

Comparison of Solid Load Cartridges and Reservoir-style Columns

Abstract

The use of solid load cartridges with RediSep columns is compared to reservoir-style columns in terms of loading capacity. The loading capacity of RediSep columns paired with solid load cartridges is limited only by the resolution between the desired compound and nearby impurities while the reservoir-style columns are limited in load capacity by the reservoir. Solid load cartridges are also easier to use and allow walk-away automation on CombiFlash[®] Rf and CombiFlash Torrent[®] systems.

Background

Adsorbing a mixture on silica provides high resolution and load capacity¹. Researchers use reservoir-style columns because the sample adsorbed on silica can be dumped in the top of the column, so the user does not need a solid load cartridge. However, the loading capacity of reservoir-style columns is limited by the amount of material that can be placed in the reservoir. This type of column also requires that the sample be loaded after equilibration, necessitating an extra step during the purification.

Experimental and Results

Crude 3-(2-nitrophenyl amino) propionitrile was adsorbed on silica (20.0% w/w). This mixture was used for all experiments. All columns were run with a hexane/ethyl acetate (EtOAc) gradient. Detection was 254 nm. Columns were equilibrated with hexane. All runs used the same gradient from 0 to 100% ethyl acetate.

Table 1: Sample Load Parameters – 100, 120 g Columns

Column Size/Type	Silica w/ Sample (g)	Sample (g)	Percent Load	Solid Load Cartridge Used
100 g Reservoir	13.0	2.60	2.6	none
120 g RediSep	15.6	3.12	2.6	25 g empty
120 g RediSep	31.2	6.24	5.2	65 g empty

1. Teledyne Isco, Inc. http://www.isco.com/WebProductFiles/Applications/101/Poster_and_Paper_Reprints/Sample_Loading_techniques_for_Large_Scale_Flash_Chromatography.pdf, accessed December, 2010

100 and 120 g Columns

A reservoir-style column containing 100 g silica was used for this experiment. The reservoir insert was determined to hold 13.0 g of silica and sample when completely filled.

The columns were run as described in Table 1. The resulting chromatograms are shown in Figure 1.

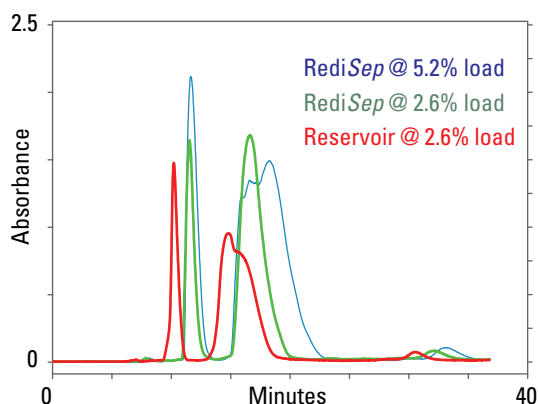


Figure 1: 100 g reservoir column with filled reservoir (red); 120 g RediSep column with solid load cartridge with same loading (green) and twice loading (blue) of reservoir column

Both types of columns purified the sample with baseline resolution. However, the RediSep column was not constrained by sample load volume limits and demonstrated separation of all compounds with twice the load. The only way to increase the amount of sample with a reservoir column would be to use a larger column with increased run time and solvent usage, or split the sample and perform multiple runs.

At a 5.2% load, the RediSep column is showing overloading, but the two major compounds still have baseline resolution. Using the RediSep column at the higher load allows the use of only one column, saving time and money (Table 2).

Table 2: Comparison of time and cost to purify 6.2 g of sample

Column Size/Type	Volume Required (liters)		Run Time (minutes)	Solvent cost (USD)
	Hexane	EtOAc		
100 g Reservoir	4.2	3.2	128	\$152
120 g RediSep	2.1	1.6	64	\$76

330 g Columns

Samples were run on either a *CombiFlash* Rf-200 or a *CombiFlash* Torrent system. The *CombiFlash* Torrent was used when the 270 g solid load cartridge was required.

