How to Evaluate Biotage® Sfär Flash Chromatography Columns

Seeing for Yourself Why You Should be Using Biotage® Sfär Flash Columns

Confidence is what you need before you separate your precious compound on a new brand of flash chromatography column. Predictably getting your compound in a pure state is what you want, and if you can get it faster and use less solvent all the better. After all, your project needs to be completed on time, and trying something new is not always appealing.

In order to take your first steps toward gaining that confidence you can follow the same procedures we use here at Biotage to evaluate columns. Below you will find results of tests we have conducted preceded by a detailed procedure.

How to compare Biotage Sfär to your current column

- Contact your Biotage representative and order the columns you wish to compare
  - Biotage® Sfär
  - Biotage® Sfär HC
- Choose a sample for evaluation from your inventory
- Split sample into four equal aliquots
- Create a suitable method for purification with your preferred gradient shape
- Purify each aliquot using a different column, be sure not to change your gradient shape
- Compare the chromatograms and purity of collected fractions

What you should expect to see

- Peaks will come out a bit later on the Sfär runs, this is due to the higher surface area, once you get used to the performance, you'll find that a steeper gradient may be preferred
- Peaks will be sharper
- You will likely see more peaks than you have seen before

Our internal comparison

We ran this procedure in our laboratory in Charlotte and recorded the results below.

Columns Used and Samples Separated

- Biotage® Sfär 60 µm (25 g)
- Biotage® SNAP KP-Sil (25 g)
- Biotage® Sfär HC 20 µm (25 g)
- Competitor's Premium Column (24 g)
- Four different samples
  - methyl and butyl paraben in acetone
  - methyl paraben, propyl paraben, quinoxaline, and 4'-methoxy acetanilide in acetone
  - Organic synthesis reaction mixture in acetone
  - Natural product extract, ~100 mg/mL in 1:1 acetone/hexane
- Same flow rate
- Same gradient based on column volumes

Media specifications

Column	Media shape	Particle size (µm)	Surface area (m^2/g)	Pore diameter (Å)	Column size (g)	Column volume (mL)
Sfär 60 µm Duo	Spherical	50 - 70	700 - 850	35 - 63	25	42
SNAP KP-Sil	Granular	40 - 63	470 - 570	45 - 65	25	33
Sfär HC 20 µm Duo	Spherical	20 - 30	700 - 850	35 - 63	25	42
Competitor's Premium Column	Spherical	20 - 30	450 - 550	60	24	36

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Our results

Test 1, Parabens

- **Sample** 200 mg/mL each methyl and butyl paraben in acetone
- **Solvent A** hexanes
- **Solvent B** ethyl acetate
- **Gradient** 5% B for 1 CV
  5-40% B in 10 CV
  40% B for 2 CV
- **Detection** λ-all 200-400 nm, 254 nm, 280 nm
- **Load** 0.5 mL (100 mg)

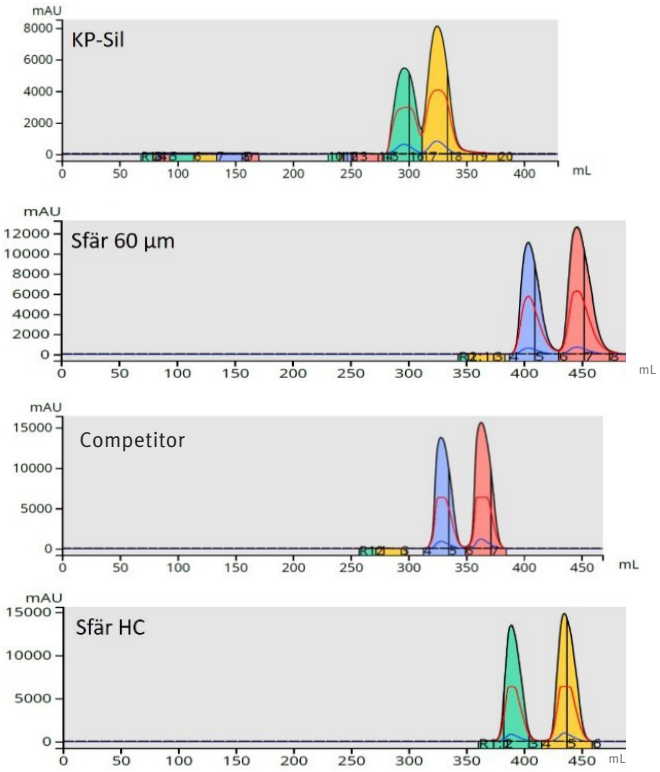

Figure 1. Column performance comparison.

