Scalability of Short RRHT Columns to Longer 3.5-µm and 5-µm Columns

Application

Pharmaceutical

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Abstract

ZORBAX particles in 1.8-, 3.5- and 5-µm diameters exhibit the same selectivity. This allows the expanding library of RRHT methods being developed today to be transferred to analytical scale methods in a straightforward fashion. It gives the method developer who is unable to upgrade to newer technology an opportunity to reproduce new methods on older technology. Several RRHT HPLC methods were easily scaled up to analytical-sized methods. These scaled up applications cover several bonded phase chemistries and gradients. All methods, including RRHT (1.8-µm columns), were carried out on an Agilent 1100 liquid chromatograph (LC). It is recommended that flow rate and data acquisition rate be examined after scaling up to optimize the method.

Introduction

Developing HPLC applications using rapid-resolution high-throughput (RRHT) columns is becoming more prevalent and routine, as faster separations are considered necessary for better laboratory efficiency. In many cases, older methods using standard analytical columns are scaled to shorter, highly efficient RRHT columns. However, longer 3.5- and 5-µm columns are well established in many labs and will likely continue to be widely used. Here we show several examples of HPLC methods performed on RRHT columns and analytical-sized columns. Although analysis times are longer, the separation is the same. Transferring a method developed on short RRHT columns to longer analytical-sized columns is straightforward, requiring little more than substituting columns. The rugged scalability of the ZORBAX particles between the 1.8-µm and the larger 3.5-µm to 5-µm columns allows the expanding library of high-throughput methods to be available for chromatographers employing the larger particle technology.

Experimental

Specific method parameters are listed in their associated figures. All separations were done on an Agilent 1100 liquid chromatograph (LC) with a binary pump, diode array detector, and semi-micro flow cell (G1315-60011). All liquid flow-path tubing connecting the 1100 modules was 0.12 mm id.

Results and Discussion

For a method to be successfully scaled down from conventional “analytical scale” technology to high-throughput technology, some general parameters must be met: The stationary phase packing must possess the same selectivity, independent of the particles’ size, to produce the same elution pattern. The HPLC instrument must be able to handle higher system backpressure due to smaller parti-

cles packed into columns and possibly higher flow rates, and the LC must have a faster data acquisition rate to accurately quantify the narrower peak widths. There may be other instrument modifications needed for scaling down across smaller id columns (4.6 mm to 2.1 mm), such as a smaller detector cell and smaller tubing to minimize extra-column volume. The focus of this work, however, is scaling up from RRHT methods to conventional analytical HPLC methods. The Agilent 1200 Rapid Resolution LC, therefore, may not be necessary; rugged, reproducible columns, though, are necessary.

Retention (k) and Selectivity (α) Factors

Column packing has many characteristics that influence the chromatographic separation: particle size, the particle size distribution, how well the column bed is packed, the surface area, the amount of bonded phase, and the chemistry (functionality) of the bonded phase. The first three characteristics are associated with the efficiency (N) term of the resolution equation:

$$\text{Resolution} = (¼)\,(\alpha - 1)\,\sqrt{N}\,\left(\frac{k}{k+1}\right)$$

These characteristics' effects on resolution are well known. For example, the fact that smaller particles increase efficiency is the basis for RRHT columns. The other mentioned characteristics are associated with the retention (k) and selectivity (α) terms of the resolution equation. If these column-packing characteristics are the same between particle sizes, then the retention, and consequently the selectivity factors, should be equivalent between particle sizes. In fact, that is what was found: Figure 1 shows very similar selectivity, scaling between 1.8-µm and 5-µm packing on two phases, ZORBAX StableBond-C8 and Eclipse XDB-C8. The differences in selectivity between the ZORBAX XDB-C8 and SB-C8 are caused by designed differences in bonding. Figure 2 is an example of ZORBAX 1.8-µm packings having the same selectivity as 3.5-µm packings. Again, the two different bonded phases exhibit different selectivity because of their different functionality, but they have essentially identical selectivity when comparing particle size. Having a variety of bonded phases available on several particle sizes is advantageous to method developers because it is a quick way to alter selectivity and resolution [1].

