

RediSep® Rf C18 Flash Column Loading Techniques

Abstract

There are several techniques for loading samples onto reusable RediSep Rf C18 flash columns. The techniques can be separated into two categories: Solid Load Cartridges and Liquid Injection. When using the solid load technique, the sample can be either wet or dry loaded.

The various loading techniques are evaluated for resolution with the same reaction mixture. The effect of air from the solid load cartridge is also explored in this application note.

Experimental

The sample purified was a crude reaction mixture of 3-(2-nitrophenyl amino) propionitrile that were previously synthesized¹. Except where noted, 0.15g sample (1% sample load) was loaded onto a 15.5g RediSep® Rf Gold C18 column (PN 69-2203-334) that was used for all experiments. The compounds were eluted with 35% acetonitrile in water followed by a gradient to 100% acetonitrile to wash the column for re-use. Other experiment details are described for each loading technique.

Results and Discussion

Solid Load Cartridges

Celite

Compounds run on reverse phase columns can be loaded on Celite 545 which absorbs weakly. The water and organic solvents used for RediSep Rf C18 is a “strong” solvent for eluting compounds from Celite so there are few, if any, issues for using this material to adsorb compounds.

“Empty” Solid Load Cartridges

In *Figure 1*, the sample was dissolved in methanol containing dichloromethane and mixed with 1.35g Celite 545 (10% total sample on Celite). A sufficient amount of dichloromethane was used to ensure dissolution of the sample. The slurry mixture was evaporated to dryness and the powder was placed in an empty 5g RediSep® solid load cartridge (PN 69-3873-235). The cartridge is described as “empty” because it contains no media until filled by the user. The purification was run as previously described. The major peaks eluted at 8 and 15 column volumes with baseline resolution.

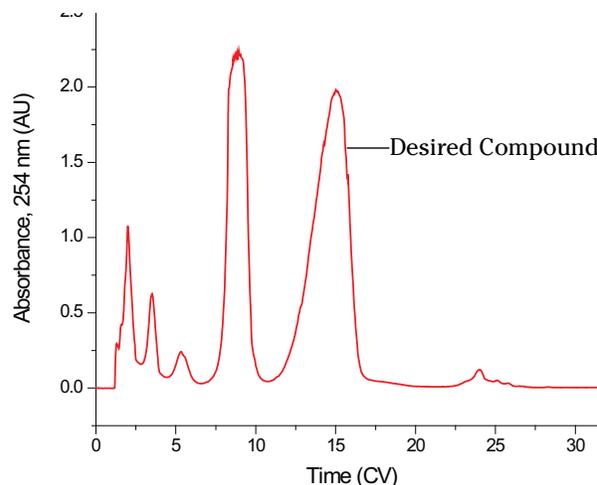


Figure 1: Sample pre-absorbed on Celite and loaded in an empty solid load cartridge

Adsorbing the compound on Celite allows other solvents to be used to dissolve your sample other than those used for the purification. Also, any volume of solvent can be used to dissolve the solvent without compromising the purification. Samples dried on Celite also avoid clogging issues due to sample precipitation.

Pre-packed Celite Cartridges

In the experiment depicted in *Figure 2*, the sample was dissolved in acetonitrile and liquid loaded onto a 5g pre-packed Celite solid load cartridge (PN 69-3873-313). Air pressure was used to push the sample and solvent near the bottom of the solid load cartridge. The cartridge was dried in a vacuum oven at 60° C for two hours prior to elution. The sample was dissolved in acetonitrile because it was found to be more soluble than in dichloromethane. Dissolving the sample in acetonitrile allowed it to be entirely loaded on the cartridge.

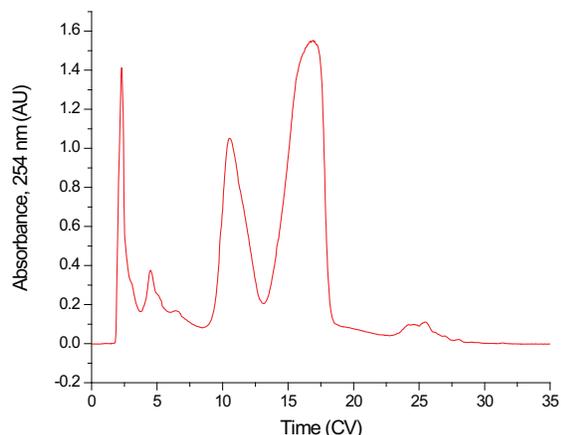


Figure 2: Reaction mixture adsorbed on pre-packed Celite solid load cartridge and dried

The peaks were not as sharp or resolved as that shown in *Figure 1*, because the solvent used to dissolve the sample was not completely evaporated. Drying the cartridge for a longer period would likely remove this residual solvent. The two major peaks still exhibit near-baseline separation.

