RediSep Gold® C18 Flash Column Loading Techniques

TELEDYNE ISCO
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Chromatography Application Note AN88

Abstract

There are several techniques for loading samples onto reusable RediSep Gold 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) proprionitrile that were previously synthesized[1]. Except where noted, 0.15 g sample (1% sample load) was loaded onto a 15.5 g RediSep 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

Silica

Pre-packed silica cartridges work very well for loading reverse phase columns. They work because water, a weak solvent for reverse phase, is a strong solvent for silica gel, causing compounds to move from the solid load cartridge and become adsorbed on the reverse phase column.

Silica columns should be conditioned with the solvent composition used at the start of the purification. Although samples can be dry loaded, very polar compounds which elute early, less than 50% B solvent, tend to show reduced resolution because these compounds are so polar that the silica needs to adsorb water before releasing the compounds.

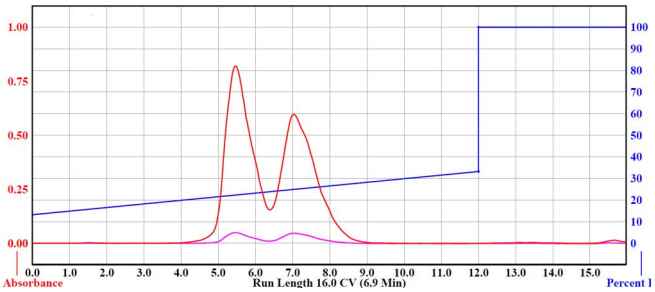

Figure 1: Catechol and resorcinol run on C18 after being dissolved in water

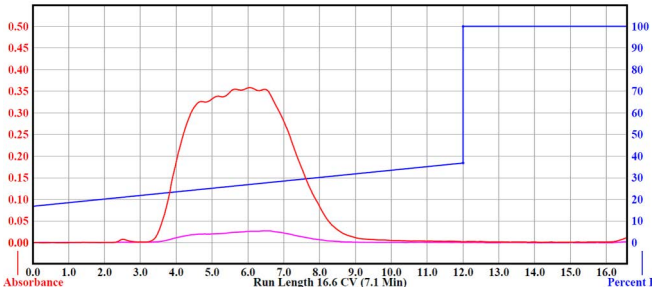

Figure 2: Catechol and resorcinol dry loaded on silica showing loss of resolution

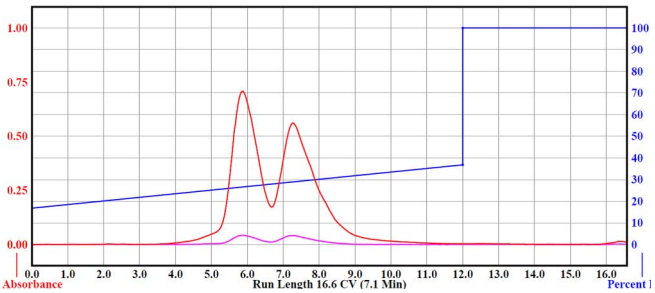

Figure 3: Catechol and resorcinol run on an equilibrated silica cartridge. The sample was dissolved in 1:1 methanol/water and shows similar resolution to a sample dissolved in water injected directly on the column (Figure 1).

The silica in the solid load cartridge also acts like a guard column, adsorbing compounds that would be irreversibly adsorbed on the reverse phase column (Figure 4)

Figure 4: Yellow material on the silica is irreversibly bound material which otherwise would be adsorbed on the reverse phase column.

To run the cartridge, use manual control to condition the cartridge with the conditioning volume listed in Table 1.

Table 1: Cartridge Conditioning Volume

Part #	g packing	Injection volume	Conditioning Volume
693873226	65	45	100
693873310	32	23	50
693873241	25	19	40
693873243	12	9	20
693873236	5	3	8
693873238	2.5	2	5

For easier injection of the sample, use P-661 Female Luer Adapter to 5/16-24 Male Tefzel™ (ETFE) available from Idex Health & Science (already installed on NextGen 100 flash systems). Next, inject the sample, see Table 1 for the volume that can be injected into various cartridges.

Figure 5: Silica solid load cartridge and cap with a Luer adapter.

Load the cartridge and cartridge on the system as usual. For systems with injection valves, the injection type should be “Solid Load” or “Solid with pause” since the flow path uses the solid load cartridge.

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 Combi*Flash*® systems show that chromatography is unchanged from air introduced by the solid load cartridge[2]. For Redi*Sep* Gold 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 buffer[3]. 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 6 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 (1.0 g, 0.10 g reaction mixture). One sample was run (Figure 6, upper image) without removing the air from the cartridge while the second separation (Figure 6, lower image) the air was removed with water.

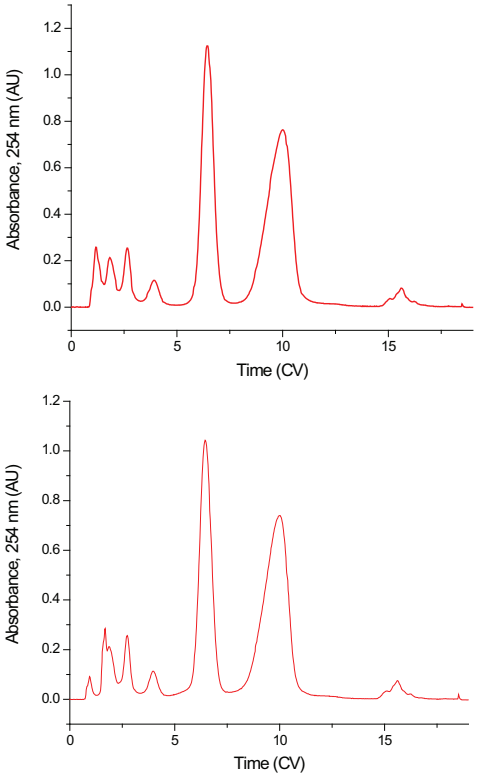

Figure 6: Sample loaded on Celite with air (top) and with air purged from cartridge (bottom)

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.

