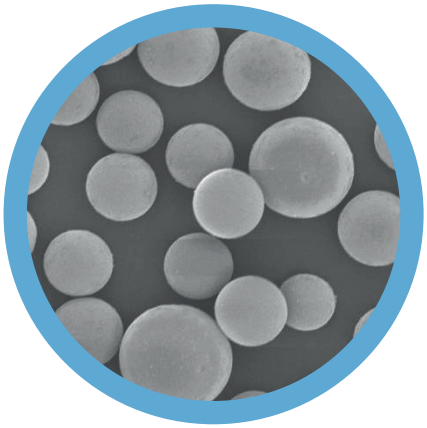
APPLICATION NOTE
#SSP002-0

SiliCycle

SiliaSphere™ PC



Advantages of SiliaSphere PC Spherical Silica over SiliaFlash Irregular Silica

There are distinct advantages of using spherical silica gel over irregular ones. This study demonstrates that not only does the spherical silica shows better performances, but it is also possible to increase the loading of a flash cartridge without adversely affecting the performance of the separation.

LEARN MORE about Silia*Sphere* PC in our brochure "*Solutions for Purification & Chromatography*."

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Advantages of Silia*Sphere* PC Spherical Silica over Silia*Flash* Irregular Silica

THE QUINOLINES SEPARATION TEST

To evaluate the performance of the spherical silica we tested it in chromatography for the separation of a mixture of quinolines (*Figure 1*). We also wanted to benchmark the performance against irregular silica and to achieve this, one of our most popular irregular silica (*PN: R10030B*) was compared to one of our most popular spherical gel (*PN: S10095D-A*). An equimolar mixture of quinolines was chromatographed on 12 g cartridges packed with those two gels. Figures 2 and 3 demonstrate the better performance of the S10095D-A spherical silica over the R10030B irregular silica. A 1% w/w ratio of total mixture/silica gel was used with the following elution conditions:

Eluant mixture: Ethyl acetate/Hexane
Gradient: 1- 3 to 20% (8 CV); 2- 30 à 45% (17CV); 3- 45% (3CV)
Flow: 12 mL/min
Wavelength: 280 nm
Elution order: 2-Chloroquinoline, 3-Bromoquinoline, 8-Chloroquinoline, 6-Nitroquinoline, 5-Nitroquinoline

Figure 1: Mixture of quinolines

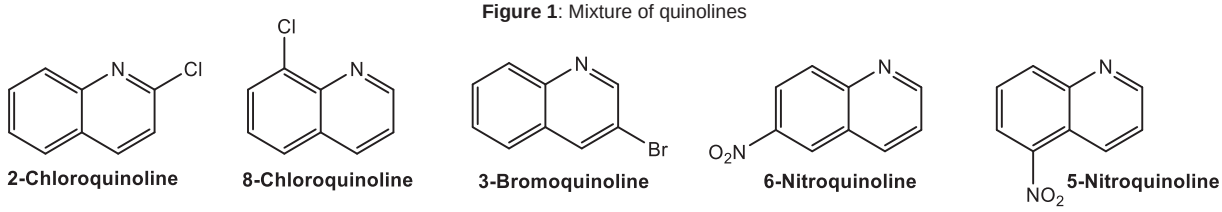


Figure 2: Silia*Flash* **F60** 40-63 mm, 60 Å irregular (*R10030B*)

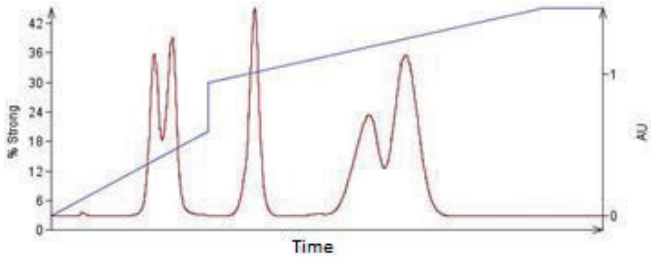
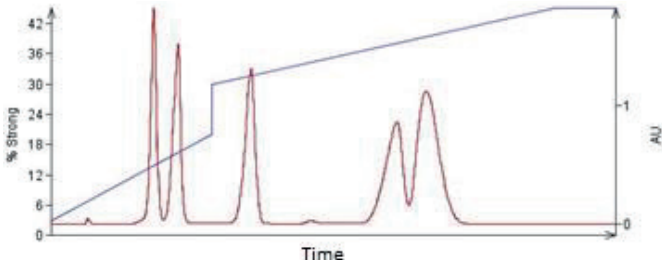