Results:

Sfär 60 µm provides more separation than the comparable particle size SNAP KP-Sil column. Sfär 60 µm provides equivalent separation performance to the 30 µm Competitor's Premium Column. In both comparisons, the increased Sfär silica surface and optimized column packing protocols generated the high separation performance.

Comparison of a Sfär HC 20 µm HC column and the high performance Competitor's Premium Column shows the Sfär HC column provides increased resolution between the peaks which increases compound purity.

Test 2, 4-component mix

- **Sample** methyl paraben, propyl paraben, quinoxaline, and 4'-methoxy acetanilide in acetone
- **Solvent A** hexanes
- **Solvent B** ethyl acetate
- **Gradient** 7% B for 1 CV
  7-50% B in 8 CV
  100% B for 3 CV
- **Detection** λ-all 200-400 nm, 254 nm, 280 nm
- **Load** 0.1 mL (30 mg)

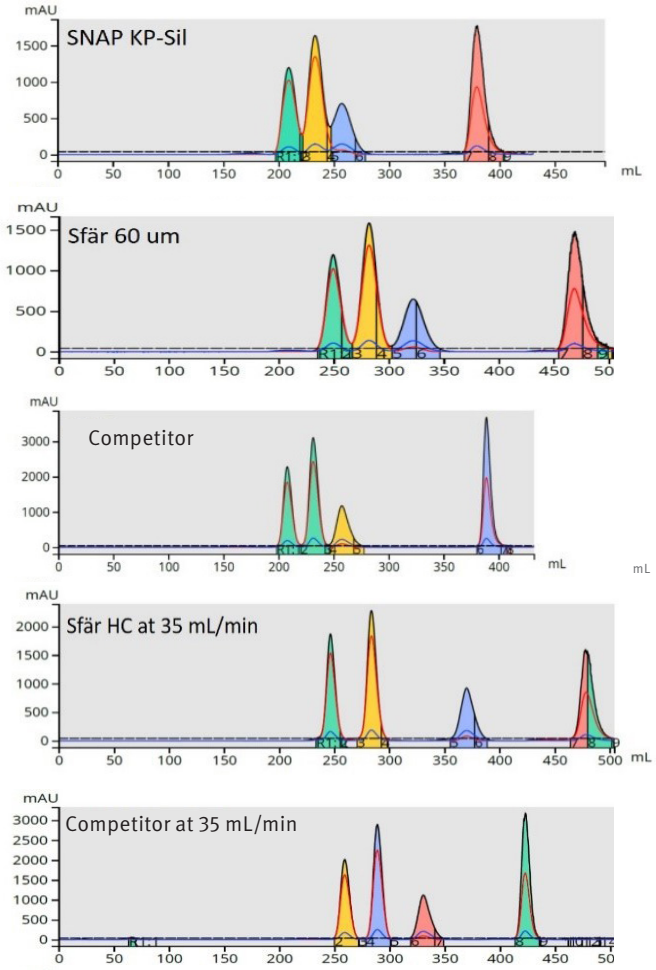

Figure 2. Column performance comparison with a 4-component mix.

Results:

Sfär 60 µm provides a better separation than SNAP KP-Sil. Sfär 60 µm matches the separation performance of the high performance Competitor's Premium Column.

In the comparison of Sfär HC and Competitor's Premium Column with 4-component mix Sfär HC shows improved separation with much improved resolution.

Test 3, Reaction Mixture

- **Sample** Microwave reaction mixture in acetone
- **Solvent A** hexanes
- **Solvent B** ethyl acetate
- **Gradient** 20% B for 1 CV
  20-40% B in 10 CV
  40% B for 2 CV
- **Detection** λ-all 200-400 nm, 254 nm, 330 nm
- **Load** 0.5 mL (123 mg)

