HPLC to Flash

TELEDYNE ISCO
Everywhereyoulook™

Chromatography Application Note AN118

Abstract

Teledyne ISCO analytical columns work very well to create methods for ACCQ*Prep*® HPLC systems from a simple scouting run. Aside from matching the Redi*Sep*® Prep stationary phase, these columns also match Redi*Sep* Gold® flash media, which means they can be used to develop methods for flash chromatography as well. Although C18 columns from other vendors may work for this purpose, there may be differences in selectivity as compared to Redi*Sep* columns, which means peaks may not elute at the expected time. The scale-up technique described in this application note works because a compound elutes at the same time at a particular solvent composition, so long as the time is defined in column volumes. An alternative method uses the ACCQ*Prep* HP150 with Redi*Sep* Prep columns and the Focused Gradient Generator built into the system to create a focused gradient from a scouting run (see Application Note AN119).

Materials required

Development of the flash method requires an analytical HPLC system and one of the columns listed in Table 1.

Column Type	Part #	Typical flow rate (mL/min)	Column Volume (mL)
RediSep Prep UHPLC C8 2x50 mm	69-2203-853	0.4	0.1
RediSep Prep UHPLC C18 2x50 mm	69-2203-854	0.4	0.1
RediSep Prep UHPLC C18AQ 2x50 mm	69-2203-855	0.4	0.1
RediSep Prep HPLC C8 4.6x150 mm	69-2203-856	1.0	1.5
RediSep Prep HPLC C18 4.6x150 mm	69-2203-800	1.0	1.5
RediSep Prep HPLC C18AQ 4.5x150 mm	69-2203-801	1.0	1.5

Table 1–Redi*Sep* Prep analytical columns

If solvent modifiers are needed, the same modifiers should be used on the HPLC and the flash system. Volatile modifiers are easier to remove from the sample after purification.

Experimental & discussion

For flash chromatography, an elution time of about 6 column volumes (CV) is preferred. As it is easy to scale-up from one column to another in units of CV, convert the uHPLC gradient to units of CV.

A three-segment linear gradient works well. The first segment is 12 CV long, with the desired compound eluting in the middle, at about 6 CV. The starting and ending solvent composition for this segment are whatever is needed to elute the compound. There is no need for an isocratic hold at the start of the run because one naturally occurs due to the HPLC system dwell volume. Depending on the size of the HPLC dwell volume relative to the column volume, peaks can elute earlier or later when running the Combi*Flash*® NextGen gradient. There is a step 0.01 minutes long followed by a wash at 100% B to purge retained compounds from the column.

Each segment length in minutes is calculated by multiplying the gradient length by the column's volume followed by dividing by the flow rate. The gradient table is made by adding the segment length to the sum of the previous times. In Table 2, segment lengths in column volumes are converted to time, and the segment lengths added to form a gradient table.

Segment length (CV)	Segment length (Minutes) 2x50 mm column (0.4 mL/min)	2x50 mm column gradient table	Segment length (Minutes) 4.6x150 mm column (1.0 mL/min)	4.6x150 mm column gradient table
12	3	3	18	18.0
0	0.01	3.01	0.01	18.01
3 (100% B)	0.5	3.5	4.5	22.5

Table 2–Suggested segment lengths for analytical columns in column volumes, converted to minutes and gradient tables that can be entered to create HPLC methods.

Once you have a working method with an acceptable resolution, it is easy to create a flash system gradient using the same gradient table, but in units of column volumes, for the Combi*Flash* instrument.

Results

The runs shown below used the gradient segments listed in Table 2. One run used a 2 x 50 mm C8 column, while the other used a 4.6 x 150 mm C18 column. Benzophenone was run with a water/acetonitrile gradient.

C8, 2x50 mm example

A method was developed on a Redi*Sep* Prep C8 2 x 50 mm column. The gradient was 40% to 50% B over 3 minutes, followed by a step to 100% B to purge contaminants from the column using a water/acetonitrile gradient.

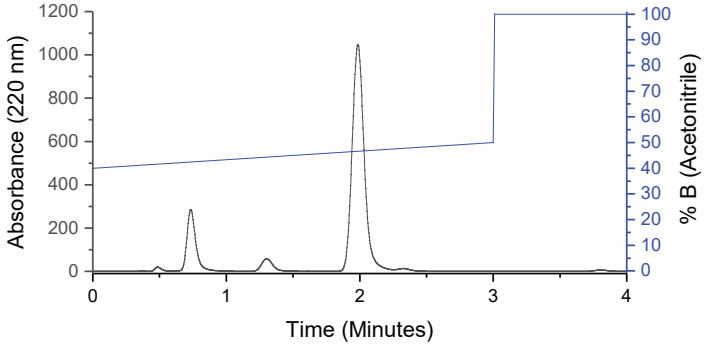

Figure 1–Benzophenone run on a Redi*Sep* Prep C8 column, 2x50 mm, 0.4 mL/min.

