

# Why Silica Column Selection Matters

## A Performance Comparison of Three Flash Column Brands

### Background

There are many companies manufacturing flash columns and media. By and large, a column from any of the manufacturers will likely perform a purification with the right method – but how well?

The silica used to pack flash columns varies in its performance, sometimes dramatically, due to differences in surface area, porosity, purity, consistency, etc. Therefore, it is important to use columns from a reliable source with consistent performance and selectivity.

In this product note, a Biotage® Sfär HC silica column is compared to two other popular brands with similar media, one from Competitor G and another from Competitor P, using two different samples - a reaction mixture and a lipid mixture.

### Materials and Methods

**Flash system:** Biotage® Selekt  
**Columns:** Biotage® Sfär HC 10 g, Competitor G 12 g  
**Sample 1:** Synthetic amide  
**Solvent A:** Hexane  
**Solvent B:** Ethyl acetate  
**Gradient:** 7–60% B in 10 CV  
**Flow rate:** Biotage® Sfär - 40 mL/min, competitors G and P – 30 mL/min (stated default flow rates)

**Sample 2:** Lipid mixture (cholesterol +  $\alpha$ -tocopherol)  
**Solvent A:** Hexane  
**Solvent B:** MTBE  
**Gradient:** 5–60% B in 10 CV  
**Flow rate:** Biotage® Sfär - 40 mL/min, competitors G and P – 30 mL/min

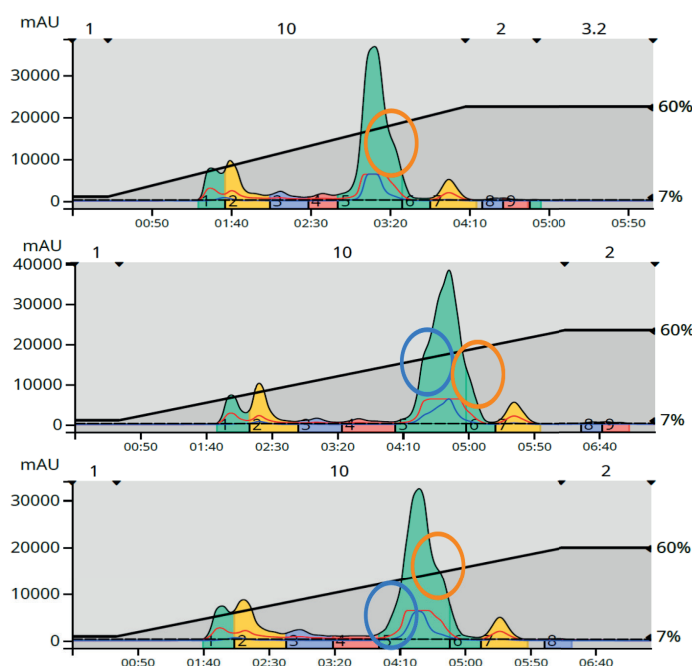
### Column Media Comparison

	Biotage® Sfär HC	Competitor G	Competitor P
Size, g	10	12	12
Column volume (CV), mL	17	17	17
Particle size, $\mu\text{m}$	20	30	25
Pore diameter, $\text{Å}$	50	60	90
Surface area, $\text{m}^2/\text{g}$	740	500	450

### Sample 1 – Reaction Mixture

This sample, a product of an isatoic anhydride, benzylamine, benzaldehyde, and acetic acid reaction in DMF, generated many by-products requiring removal by flash chromatography. Dry loading was used to eliminate the DMF reaction solvent using silica as the sorbent and a Biotage® V-10 Touch to evaporate the solvent. Each column was loaded with 415 mg of the reaction mix.

The purification (7–60% ethyl acetate in hexane over 10 column volumes) results highlight some of the performance differences between the three brands such as purification speed and resolution of the product peak from impurities, Figure 1.

