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

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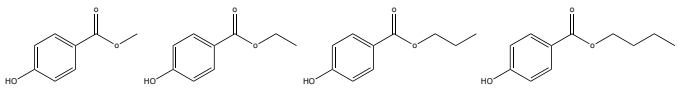
Introduction

The newly released Biotage® Selekt flash 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 enable a user to perform chromatography using not only dry-packed, single-use plastic flash columns containing small (≥20 µm) spherical silica particles, but also semi-preparative, slurry-packed HPLC columns for multiple use with smaller (≤20 µm) spherical silica particles.

In this application note, we show the separation of a four-component mixture using a semi-preparative, slurry-packed reversed phase HPLC column with 15 µm spherical silica particles and a Biotage® Selekt instrument.

Experimental and Results

A sample of equal amounts of four similar compounds, methyl 4-hydroxybenzoic acid **(1)**, ethyl 4-hydroxybenzoic acid **(2)** propyl 4-hydroxybenzoic acid **(3)** and butyl 4-hydroxybenzoic acid **(4)**, was separated using a commercially available C18 15 µm Silica 250 x 21.2 mm column on a Selekt instrument.



1 2 3 4

Figure 1. Compounds separated.

Sample Solution

» Methyl 4-hydroxybenzoic acid **(1)**	1.50 g
» Ethyl 4-hydroxybenzoic acid **(2)**	1.50 g
» Propyl 4-hydroxybenzoic acid **(3)**	1.50 g
» Butyl 4-hydroxybenzoic acid **(4)**	1.50 g
» Acetonitrile	37.5 mL
» Water	approximately 9 mL

The material was dissolved in 37.5 mL acetonitrile in a 50 mL measurement flask and the solution was made up to 50 mL with approximately 9 mL water. This gave a total concentration of 0.12 g/mL.

The semi-preparative HPLC column (slurry-packed in water–acetonitrile 30–70) 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: 15 µm C18 250 x 21 mm
- Column Volume: 85 mL
- Equilibration Flow Rate: 35 mL/min
- Equilibration Pressure: 30 bar
- Equilibration Length: 3 CV
- Default Flow Rate: 35 mL/min
- Max Pressure: 30 bar

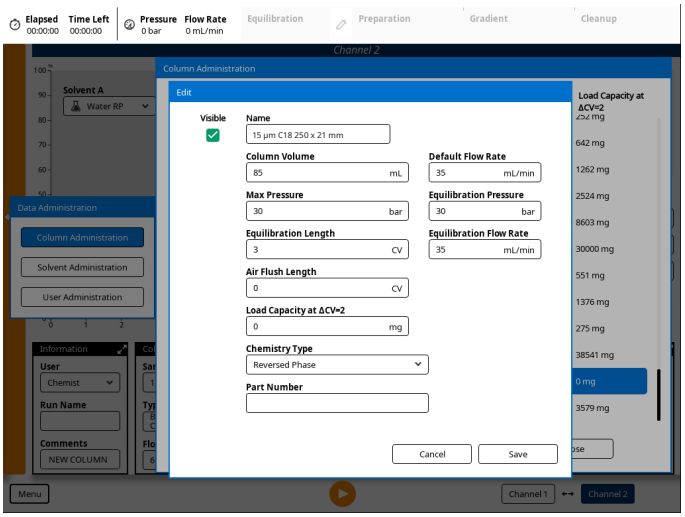


Figure 3. Column edit screen on Biotage® Selekt.

The HPLC column was washed twice with 85 mL water–acetonitrile 60–40 at 40 mL/min (this gave a back-pressure of approx. 30 bar) and then equilibrated with 3 CV water–acetonitrile 80–20 at 35 mL/min (approx. 30 bar).

3.0 mL sample (0.36 g, approx. 0.72% loading) was added via the sample loop and the separation was executed using the method below.

Chromatography Conditions

- Solvent A: Water, Solvent B: Acetonitrile
- Column equilibration: 20% B, 3 CV, 35 mL/min
- Gradient run: 20% B for 1 CV, 20–60% B for 6 CV, 60% B for 1 CV
- UV1: 254 nm (Collect), UV2: 280 nm (Monitor), Baseline Correction: On, Threshold: 150 mAU
- Column: 15 µm C18 250 x 21.2 mm
- Flow rate: 35 mL/min

Chromatogram

The separation worked smoothly, and the pressure did not exceed 30 bar during the chromatography, owing to the newly implemented pressure regulated equilibration method.[1] The compounds were separated with excellent baseline separation (Figure 4).

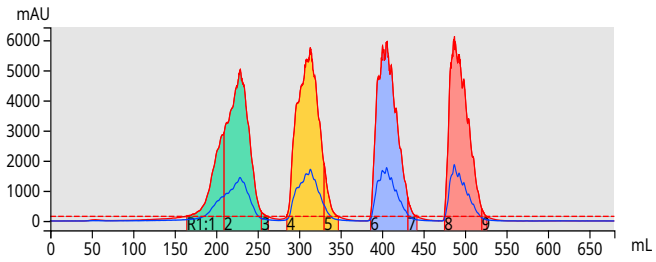


Figure 4. Chromatogram of the separation.

Conclusions

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

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

References

1. Patent application P11935EP00

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