Methanol/water and water sample injections

An example of direct column injection is that of vitamin B_{12} in Figures 7 and 10. A 10 mg sample was run for each experiment. The first example was dissolved in 1.0 mL of 1:1 methanol to water and the second was dissolved in 1.0 mL 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 210 nm.

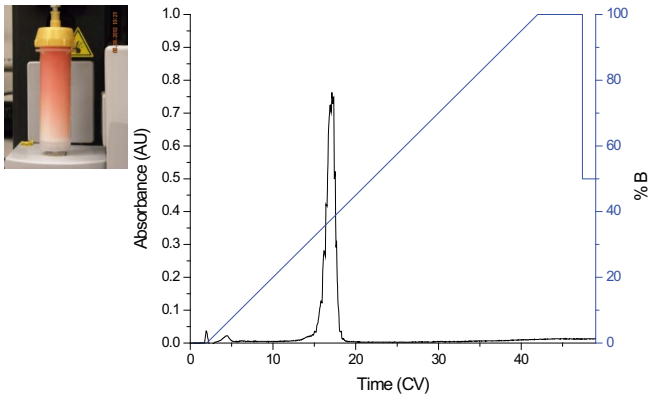

Figure 7: Vitamin B_{12} dissolved in 50% methanol in water. Inset shows the compound elution after 1 CV elution.

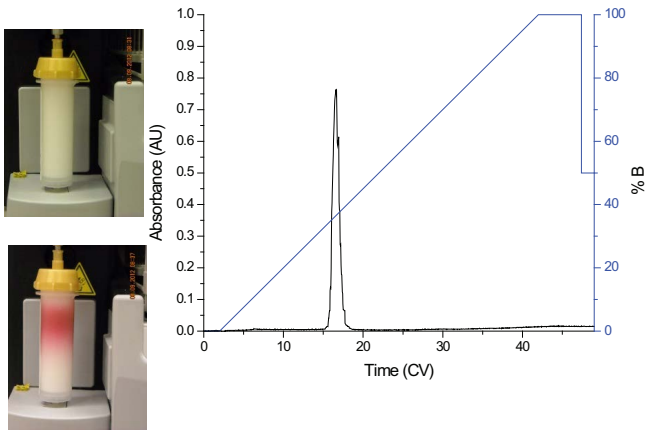

Figure 8: Vitamin B_{12} dissolved in 100% water. Top inset shows compound retained under the Gold Cap after 1 CV elution. Bottom inset shows tight band eluting later in the purification.

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 7). 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.

Dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) sample injections

DMSO and DMF are widely used to dissolve samples for column chromatography because the majority of compounds are soluble in these solvents allowing a large mass of sample to be injected in a small volume. These solvents are "weaker" than the common "B" solvents used in reverse phase chromatography such as methanol and acetonitrile and so reduces peak splitting. However, DMSO and DMF are stronger than water in reverse phase, so they solvents contribute to peak broadening in highly aqueous mobile phases, less than 50% organic solvent (Figure 9). Compounds eluting near the void volume (one column volume) will be mixed with DMSO/DMF and will be more difficult to evaporate.

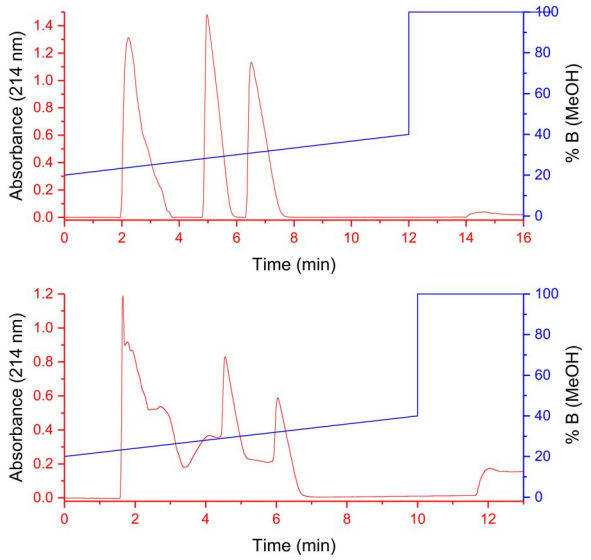

Figure 9: Catechol and resorcinol dissolved in DMSO. 2.5 mL injection (top), 3.5 mL injection (bottom)

Some tips for DMSO/DMF injection include:

- If the compound elutes in less than 30% organic solvent, try dissolving it in plain water. Some Combi*Flash* systems are able to calculate focused gradients (see TN-62 on the Teledyne ISCO web site). If the focused gradient end-point is less than 30% organic solvent, the compound likely dissolves in water.
- Make the sample as concentrated as possible in DMSO or DMF to minimize the volume of injection solvent.
- Try diluting the sample with water until it is barely cloudy. This makes the injection solvent weaker compared to the mobile phase and less likely to cause peak broadening.

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 Redi*Sep* Gold 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*® NextGen 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

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3. Dolan, J. *HPLC Solutions.* Separation Science. http://www.sepscience.com/emails/HPLCsoldemo.pdf (accessed 23 Dec 2011).
4. Teledyne Instruments, Inc. *Flash method development in a flash*. Technical Note TN62.

Teledyne ISCO Application Notes can be found at https://www.teledyneisco.com/chromatography/application-notes. Technical Notes can be found at https://www.teledyneisco.com/chromatography/technical-notes.

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