Scalability of an Isocratic Method

The reproducible selectivity among the several ZORBAX particle sizes makes the scalability of RRHT methods to analytical methods easy and straightforward by simply substituting columns. Figure 3 is an overlay of an RRHT method for sunscreen lotion active ingredients, scaled up to a 100-mm 3.5-µm Eclipse Plus-C18 column and then to a longer 150-mm 5-µm column. Because the different-sized ZORBAX particles have very similar retention and selectivity factors, the separation is practically identical. System pressure, as expected, is lessened as particle size is increased. This RRHT method, nonetheless, is easily carried out on an LC system without any worries about high pressure.

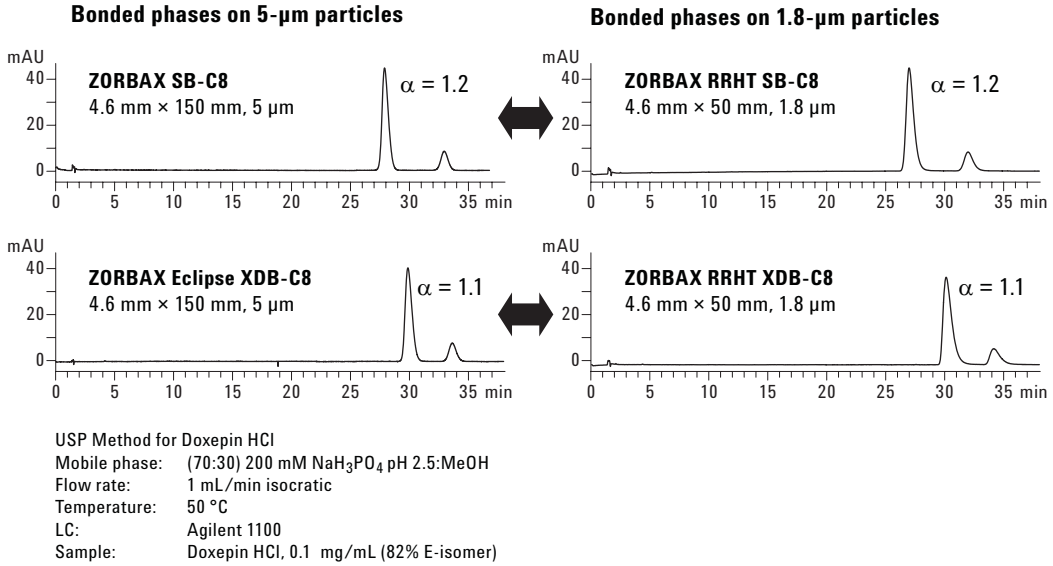
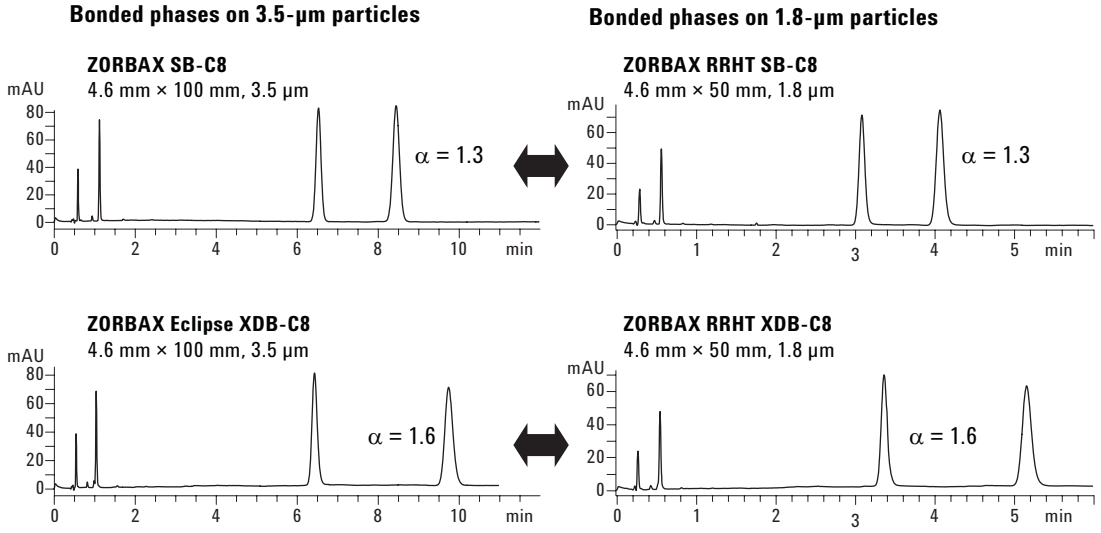


