Loading Capacity of Biotage® Sfär Silica vs Biotage® SNAP KP-Sil Columns

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Introduction

It is well known that in flash chromatography, the shape and size of the silica material, irregular or spherical, large or small particles, affects the amount of material that can be purified on a flash column. The smaller, more spherical particles and the higher the surface area, the more material can be separated.

In this application note, we compare the amount of a three-component mixture that can be separated on 25 g columns with different silica material, namely Biotage® SNAP KP-Sil (average 50 µm irregular particles), Biotage® Sfär Silica (60 µm spherical particles) and Biotage® Sfär Silica HC (20 µm spherical particles) on a Biotage® Selekt flash purification instrument.

Experimental

A 10 g sample of three similar compounds, dimethyl phthalate, diethyl phthalate and dibutyl phthalate (Figure 1) was separated on three different 25 g columns:

- Biotage® SNAP KP-Sil
- Biotage® Sfär Silica D
- Biotage® Sfär Silica HC D

Chromatography was done using a Biotage® Selekt instrument and the chromatograms were compared.

The criterion for separation used was to obtain separation between peak 2 and peak 3 using a threshold set at 100 mAU at 254 nm on the Biotage® Selekt.

Sample Solution

- Dimethyl phthalate 10.0 g
- Diethyl phthalate 10.0 g
- Dibutyl phthalate 10.0 g
- n-Heptane, pa 60 mL
- Ethyl acetate, pa 10 mL

4.0 mL sample was concentrated on a rotary evaporator and the residue was dried under vacuum for 2 hours. The dried material weighed 1.36 g, which gives a concentration of 341 mg/mL.

Chromatography Conditions

- A: n-Heptane, B: Ethyl acetate
- Column Equilibration, Biotage® SNAP KP-Sil: 15 % B, 3 CV
- Column Equilibration, Biotage® Sfär Silica D and Sfär Silica HC D: 15 %B, 2 CV
- Gradient run: 15 % B 1 CV, 15–50 % B 4 CV, 50 % B 1 CV
- UV signals: 254 nm (collect, red), 280 nm (show, black), Baseline Correction: On, Threshold: 100 mAU
- SNAP KP-Sil 25 g flow rate: 75 mL/min
- Sfär Silica D and Sfär Silica HC D, 25 g flow rate: 80 mL/min

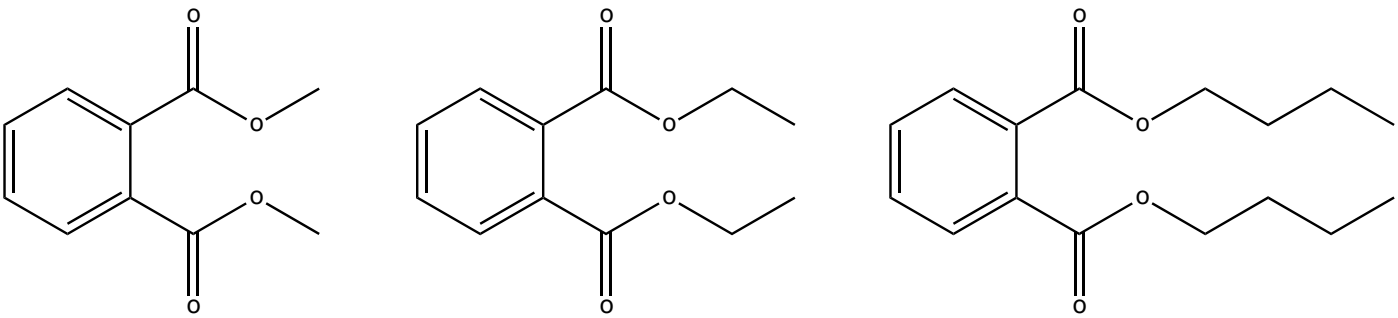

Figure 1. Sample compounds from left to right: dimethyl phthalate, diethyl phthalate and dibutyl phthalate.

Results

The chromatography conditions for separation of the three components were initially optimized using 25 g Biotage® SNAP KP-Sil columns (Figure 2). The maximum loading that resulted in peak separation under the conditions used was found to be 0.682 % (0.5 mL, 170 mg).

Using the same conditions on a 25 g Biotage® Sfär Silica D column (Figure 3), the loading could be increased four times to 2.73 % (2.0 mL, 682 mg).

Repeating the experiment using a 25 g Biotage® Sfär Silica HC D column (Figure 4), the loading could be further increased to 6.82 % (5.0 mL, 1705 mg), 2.5 times higher compared to the 25 g Sfär Silica D column, and 10 times compared to the 25 g SNAP KP-Sil column.

Conclusions

In this application note, we compare the amounts of a three-component mixture that can be separated on columns of the same size, 25 g, but with different Silica material: SNAP KP-Sil (average 50 µm irregular particles), Sfär Silica (60 µm spherical particles) and Sfär Silica HC (20 µm spherical particles) using a Biotage® Selekt instrument.

As can be seen from the table below, the smaller and more spherical the silica particles are, the more material can be separated on the flash chromatography column.

Cartridge type	Material type	Sample volume	Sample mass	Loading (%)
(KP-Sil)	45–60 µm Irregular	0.5 mL	170 mg	0.68
(Sfär)	60 µm Spherical	2.0 mL	682 mg	2.73
(Sfär HC)	20 µm Spherical	5.0 mL	1705 mg	6.82

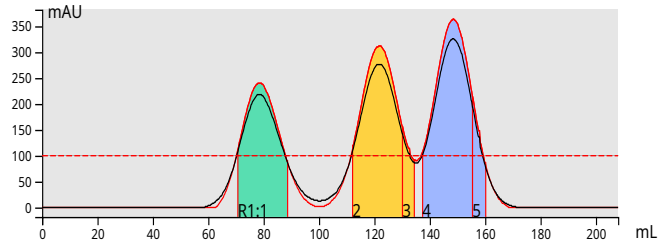

Figure 2. Chromatogram using a Biotage® SNAP KP-Sil column.

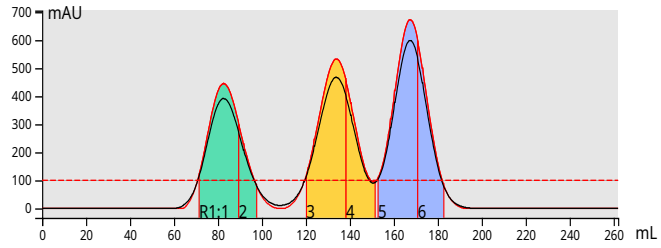

Figure 3. Chromatogram using a Biotage® Sfär Silica D column.

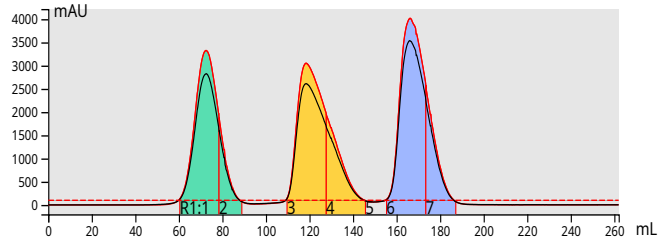

Figure 4. Chromatogram using a Biotage® Sfär HC D column.

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