

Why C18 Column Selection Matters

A Performance Comparison of Three Flash Column Brands

Background

When it comes to chromatography, no two media are the same. Silica from one flash column manufacturer will likely be different from another due to their media source's manufacturing process and raw material quality. The same is true with bonded silica such as C18. Some flash column suppliers buy C18 bonded silica from a chromatography media supplier while others, such as Biotage, synthesize their own flash C18 media, and thus, better control quality, performance, and supply issues.

This product note compares a Biotage® Sfär C18 to two well-known competitive brands using an amide reaction mixture and a blend of seven different compounds.

Materials and Methods

Flash system:	Biotage® Selekt
Columns:	Biotage® Sfär C18 12 g, Competitor G C18, 15.5 g, Competitor P C18, 12 g
Solvent A:	Water
Solvent B:	Methanol
Sample 1:	Synthetic amide
Gradient:	Isocratic, 40% methanol
Flow rate:	30 mL/min, all columns
Sample 2:	7-component mix in DMSO
Gradient:	45–80% B in 10 CV
Flow rate:	30 mL/min, all columns

Column Media Comparison

	Biotage® Sfär C18	Competitor G C18	Competitor P C18
Size, g	12	15.5	12
Column volume (CV), mL	17	13.5	17
% Carbon	18	15	21
Particle size, µm	28	30	25
Pore diameter, Å	100	100	90
Surface area, m ² /g	400	300	450

Sample 1 – Reaction Mixture

One interesting item to note is the column volume (CV) of competitor G, stated as 13.5 mL. This seems low as CV values typically are 40% to 50% larger than the amount of media in the column. The impact of this discrepancy can be seen with isocratic purification of the amide reaction mixture, Figure 1.

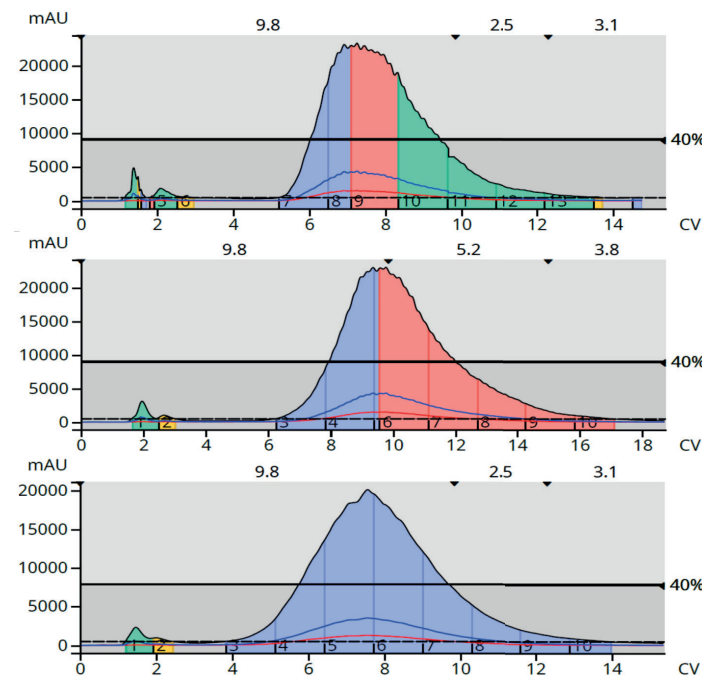


Figure 1. Comparison of three C18 column brands for the isocratic purification of a synthetic amide. Top - Biotage® Sfär C18. Middle - Competitor G C18. Bottom - Competitor P C18. Although the % carbon and surface area for Competitor G are the lowest of the three brands, its compound retention is the longest indicating the stated column volume is incorrect.

The Biotage® Sfär C18 and Competitor P columns have published column volumes of 17 mL with surface areas >400 m²/g and carbon content between 18% and 21%. These two brands elute the product between 6.5 and 8 CV. Competitor G, with its stated 13.5 mL CV, 300 m²/g surface area, and 15% carbon content, requires nearly 10 CV to elute the compound, indicating the true CV for Competitor G's column is much higher than the stated 13.5 mL.

Another difference seen with the chromatography is the peak bandwidth. While the Biotage® Sfär column eluted the product in a peak volume of ~8.5 CV (144.5 mL), the column from Competitor G required ~ 11 CV (148.5 mL) and Competitor P ~10 CV (170 mL). This is significant because peak volume impacts evaporation time and therefore, workflow.

Sample 2 - 7-Component Mix

With chromatography, a good measure of column performance is peak capacity. The better the column, the more compounds, or peaks, that can be separated. To test the three column brands for peak capacity, each column was loaded with 125 mg of a 7-component mixture and purified with the identical gradient.

From the peak capacity perspective, only the Biotage® Sfär C18 column fully resolved all seven components, plus the DMSO dissolution solvent, indicating it has the most efficient purification capabilities, Figure 2.

Conclusions

The data show that flash chromatography C18 media varies from vendor to vendor and those differences can have a significant impact on purification results. Biotage Sfär C18 columns separate compounds more completely with less solvent than other column brands.

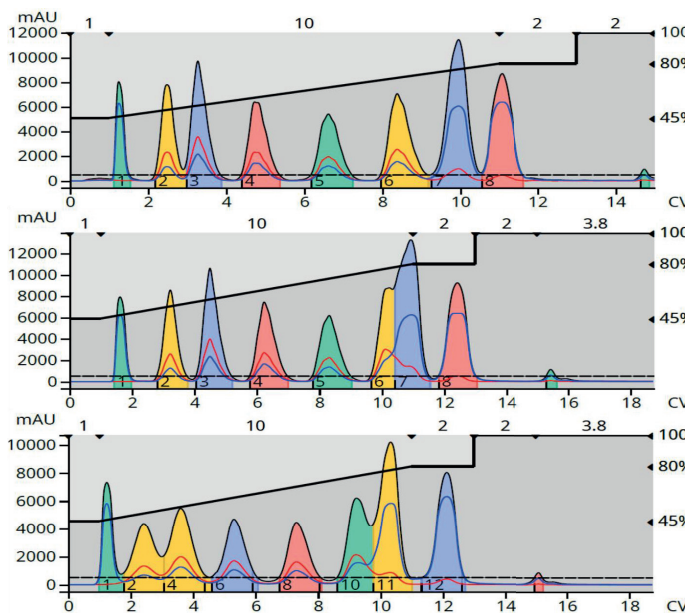


Figure 2. Performance comparison of three C18 flash column brands with a 7-component mixture dissolved in DMSO (first peak). Top - Biotage® Sfär C18, Middle - Competitor G C18, Bottom - Competitor P C18.

EUROPE

Main Office: +46 18 565900
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: +46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA

Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 800 446 4752
Order Fax: +1 704 654 4917
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
us-1-pointsupport@biotage.com

JAPAN

Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA

Tel: +86 21 68162810
Fax: +86 21 68162829
cn_order@biotage.com
cn-1-pointsupport@biotage.com

KOREA

Tel: +82 31 706 8500
Fax: +82 31 706 8510
korea_info@biotage.com
kr-1-pointsupport@biotage.com

INDIA

Tel: +91 11 45653772
india@biotage.com

Distributors in other regions
are listed on www.biotage.com

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