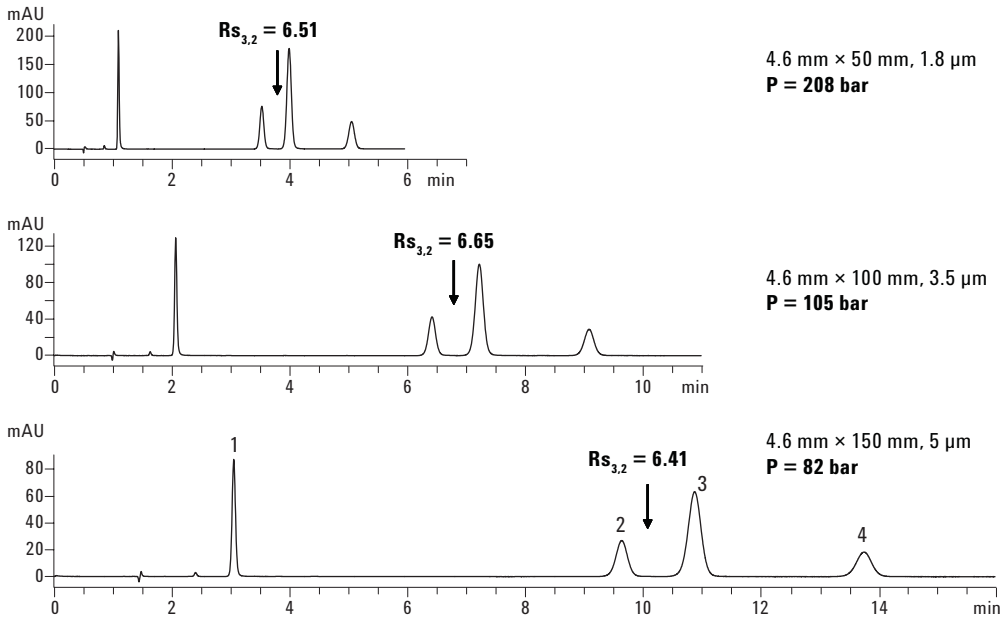
Figure 1. Reproducibility of selectivity (α) between 5-µm and 1.8-µm particles.



USP Method for ibuprofen oral suspension

Mobile phase:	(63:37) water:acetonitrile + 1.8 mL H_3PO_4
Flow rate:	2.0 mL/min isocratic
Temperature:	Ambient
LC:	Agilent 1100
Sample:	Children's ibuprofen oral suspension, with benzophenone as an internal standard prepared as described in USP

