APPLICATION NOTE

#Appn_SS008-0

SiliaSep™

Purification of a Heterocyclic Mixture Using SiliaSep PREMIUM Cartridges

Natural product research needs efficient purification techniques for the characterization and isolation of molecules of interest. This study involves purifying a mixture of apolar lipophilic heterocyclic compounds, prevalent in natural products, by normal phase chromatography using SiliaSep PREMIUM cartridges (*containing spherical silica gel*) and aims to compare the separation results from different brands of cartridges.

LEARN MORE about SiliaSep in our brochure “Solutions For Purification & Chromatography”.

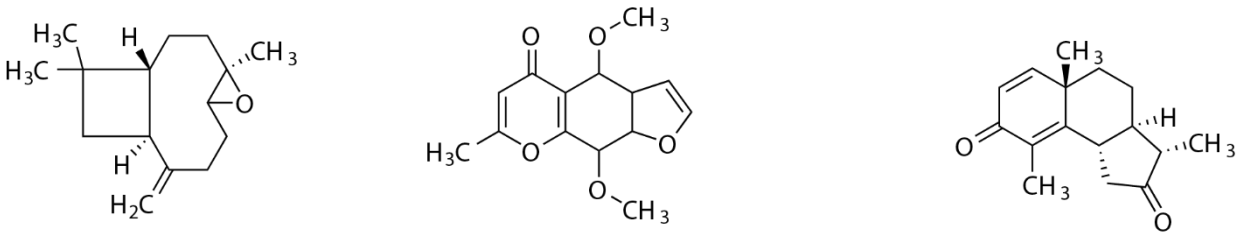
Molecules of interest in pharmaceutical research are often complex structures that come with purification challenges. To solve these difficulties, flash chromatography is the go-to method for separating target compounds.

The convenience of normal phase silica gel is notable for preparative chromatography since the low boiling point of the eluants allows easier mobile phase removal after the chromatographic run. Due to their usefulness and popularity, many manufacturers offer flash columns with similar specifications for flash chromatography. That being said, it is important to keep in mind that protocols may not be transferable, and work may need to be optimized when changing cartridge manufacturers.

SiliCycle's R&D team wanted to see how the results obtained with a protocol optimized for one brand's column would change by using similar pre-packed columns from different manufacturers for the same separation. Bare silica gel was used to purify a commercially available apolar lipophilic heterocyclic solution from plants extracts and oils (*Figure 1*). Performance was determined by analyzing peak separation and resolution for equivalent spherical silica gels of different brands.

In addition, an initial run was performed between SiliCycle's Silia*Sep* PREMIUM cartridge (*spherical silica*) and Silia*Sep* cartridge (*irregular silica*) to confirm that spherical gel would provide better separation for this mixture.

