Redi*Sep*® Column Stacking

to improve resolution of normal phase flash chromatography

David Trail, Veronica Thomason, John Urh
Teledyne ISCO, Inc.

TELEDYNE ISCO
Everywhereyoulook™

Chromatography Application Note AN12

Overview

Purification of compounds that are difficult to separate by flash chromatography ($\Delta R_f \leq 0.2$ between spots on TLC) often results in additional steps such as subsequent purification by preparative scale HPLC.

It is possible to reduce the amount of additional work required for purification by simply stacking several prepacked Redi*Sep* columns end to end on a Combi*Flash*® automated flash chromatography instrument. In liquid chromatography, chemical species are separated on the basis of their difference in velocity as they move through the column. Increasing column length can significantly increase resolution.

By stacking columns end to end the length to diameter (L to D) ratio is increased so that no major changes to the media and solvent system are necessary. Often this increased L to D is sufficient to provide successful separation of difficult mixtures due to close compounds retention time that is not obtained on a single column. The difference in velocity is linear and by adding length, two closely eluting species can be separated. The data illustrates the linear relationship between of resolution and overall column length.

Method

To investigate the effectiveness of Redi*Sep* column stacking as a solution for increasing the resolution of closely eluting compounds, nine purification runs were performed on an automated flash chromatography system. Table 1 summarizes the experimental conditions. The first purification run used a 4-gram Redi*Sep* normal phase silica column. Each subsequent purification run added one 4-gram column in series until the stationary phase consisted of a stack of nine 4-gram columns.

For comparison purposes, two additional purifications were performed with different size columns. All other conditions for the comparison runs were the same. One of these comparison runs used a single column holding 12 grams of normal phase silica, the equivalent mass of three stacked 4-gram columns. The other comparison run used a single 40-gram normal phase column to approximate the stationary phase of nine stacked 4-gram columns.

Table 1: Experimental Parameters

Instrumentation:	Teledyne Isco Combi*Flash*® Sq 16x System with Column Stacker[a]	
Columns	Teledyne Isco Redi*Sep* Normal Phase Columns: 4g, 12g, 40g sizes	
Wavelength	254 nm	
Isocratic Mobile phase:	Solvent A: Hexane, 80% Solvent B: Ethyl acetate, 20%	
Sample	54 mg/mL Acetophenone, 4-methoxy Acetophenone	
Sample Inject Volume	4g column runs: 12g column runs: 40g column runs:	1 mL 2 mL 4 mL
Flow Rate:	18 mL/minute	

a. The Column Stacker (Figure 1) is an accessory for Combi*Flash* instruments. The accessory allows quick and easy column stacking for up to nine 4-gram Redi*Sep* columns. The Column Stacker also will accommodate as many as six 12-gram Redi*Sep* columns, or three Redi*Sep* columns ranging in size from 40 to 330 grams.

Figure 1: Teledyne Isco Redi*Sep* column stacker

Results

Chromatograms (absorbance unit traces) for the nine purifications using stacked columns and the two using larger, single columns are illustrated in Figure 2. The chromatograms for the stacked 4-gram columns clearly show the increased retention time resulting in improved peak-to-peak resolution. This demonstrates that increasing the L to D ratio by stacking columns can improve resolution, without the need to adjust other method parameters.