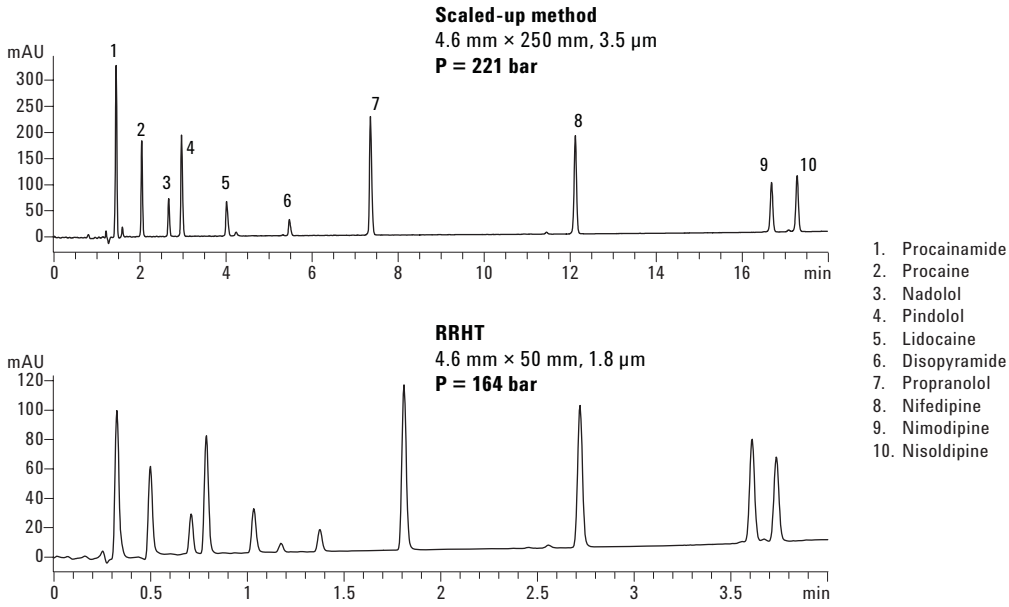
Figure 2. Reproducibility of selectivity (α) between 5-µm and 1.8-µm particles.



Columns:	ZORBAX Eclipse Plus-C18, as described below
Mobile phase:	A: water, B: MeOH, (15:85)
Temperature:	25 °C
Flow rate:	1 mL/min
Detection:	310, 4 nm, 0.5 s response time, semi-micro flow cell
Sample:	Sunscreens

1. 2-hydroxy-4-methoxybenzophenone
2. Padimate-O
3. 2-ethylhexyl trans-4-methoxycinnamate
4. 2-ethylhexyl salicylate

Figure 3. Scalability of ZORBAX Eclipse Plus-C18.

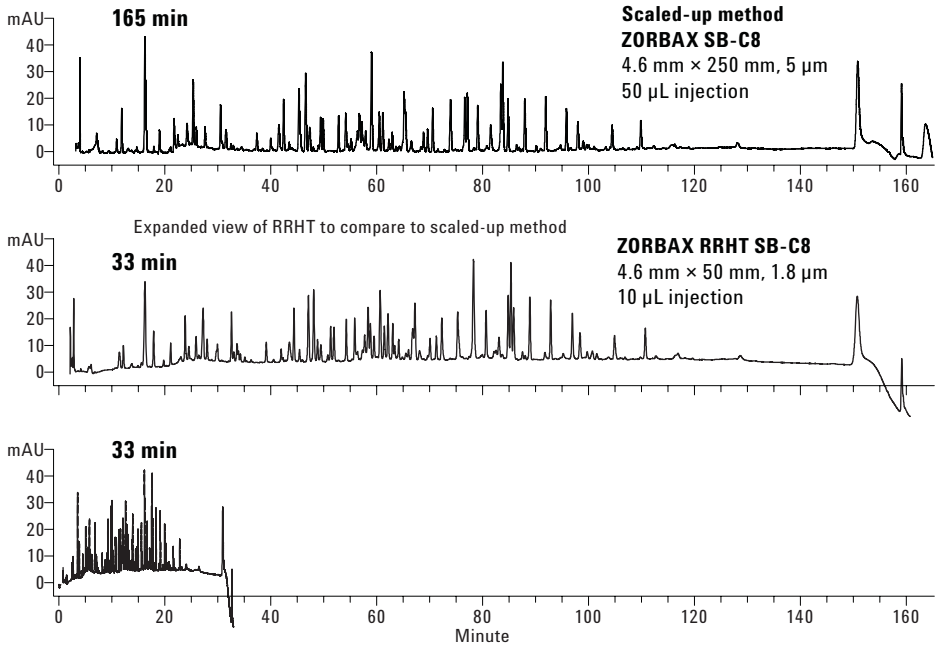


Columns: ZORBAX SB-C18
Mobile phase: A: 0.1% TFA, 5% MeCN, (v/v)
B: 0.08% TFA, 95%, 95% MeCN (v/v)
Sample: 0.1 mg/mL each × 0.5 µL injection
Temperature: 60 °C
Flow rate: 2 mL/min gradient
Detection: 230,16 nm Ref = 360,100
Response time: 0.5 s
LC: Agilent 1100

