

# Scalability of Agilent Columns Across HPLC and UHPLC Instruments

# **Application Note**

Pharmaceutical

# 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.



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# **Experimental**

# **LC Method Parameters**

An Agilent 1200 RRLC, Agilent 1290 Infinity UHPLC, Agilent 1290/6410 Infinity UHPLC/MS 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 <sub>g</sub> ) 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 $\mu g/mL$ (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**

 $\begin{aligned} k^* &= (t_g F)/(d/2)^2 L(\Delta\%B) \\ \text{where:} \quad t_g = \text{gradient time} \\ F &= \text{flow rate} \\ d &= \text{column internal diameter} \\ L &= \text{column length} \\ \Delta\%B &= \text{change in organic content} \\ across gradient segment \end{aligned}$ 

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

#### **Equation 2**

$$\begin{split} t_{g2} &= (t_{g1}d_2^{-2}L_2F_1)/(d_1^{-2}L_1F_2) \\ \text{where:} \quad t_{g1} \text{ and } t_{g2} = \text{original and new gradient times} \\ F_1 \text{ and } F_2 = \text{original and new flow rates} \\ d_1 \text{ and } d_2 = \text{original and new column internal diameters} \\ L_1 \text{ and } L_2 = \text{original and new column lengths} \end{split}$$

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.

#### Critical Parameters of Agilent LC Systems Table 1.

	Agilent 1100/1200 Series 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	
Delay volume (μL)	600–900	600–800	600-800 (120*)	10–110	
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	100	
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		50300
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 <sup>^</sup>	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  $5-\mu m$  column is shortened from 250 mm to 150 mm, and when transferred to a 3 mm × 100 mm, 3.5- $\mu m$  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 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 the Agilent 1200 RRLC system with a smaller dimension column (in this case 3 mm × 100 mm,  $3.5 \mu$ m) 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's ALS autosampler can be used, as shown in the bottom chromatogram in Figure 2.

#### **Method Transfer across Agilent Instruments**

Transferring the method from a 1200 RRLC to an Agilent 1290 Infinity UHPLC yields very similar results with the 3.5- $\mu$ m column, as shown in Figure 3, when the automatic delay volume reduction feature of the 1200's ALS is used. As shown in Table 1, the delay volume of a 1290 Infinity UHPLC is substantially lower than the 1200 RRLC. Conversely, if a method originally developed on a 1200 needed to be transferred to a 1290, a larger delay volume would be needed. For this, an isocratic hold can be added to the beginning of the gradient program, or extra capillary tubing can be added to the system between the pump and ALS to compensate for the extra system delay volume.



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 RRLC without (top) and with (bottom) the delay volume reduction function on the autosampler.



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

#### **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 UHPLC.

#### **Method Transfer with Different Agilent Detectors**

Selectivity is maintained when transferring this method from an Agilent 1290 Infinity UHPLC with UV detection to a 1290 Infinity UHPLC 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 UHPLC with UV detection with the Agilent 1290/6410 Infinity UHPLC/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 UHPLC/MS.

### Agilent Columns on a non-Agilent Instrument

Agilent's RRHD columns can be run not only with the Agilent 1290 Infinity UHPLC, 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 UHPLC 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 RRLC systems to Agilent 1290 Infinity UHPLC systems. However, there is a noticeable difference in early eluting peaks that is corrected by the automatic delay volume reduction function in the Agilent ALS autosampler.

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 coeluting 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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