

# Biotage<sup>®</sup> Scaling Columns

Tools for Flash Chromatography Method Development

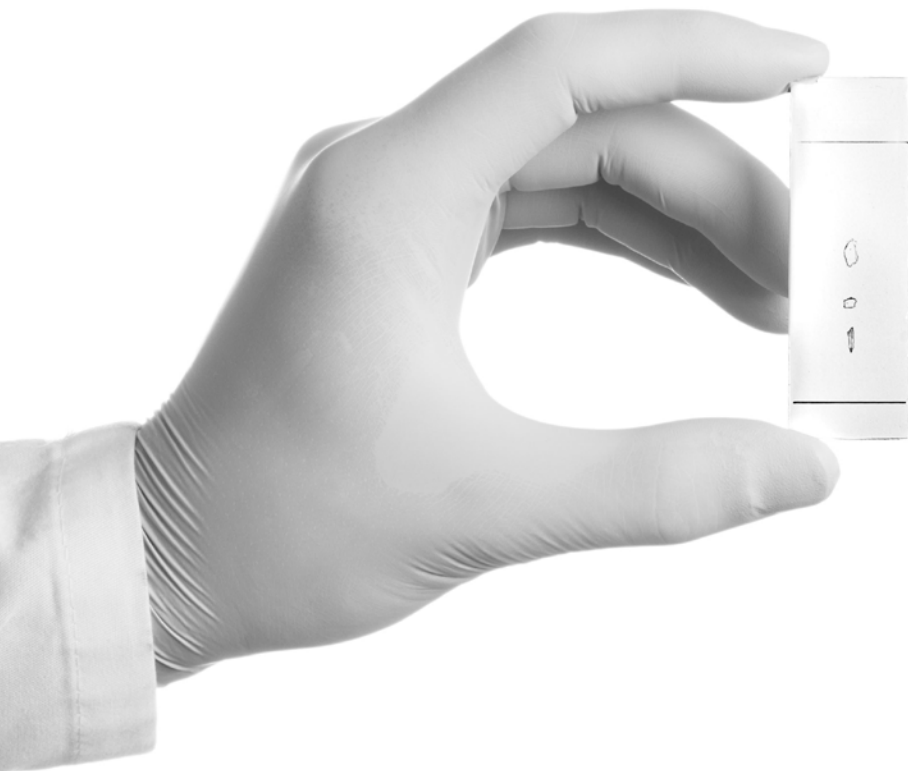


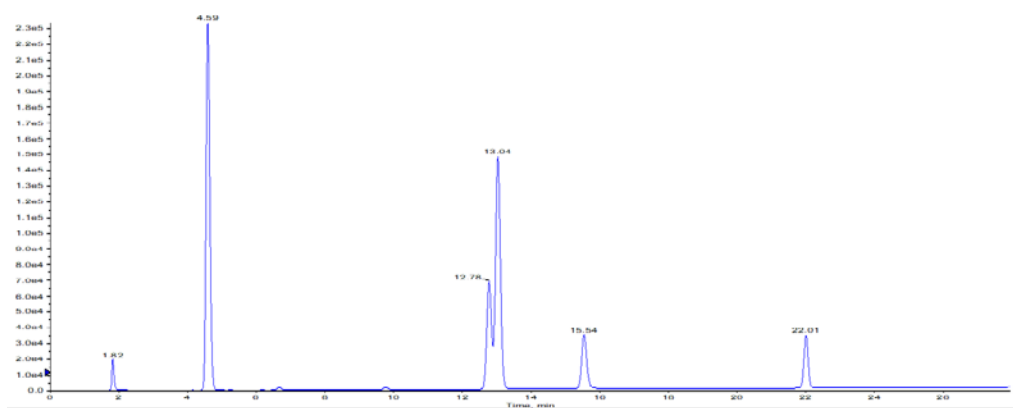
## The Alternative to TLC

Flash Chromatography method development has historically been done using TLC plates. While this technique works in normal phase (silica, amine-functionalized silica), differences in media properties between the TLC and flash column in reversed phase can provide different selectivity and not provide accurate method information. For reversed phase chromatography, TLC is quite limited and not very useful due to poor water wettability. An alternative approach is provided by scaling columns.

