

# Method Transfer to RediSep® Gold C18 8.6 kg columns



Chromatography Application Note  
AN121

## Abstract

Teledyne ISCO's new 8.6 kg RediSep® Gold C18 column (PN 69-2203-900) is designed to maximize throughput of large-scale processes by providing fast and easy purification of up to 860 grams (sample dependent), including peptides, synthesized compounds, and natural products. The smaller spherical particle size provides superior resolution and higher loading capacity than other C18 flash media. RediSep columns are easily connected to other brands of chromatography systems as well, and methods using other C18 columns can easily be transferred to the 8.6 kg RediSep. The key factor to consider when migrating methods is column volume (CV). This application note provides a step-by-step procedure for transferring an existing method from any similar media or column size to the larger 8.6 kg RediSep Gold C18 column.

## Scale-up

The best way to scale-up is to work in column volumes. Within the same stationary phases, a compound elutes

at a consistent column volume if the gradient method is run as column volumes, regardless of the column size.

## Scale-up from pre-packed columns

The column volume of a pre-packed column is provided by its manufacturer. Since many instruments allow the gradient method to be programmed in terms of column volumes, it is easy to set up a run for the 8.6 kg column using CV.

If the existing gradient method is run as minutes, use the time, flow rate, and original column volume to convert to time as column volumes. As many systems are not programmed to run columns as large as 8.6 kg columns, the run time will then need to be converted back to minutes to program that system. A spreadsheet, such as the one below, makes the calculations easy.

	A	B	C	D	E	F	G	H	I	J	K
1	Time (minutes)	% B	Flow Rate	Segment length (Minutes)	Original column volume	Original column volume	Segment length (CV)	New Column volume	New Flow Rate	New segment length (minutes)	New time (minutes)
2	0	40	500								0
3	5	40	500	5	2500	3100	0.81	7500	850	7.12	7.12
4	60	50	500	55	27500	3100	8.87	7500	850	78.27	85.39
5	65	100	500	5	2500	3100	0.81	7500	850	7.12	92.50
6	80	100	500	15	7500	3100	2.42	7500	850	21.35	113.85

Figure 1- sample scale-up spreadsheet

## Method Transfer Procedure

1. Figure 1 is an example spreadsheet used for performing this procedure.
2. Set up the time for each segment in column A. Enter the initial starting conditions as shown in Row 2.
3. Enter the % B solvent for each segment in Column B. These are not used for the calculation, but the information is handy when programming the new method.
4. Enter the flow rate for the existing method into column C.
5. Column D is where each segment length is calculated. Start in the row containing the first gradient point after the initial set of conditions. Subtract the value in column A, previous row, from the value in column A, current row. As an example,  $D3 = A3 - A2$ , which yields 5 minutes. Filling down with this formula calculates the other cells' values.
6. Calculate the volume of solvent used by multiplying the flow rate (column C) by the segment length (column D).
7. Enter the column volume (CV) of the column currently being used into spreadsheet column F.
8. In column G, calculate the column volume for each segment by dividing column F by column E. If running a CombiFlash<sup>®</sup> Torrent or Torrent AQ, the column volumes can be entered directly into the system, so long as the software supporting the 8.6 kg column has been installed.
9. To convert the method segment lengths from CV to time, enter the CV for the 8.6 kg column into column H; the correct value of 7500 mL was entered for the example.
10. Enter the flow rate that your system can run into column I; the 8.6 kg column runs at 850 mL/min on a CombiFlash Torrent, but other systems may run at a slower flow rate.
11. Column J determines the segment length in minutes, calculated by multiplying column G times column H, and then dividing by column I.
12. If needed, you may convert the segment lengths to minutes. Enter '0' into cell K2. Add the value in the cell above the current row in column K to the cell in the current row in column J, then fill down.

## Scale-up from Radial Compression Modules

Radial compression modules are often run in terms of volume rather than time because the pressure is supplied by air. If your radial compression module is listed below, multiply the volume of solvent in the original method by the Scaleup Ratio in Table 1 to get the new volume of solvent for each step.

Radial Compression Module	CV (mL)	Scaleup Ratio
75 M	500	15
75L	1000	7.5
150M	4300	1.74
150L	8600	0.87

**Table 1- Column volumes of some popular radial compression modules**

## Scale-up from DAC columns

Dynamic Axial Compression (DAC) columns are scaled up similarly to a pre-packed column. However, the column volume is unknown due to differences in media, fill height, and packing pressure. The column volume for these is determined by measuring the void volume with a non-retaining compound. Salts such as nitrate or iodide salts work well, with detection at 210 nm. Set the column to run at 10% to 20% B solvent (the exact solvent composition doesn't matter). Inject some salt dissolved in water (10 mg/mL works fine) and note the time of the peak eluting at the void; the front of the peak works as reference point. Multiply the elution time by the flow rate to obtain the column volume. You can then use this column volume in a spreadsheet like that depicted in Figure 1.

## Conversion from other C18 materials

When changing from another brand of column, the selectivity between the C18 medias may be different. You can fine-tune a method on a 450g RediSep Rf Gold C18 column (PN 69-2203-369) from your existing method from the alternate brand's column as a starting point. Using a smaller column uses less time, solvent, and sample than fine tuning the method on the 8.6 kg column. Once the method is optimized, it can be scaled as described above.

## Conclusion

The RediSep Gold C18 8.6 kg column easily purifies large samples without expensive and heavy column hardware. The column can be connected to a pressurized solvent source or automated flash system and operated with full view of the separation. Method conversion from a Biotage or DAC column is easily calculated based on equivalent column volumes. The calculations to scale it up are simple and can be implemented in a spreadsheet.

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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