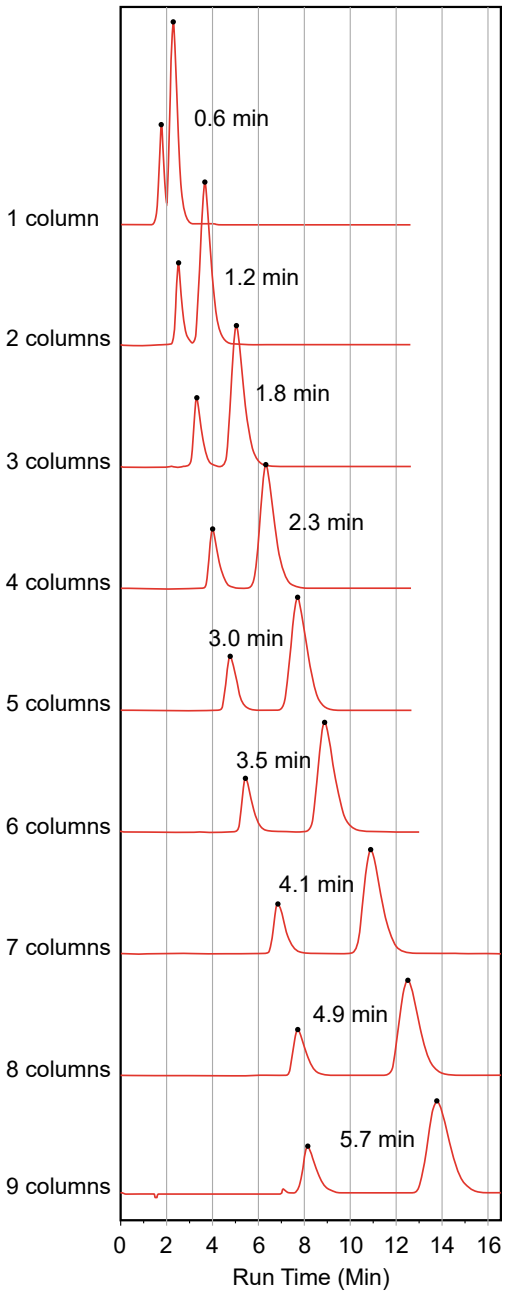

Figure 2: Peak-to-peak resolution of multiple stacked 4g columns

The three stacked 4-gram columns produced a peak-to-peak resolution time of 1.8 minutes (Figure 3). A single 12-gram column with a lower L to D ratio, produced a lower resolution of 1.2 minutes (Figure 4). Also, note that the baseline separation of the single 12-gram column provides little margin for error in peak collection. The 3-column stack produced better baseline separation of the peaks.

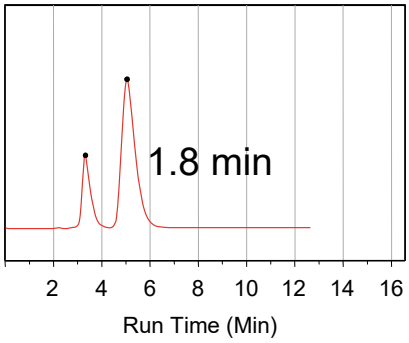

Figure 3: Three 4g columns stacked

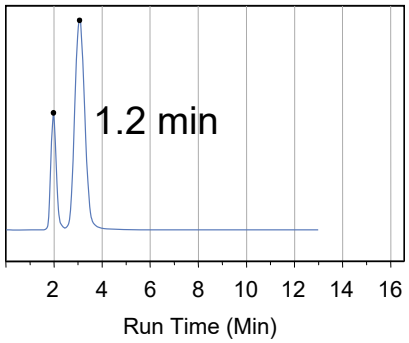

Figure 4: A single 12g column

A stack of nine 4-gram columns produced a peak-to peak resolution of 5.7 minutes (Figure 5); the single 40-gram column produced 3.4 minutes between peaks (Figure 6). Comparing the baseline separation between these two runs shows advantages of using the greater L to D ratio offered by stacking smaller diameter columns.

It should be noted that when stacking columns there is a small void volume between columns. This void volume had little effect on resolution as it increased linearly from one to nine columns (Figure 7).

This linear increase suggests that with a known peak-to-peak separation time of a single-column run, the approximate resolution from stacked columns can be extrapolated by multiplying the separation time from a single-column run by the number of stacked columns.

The technique of adding columns to increase resolution gives the chemist a handy tool for separating closely eluting species.

The improved resolution through column stacking also supports greater sample loading capacities to purify more sample in a single run.

