The Effect of Wetting and Equilibrating C18 RP Columns at Higher Flowrate and Pressure in Flash Chromatography

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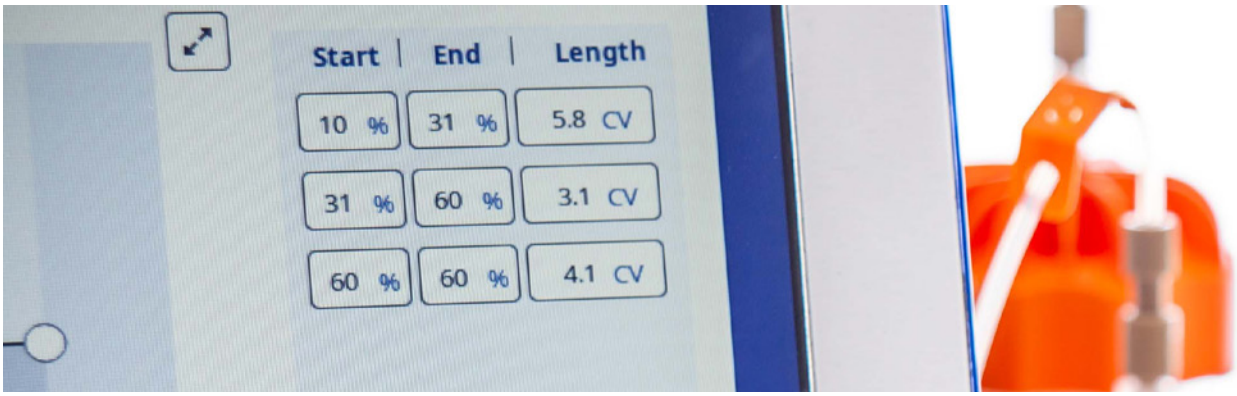


Introduction

Reversed phase flash chromatography columns are packed and transported with dry media and the need of efficient wetting (removal of air) and equilibration of the column prior to adding the sample to be purified is crucial for efficient chromatography.

Air bubbles disturb the separation and are generally formed and released during the mixing of water and an organic solvent, typically methanol or acetonitrile. Another source of air bubbles is air released from the media, if inefficient wetting and equilibration is obtained. With the smaller particles and the higher surface area in this media, higher flowrate and/or pressure is needed for efficient wetting of the column. Trapped air or dry patches causing irregularities in the chromatogram may increase the risk for erroneous fractionation. This risk is higher if compounds with low UV absorption are separated.

In this application, we present the results in chromatography achieved, when C18 columns of different sizes are wetted and equilibrated at different flowrates/pressures, but the chromatography is run at the same flowrate.

Experimental

The Biotage® Selekt instrument enables flowrates up to 300 mL/min and pressures up to 30 bar, compared to the Isolera instrument where the corresponding values are 200 mL/min and 10 bar. Having the same C18 media, Biotage® SNAP Ultra C18 columns can be run at maximum 7 bar, whilst Biotage® Sfär C18 D columns can be run at 17 bar due to improved construction and reinforced cap and bottom on the Sfär column.

A sample of equal amounts of two similar compounds, dimethyl phthalate and dibutyl phthalate (Figure 1) were separated using SNAP Ultra C18 and Sfär C18 D columns of different sizes on a Selekt instrument and the chromatograms were compared.

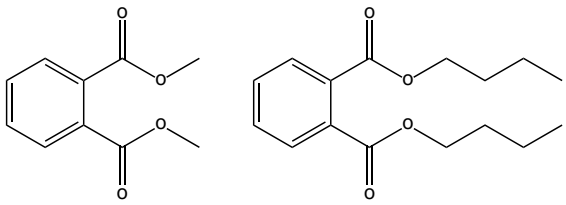


Figure 1. Structure formulas of dimethyl phthalate (left) and dibutyl phthalate (right).

