Scalability of Columns Across HPLC and UHPLC Instruments

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Introduction and Experimental Design

Abstract

LC instrumentation and column technology is continually improving (higher throughput, higher resolution, and higher sensitivity). Transferring a LC method from one instrument to a different instrument ideally is a straightforward process. Columns specifically designed for one instrument, however, often are not recommended or necessary for another instrument. Newer sub-2-micron columns are designed to withstand higher pressures than the limits of traditional HPLC systems, resulting in the inability to use the smaller particles to their full potential. Also, differences in system delay volume and extra-column volume could impact column performance from one instrument to another, most notably with small internal diameter columns. Scalability between column dimensions, especially particle size, therefore, is paramount to ensure straightforward method transfer. The flexibility of ZORBAX columns in several column configurations and stationary phases is demonstrated by transferring an LC method across several instrument types including a non-Agilent LC.

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 must be maintained, while varying these column conditions so as not to lose selectivity while scaling the gradient.

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

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

Instrument ⇔ Column Compatibility

Column Type	UHPLC						UHPLC	HPLC
Particle	1.8-µm						Superficially Porous	3.5-5-µm
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
1290 Infinity- 1200 bar									
1260 Infinity/1200 RRLC - 600 bar									
1100/1200 Series - 400 bar									

Critical Parameters with Agilent LC Systems

	1100/1200 Series HPLC	1200 Series RRLC (Std.)	1260 Infinity Binary LC (*Optimized for 2.1 mm ID)	1290 Infinity Binary LC
Pressure Limit	400 bar	600 bar	600 bar	1200 bar
Max Flow Rate	5 mL/min	5 mL/min	5 mL/min	5 mL/min
Delay Volume	600-900 µL	600-800 µL	600-800 µL (120 µL*)	10-110 µL
Capillary ID	0.17 mm	0.17 mm	0.17 mm (0.12 mm*)	0.12 mm
Disp. Vol. w/o Cell	15 µL	15 µL	15 µL (7.5 µL*)	7.5 µL
Injection Principle	Variable Loop	Variable Loop	Variable Loop	Variable Loop
Inj. Vol. (Std./Ext.)	100/1500 µL	100/1500 µL	100/1500 µL	100 µL
Area RSD	<0.25%	<0.25%	<0.25%	<0.25%