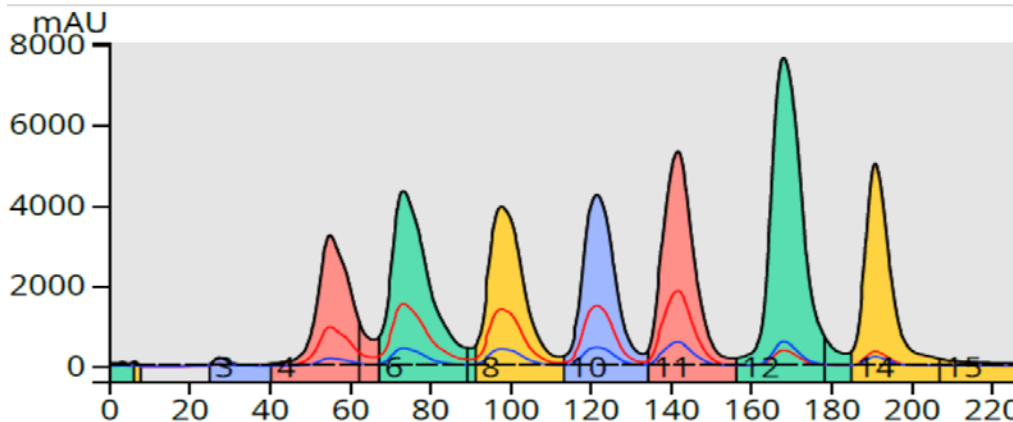
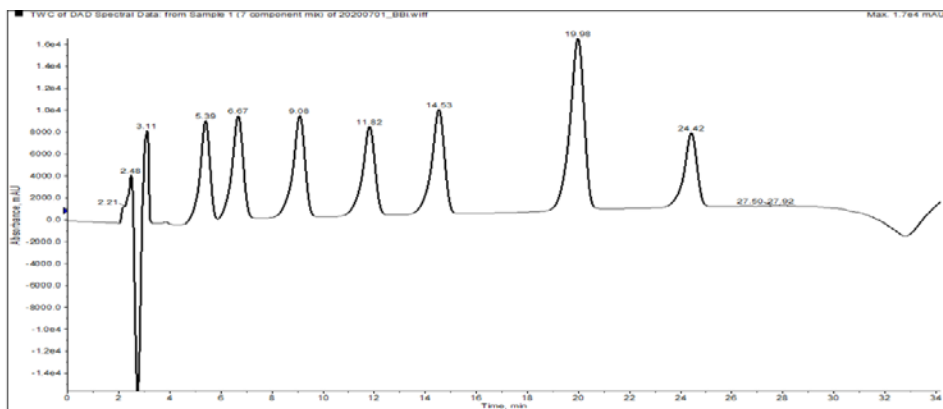
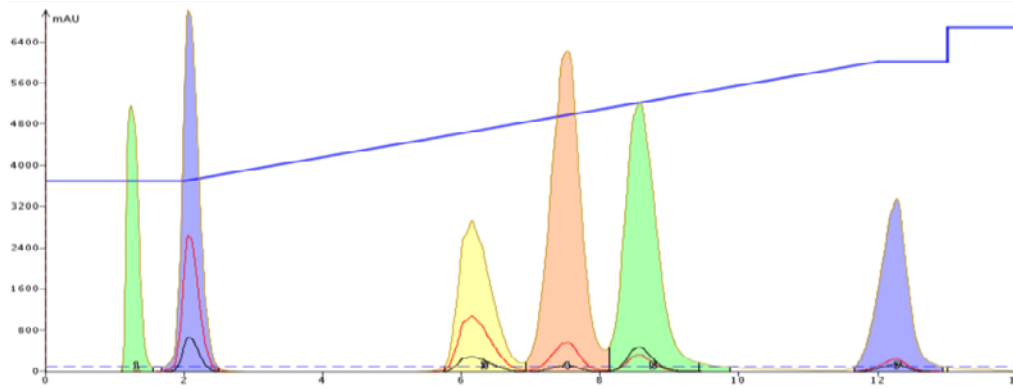
Commercially available reversed-phase HPLC columns can be used for flash chromatography method development, but differences in media chemistry (surface area, porosity, % carbon, etc.) can lead to altered selectivity compared to the flash column, Figure 1.