The separation depicted in *Figure 3* differs from *Figure 1* in that the sample was dissolved in 2mL of 1:1 acetonitrile to water and loaded onto the 5g pre-packed Celite cartridge without drying.

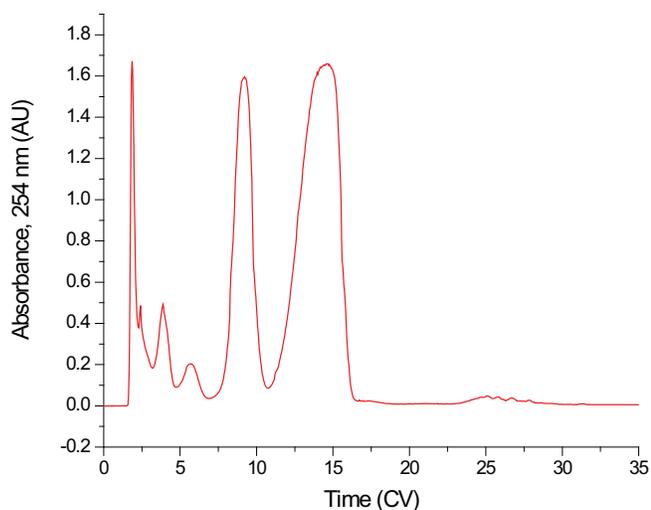


Figure 3: Sample dissolved in 1:1 acetonitrile: water and run on a pre-packed Celite solid load cartridge without drying

Both the peak shape and resolution are improved for the run depicted in *Figure 3* compared to that in *Figure 2*, because there was less of the strong solvent used to dissolve the sample; however, both runs are inferior to the sample dried on Celite (*Figure 1*).

RediSep Rf C18 Solid Load Cartridges

The experiments in this section used a 5g RediSep[®] Rf C18 solid load cartridge (PN 69-3873-237). The RediSep Rf C18 solid load cartridge is reusable and acts as a pre-column that prevents non-eluting compounds from becoming trapped on the main column (*Figure 4*).



Figure 4: RediSep C18 solid load cartridge "catches" retained compounds extending the life of the main C18 column

The RediSep Rf C18 solid load cartridge also adds more C18 packing which allows more compound retention. RediSep Rf C18 solid load cartridges should be conditioned in a similar fashion to C18 columns by washing them with the strong organic solvent followed by washing with water. The amount of solvent for each wash, in milliliters, is twice the weight of the cartridge. For example, a 5g cartridge is conditioned with at least 10mL of each solvent.

Sample Loading with Strong Solvent in Non-conditioned Cartridge

Although the RediSep Rf C18 solid load cartridge should be conditioned prior to use, the conditioning may be performed during sample load if the sample is dissolved in an organic solvent.

The disadvantage is that the chromatography may suffer as depicted in *Figure 5*, where the sample was dissolved in 2mL acetonitrile and the sample and solvent were pushed to the bottom of the solid load cartridge with air. The acetonitrile in the solid load cartridge is able to carry the compound through the column with reduced interaction with the RediSep Rf C18 packing.

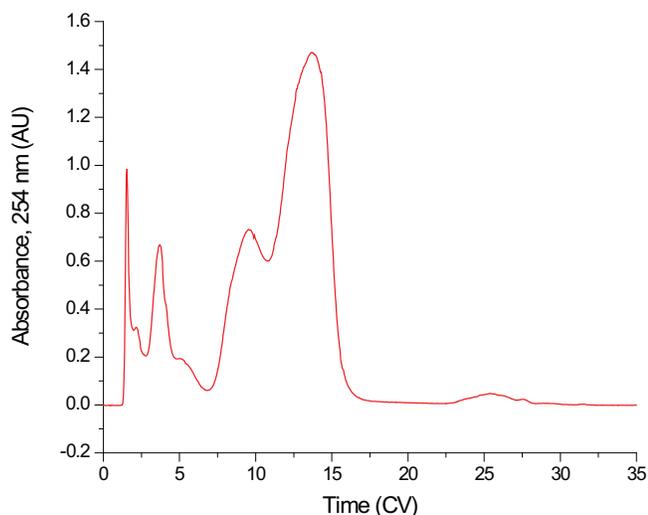


Figure 5: Sample loaded in strong solvent on unconditioned C18 solid load cartridge

Sample Loading with Strong Solvent in Conditioned Cartridge

The purification in *Figure 6* was run similarly to that of *Figure 5* except that the cartridge was conditioned with 10mL acetonitrile followed by 10mL water. This was done by placing the cartridge on the Rf 200 and running the solvent in manual mode.

