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

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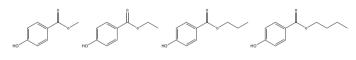
Introduction

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 fourcomponent mixture using a semi-preparative, slurry-packed reversed phase HPLC column with 15 μ 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 a commercially available C18 15 µm Silica 250 x 21.2 mm column on a Selekt instrument.



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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
- » Water

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37.5 mL
approximately 9 mL
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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 wateracetonitrile 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

Elapsed Time Left Pressure Flow Rate Equilibration

- » Equilibration Pressure: 30 bar
- » Equilibration Length: 3 CV
- » Default Flow Rate: 35 mL/min
- » Max Pressure: 30 bar

00:00:00 00:00:00 0 bar	0 mL/min	<i>w</i>			
		Channel 2			
100 m Colur					
90 - Solvent A	dit				Load Capacity at ∆CV=2
80-		Name			252 mg
70 -	\checkmark	15 µm C18 250 x 21 mm			642 mg
		Column Volume	Default F		
60 -		85 mL	35	mL/min	1262 mg
50-		Max Pressure	Equilibra	tion Pressure	2524 mg
Data Administration		30 bar	30	bar	8603 mg
Column Administration		Equilibration Length	Equilibra	tion Flow Rate	abusing
		3 CV	35	mL/min	30000 mg
Solvent Administration		Air Flush Length			551 mg
User Administration		0 CV			1376 mg
		Load Capacity at ∆CV=2			isvoring
ò i ż		0 mg			275 mg
Information 🖍 Col		Chemistry Type			38541 mg
User		Reversed Phase	×		
Chemist V		Part Number	_		0 mg
Run Name Tyr					3579 mg
Comments			Cancel	Save	ose
NEW COLUMN 6			correct!		
Menu				Channel 1 +	+ Channel 2

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).

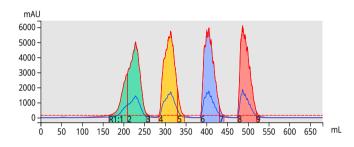


Figure 4. Chromatogram of the separation.

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 P11935EPoo

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Literature Number: AN139

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