Table 3: Loading parameters – 330 g columns

Column Size/ Type	Silica w/ Sample (g)	Sample (g)	Percent Load	Solid Load Cartridge Used
330 g Reservoir	38.6	7.7	2.3	none
330 g RediSep	38.6	7.7	2.3	65 g empty
330 g RediSep	66.0	13.2	4.0	270 g empty (on Torrent)
330 g RediSep	78.2	15.6	4.7	270 g empty (on Torrent)

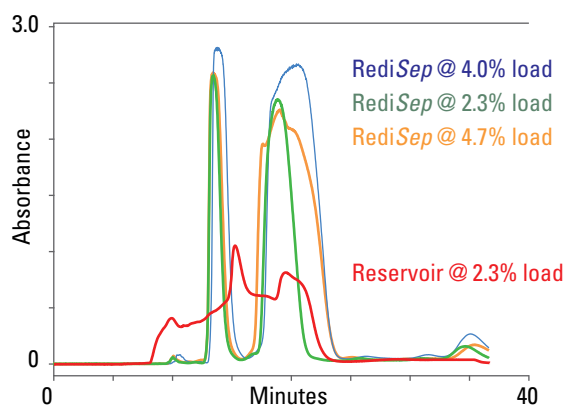


Figure 2: 330 g column runs Reservoir-style column with 38.6 g (2.3%) sample load compared to RediSep column at varying sample loads.

In this case, the reservoir column performed poorly compared the RediSep column. The RediSep column was not limited by either resolution or solid load cartridge capacity—it was always possible to increase loading by using a larger solid load cartridge.

The reservoir columns are subject to leaking if a small amount of silica gel is left on the column seal to the cap. In addition, the reservoir needs to be drained after equilibrating the sample. RediSep Rf solid load cartridges allow automatic injection and walk-away automation on *CombiFlash* instruments.

The reservoir columns are also limited to ~100 psi pressure due to the screw cap design. RediSep columns are rated to 200 psi, allowing higher flow rates.



Figure 3: Reservoir Column showing equilibration solvent which requires removal before loading sample

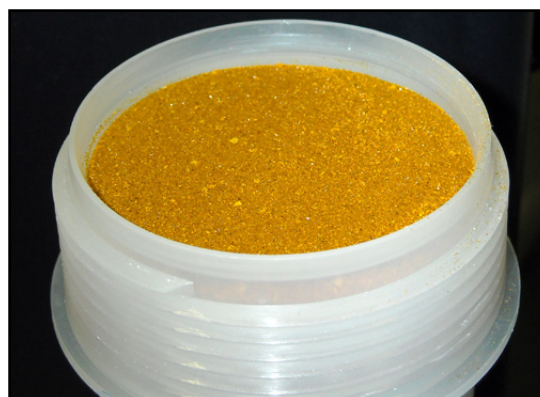


Figure 4: Sample Adsorbed on Silica added to the reservoir column up to the fill mark



Figure 5: Leakage and Sample Loss Evident around the threads of the reservoir-style column

Table 4: Comparison of time and cost to purify 6.2 g of sample

Column Size/ Type	Volume Required (liters)		Run Time (minutes)	Solvent cost (USD)
	Hexane	EtOAc		
330 g Reservoir	10	7.4	183	\$364
330 g RediSep	5.0	3.7	92	\$182

Conclusion

RediSep Rf columns and cartridges allow sample loading limited only by the resolution of the compound and impurities. Reservoir columns are limited by the capacity of sample that can be loaded into the reservoir. Solid Load Cartridges are easier to use because there is no need to drain the reservoir after equilibration. In addition they are less subject to leakage and sample loss and can run at higher back pressures. The compound can automatically be loaded after column equilibration when solid load cartridges are used; reservoir style columns require user intervention to load the sample after column equilibration.

The increased loading capacity of RediSep columns when used with solid load cartridges allows time and solvent savings over the use of reservoir columns.

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