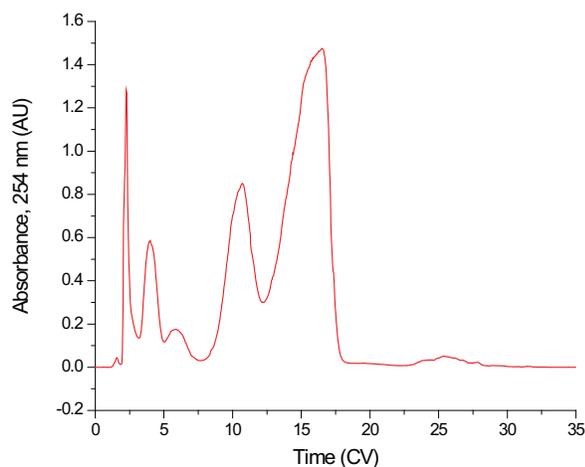


Figure 6: Sample in strong solvent eluted from properly conditioned C18 solid load cartridge

The properly conditioned solid load cartridge in *Figure 6* exhibits improved resolution of the two main peaks compared to the unconditioned cartridge in *Figure 5* due to the additional separation in the RediSep Rf C18 cartridge.

Sample Loading with Weaker Solvent in Conditioned Cartridge

The sample, depicted in *Figure 7*, was dissolved in 2mL 1:1 acetonitrile to water. This solvent mixture contains half the amount of strong solvent that used in *Figure 6*.

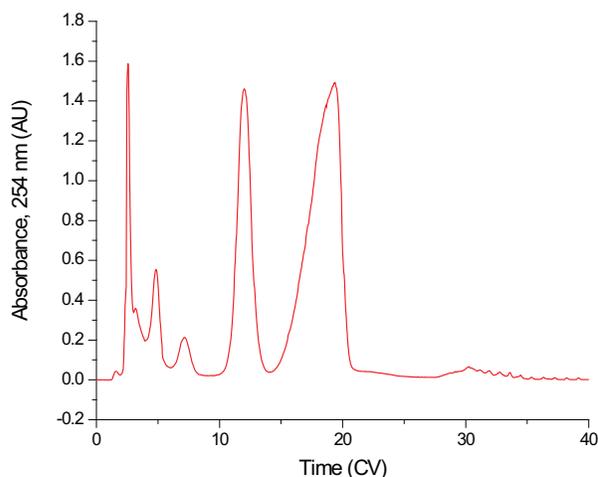


Figure 7: Sample loaded with 50% strong solvent on a conditioned solid load C18 cartridge

The addition of water, weakening the solvent system, greatly improved the chromatography.

Effects of Air on the Column from the Solid Load Cartridge

When using solid load cartridges, especially when the sample has been dried onto the sorbent, air is pushed onto the column at the start of the purification. With traditional glass columns, channeling is observed if the column is allowed to run dry. Separations with silica columns on the CombiFlash[®] systems show that chromatography is unchanged from air introduced by the solid load cartridge². For RediSep Rf C18 columns, John Dolan has indicated that a small amount of air does not cause issues with retention or resolution unless the column is allowed to dry while containing a buffer³. For the short amount of time it takes to purge air from the solid load cartridge, drying the column does not affect resolution.

The experiments in *Figure 8* were run with identical samples. The crude reaction mixture was adsorbed onto Celite 545 to create a 10% load (w/w) of sample on Celite. This material was placed in two separate solid load cartridges (1.0g, 0.10g reaction mixture). One sample was run (*Figure 8* upper image) without removing the air from the cartridge while the second separation (*Figure 8* lower image) the air was removed with water.

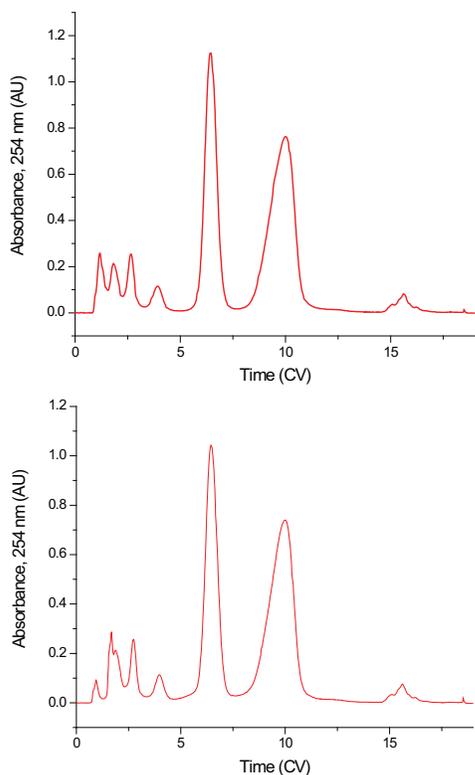


Figure 8: Sample loaded on Celite with air (top) and with air purged from cartridge (bottom)

Liquid Injection

Liquid injection is commonly used because it is convenient and fast; there is no cartridge to condition and no time required to dry the sample. When injecting directly on the column insoluble material is placed on the head of the column, unless the sample is filtered, reducing the life of the column.