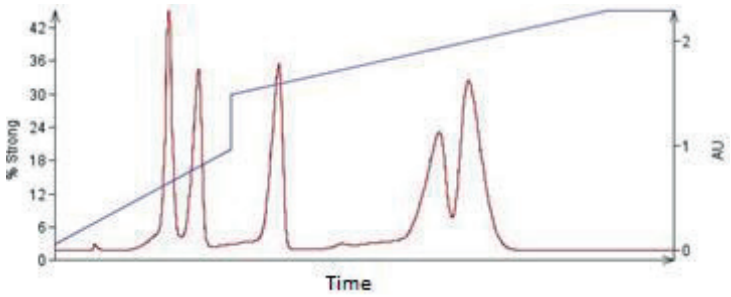
Figure 3: Silia*Sphere* **PC**, 25 µm, 90Å spherical (*S10095D-A*)



We can see on Figure 2 and 3 that spherical silica gives a better separation than regular silica and is a better solution for difficult separations.

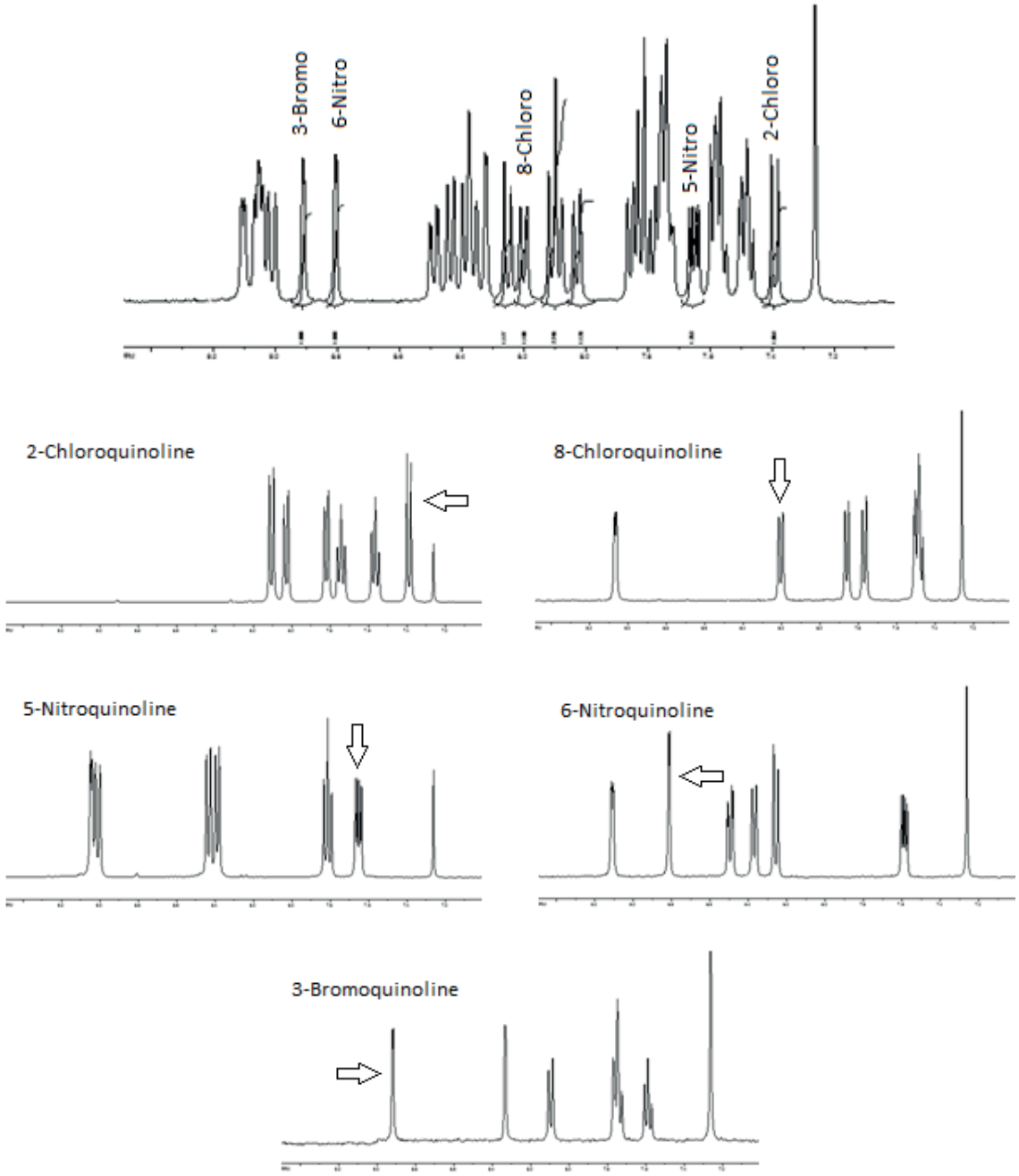
Furthermore, it is interesting to observe that increasing loading from 1.0 to 1.5% doesn't affect the separation on S10095D-A (Figure 4).