Because method development is important for optimal flash purification, Biotage created Scaling Columns. Scaling Columns are HPLC columns packed with the same media used in Biotage flash columns. The columns are designed for use on an HPLC for method development. Methods optimized using scaling columns are directly transferrable to flash chromatography using the same media and gradient, and eliminate selectivity differences, Figure 2.





**Figure 1.** Selectivity differences between a commercially available C18 HPLC column (top) and a Biotage® Sfär C18 column (bottom) are clearly evident which can present scale-up issues.



**Figure 2.** Method development using a Biotage® scaling column (top) provides identical selectivity to the Biotage® Sfär C18 column (bottom) which simplifies method transfer and scale-up.

# Guidelines for Converting HPLC Methods to Flash

Method development with reversed-phase HPLC can be as easy as creating a gradient method on an HPLC system and duplicating it on a flash system. The best results are achieved when your HPLC column is packed with the same media as the flash column (Biotage® scaling columns). Using a scaling column for method development eliminates any separation quality differences in resolution/efficiency impact on the separation related to particle size and media chemistry differences.

Using an off-the-shelf, commercially available HPLC column will certainly point you in the right direction regarding the gradient method but due to brand to brand media selectivity differences, these results may not translate as well to the flash method as you might want.

## Scaling Column-based Method Development

<b>Column size (mm x mm)</b>	4.6 x 250 (~2.5 grams)
<b>Media</b>	C18 or KP-NH
<b>Particle size (µm)</b>	C18: 25 KP-NH: 50
<b>Column volume (mL)</b>	2.35
<b>Flow rate (mL/min)</b>	1
<b>Linear velocity (cm/min)</b>	10.6
<b>Detection</b>	Diode array
<b>Solvent A</b>	C18: Water KP-NH: Hexane
<b>Solvent B</b>	C18: Methanol or acetonitrile KP-NH: Ethyl acetate
<b>Equilibration</b>	10% B for 7 min. (3 CV)
<b>Gradient segment 1</b>	10% B for 2.35 min. (1 CV)
<b>Gradient segment 2</b>	10% to 100% B in 23.5 min. (10 CV)
<b>Gradient segment 3</b>	100% B for 4.70 min. (2 CV)
<b>Run time, gradient (min)</b>	30.55

## On the HPLC

Create a 3-segment scaling column linear gradient using the method suggested below based on column volumes. The scaling column's column volume (CV) is the void time ( $t_0$ ) multiplied by the flow rate and is typically 2.35–2.6 mL.

### Equilibration

10% B for 3 CV at 1 mL/min (~7 min.)

### Segment 1

10% B for 1 CV at 1 mL/min (~2.35 min.)

### Segment 2

10% B to 100% B in 10 CV at 1 mL/min (~23.5 min.)

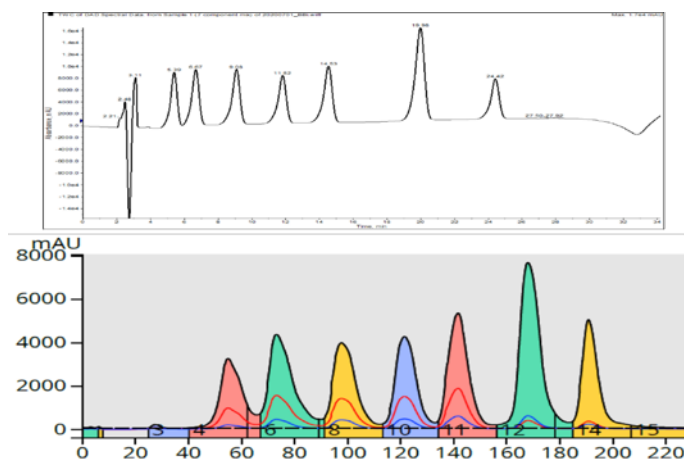
### Segment 3

100% B for 2 CV at 1 mL/min (~4.7 min.)

