Redi*Sep*® Column Stacking

A useful technique for difficult sample separation

Mikael Mahler, Veronica Thomason
Teledyne ISCO, Inc.
Yoshio Furusho, Yoshie Tanaka
Artificial Helix Architecture Group, Yashima Super-Structured Helix Project, ERATO,
Japan Science & Technology Agency
Nagoya, Japan

Chromatography Application Note AN13

Overview

Flash chromatography purification of samples where TLC products spots are very close or overlapping are usually difficult to separate. In this context, it sometimes require several manual glass column chromatography purifications before obtaining a satisfactory yield of isolated products.

This application will describe exploitation of Redi*Sep* column stacking as a technique for resolving difficult compounds to separate.

Background

Sample mixtures containing two or more compounds which TLC spots under best solvent condition (*i.e.* optimal selectivity) show ΔR_f<0.20 represent challenging purification by flash chromatography.

Several time consuming manual flash chromatography purifications are usually necessary in order to achieve separation results ranging from medium to poor yields in pure single products isolated.

The combination of automated flash chromatography instrumentation and column stacking technique offers an attractive alternative in terms of time efficiency and increased resolution for difficult separations.

The column stacking technique consists of connecting several Redi*Sep* columns in series on a Teledyne Isco Combi*Flash* instrument, thus providing increased column length. A column stacking accessory for the Combi*Flash* instruments conveniently stacks Redi*Sep* columns (Figure 1).

Figure 1: Teledyne Isco Redi*Sep* column stacker

Results and discussion

Palladium catalyzed coupling reaction of 1-bromo 4-iodobenzene with (trimethylsilyl)acetylene led to a mixture of mono- and di-trimethylsilylacetylene products (Figure 2).

The products mixture is showing poor resolution by TLC under various solvent conditions, representing a challenging purification by flash chromatography (Figure 3).

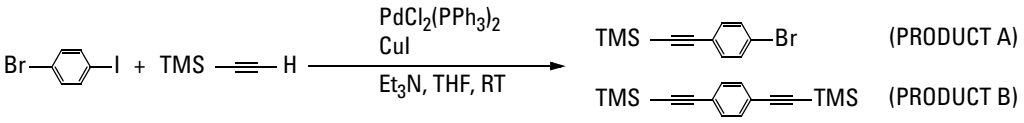

Figure 2: Reaction scheme

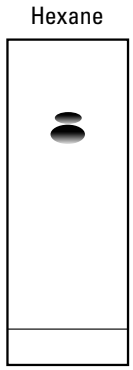

Figure 3: A & B products TLC in hexane

The separation of this mixture with the column stacking technique was examined.

The mixture was subjected to purification using one 4g Redi*Sep* normal phase column (Table 1).

The resulting chromatogram (Figure 4) shows the products peaks being slightly overlapped and that the baseline is not fully resolved. Therefore some cross-contamination may be possible.

An identical mixture was next subjected to purification using six stacked 4g Redi*Sep* normal phase columns (Table 2).

The resulting chromatogram (Figure 5) shows the product peaks being clearly separated with a well resolved baseline. Consequently, both fractions obtained contain pure single product.

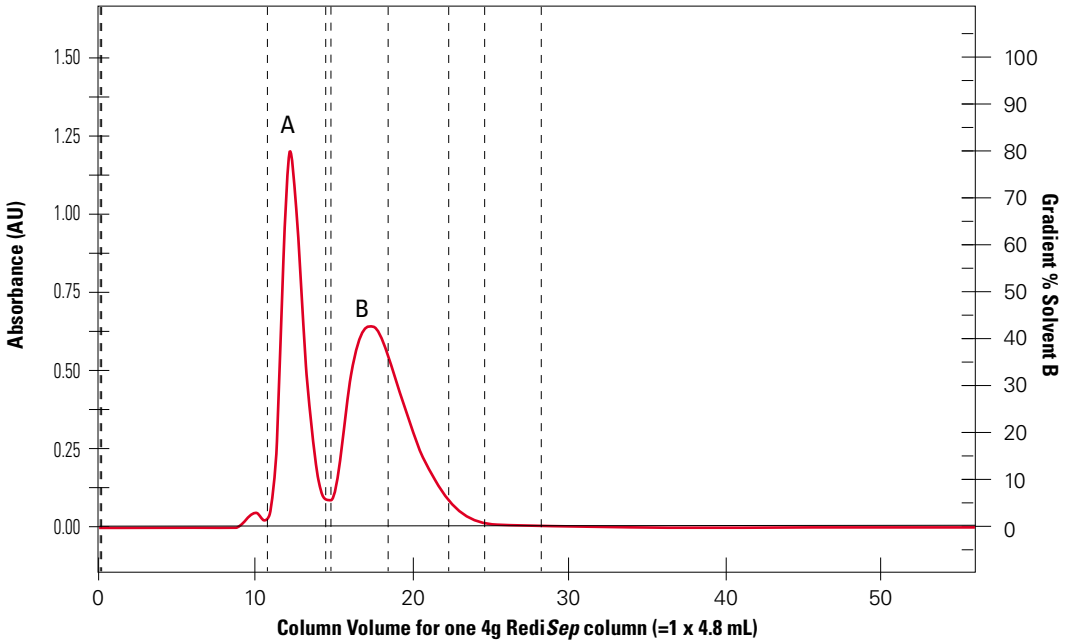

Figure 4: Separation of products A (left peak) & B (right peak) with one 4g Redi*Sep* normal phase column