LC Method Parameters

•Mobile Phase A: 0.2% formic acid in water; B: acetonitrile
•Gradient: 15-95% B
•DAD: Sig = 260,4 nm; Ref = Off
•MS: Positive ESI, MS Scan mode, 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; Source: 350 °C, 12 L/min, 50 psi, 3500 V
•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)
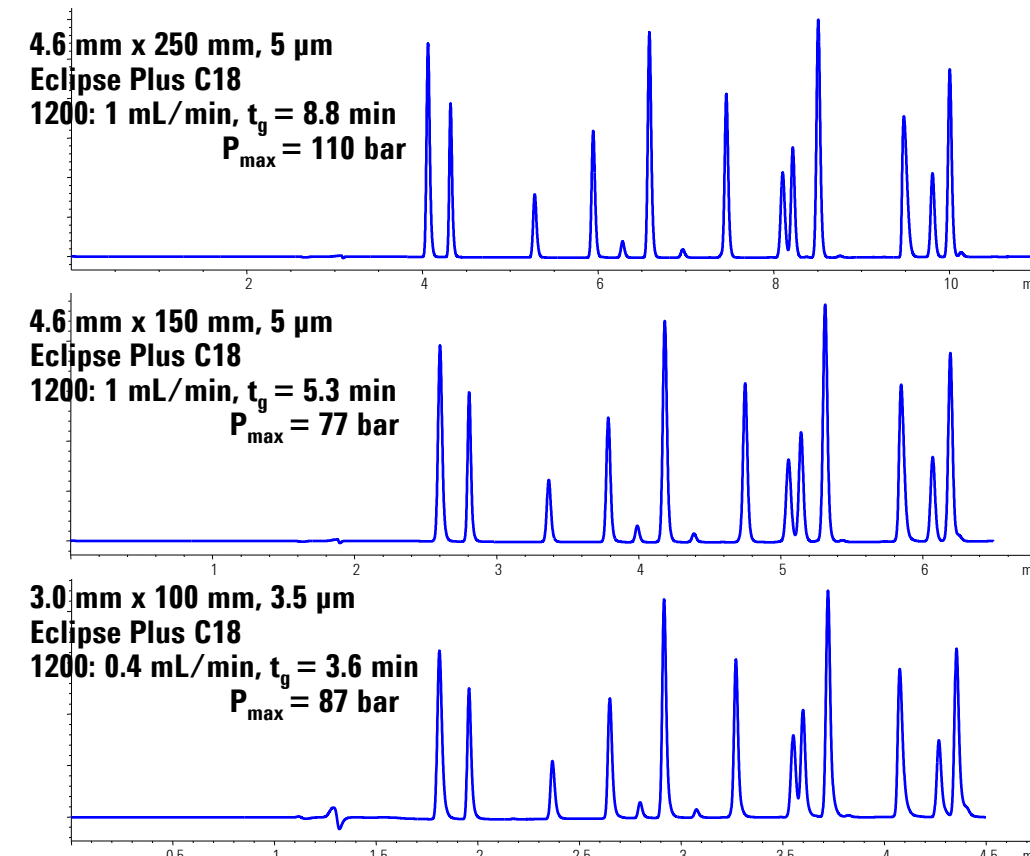•Sample: 0.01 mg/mL (UV) & 1 µg/mL (MS) each in water

Agilent Columns Used

4.6 mm x 250 mm	5-µm	ZORBAX Eclipse Plus C18	959990-902
4.6 mm x 150 mm	5-µm	ZORBAX Eclipse Plus C18	959993-902
3.0 mm x 100 mm	3.5-µm	ZORBAX Eclipse Plus C18	959961-302
3.0 mm x 100 mm	1.8-µm	ZORBAX RRHD Eclipse Plus C18	959758-302
3.0 mm x 50 mm	1.8-µm	ZORBAX RRHD Eclipse Plus C18	959757-302
3.0 mm x 100 mm	2.7-µm	Poroshell 120 EC-C18	695975-302
3.0 mm x 50 mm	2.7-µm	Poroshell 120 EC-C18	699975-302

Instruments Used

Agilent 1200 RRLC, Agilent 1290 Infinity UHPLC, Agilent 1290/6410 Infinity UHPLC/MS, non-Agilent UHPLC
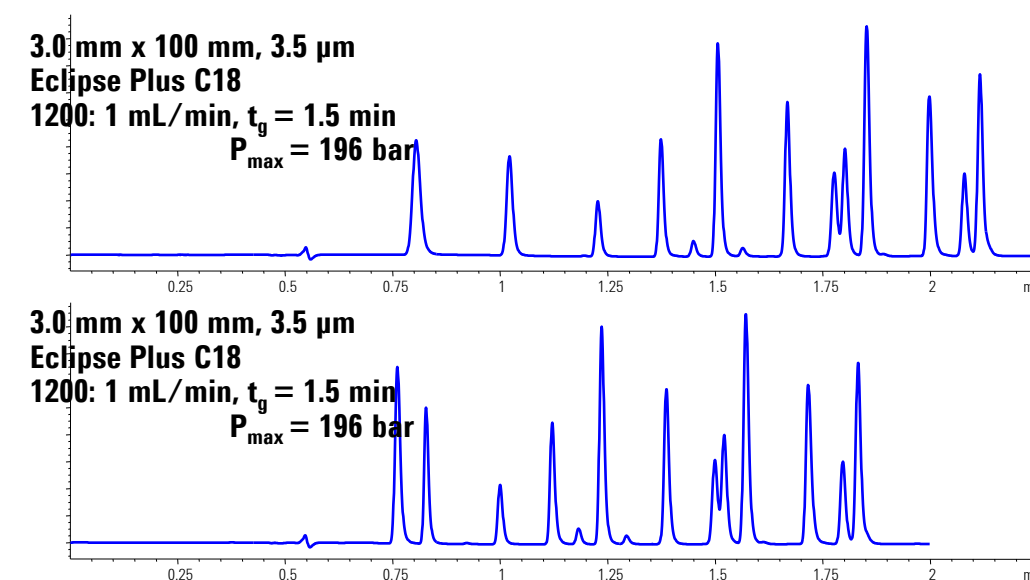
Scalability Across Columns and Instruments