If your compounds elute too early, reduce the end % B to 50% and run the new gradient (do not change the run time or flow rates).

If your compounds elute late in the gradient, increase the start % B to 50% and run the new gradient (do not change the run time or flow rates).

Continue this process until you have an acceptable separation then transfer the method to your flash system using either a 6- or 12-gram C18 column (5- or 11-gram KP-NH), Figure 3.



**Figure 3.** A method developed on an HPLC using a C18 scaling column (top) and the flash chromatography results using the same method on a 12-gram Sfär C18 column (bottom). The separations are nearly identical.

## Determining Loading Capacity

1. Challenge the flash column's loading capacity until your target compound has achieved baseline resolution from its nearest neighbors, this will be your loading limit which can be scaled up to any size flash cartridge.
2. To scale-up the purification, choose the proper column size for the amount of material you need to purify, check Table 1.
3. Set your larger-scale flash column flow rate to match the small-scale column linear velocity using Table 2.

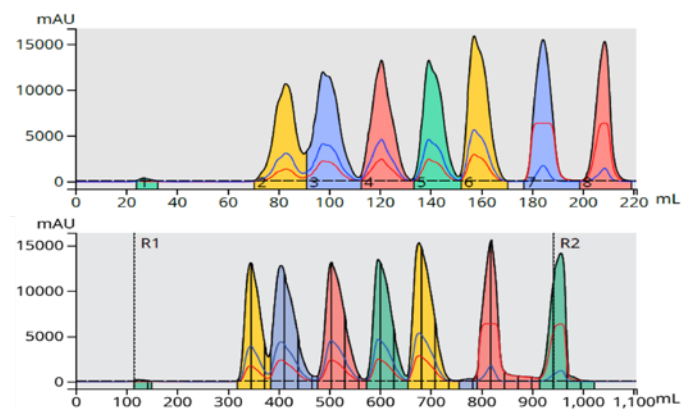
Column size	Scale factor
6-gram	1
12-gram	2
30-gram	5
60-gram	10
120-gram	20
240-gram	40
400-gram	67

