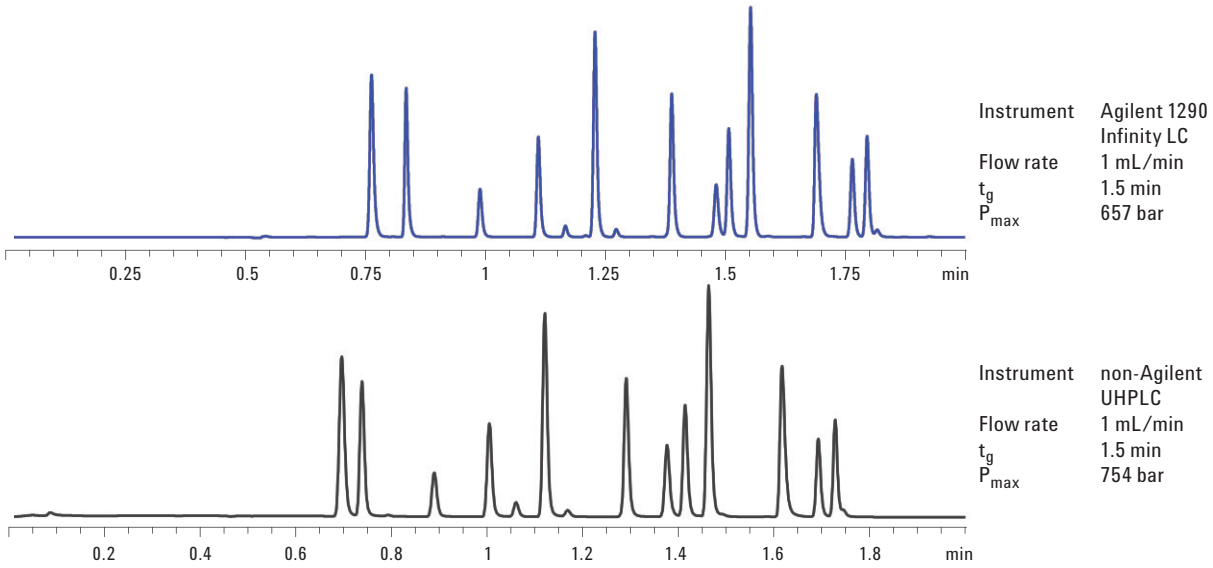

Figure 7. Using an Agilent ZORBAX RRHD Eclipse Plus C18 3.0 mm × 100 mm, 1.8-µm column to compare separation performance on an Agilent 1290 Infinity LC and a non-Agilent UHPLC.

Conclusions

Agilent ZORBAX columns offer the same selectivity across multiple particle sizes, including 5, 3.5 and 1.8 µm. The Agilent Poroshell 120 column has similar selectivity to the Agilent ZORBAX columns, with efficiency close to that provided by 1.8-µm particles, while generating substantially lower pressure due to its larger 2.7-µm particles. Scaling gradient methods according to column volume preserves selectivity when transferring methods, and methods can be easily transferred from Agilent 1200 Series RRLC systems to Agilent 1290 Infinity LC systems. Investigate ISET for more information on new technology from Agilent that makes this process easier.

Transferring methods to MS is easy, as it has no significant effect on the chromatography, other than a small increase in extra-column volume. Using MS detection delivers fast analyses because of its more selective nature when detecting co-eluting peaks.

Agilent ZORBAX RRHD columns, with their 1200 bar pressure limits, are easy to run on a non-Agilent UHPLC without significant method modification.

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