5-µm ZORBAX vs. 3.5-µm ZORBAX Columns on an Agilent 1200 RRLC

4.6 mm x 250 mm, 5 µm
Eclipse Plus C18
1200: 1 mL/min, t_0 = 8.8 min
P_{max} = 110 bar

4.6 mm x 150 mm, 5 µm
Eclipse Plus C18
1200: 1 mL/min, t_0 = 5.3 min
P_{max} = 77 bar

3.0 mm x 100 mm, 3.5 µm
Eclipse Plus C18
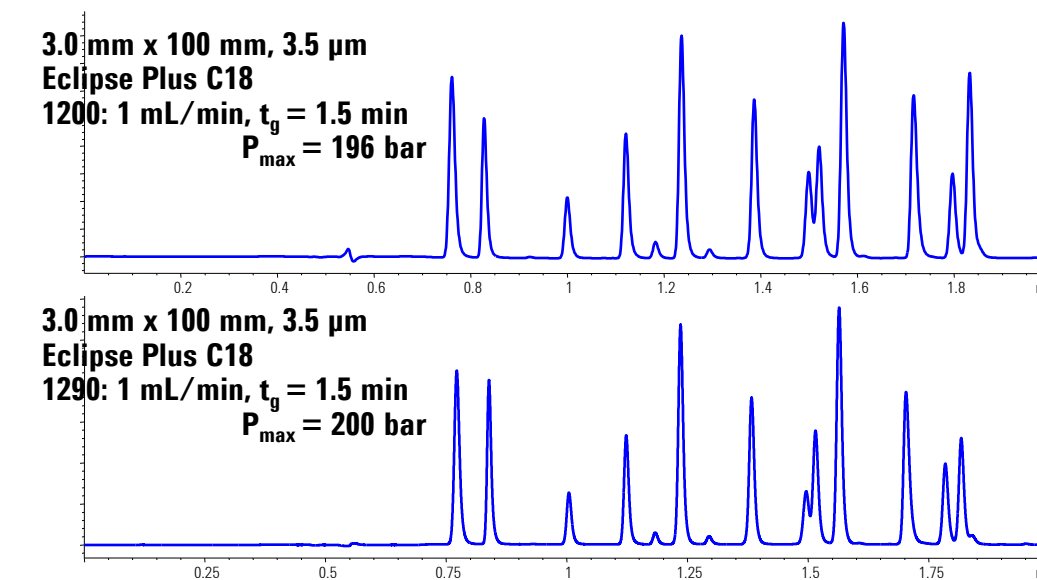1200: 0.4 mL/min, t_0 = 3.6 min
P_{max} = 87 bar

Selectivity is maintained when the 5-µm column is shortened from 250 mm to 150 mm, and when transferred to the 3.5-µm column. Some resolution, however, is lost with the shorter columns, most notably with ibuprofen, the last peak to elute.

