

# 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 ( $\geq 20 \mu\text{m}$ ) 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 four-component mixture using a semi-preparative, slurry-packed reversed phase HPLC column with  $15 \mu\text{m}$  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 an Interchim PuriFlash Prep  $15 \mu\text{m}$  Silica 250 x 21.2 mm column on a Selekt instrument.

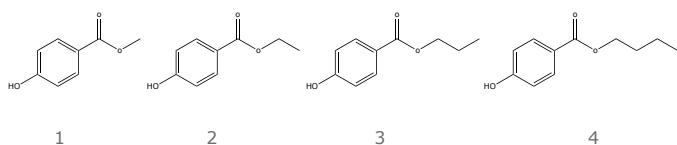


Figure 1. Compounds separated.

### Sample Solution

» Methyl 4-hydroxybenzoic acid ( <b>1</b> )	1.50 g
» Ethyl 4-hydroxybenzoic acid ( <b>2</b> )	1.50 g
» Propyl 4-hydroxybenzoic acid ( <b>3</b> )	1.50 g
» Butyl 4-hydroxybenzoic acid ( <b>4</b> )	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: Interchim 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

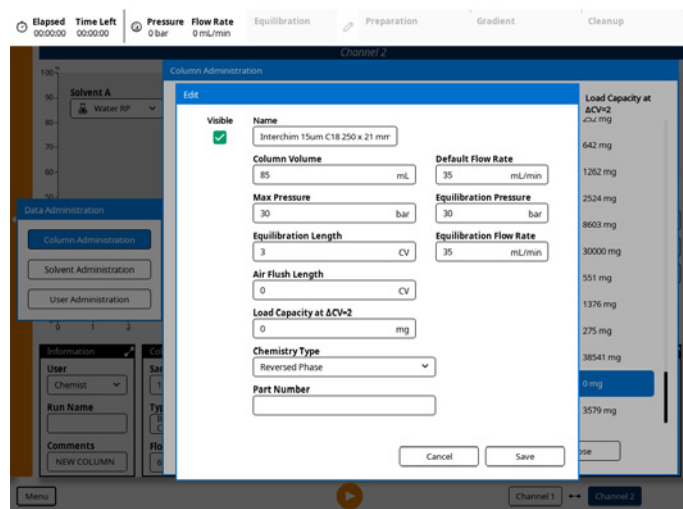


Figure 3. Column edit screen on Biotage® Selekt.

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: Interchim 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.<sup>1</sup> The compounds were separated with excellent baseline separation (Figure 4).

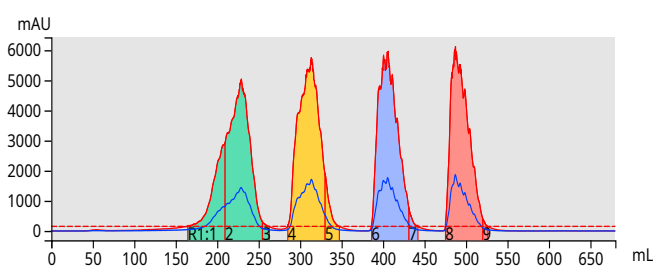


Figure 4. Chromatogram of the separation.

## 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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