The compound was found to elute at 2 minutes or 7.9 CV. The sample was then run on a 50 g Redi*Sep* Rf Gold C8 column (PN 69-2203-712) using the same gradient, defined in column volumes.

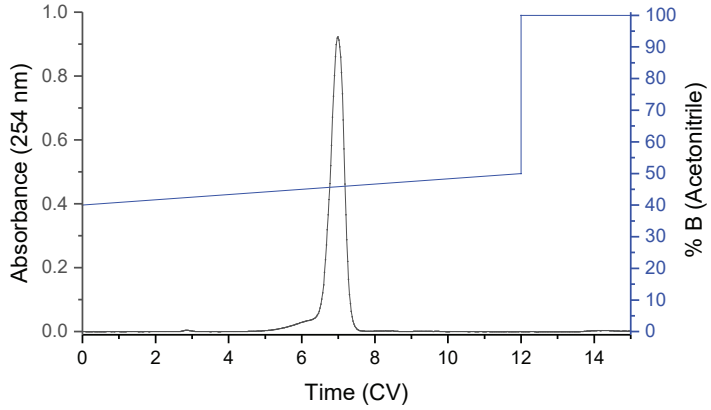

Figure 2–Benzophenone run on a Redi*Sep* Rf Gold C8 column, 50 g, run with the same gradient used for Figure 1

The elution from the flash column was very close to that from the uHPLC column, showing that a method can be transferred in this manner.

C18, 4.6 x 150 mm example

A method was developed on a Redi*Sep* Prep C18 4.6 x 510 mm column using water/acetonitrile. The gradient was 50% to 60% B over 18 minutes, followed by a step to 100% B to wash contaminants from the column. C18 is more retentive than C8, so the solvent system needs to be stronger to cause elution at the same retention time in column volumes.

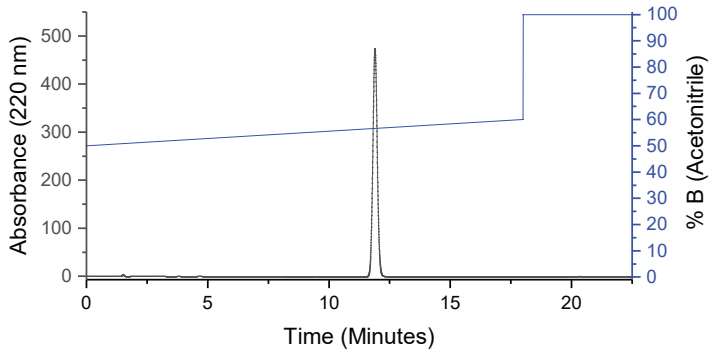

Figure 3–Benzophenone run on a Redi*Sep* Prep 4.6 x 150 mm C18 column, 1.0 mL/min

The compound eluted at 11.9 minutes, or 7.9 CV. The run was transferred to a 50 g Redi*Sep* Rf Gold C18 column (PN 69-2203-336) in water/acetonitrile.

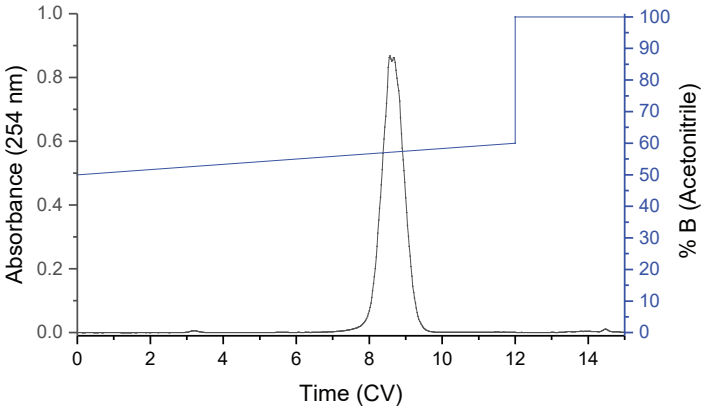

Figure 4–Benzophenone run on a Redi*Sep* Rf Gold C18 column, 50 g, run with the same gradient used for Figure 3, in column volumes.

Again, the flash column has excellent agreement compared to the analytical HPLC retention.

It may be noted that the flash run derived from the uHPLC column eluted slightly earlier than the predicted retention, while the HPLC predicted a slightly earlier elution than the actual flash run. This is because system dwell volumes were not considered in the calculations. The uHPLC and HPLC columns were run on the same analytical instrument. The analytical system dwell volume was large compared to the uHPLC column, but small relative to the 4.6 mm column, so the gradient was delayed to a different extent for the different runs. It is best to cause the desired compound to elute towards the middle of the gradient in the analytical run to allow for some variation in retention from dwell

volume between different instruments.

Conclusion

Redi*Sep* Prep columns can be used reliably to develop methods for flash chromatography. The 2 x 50 mm columns allow very rapid method development, with the run complete within 5 minutes. Both columns require minuscule amounts of sample, saving nearly all of the sample for purification instead.

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

March 2021