

How Important is Flow Rate in Flash Chromatography?

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Chemists often wonder about the influence of flow rate on their flash chromatography. Typically, the questions are:

1. If I slow the flow rate will my separation improve?
2. If I speed the flow rate will my separation improve and finish sooner?

This Application Note answers both questions.

The influence of linear velocity on the chromatographic separation, especially in regards to purification scale-up is an important variable. Flow rate impacts linear velocity which influences mass transfer kinetics and the separation, regardless of column size.

For every particle size media, there is an optimal linear velocity, or flow rate.

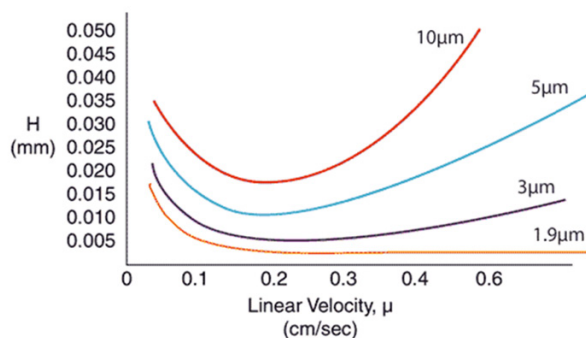


Figure 1. Linear velocity impact on separation performance and indicated by H, or height equivalent to a theoretical plate. Smaller particles have larger optimal linear velocities and are impacted less by increased flow rate.

Flash chromatography columns are packed with larger particle size media than those in Figure 1 meaning that straying from the optimal flow can have serious consequences on the purification quality. But what does this translate to in regard to reality? Do I need to have the flow rate set precisely to the optimal conditions? How much leeway is there?

The impact that flow rate has was determined, using a 12-gram Biotage® Sfär C18 column and a two-component sample. The same separation using the same method was utilized but with increasing flow rates starting at 20 mL/min and ending at 50 mL/min. The results indicated that, at least with this easy separation, flow rate (linear velocity) really had little impact on the separation, Figure 2.

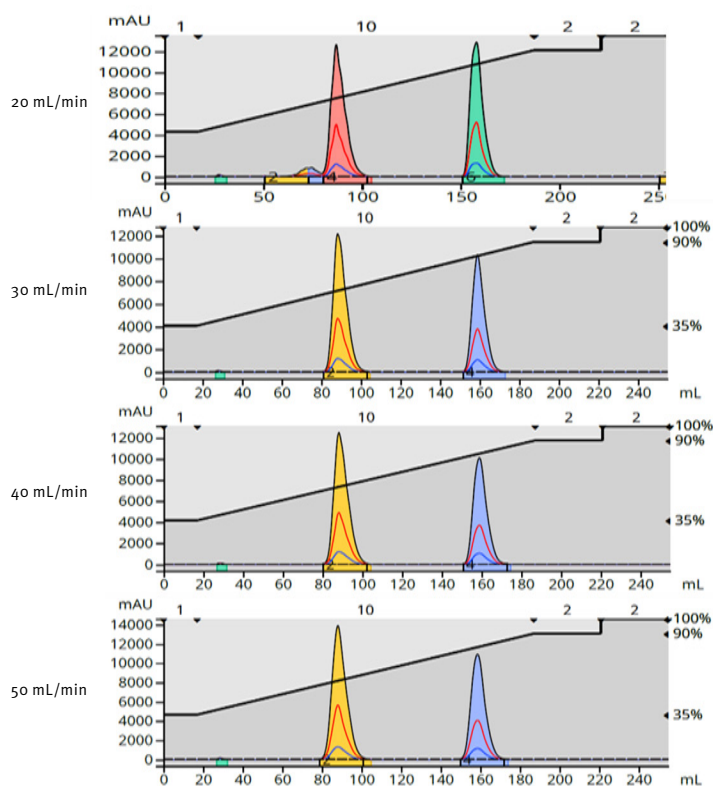


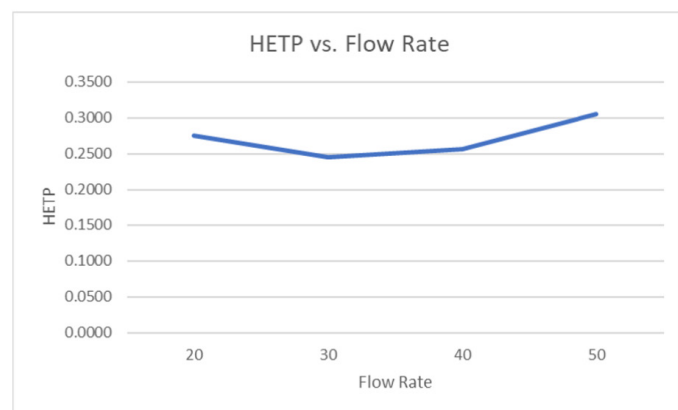
Figure 2. Impact of increasing flow rate on a binary component sample separation.

What this does show is that peak width decreased between 20 and 30 mL/min, especially for the first eluting compound. Though hard to see in the chromatograms, peak widths did increase at both 40 and 50 mL/min. This peak width analysis data is expressed in terms of height equivalent to a theoretical plate or HETP, which is a measure of peak width at a specific peak retention time or volume, Table 1.

Table 1. Impact of flow rate on HETP (Height Equivalent to a Theoretical Plate). Lower number equal better performance.

Flow Rate (mL/min)	HETP 1	HETP 2
20	0.2753	0.0619
30	0.2452	0.0616
40	0.2564	0.0771
50	0.3048	0.0801

Plotted as in Figure 1, the data show the optimal flow rate to be 30 mL/min, Figure 3. However, in this instance, performing the separation at 50 mL/min has no negative impact and speeds the purification. More challenging purifications, however, will greatly benefit from operating at the optimal flow rate, especially when there is a need to scale-up the purification.

**Figure 3.** Flow rate impact on HETP, or separation efficiency. Lower values equal better performance.

So, how do you calculate linear velocity?

It's quite simple, just divide the column volume by flow rate then divide column length or bed depth by the value in the first calculation.

Equation 1. CV (mL)/FR (mL/min) = minutes

Equation 2. BD (mm)/minutes = mm/min

Keep this in mind when scaling up a purification, Table 2.

Table 2. Biotage® Sfär Column dimensions.

Column	ID (cm)	Length (cm)
5 g	1.64	4.30
10 g	2.00	5.60
25 g	3.02	7.50
50 g	3.94	7.80
100 g	3.94	15.80
200 g	5.92	14.50
350 g	5.92	25.00
750 g	8.20	29.10
1500 g	10.70	32.80

If you are interested in learning more about flash chromatography, visit the Biotage blog site at biotage.com.

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