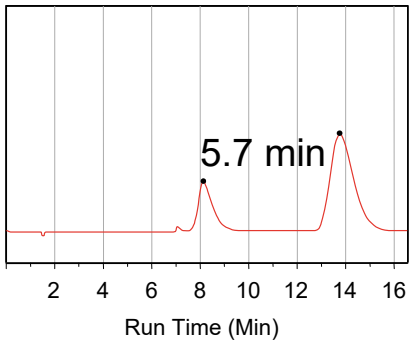

Figure 5: Nine 4g columns stacked

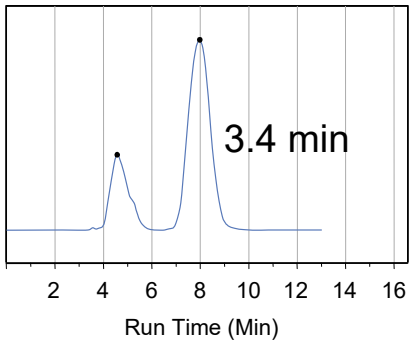

Figure 6: A single 40g column

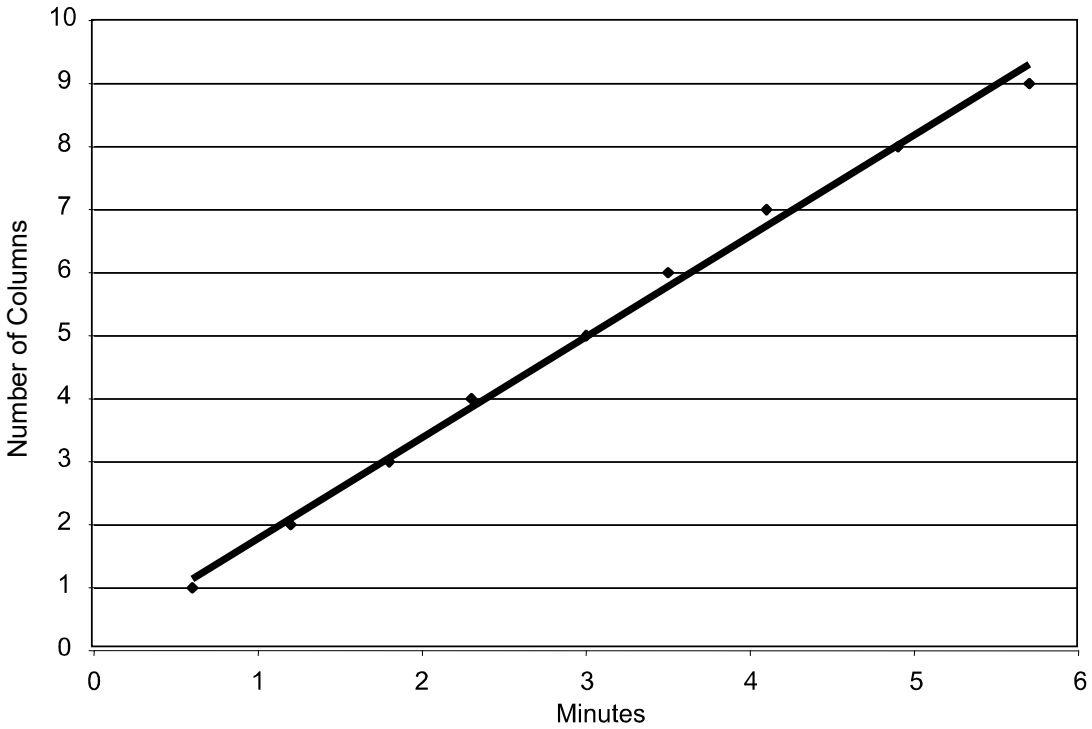

Figure 7: Linear relationship between number of stacked columns and peak-to-peak resolution times

Conclusion

Column stacking is a useful tool to achieve significantly greater resolution. As a result, column stacking provides chemists a convenient technique to separate difficult samples without significantly changing methods.

Stacking columns end-to-end provides greater resolution with each added column. Columns are added as required until the desired separation is achieved.

Last modified November 8, 2012

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