Effect of System Delay Volume on a 3.0 mm x 100 mm, 3.5-µm ZORBAX Column

3.0 mm x 100 mm, 3.5 µm
Eclipse Plus C18
1200: 1 mL/min, t_0 = 1.5 min
P_{max} = 196 bar

3.0 mm x 100 mm, 3.5 µm
Eclipse Plus C18
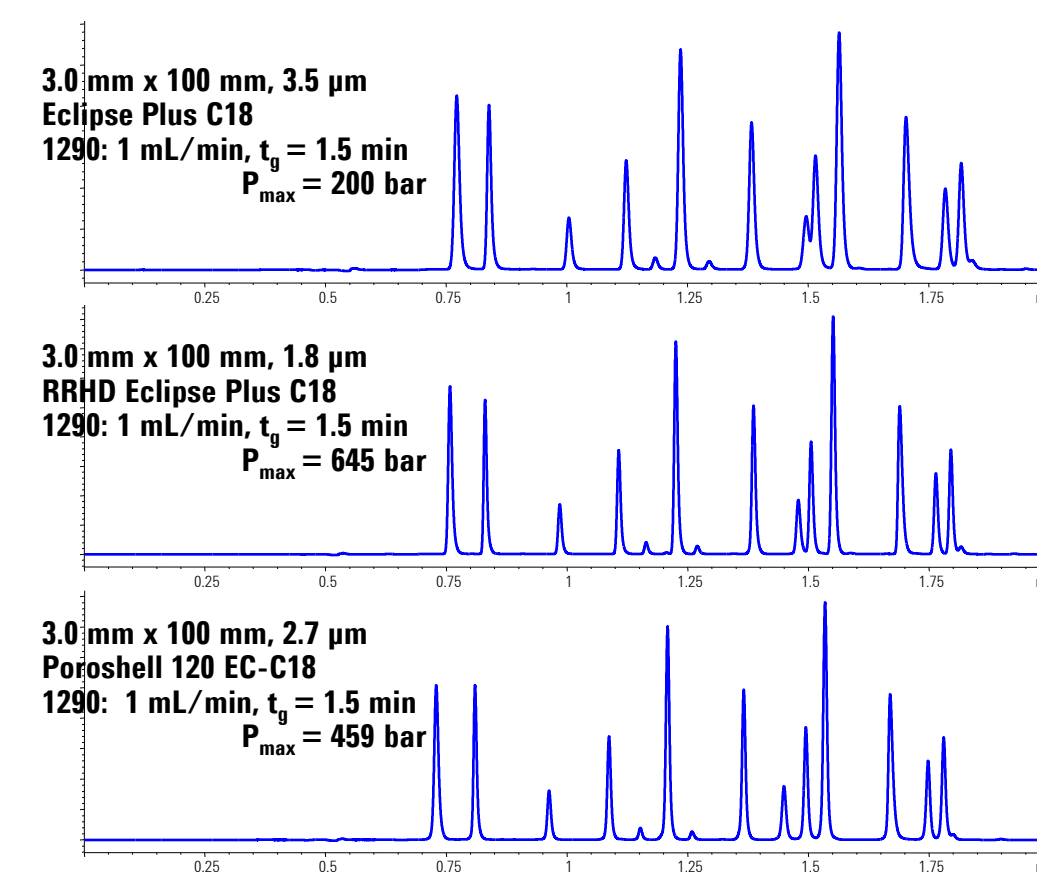1200: 1 mL/min, t_0 = 1.5 min
P_{max} = 196 bar

A manual change in system delay volume is the cause of the pronounced difference in the early eluting peaks. The relatively large delay volume of the 1200 RRLC system with a smaller dimension column (in this case 3 x 100 mm) causes delayed elution of all peaks. In order to make the chromatography more similar to the larger dimension columns shown above, the automatic system delay volume reduction feature of the 1200's ALS may be utilized (as shown in the bottom chromatogram).