Figure 4 : 1.5x Loading using Silia*Sphere* **PC** (*S10095D-A*)



The NMR^{1}H spectra in Figure 5 confirms the identity and relative purity of each separated quinoline, using silica gel S10095D-A. Peaks highlighted are the ones used to distinguish a specific quinoline in the crude NMR^{1}H spectrum.

Figure 5 : NMR^{1}H ($CDCl_3$) of Quinolines mixture, and purified compounds

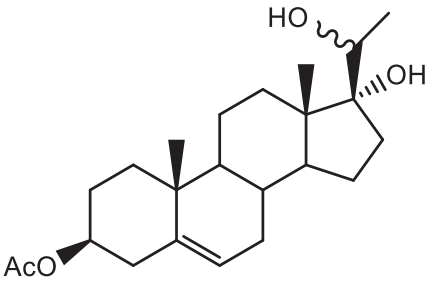


THE STEROID'S STEREOISOMERS SEPARATION TEST

We also studied the capabilities of spherical silica for the separation of more complex mixtures such as stereoisomers of steroid skeletons (*Figure 6*). The gain in performance using the spherical gel (*S10095D-A*) over the irregular gel (*R10030B*) was notable for the separation of these stereoisomers. The mixture was injected in flash chromatography on glass column using those two silicas. A portion of 200 mg of mixture was solubilized in 3 mL of toluene and loaded on 10 g of silica (*2% loading*). Elution was done using ethyl acetate/toluene using the following gradient:

Gradient:
1- 0% (100% Toluene) for 1 CV
2- 5% Ethyl Acetate in Toluene for 2 CV
3- 10% Ethyl Acetate in Toluene for 2 CV
4- 20% Ethyl Acetate in Toluene for 1 CV

Figure 6: mixture of stereoisomers



20α-OH : 20β-OH
76 : 24

As shown in Table 1, spherical beads (*S10095D-A*) proved to be far more efficient than irregular ones (*R10030B*). It was also demonstrated that increasing the loading percentage from 2% to 3% with spherical beads does not affect the separation performance (*Table 2*).