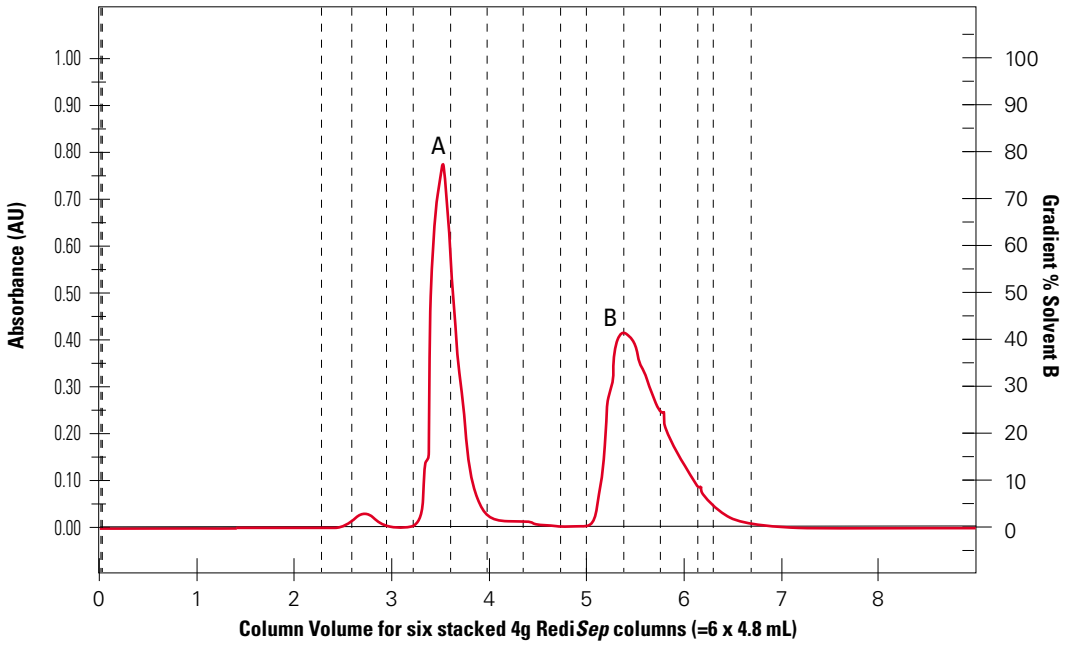

Figure 5: Separation of products A (left peak) & B (right peak) with six 4g Redi*Sep* normal phase columns

The advantage gained using this technique is increased column length while column diameter and column loading remain identical to a single column run (*i.e.* 1% w/w sample/1 column).

Considering a Redi*Sep* column of greater size (*e.g.* 1×12g column) compared to several smaller-sized stacked columns (*e.g.* 3×4g Redi*Sep* column), the column of greater size holds a larger diameter which could potentially generate a dilution problem for low concentration samples therefore leading to undetected sample. A lower resolution was also noted (*c.f.* Application Note AN12).

Increased column length provides extended interaction between sample products, stationary phase and mobile phase. As illustrated by the example described in this paper, the exposure over a longer column run length increases the resolution of the separation as the sample's products have more run time to proceed with the separation.

The suggested parameters to choose when operating stacked columns are:

Run length: Factor the time or Column Volume (CV) units used during the gradient profile of the chromatogram of a single column to the number of columns stacked onto the instrument.

Example:
10 CV run length with one single 4g Redi*Sep* column corresponds to 3×10 CV= 30 CV run length with three stacked 4g Redi*Sep* columns.

Flow rate: It is recommended to lower flow rate by 30% when stacking column compared to a single column default flow rate.

Sample loading method: Solid loading sample technique should be used with difficult purification due to TLC spot closeness. 1% loading of single column weight is recommended.

Experimental

Table 1: Method Parameters

Instrumentation:	Teledyne Isco Combi*Flash*® Companion™ 4x	
Column	1 × 4g Normal Phase Redi*Sep*	
Sample Loading Method	40 mg pre-loaded on celite (J.T. Baker celite 503)	
Wavelength	254 nm	
Mobile phase:	Solvent A: Hexane	
Flow Rate:	18 mL/minute	
Equilibration Volume:	3 column volumes	
Gradient:	% Solvent B	CV_1 (1 CV_1 = 1 × 4.8 mL)
	0	Initial
	0	56.0

Table 2: Method Parameters

Instrumentation:	Teledyne Isco Combi*Flash*® Companion™ 4x	
Column	6 × 4g Normal Phase Redi*Sep*	
Sample Loading Method	40 mg pre-loaded on celite (J.T. Baker celite 503)	
Wavelength	254 nm	
Mobile phase:	Solvent A: Hexane	
Flow Rate:	12 mL/minute	
Equilibration Volume:	18 column volumes	
Gradient:	% Solvent B	CV_2 ($1CV_2 = 6 \times 4.8$ mL)
	0	Initial
	0	9.0

Conclusion

The separation of a mixture of products difficult to purify due to close physico-chemical properties was successfully separated using stacked Redi*Sep* columns in conjunction with automated flash chromatography instrumentation.

Column stacking technique is a useful purification tactic available to chemists facing difficult samples separation with tight or overlapping TLC products spots.

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Teledyne ISCO
P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3091
www.teledyneisco.com