

Large scale purification on 7.0 kg RediSep® silica columns



Chromatography Application Note
AN137

Abstract

Sometimes, a lot of sample needs to be purified using silica. RediSep 7.0 kg large columns can purify sample loads of a kilogram with sufficient resolution between the desired compound and the nearest impurity.

For example, purification using the Focus Gradient Generator in the CombiFlash® NextGen PeakTrak™ software allows for fast and easy calculation of a gradient that is easily scaled up for transfer to the CombiFlash Torrent®.

Types of RediSep silica in 7.0 kg

The 7.0 kg columns are listed in the table below. For the first column purchased, a tubing kit needs to be bought too, but this kit can be reused for subsequent columns.

Part number	Description	Typical uses
69-2203-921	7.0 kg, RediSep Gold Silica Gel Disposable columns, pkg. of 1.	Use this column when higher resolution and higher loading is needed
69-2203-922	7.0 kg, RediSep Silver Silica Gel Disposable columns, pkg. of 1.	This is a general-purpose column
60-2207-420	Large column tubing kit, for connection of 7.0 kg columns or larger to the CombiFlash Torrent.	This must be ordered separately for the 7.0 kg Silica column. This can be reused for subsequent columns

Why use these columns?

The 3.0 kg silica columns are run at 900 mL/minute, while the maximum flow rate for the Torrent is 1000 mL/minute. The 3.0 kg RediSep column volume is 4800 mL, compared with 10,048 mL for the 7.0 kg column. That means that running 7.0 kg column at a higher flow rate passes a CV worth of solvent faster than using two 3.0 kg columns. Also, it increases the loading capacity of the sample by about 15% on the 7.0 kg column versus using two 3.0 kg columns. However, the method and system are set up only once rather than twice for the sample, reducing errors and paperwork in GMP environments. The increased sample loading on a single 7.0 kg column versus two 3.0 kg columns, when combined with the higher flow rate (1000 mL/min), allows 1.2 times the throughput.

Method development and scaleup

PeakTrak on Teledyne ISCO CombiFlash NextGen chromatography systems feature the Focus Gradient Generator, which calculates focused preparative gradients from scouting runs. Figure 1 depicts a scouting run where the desired compound elutes at 10 column volumes. All runs use hexane and ethyl acetate with RediSep Gold silica columns. All columns were run using the methods built into PeakTrak. All runs used column volumes as the time axis.

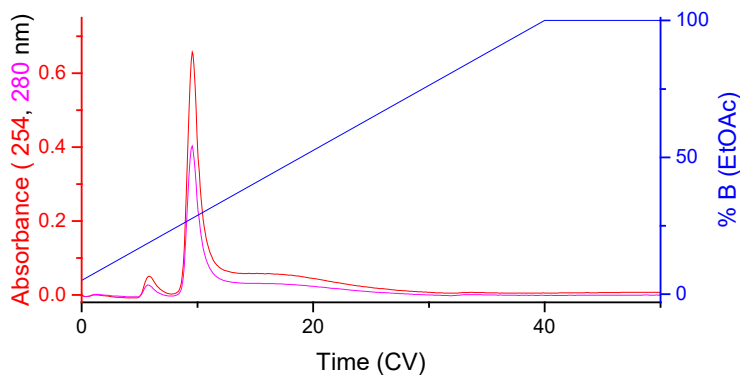


Figure 1. Scouting gradient of a mixture on a CombiFlash NextGen system using a 4 g RediSep Gold silica column.

After the scouting gradient was run, the Focus Gradient Generator on the NextGen was used to calculate the preparative gradient method. The chromatogram of the resulting run on a 40 g RediSep Gold Silica column (Figure 2) shows the desired compound eluting in ~4.5 CV.

The preparative method was transferred to the Torrent and to the 7.0 kg RediSep Gold Silica column. As the focused preparative method proposed on the NextGen is in column volumes, the same gradient length (in CVs) and the same starting and ending solvent composition can be used on the Torrent as on the NextGen for the 40 g column.

After the second peak eluted, the run was finished because the 40 g run showed that all the target material had eluted. Ending the run saved time and solvent. This run loaded

210 g sample (2% loading), but there is enough resolution to load even more—potentially a 5% load of 350 grams of sample. The scouting gradient in Figure 1 shows the peaks relatively close to one another. With more resolution between the compounds, it is possible to load a kilogram at one time.

Conclusion

The 7.0 kg columns are useful when one needs to purify kilograms of compounds in a short time. Such a column increases throughput during the actual run by 1.2x when using the maximum flow rate of the Torrent system. Additionally, it reduces setup-time compared to running multiple smaller columns, which reduces errors and documentation needs in GMP situations.

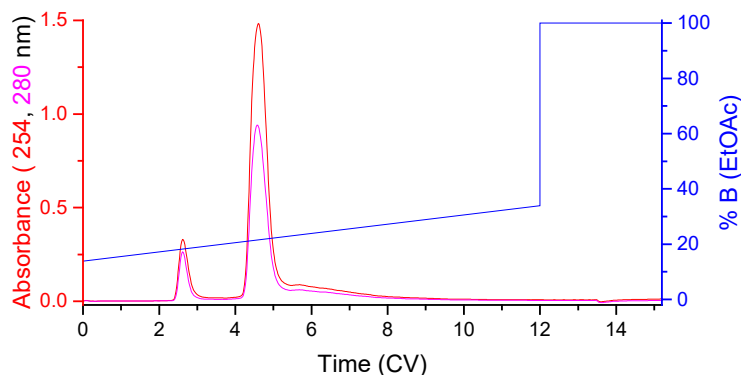


Figure 2. Preparative focused gradient run on a 40 g RediSep Gold Silica column on the CombiFlash NextGen system. (The gradient was focused on the second eluting peak from the scouting run in Figure 1.)

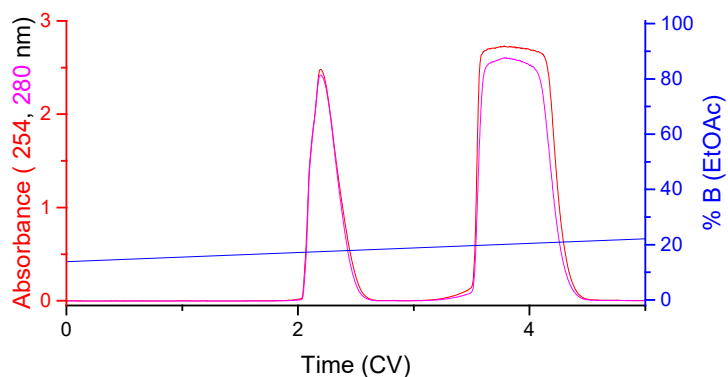


Figure 3. Run from Figure 2 scaled from a 40 g column to a 7.0 kg column on the CombiFlash Torrent system.

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