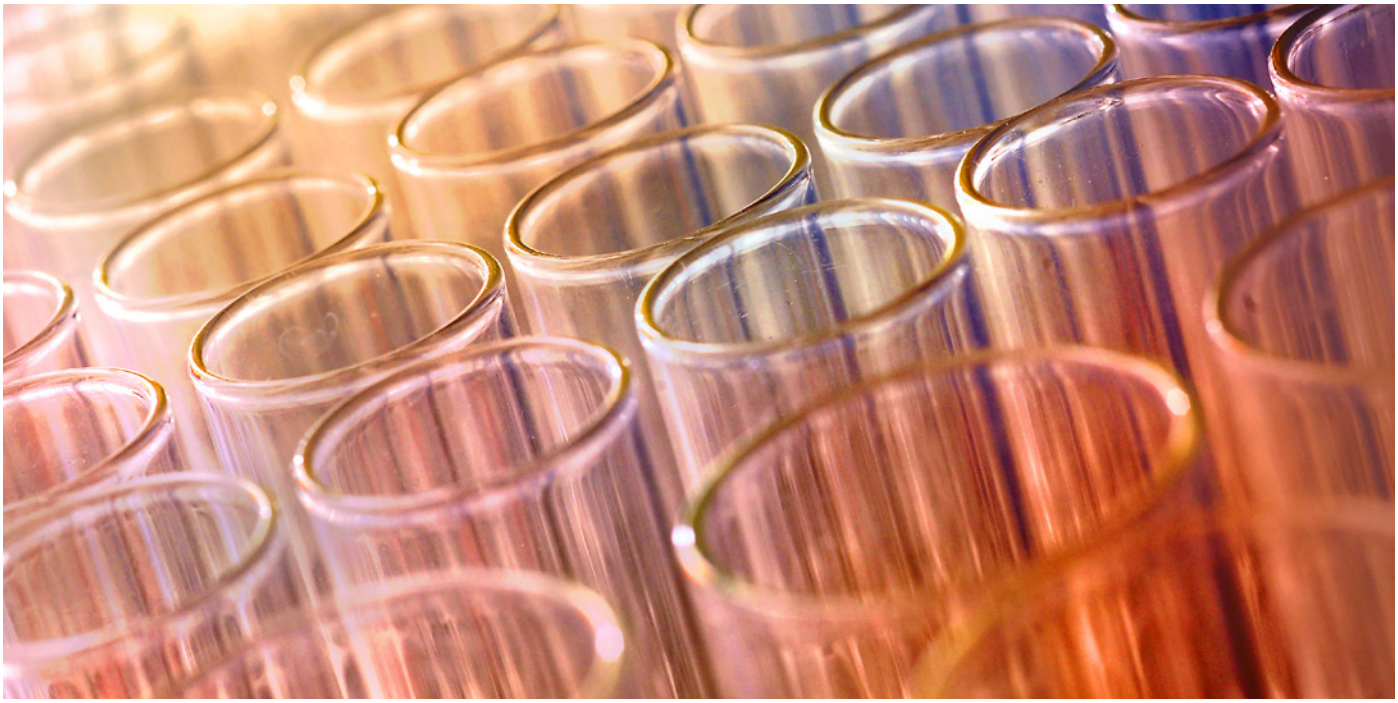
Sample Solution

- Dimethyl phthalate 2.0 g
- Dibutyl phthalate 2.0 g
- Water 2 mL
- Acetonitrile 12 mL

Concentration: 0.25 g/mL, Loading: 1% (by mass)

Chromatography – Conditions

- Wetting: 100% acetonitrile, 5 CV
- A: water, B: acetonitrile
- Equilibration: 90 %B, 3 CV
- Gradient run: 90 %B 1 CV, 90–100 %B 0.5 CV, 100 %B 2 CV
- 254 nm (Show), 280 nm (Show), No fraction collection