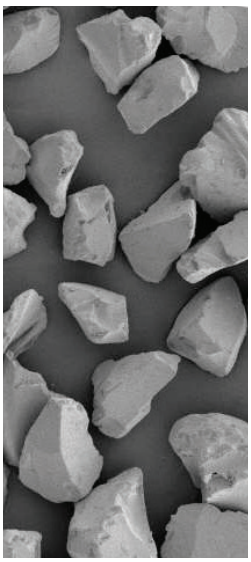
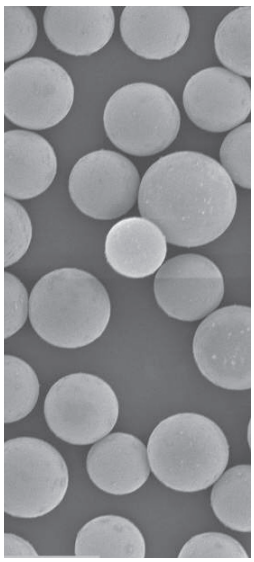


Table 1: Efficiency of separation of steroidal isomers using spherical and regular silica

Efficiency of Separation of Steroidal Isomers				
	Isolated Weight (mg)		Isolated Yield (%)	
	α	β	α	β
R10030 B	78	26	39	13
S10095D-A	144	46	72	23
Theoretical Ratio	***152***	***48***	***76***	***24***

Table 2: Effect of loading variation on spherical silica (*S10095D-A*)

Effect of Loading Variation on Spherical Silica				
	Isolated Weight (mg)		Isolated Yield (%)	
	α	β	α	β
2% w/w load	144	46	72	23
3% w/w load	139	46	70	23
Theoretical Ratio	***152***	***48***	***76***	***24***



This experiment confirms that using spherical gels improves greatly the separation performance and that increasing the loading from 2% to 3% doesn't affect the separation efficiency.

THE DYE MIXTURE TEST

Qualitative visual tests were also performed on Silia*Sep*™ 12g cartridges packed with R10030B and Silia*Sep*™ **PREMIUM** packed with S10095D-A. Portions of 0.25 mL of commercial three-components mixture (*CAMAG Test Dye Mixture I*) of dyes were injected on pre-conditioned cartridges. Products were eluted with a mixture of ethyl acetate/hexane (*15 to 45% on 10 CV at 12 mL/min*). Figures 7 to 10 show the separation at 3 CV and 7 CV, on both tested silicas.

Figure 7: R10030B at 3 CV

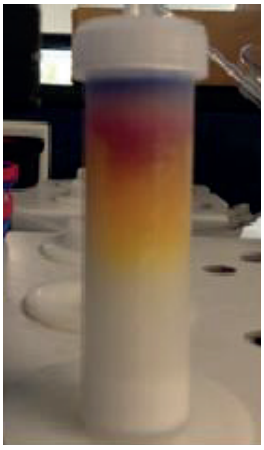


Figure 8: S10095D-A at 3 CV

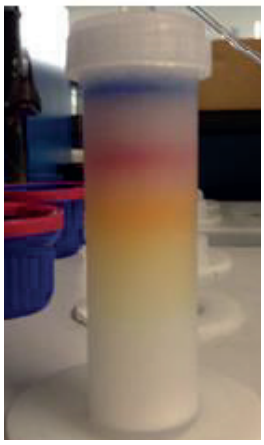
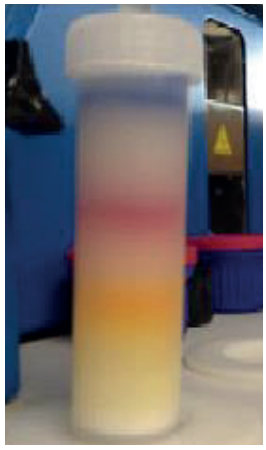


Figure 9: R10030B at 7 CV

Figure 10: S10095D-A at 7 CV



Conclusion

There's a gain in separation using Silia*Sphere* PC spherical beads over Silia*Flash* irregular gels, when it comes to performing difficult separations.

Based on quinolines and stereoisomers separations, loading on Silia*Sphere* PC S10095D-A can be increased by 50% without decreasing the silica's high efficiency.

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