Figure 1: Heterocycles present in the sample mixture



Caryophyllene oxide | Khellin | α-Santonin

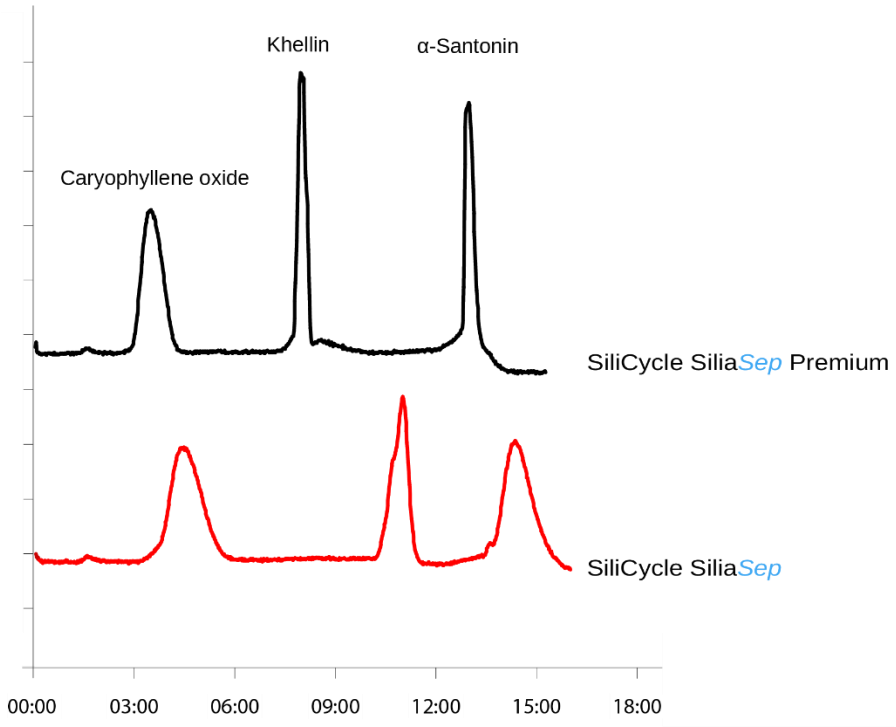
Table 1: Chromatographic conditions

Chromatographic Conditions	
Parameter	**Value**
CARTRIDGE	#1: Silia*Sep* PREMIUM Flash Cartridge, Silica-Based, 25 µm, 90 Å #2: Silia*Sep* Flash Cartridge, Silica-Based, 40 - 63 µm, 60 Å
PART NUMBER	#1: FLH-10095D-A-ISO40 #2: FLH-R10030B-ISO40
GRADIENT	1. 100 % Dichloromethane (*1.25 CV*) 2. 99.4 % Dichloromethane, 0.6 % Isopropanol (*11 CV*) 3. 97 % Dichloromethane, 3 % Isopropanol (*7 CV*)
TEMPERATURE	25°C
FLOW RATE	60 mL/min
DETECTOR	UV at 215 nm
INJECTION	0.5 of a solution of the mixture in methanol

Abbreviation used: CV = Column Volume

Comparison Between SiliCycle's Silia*Sep* PREMIUM and Irregular Cartridges

Figure 2: Separation of the mixture using Silia*Sep* and Silia*Sep* PREMIUM (*the elution order is Caryophyllene oxide, Khellin, α-Santonin*)



Spherical silica gel offers improved separation efficiency, better resolution, and greater loading capacity compared to irregular gel, especially for compounds that are considered harder to separate. As seen in Application Note Appn_SSp003-0, the use of spherical gel allowed greater loading capacity, superior resolution, and more efficient separation. The better separation and narrower and more separated bands of the Silia*Sep* PREMIUM cartridge shown in Figure 2 confirm these results with the natural products sample mixture.

For this reason, spherical gel was chosen for the remainder of the experiment. The same method and protocol were applied to five different brands and their pre-packed cartridges (*the most comparable in shape, particle size and pore diameter*).

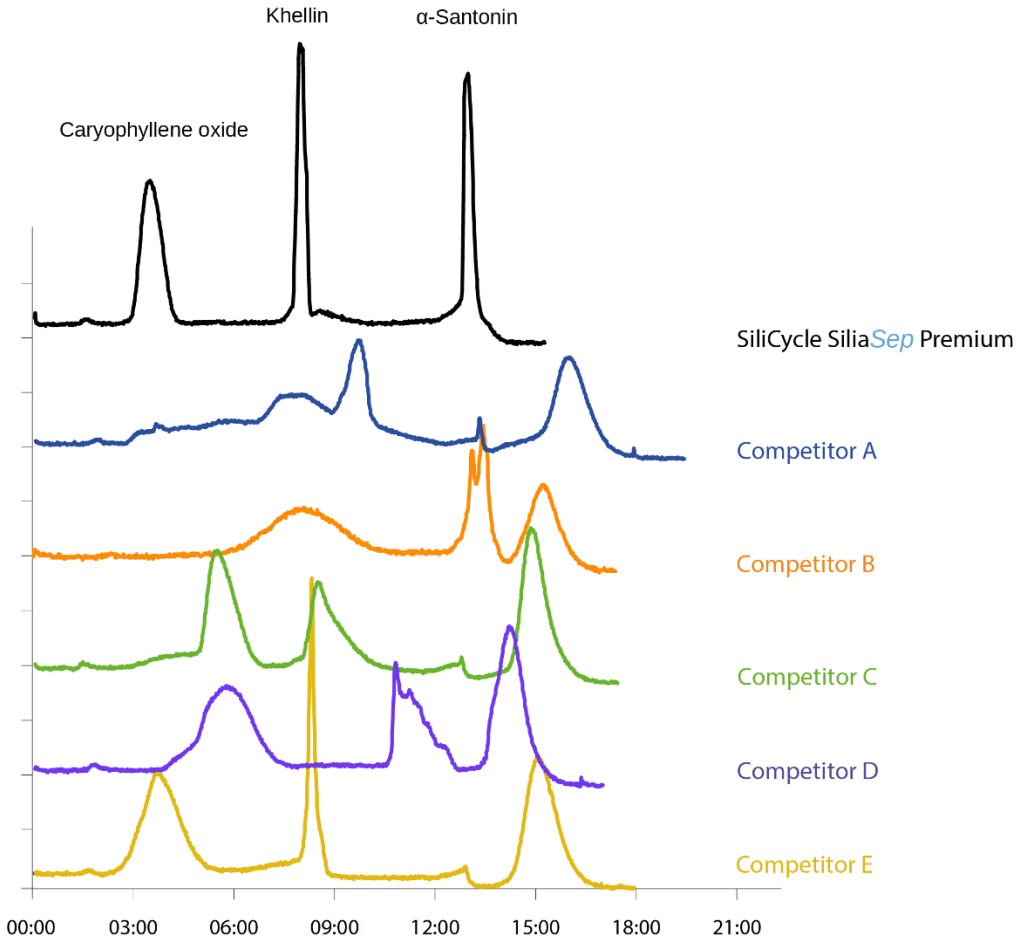
Comparison of Different Brands of Spherical Silica Gel