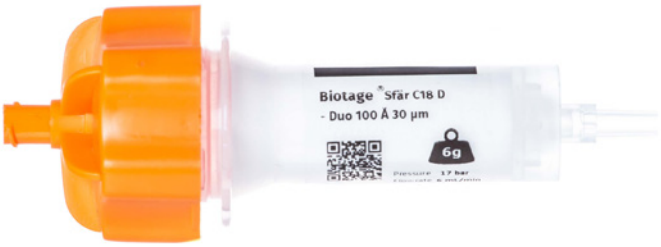
Results

Biotage® Sfär C18 D 6 g

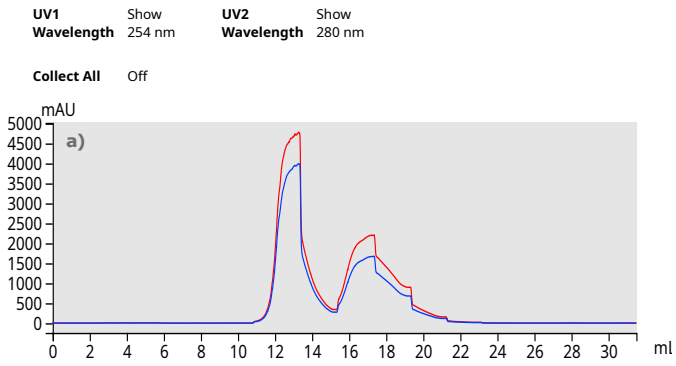
Here, we compare the wetting and the equilibration of Biotage® Sfär C18 D 6 g columns at different flowrates. The actual separation on the two different columns was performed at the same flowrate.

In the first run, a Sfär C18 D 6 g cartridge was wetted, equilibrated and run, all at 6 mL/min, and 0.25 mL sample (70 mg) was separated (Figure 2a).

In the second run, a Sfär C18 D 6 g cartridge was wetted at 300 mL/min, equilibrated at 150 mL/min and run at 6 mL/min. Again, 0.25 mL sample (70 mg) was separated (Figure 2b).



In the first run (Figure 2a), air bubbles released during the chromatography reach the UV cell and give disruptions in the UV curve. In the chromatogram in Figure 2b), the UV curve is smooth, since no air bubbles are released from the column.



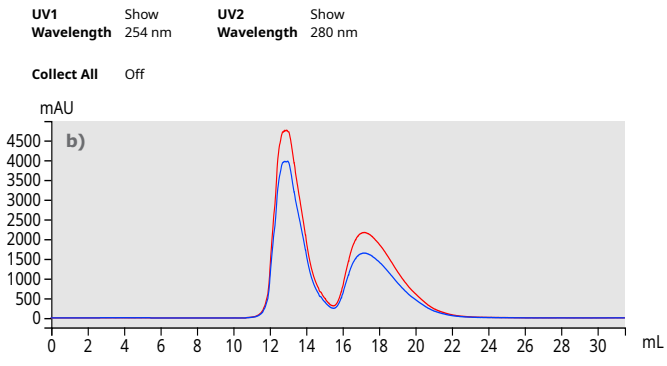


Figure 2a). Biotage® Sfär C18 D 6 g wetted and equilibrated at 6 mL/min.

Figure 2b). Biotage® Sfär C18 D 6 g wetted at 300 mL/min and equilibrated at 150 mL/min.

Comparison of Biotage® SNAP Ultra C18 and Biotage® Sfär C18 D 12g

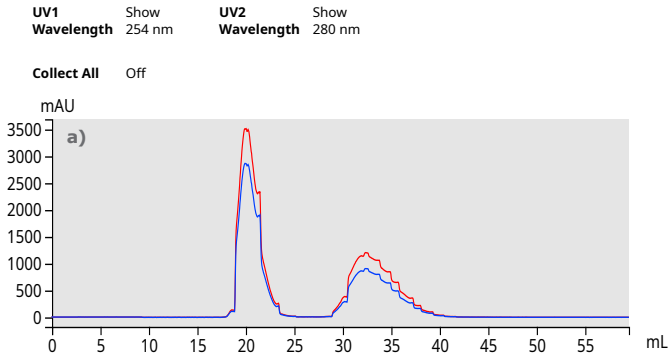
In this section, we compare columns with the same separation media, but wetted and equilibrated at different flowrates. The actual separation on the two different columns was performed at the same flowrate.