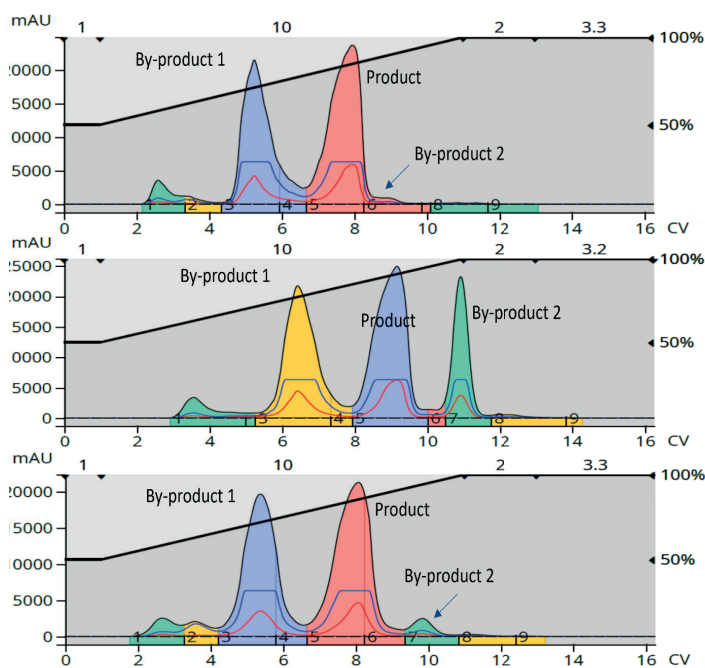


**Figure 1.** Chromatographic comparison with a reaction mixture. Top – Biotage® Sfär HC. Middle - Competitor G. Bottom - Competitor P. Orange and blue circles show the presence of partially resolved by-products/impurities of which only one is found with the Sfär column's purification.

There is at least one partially resolved by-product as evidenced by the shoulders on the back (orange circles) and front (blue circles) of each column's product peak.

The Sfär column eluted the product in a tighter band (less solvent/time) than the competition and appears to have resolved the leading-edge by-product since there is no visible shoulder.

Each product peak's purity was determined by each brand's reversed-phase flash chromatography column, Figure 2.

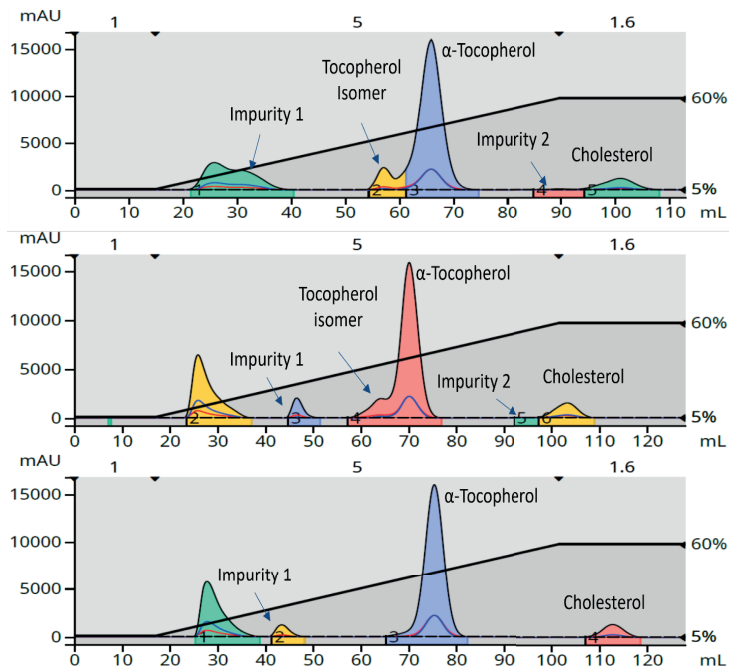


**Figure 2.** Reversed-phase purification of the product peak isolated from the normal phase purification of the reaction mixture. Top - Biotage® Sfär. Middle - Competitor G. Bottom - Competitor P.

Reversed-phase chromatography separated the partially resolved by-products - by-product 1, found in all the normal-phase purifications, and by-product 2, seen with the normal-phase purification results of competitors G and P. The small by-product 2 peak seen with the Biotage® Sfär C18 column purification proves the Biotage® Sfär silica column purified the crude reaction better than the either competitor.

### Sample 2 – Lipid Mixture

Lipids are a class of hydrophobic compounds that can be challenging to purify by normal-phase chromatography. In this example, a mixture of cholesterol and 95% pure  $\alpha$ -tocopherol (1:1) dissolved in dichloromethane was used to see how the silica columns from the three manufacturers performed, Figure 3.



**Figure 3.** Lipid mixture purification comparison. Top - Biotage® Sfär HC. Middle - Competitor G. Bottom - Competitor P. Only the Sfär HC column provided adequate resolution of isomers and impurities.

The results show that the Biotage® Sfär HC column provided the best separation of the tocopherol isomer from  $\alpha$ -tocopherol and cholesterol from its impurity. Competitor P provided no separation of either the tocopherol isomer or the cholesterol impurity.

### Conclusion

Chromatographic silica from different sources will not have the same properties or separation performance. Silica with a small particle size and high surface area, such as the Biotage® Sfär HC silica, maximizes compound contact with the media enabling separations not achievable on other silica brands.

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