**Table 1.** Scale up factors.

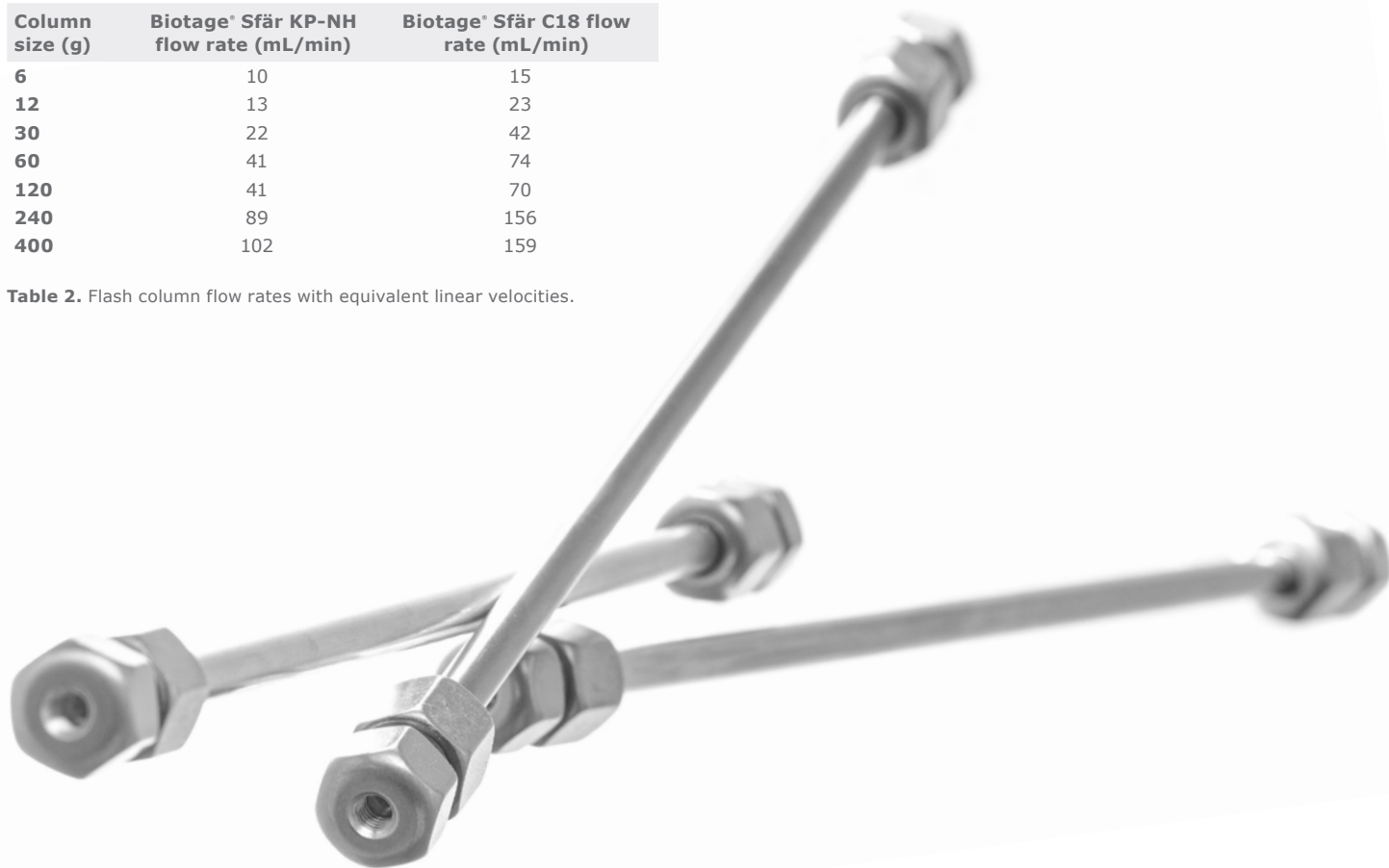
Column size (g)	Biotage® Sfär KP-NH flow rate (mL/min)	Biotage® Sfär C18 flow rate (mL/min)
6	10	15
12	13	23
30	22	42
60	41	74
120	41	70
240	89	156
400	102	159

**Table 2.** Flash column flow rates with equivalent linear velocities.

If you follow this process you should find success scaling up a method, Figure 4.