Agilent 1200 RRLC vs. Agilent 1290 Infinity UHPLC with a 3.5-µm ZORBAX Column

3.0 mm x 100 mm, 3.5 µm
Eclipse Plus C18
1200: 1 mL/min, t_0 = 1.5 min
P_{max} = 196 bar

3.0 mm x 100 mm, 3.5 µm
Eclipse Plus C18
1290: 1 mL/min, t_0 = 1.5 min
P_{max} = 200 bar

Transferring the method from a 1200 RRLC to a 1290 Infinity UHPLC yields very similar results with this 3.5-µm column. It should be noted the automatic delay volume reduction feature of the 1200's ALS is used in this example. If a larger delay volume were needed to transfer a method, an isocratic hold may be added to the beginning of the gradient program, or extra capillary tubing may be added to the system between the pump and ALS to compensate for the extra system delay volume.
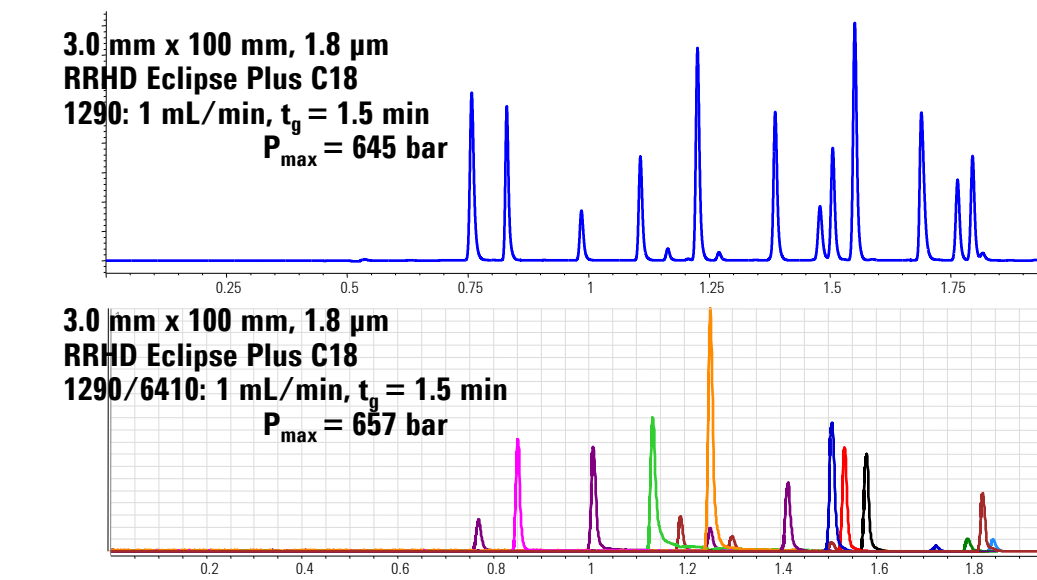
3.5-µm ZORBAX vs. 1.8-µm ZORBAX vs. 2.7-µm Poroshell 120 on an Agilent 1290 Infinity UHPLC

3.0 mm x 100 mm, 3.5 µm
Eclipse Plus C18
1290: 1 mL/min, t_0 = 1.5 min
P_{max} = 200 bar

3.0 mm x 100 mm, 1.8 µm
RRHD Eclipse Plus C18
1290: 1 mL/min, t_0 = 1.5 min
P_{max} = 645 bar

3.0 mm x 100 mm, 2.7 µm
Poroshell 120 EC-C18
1290: 1 mL/min, t_0 = 1.5 min
P_{max} = 459 bar