An example of direct column injection is that of vitamin B₁₂ in *Figures 9* and *10*. A 10mg sample was run for each experiment. The first example was dissolved in 1.0mL of 1:1 methanol to water and the second was dissolved in 1.0mL water. Both experiments were run with the same gradient (0-100% B). A was 0.1% formic acid in water; B was 0.1% formic acid in methanol. Absorbance was measured at 210nm.

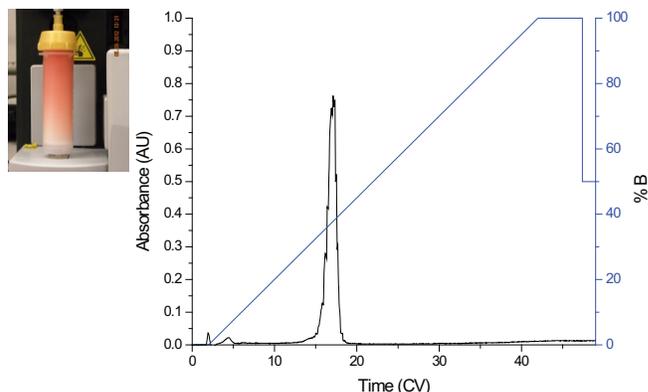


Figure 9: Vitamin B₁₂ dissolved in 50% methanol in water. Inset shows the compound elution after 1CV elution.

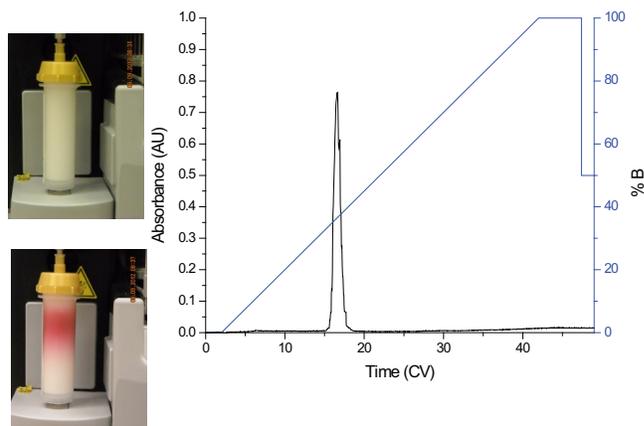


Figure 10: Vitamin B₁₂ dissolved in 100% water. Top inset shows compound retained under the Gold Cap after 1CV elution. Bottom inset shows tight band eluting later in the purification.

For the sample dissolved in the water/methanol mixture, the methanol dragged the compound down the length of the column during the early portion of the run (*Figure 9*). This is visible in the chromatogram as a minor peak at 2 CV, as well as shown by the bright red color throughout the length of the column. The sample dissolved in water is visible as a narrow band and exhibits improved peak shape.

Conclusion

Dry loading a sample using Celite provides the best resolution, followed by loading a sample dissolved in a minimal amount of strong solvent onto a properly conditioned RediSep Rf C18 solid load cartridge. When using a solid load cartridge, air from the cartridge shows no negative impact on the separation. Solid load cartridges capture insoluble and highly retained material which extends the life of the column. Solid load cartridges also allow complete walk-away automation with the Combi-Flash[®] Rf-200 because the sample is automatically eluted onto the column after the column is equilibrated.

For liquid injections, using a minimal amount of strong organic solvent provides the best resolution. Liquid loading onto solid load cartridges is convenient and still allows samples to be automatically loaded onto the column after equilibration.

References

1. http://www.isco.com/WebProductFiles/Applications/101/Application_Notes/AN83_Multi-step_Synthesis_and_Purification.pdf retrieved 23 Dec 2011
2. http://www.isco.com/WebProductFiles/Applications/101/Application_Notes/AN29_Overview_of_Silica_Column_Sample_Loading_Techniques.pdf retrieved 23 Dec 2011
3. <http://www.sepscience.com/emails/HPLCsoldemo.pdf> retrieved 23 Dec 2011

Teledyne Isco

P.O. Box 82531, Lincoln, Nebraska, 68501 USA
Toll-free: (800) 228-4373 • Phone: (402) 464-0231 • Fax: (402) 465-3091
E-mail: IscoInfo@teledyne.com

Teledyne Isco is continually improving its products and reserves the right to change product specifications, replacement parts, schematics, and instructions without notice.