RRHT column	
minute	%B
0	12.5
3.5	60
4	60
4.01	12.5

Analytical column	
minute	%B
0	12.5
17.5	60
20	60
20.01	12.5

Figure 4. Gradient scalability of 1.8-µm to 3.5-µm columns.



Mobile phase: A: 0.1% TFA
B: 0.08% TFA in acetonitrile
Temperature: 50 °C
Flow rate: 1 mL/min gradient
Sample: BSA tryptic digest

RRHT column	
minute	%B
0	2
30	50
31	75
32	75
33	2

Analytical column	
minute	%B
0	2
150	50
155	75
160	75
165	2

Figure 5. Gradient scalability of 1.8-µm to 5-µm columns.

Scalability of Gradients

Two examples of gradient scale-up are shown in Figures 4 and 5, where analyses performed on short RRHT columns are transferred to longer columns. In addition to simply substituting columns to scale up the method, the gradient retention factor (k*) or gradient profile must be maintained. The gradient retention factor is defined:

$$\mathbf{k^* = t_G \times F/\Delta\Phi \times V_c}$$

Where:
t_G = Gradient time
F = flow rate
$\Delta\Phi$ = change in organic volume during the gradient
V_c = column volume

In order to keep the same gradient profile, the right side of the equation must be equivalent for both columns. The scale up in Figures 4 and 5 involves columns of different volumes (same diameter but different lengths), so one of the other variables in the equation must be changed proportionally to maintain k*. One way to keep the ratio equal is to multiply the gradient time by 5, since the analytical column volume is 5 times more than that of the RRHT column. The other options, multiply the flow by 5 or divide $\Delta\Phi$ by 5, are plainly impractical. Again, for both the cardiac drugs and the peptide digest separations, because the internal diameters of the RRHT and analytical-sized columns are the same, t_G is scaled proportionally to column length, resulting in similar chromatograms on different time scales (x-axis).

Optimizing Methods Designed for 1.8-µm Columns for 5-µm Columns

The column scalability examples presented above didn't need fine tuning or optimizing for sufficient resolution. One parameter that could be evaluated to improve the method, however, is flow rate. Operating at the optimum flow rate does improve efficiency and therefore does improve resolution. The optimum flow rate, more specifically the optimum mobile phase linear velocity, is described by van Deemter Plots for small porous particles.

Figure 6 is an overlay of typical van Deemter plots for 1.8-, 3.5-, and 5-µm porous particles. For each particle size the optimum linear velocity, u, is different (linear velocity is converted to flow rate). The optimum value is where H, the height equivalent of a theoretical plate, is smallest (plate number, N = column length/H). Operating so H is a small value means higher N, or column efficiency. The optimum value of u is highly dependent on specific operating conditions, such as mobile phase composition and temperature, so it has to be determined experimentally. The ideal u for 1.8-µm particles, however, is higher than the ideal u for 3.5-µm particles, which in turn is higher than that for 5-µm particles. Operating the flow rate near the ideal u, then adjusting it for maximum N for a particular method, is one way to optimize a scaled up or scaled down method.

Another method parameter to consider when converting 1.8-µm methods to 3.5-µm and 5-µm methods is detector data sampling rate. This is named "peak width" in ChemStation's diode array and multiple wavelength detector settings. To get optimum results, choose a peak width setting as close as possible to the narrowest peak of interest [2]. Because peak widths are narrower when eluted by an RRHT column (note the difference in peak widths between the RRHT and analytical column in Figure 3), not optimizing the sampling rate results in more data points per time unit than is necessary when using analytical-sized columns. This can lead to noisy baselines or unnecessarily large data files.