In the first run, a SNAP Ultra C18 12 g cartridge was wetted, equilibrated and run, all at 12 mL/min, and 0.5 mL sample (140 mg) was separated (Figure 3a).

In the second run, a Sfär C18 D 12 g cartridge was wetted at 300 mL/min, equilibrated at 150 mL/min and run at 12 mL/min. Again, 0.5 mL sample (140 mg) was applied (Figure 3b).

In the chromatogram in Figure 3a), the air bubbles released during the chromatography, reach the UV cell and give disruptions in the UV curve.

In the chromatogram in Figure 3b), the UV curve is smooth, since no air bubbles are released from the column.



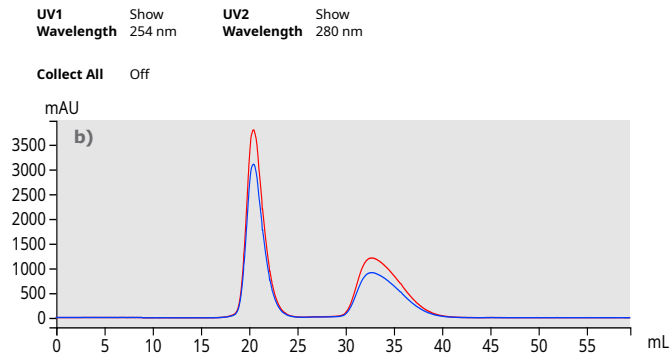
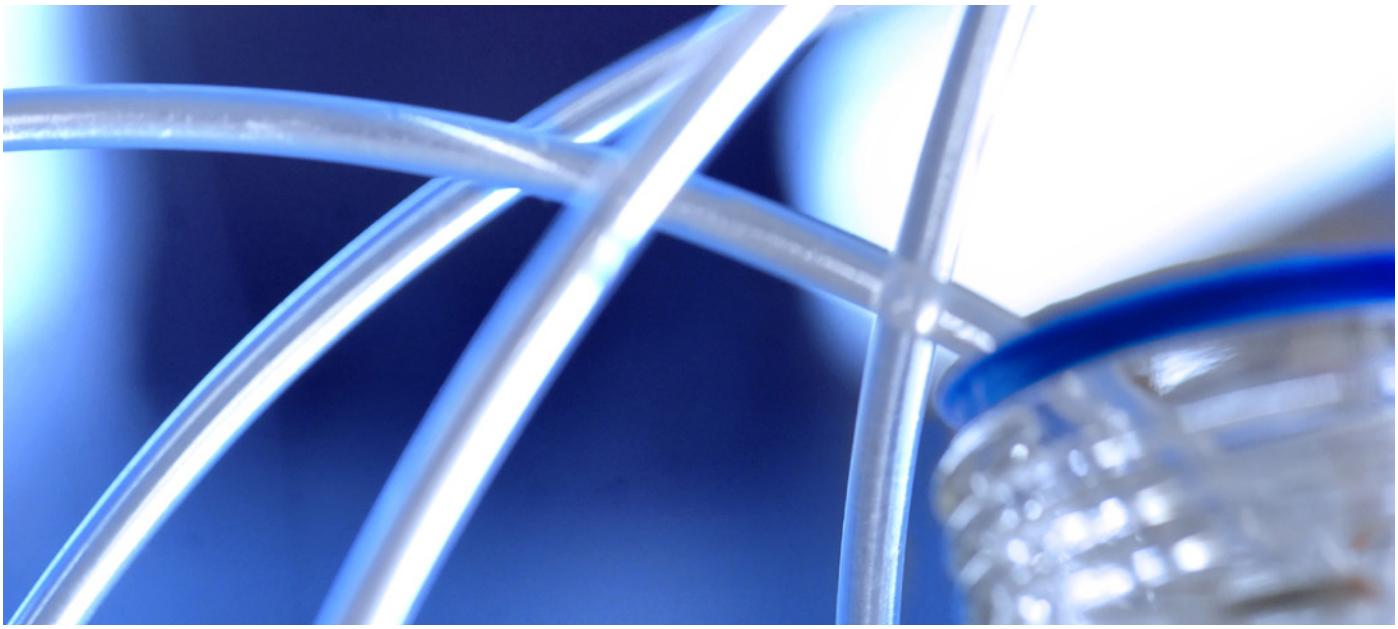


Figure 3a). Biotage® SNAP Ultra C18 12 g wetted and equilibrated at at 12 mL/min.