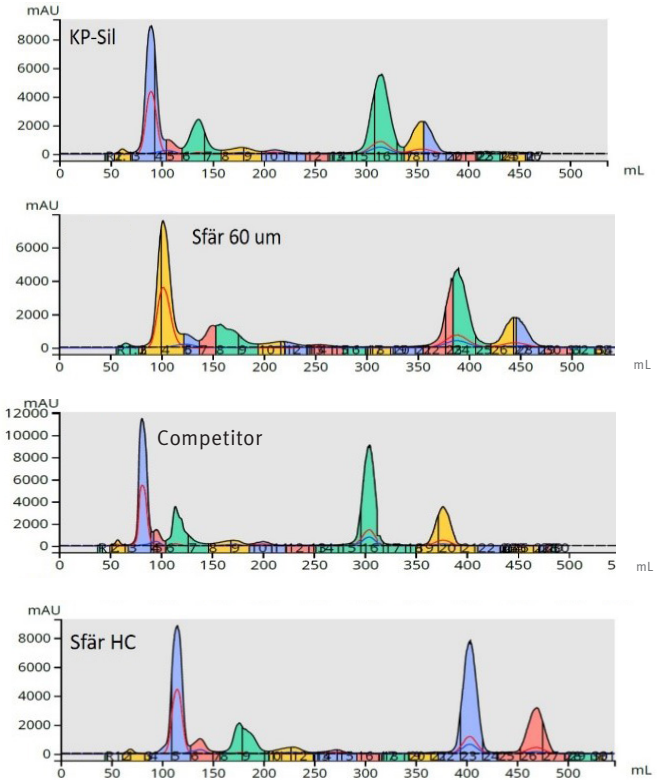

Figure 3. Column comparison with a MW reaction mixture

Results:

Similar particle size columns show Sfär 60 providing a slightly better separation of last two compounds and with a peak eluting around 150-mL. The Sfär 60 µm column also provides some separation enhancement early in the purification but the Competitor's Premium Column separates the last two peaks better due to a difference in media selectivity. Sfär HC provides improved separation of early eluting compounds and a full separation of the last two compounds.

Test 4, Natural Product Extract

- **Sample** Natural product extract, ~100 mg/mL in 1:1 acetone/hexane
- **Solvent A** hexanes
- **Solvent B** ethyl acetate
- **Gradient** 10% B for 1 CV
  10-80% B in 10 CV
  80% B for 2 CV
- **Detection** Collect: λ-all 200-400 nm + ELSD (Neb30, Evap 30, Gas 2 L/m, LED 10)
- **Load** 0.2 mL (~20 mg)

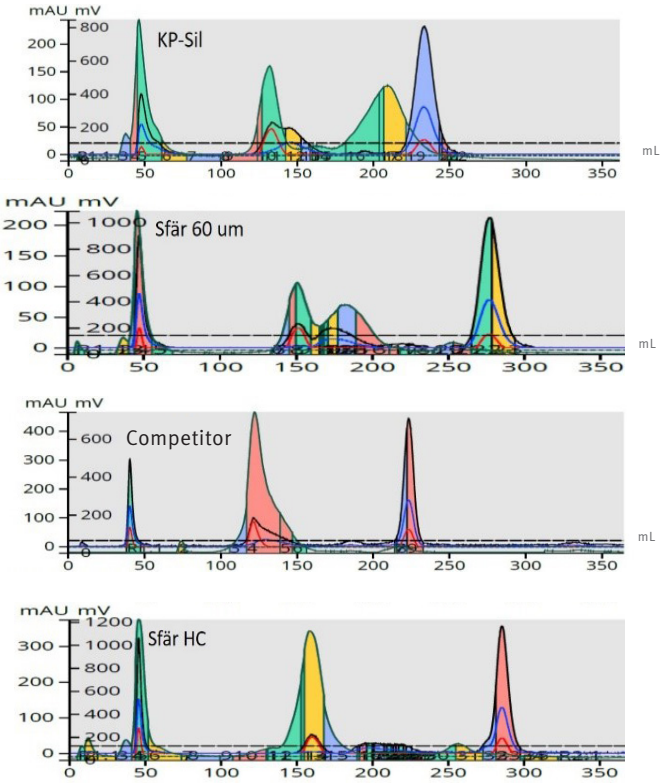

Figure 4. Column comparison of a natural product extract using both diode array UV and ELSD for fractionation.

Results:

KP-Sil and Sfär 60 um provide different separation profiles with the broad ELSD peak (green trace) being less retained on the Sfär column. The same Sfär column provides a better separation than the high-performance Competitor's Premium Column , which cannot resolve compounds eluting in the middle of the purification.

Sfär HC generates a superior separation especially with the compounds eluting in the middle and at the end of the purification. The ELSD peak fully resolved with Sfär HC co-elutes with the Competitor's Premium Column's last eluting peak.

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