Totally porous 1.8-µm and 3.5-µm ZORBAX columns yield the same selectivity, while superficially porous 2.7-µm Poroshell 120 results in very similar selectivity as a result of similar bonding chemistry. Both the 1.8-µm ZORBAX and 2.7-µm Poroshell 120 provide better resolution than the 3.5-µm ZORBAX column of the same dimension.
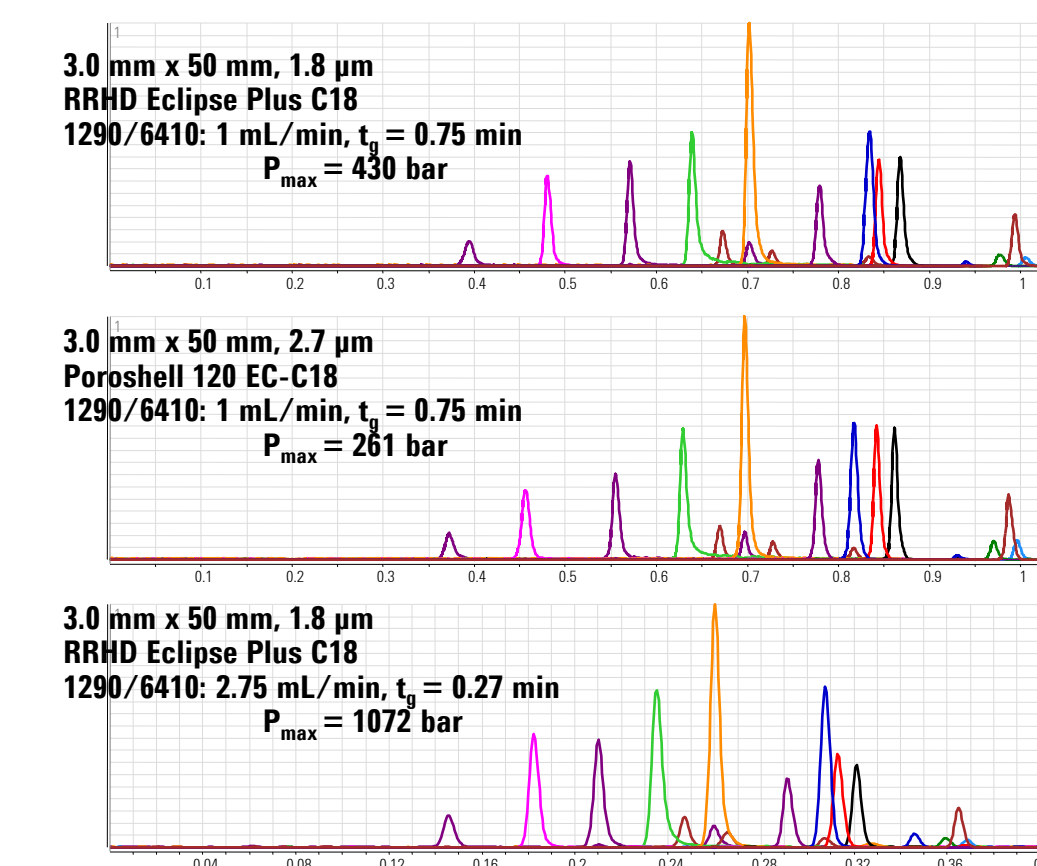
Scalability Across Columns and Instruments

Agilent 1290 Infinity UHPLC vs. Agilent 1290/6410 Infinity UHPLC/MS with a 1.8-µm ZORBAX Column

3.0 mm x 100 mm, 1.8 µm
RRHD Eclipse Plus C18
1290: 1 mL/min, t_0 = 1.5 min
P_{max} = 645 bar

