

Using a Semi-Preparative Normal Phase HPLC Column on Biotage® Selekt

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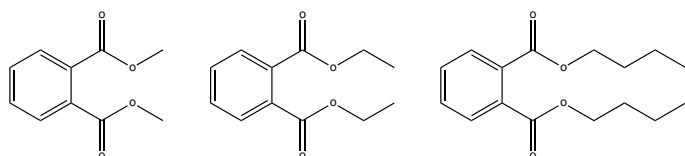
Introduction

The newly released Biotage® Selekt chromatography instrument can be run at a maximum flowrate of 300 mL/min or a maximum pressure of 30 bar. These high flowrates and pressures enables a user to do chromatography using not only dry-packed, single-use plastic flash columns containing small ($\geq 20 \mu\text{m}$) spherical silica particles, but also semi-preparative, slurry-packed HPLC columns for multiple use with smaller ($\leq 20 \mu\text{m}$) spherical silica particles.

In this application note, we show the separation of a three-component mixture using a semi-preparative slurry-packed HPLC column with $15 \mu\text{m}$ spherical silica particles and a Biotage® Selekt instrument.

Experimental

A sample of equal amounts of three similar compounds, dimethyl phthalate, diethyl phthalate, and dibutyl phthalate (Figure 1), was separated using an Interchim PuriFlash Prep $15 \mu\text{m}$ Silica 250 x 21.2 mm column on a Biotage® Selekt instrument.



1) Dimethyl phthalate 2) Diethyl phthalate 3) Dibutyl phthalate

Figure 1. Compounds separated.

Sample Solution

- » Dimethyl phthalate (1) 5.0 g
- » Diethyl phthalate (2) 5.0 g
- » Dibutyl phthalate (3) 5.0 g
- » n-Heptane 10 mL
- » Ethyl acetate 2 mL

The material was dissolved in 10 mL n-heptane in a 25 mL measurement flask and the solution was made up to 25.0 mL with 2 mL ethyl acetate. This gave a total concentration of 0.60 g/mL.

The semi-preparative HPLC column (slurry-packed in ethanol) was attached to the Selekt instrument equipped with a 3 mL sample loop with an inner diameter of 1.2 mm and a manual injection valve using stainless steel tubing with 0.5 mm inner diameter (Figure 2).



Figure 2. The semi-preparative HPLC column setup on Biotage® Selekt.

A new column, with the following characteristics, was created in the Column Administration section of the Selekt software (Figure 3):

- » Name: Interchim 15 µm Silica 250 x 21 mm
- » Column Volume: 80 mL
- » Equilibration Flow Rate: 120 mL/min
- » Equilibration Pressure: 30 bar
- » Equilibration Length: 3 CV
- » Default Flow Rate: 100 mL/min
- » Max Pressure: 30 bar

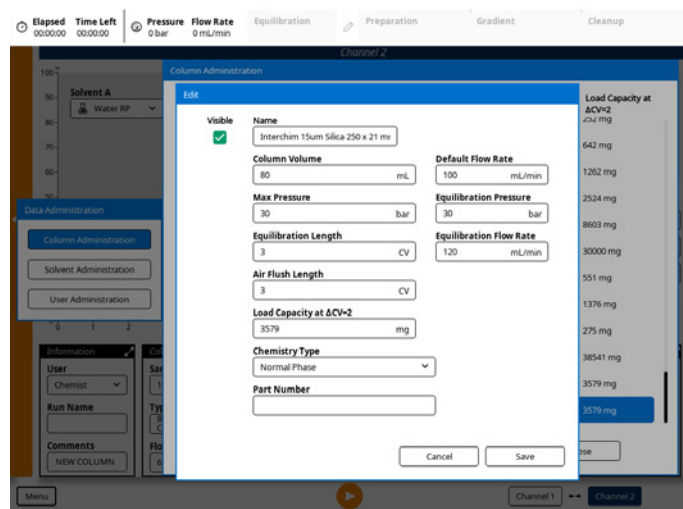


Figure 3. Column edit screen on Biotage® Selekt.

The HPLC column was washed twice with 80 mL n-heptane–ethyl acetate 1:1 at 100 mL/min (this gave a back pressure of approx. 22 bar), and then equilibrated with 3 CV n-heptane–ethyl acetate 85:15 at 120 mL/min (also approx. 22 bar).

3.0 mL sample (1.80 g, approx. 3.6% loading) was added via the sample loop and the separation was executed using the method below.

Chromatography Conditions

- » Solvent A: n-Heptane, Solvent B: Ethyl acetate
- » Column equilibration: 15% B, 3 CV, 120 mL/min
- » Gradient run: 15% B for 1 CV, 15–50% B for 4 CV, 50% B for 1 CV
- » UV1: 254 nm (Monitor), UV2: 280 nm (Monitor), λ-All: 200–400 nm (Collect), Baseline Correction: On, Threshold: 200 mAU
- » Column: Interchim 15 µm Silica 250 x 21.2 mm
- » Flow rate: 100 mL/min

Results

The separation worked smoothly and the pressure did not exceed 21 bar during the chromatography. The compounds were separated with excellent baseline separation (Figure 4).

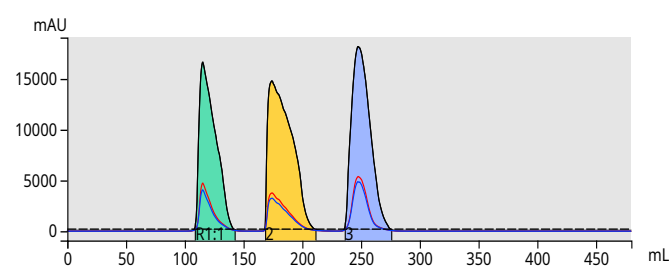


Figure 4. Chromatogram of the separation.

Conclusions

In this application note, we show that a semi-preparative slurry-packed HPLC column with 15 µm spherical silica particles can easily be used on a Selekt instrument.

The separation of a three-component mixture using this setup worked smoothly.

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