Figure 3b). Biotage® Sfär C18 D 12 g wetted at 300 mL/min and equilibrated at 150 mL/min.



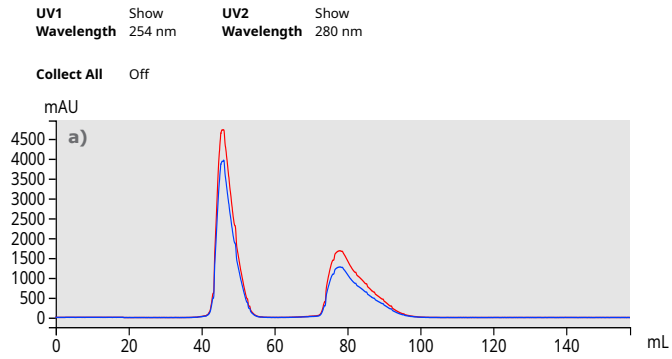
Comparison of Biotage® SNAP Ultra C18 and Biotage® Sfär C18 D 30 g

In this final section, we again compare columns with same separation media, but the wetting and the equilibration of the columns are performed at different flowrates. The actual separationon on the two different columns, has been performed at same flowrate.

In the chromatograph to the left (Figure 4a), a SNAP Ultra C18 30 g cartridge was wetted, equilibrated and run, all at 25 mL/min, and 1.3 mL sample (375 mg) was separated.

In the chromatogram to the right (Figure 4b), a Sfär C18 D 30 g cartridge was wetted at 300 mL/min, equilibrated at 150 mL/min and run at 25 mL/min. Again, 1.3 mL sample (375 mg) was applied.

The chromatogram in Figure 4a) and Figure 4b) are very similar. The disruption on the UV-curve, caused by air bubbles released during the chromatography, is minimal, since the amount of compounds is much higher compared to small air bubbles.



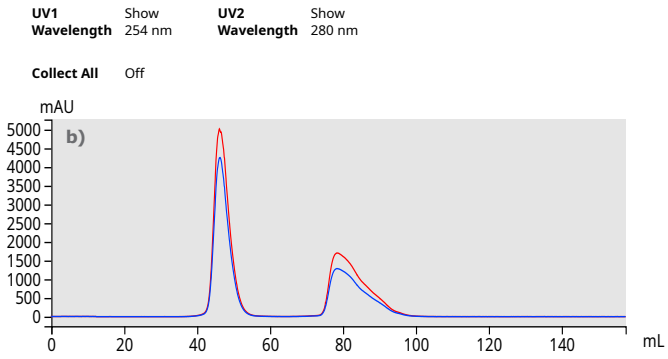
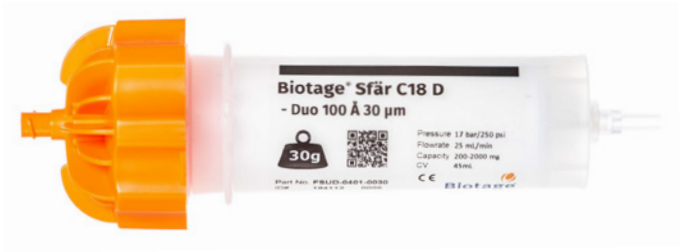


Figure 4a). Biotage® SNAP Ultra C18 30 g wetted and equilibrated at 25 mL/min.

Figure 4b). Biotage® Sfär C18 D 30 g wetted at 300 mL/min and equilibrated at 150 mL/min.



Conclusions

Wetting and equilibration of C18 columns for flash chromatography are best performed at the highest possible pressures/flowrates in order to remove the air trapped inside the porous material. That will make more C18-sites activated and accessible during the chromatography run.

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