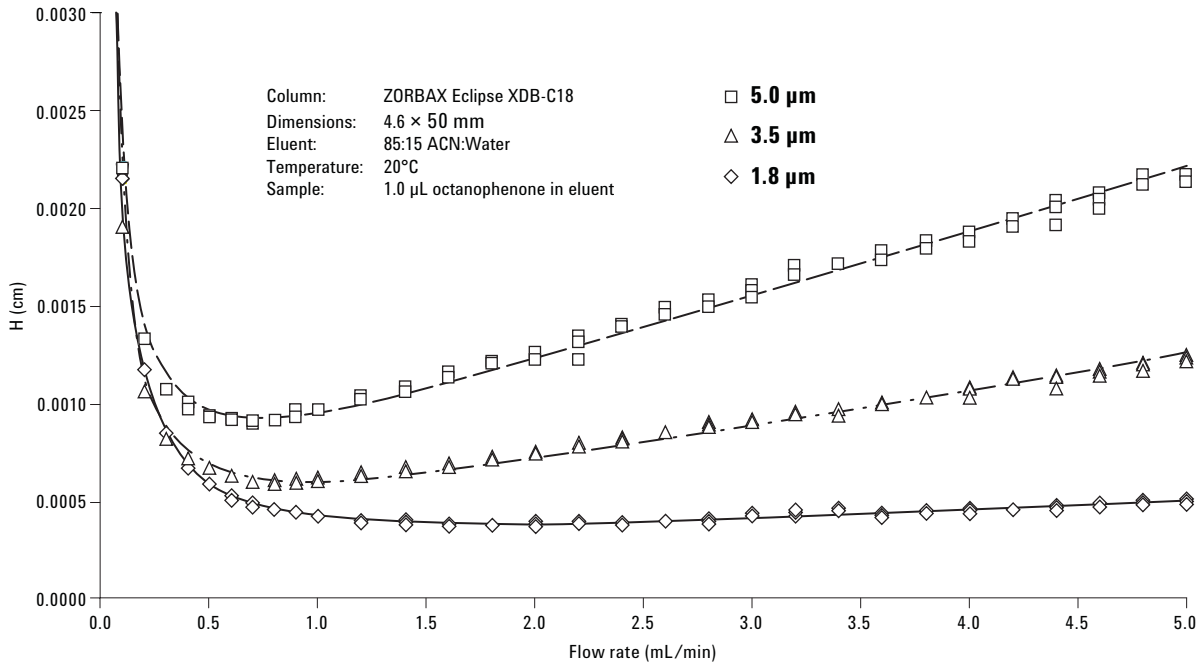


Figure 6. van Deemter plots of 1.8-, 3.5-, and 5-µm particles.

Conclusions

High-throughput methods and their associated technology are becoming widely accepted in HPLC labs. Conventional analytical-sized columns, however, are still well received and established. Transferring methods between the two technologies is straightforward when ZORBAX columns are used, especially when scaling up from RRHT to analytical columns. Here, using a variety of bonded phases, five different HPLC applications, isocratic analyses, and gradients were successfully performed on short RRHT columns and also on longer conventional columns. The only modification was substituting columns. This is possible because ZORBAX columns have very similar retention and selectivity, independent of particle size. The RRHT applications work on existing technology; upgrading instrumentation is not needed. Two method parameters, flow rate and data acquisition rate, though, should be fine tuned for their specific column configuration to improve chromatographic performance and the overall method.

References

1. J. W. Henderson, Jr. and W. J. Long, "Exploiting RRHT Columns with Different C18 Selectivities to Quickly Develop Methods for Endocannabinoids," Agilent Technologies publication 5989-6128EN, Jan 2007.
2. J. W. Henderson, Jr. and W. J. Long, "Optimize Data Sampling Rate to Take Advantage of RRHT Columns," Agilent Technologies publication 5989-5810EN, Nov 2006.

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