The specifications of the cartridges used in this experiment are shown in Table 2.

Table 2: Cartridges and silica gels comparison

Cartridge and Silica Gels Comparison			
	Cartridge Size (*g*)	Particle Size (*µm*)	Pore Diameter (*Å*)
Silia*Sep* PREMIUM	40	25	81.5
Competitor A	40	20 - 35	67.6
Competitor B	50	25	49.9
Competitor C	40	30	62.8
Competitor D	40	25	88.1
Competitor E	40	30	67.7

Figure 3: Separation of the mixture using cartridges from different manufacturers



As observed in Figure 3, four of the five competitor's cartridges offered poor performance, with only competitor E providing an acceptable separation of the compounds following the protocol presented in Table 1.

Competitor A's cartridge produced a chromatogram with a shoulder on the second peak and no return to the baseline. The absence of a straight baseline may suggest column overload occurred. Having two cartridges of comparable properties but a difference in loading capacity is an important factor in column comparison. Greater loading brings about solvent and time savings due to fewer runs needed for a given amount of sample. In the case above, this result suggests that SiliCycle's Silia*Sep* PREMIUM cartridges have greater loading capacity than this competitor.

In competitor B's chromatogram, broad peaks and peak splitting were observed in addition to longer retention times with poor separation between the peaks. The analyte's concentration in the injection volume exceeding the adsorption capacity of the stationary phase may contribute to shift in retention time and undesirable peak shape. This again could be a sign of cartridge overload.

Competitor C's chromatogram displayed baseline drifts and tailing. Tailing is a consequence of some molecules being more retained than the rest (*more interaction with the stationary phase*). Slow equilibration of the stationary phase could be a cause, and this as well could be due to cartridge overload.

The fourth competitor's chromatogram (*competitor D*) showed that all peaks were broader. Tailing on the second and fronting on the last peaks made the separation incomplete.

Lastly, competitor E had some broader peaks (*first and third*) with one sharp second peak. However, these peak shapes were good enough to provide a complete separation. Hence this cartridge offered the most acceptable separation of the compounds after SiliCycle's.

It must be noted that the run conditions were optimized on SiliCycle's Silia*Sep* PREMIUM cartridges and not individually on each of the competitors' cartridges. The results obtained using this protocol could be different with other conditions. With this in mind, conditions should not be replicated when changing supplier without taking the time to optimize the protocol.

Many factors can make or break a good separation, the choice of sorbent and its properties accounts for several of them. The shape of gel (*i.e. irregular or spherical*) is one to consider as was seen in Figure 2, since spherical provides better separation efficiency and resolution for more difficult compounds. As mentioned previously, loading capacity is another factor: greater loading capacity of the sorbent allows more efficient separation. Wide particle size distribution results in poor bed structure, and preferential paths may be followed by the compounds resulting in uneven peaks. Even the packing of the sorbent should be considered, as non-homogenous packing can have the same result as wide particle size distribution. All these criteria should be considered when sourcing silica gel cartridges to improve chromatographic results.

Conclusion

In conclusion, some chromatographic challenges can simply be solved by optimizing the run conditions, however sometimes only a change in supplier can improve chromatographic results. As seen above, not all cartridges provide the same separation under the same conditions although very similar in terms of listed properties. Complications associated with wide particle size distributions, poor packing, low loading capacity, or other problems inherent to the packing material can creep up and are best to avoid if possible. In the case of the separation of heterocyclic compounds, SiliCycle's narrow particle size distribution, uniform packing method, and greater loading capacity made Silia*Sep* PREMIUM cartridges the best in terms of separation efficiency among the six cartridges tested.

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