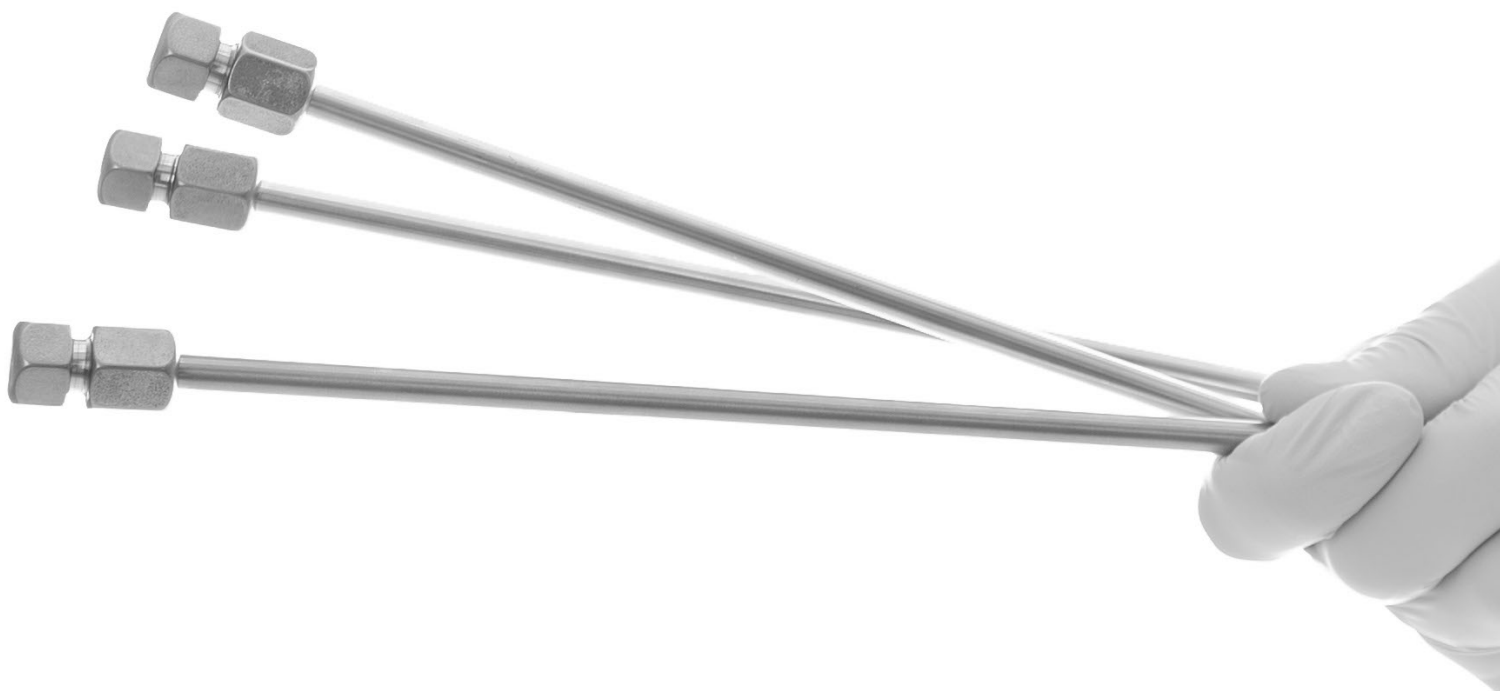


**Figure 4.** Direct flash chromatography scale-up from a 12-gram C18 column with a 140 mg load (top) to a 60-gram C18 column with a 700 mg load (bottom).



# Ordering Information

Part number	Description	Quantity	Part number	Description	Quantity
<b>Scaling Columns</b>					
<b>S1UL-0401-93050</b>	Biotage® HP-Sphere C-18 (30 µm) scaling column 4.6 x 250 mm	1	<b>FSAD-0909-0005</b>	Biotage® Sfär KP-Amino D Duo 50 µm 5 g	20
<b>S1N0-0909-93050</b>	Biotage® KP-NH (50 µm) scaling column 4.6 x 250 mm	1	<b>FSAD-0909-0011</b>	Biotage® Sfär KP-Amino D Duo 50 µm 11 g	20
<b>Lab Scale Flash Columns</b>					
<b>FSUD-0401-0006</b>	Biotage® Sfär C18 D Duo 100 Å 30 µm 6 g	2	<b>FSAD-0909-0028</b>	Biotage® Sfär KP-Amino D Duo 50 µm 28 g	20
<b>FSUD-0401-0012</b>	Biotage® Sfär C18 D Duo 100 Å 30 µm 12 g	2	<b>FSAD-0909-0055</b>	Biotage® Sfär KP-Amino D Duo 50 µm 55 g	10
<b>FSUD-0401-0030</b>	Biotage® Sfär C18 D Duo 100 Å 30 m 30 g	2	<b>FSAD-0909-0110</b>	Biotage® Sfär KP-Amino D Duo 50 µm 110 g	10
<b>FSUD-0401-0060</b>	Biotage® Sfär C18 D Duo 100 Å 30 µm 60 g	2	<b>FSAD-0909-0220</b>	Biotage® Sfär KP-Amino D Duo 50 µm 220 g	4
<b>FSUD-0401-0120</b>	Biotage® Sfär C18 D Duo 100 Å 30 µm 120 g	2	<b>FSAD-0909-0380</b>	Biotage® Sfär KP-Amino D Duo 50 µm 380 g	4
<b>FSUD-0401-0240</b>	Biotage® Sfär C18 D Duo 100 Å 30 m 240 g	1	<b>Scale-up Flash Columns</b>		
<b>FSUD-0401-0400</b>	Biotage® Sfär C18 D Duo 100 Å 30 µm 400 g	1	<b>FSUL-0401-0950</b>	Biotage® SNAP Ultra C18 950 g	1
			<b>FSUL-0401-1850</b>	Biotage® SNAP Ultra C18 1850 g	1





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