3.0 mm x 100 mm, 1.8 µm
RRHD Eclipse Plus C18
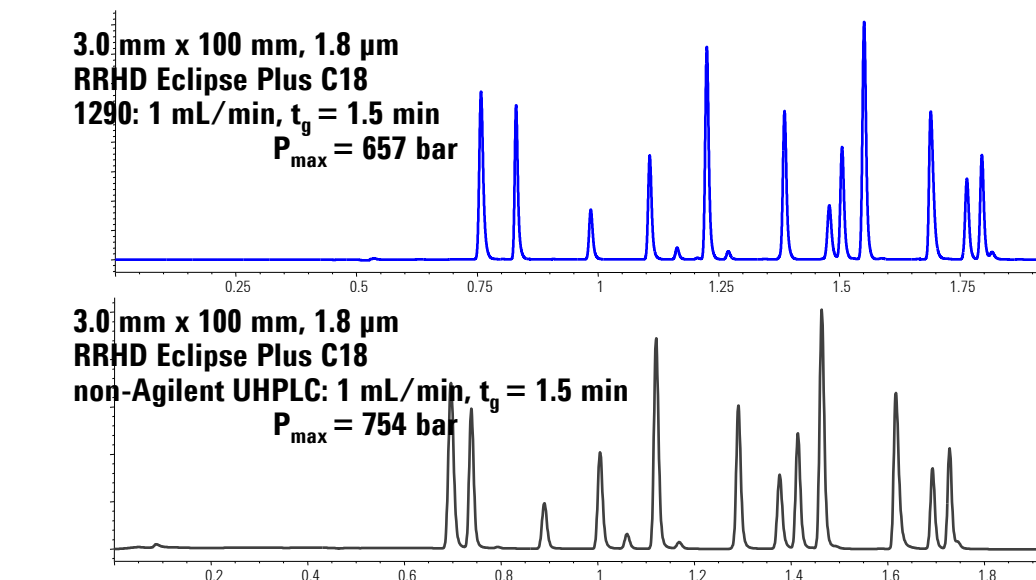1290/6410: 1 mL/min, t_0 = 1.5 min
P_{max} = 657 bar

Selectivity is maintained when transferring this method from a 1290 Infinity UHPLC with UV detection to a 1290 Infinity UHPLC with MS detection. 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.

High Throughput Methods with an Agilent 1290/6410 Infinity UHPLC/MS

3.0 mm x 50 mm, 1.8 µm
RRHD Eclipse Plus C18
1290/6410: 1 mL/min, t_0 = 0.75 min
P_{max} = 430 bar

3.0 mm x 50 mm, 2.7 µm
Poroshell 120 EC-C18
1290/6410: 1 mL/min, t_0 = 0.75 min
P_{max} = 261 bar

3.0 mm x 50 mm, 1.8 µm
RRHD Eclipse Plus C18
1290/6410: 2.75 mL/min, t_0 = 0.27 min
P_{max} = 1072 bar

Due to the highly selective nature of MS detection, this analysis can be sped up, taking full advantage of the high pressure limit of the RRHD column and the low back pressure generated by the Poroshell 120 column. The RRHD analysis can be sped up significantly more, resulting in a 0.4 minute run time for these 15 compounds.

Agilent 1290 Infinity UHPLC vs. non-Agilent UHPLC with a 1.8-µm ZORBAX Column

3.0 mm x 100 mm, 1.8 µm
RRHD Eclipse Plus C18
1290: 1 mL/min, t_0 = 1.5 min
P_{max} = 657 bar

3.0 mm x 100 mm, 1.8 µm
RRHD Eclipse Plus C18
non-Agilent UHPLC: 1 mL/min, t_0 = 1.5 min
P_{max} = 754 bar

Agilent's RRHD columns can be run not only with Agilent's 1290 Infinity UHPLC, but with a non-Agilent UHPLC as well. The overall analysis is similar, but could use slight modifications to resolve the last peak, ibuprofen. Smaller ID capillary tubing in the non-Agilent UHPLC is likely the cause of increased system pressure and reduced delay volume causing all peaks to elute earlier.

Conclusions

Agilent ZORBAX columns offer the same selectivity in multiple particle size options, including 5, 3.5 and 1.8-µm

Poroshell 120 has similar selectivity as compared to the ZORBAX columns with efficiency close to 1.8-µm, 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

Methods can easily be transferred from 1200 RRLC systems to 1290 Infinity UHPLC systems; however there is a noticeable difference in early eluting peaks which the automatic delay volume reduction function in the ALS corrects

Transferring methods to MS is easy, as it has no significant effects on the chromatography, other than a small increase in extra column volume

Using MS detection can allow for fast analyses because of its more selective nature when detecting co-eluting peaks

Agilent ZORBAX RRHD columns, with their 1200 bar pressure limits can easily be run on a non-Agilent UHPLC without significant method modification