

Overview of Silica Column Sample Loading Techniques

Abstract

There are a variety of methods available to the chemist to load samples on to flash chromatography columns. The advantages and disadvantages of liquid loading and solid loading (by adsorbing the sample onto silica or other materials) are discussed with example chromatograms for comparison of the techniques. The effect of air introduced into the column from solid load cartridges is also discussed.

Background

There are several methods to load sample onto flash chromatography columns. These methods can be divided into dry load and wet load techniques. Dry loading involves dissolving the sample in a strong solvent followed by adsorbing the sample onto silica or another material such as celite followed by removing the solvent. Wet loading also starts with dissolution of the sample, but the mixture is injected directly into the column.

There are several techniques for both wet and dry loading. Each technique has advantages and disadvantages. The advantages and disadvantages are more critical for larger scale where, due to the larger column sizes and increased sample volume, back pressure increases.

3-(2-nitrophenyl amino) propionitrile is used for experiments because it is a reasonable model for many compounds. The product and starting material elute fairly close to each other allowing a better comparison of different loading techniques as a function of purity.

Synthesis of 3-(2-nitrophenyl amino) propionitrile

2-Nitroaniline (304 g) was dissolved in 1500 mL reagent alcohol. Triton B (45 mL) was added and the mixture was heated to reflux. Acrylonitrile (420 mL) was added with stirring. The mixture was stirred overnight at reflux. The alcohol was evaporated. A tarry mixture (~550 g) was obtained.

Chromatography

Except where noted, 6.8 g of crude 3-(2-nitrophenyl amino) propionitrile was used. The sample was run on a 330 g RediSep® Rf silica column (69-2203-330). The samples were run on a CombiFlash Torrent® system (68-5240-003) using the standard gradient method and flow rate (200 mL/min) for that column in the PeakTrak® software or a CombiFlash® Rf 200 system (68-5230-006). Solvent A (starting solvent) was hexanes; Solvent B was ethyl acetate. Peaks were collected at 254 nm. Fractions were collected into 25 mm tubes with a Foxy® R2 frac-

tion collector (68-2130-007). The sample mass is ~2% sample load with respect to the mass of silica in the column. The flash runs gave 4.8–5.3 g total sample corresponding to 71–77% recovery by mass.

HPLC was run on an Isco HPLC system using a C18 column (Restek, C18, 250×4.6 mm, 5 μ, 1.00 mL/min, 50–100% MeOH in water over 15 min).

Further experimental conditions are listed with each run.

Empty Solid Load Cartridges

Silica

Experimental – Crude sample (6.8 g) was dissolved in 50 mL methanol. This was added to 30 g silica (75–150 μ particle size, 60-3874-09) and dried with a rotary evaporator. The contents were placed in a 65 g solid load cartridge (69-3873-225).

Results – There was baseline separation between the first and second peaks on the flash system. There was slight tailing of the first peak into the second peak, demonstrated by HPLC data.

The sample load onto the silica in the solid load cartridge was 22%, approximating the 15–20% load that is typical for a solid load cartridge due to the capacity of silica.

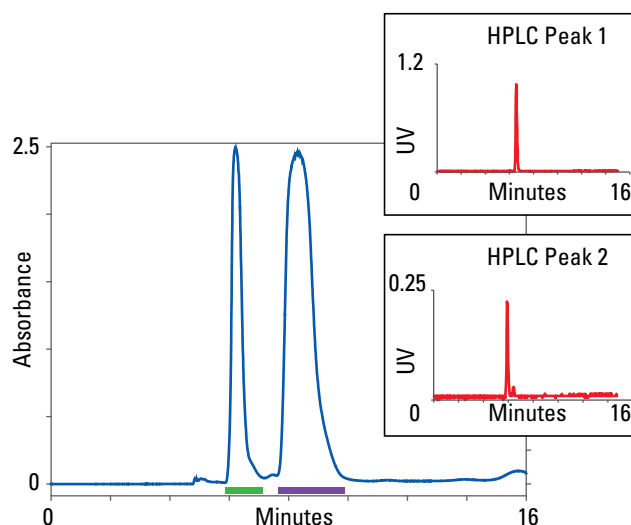


Figure 1: Purification of 3-(2-nitrophenyl amino) propionitrile adsorbed on silica and placed in an empty solid load cartridge

Celite

Experimental – Crude sample (10.00 g) was dissolved in a mixture of dichloromethane and methanol and mixed with 90.00 g Celite 545. Methanol was added to make a slurry to ensure the reaction mixture was evenly dispersed on the Celite. The solvent was evaporated on a rotary evaporator to make a free-flowing powder. In a similar fashion, 2.00 g of reaction mixture was adsorbed 8.00 g of silica gel (75–150 μ particle size, 60-3874-091) for a comparison between the two adsorbants.

8.0 g of the Celite/crude reaction mixture was loaded into a 25 g Solid Load Cartridge (69-3873-220) and run with a standard solvent method on a 40 g RediSep Rf silica column (69-2203-380). For the sample adsorbed on silica, 4.00 g of the silica/reaction mixture was loaded into a 5 g solid load cartridge (69-3873-217) and run on a 40 g column in the same fashion as the Celite sample.

Results – Loading the sample on either silica or Celite at the sample column load (~2%) gave essentially the same results. The elution times and resolution are similar whether the sample was adsorbed on celite or silica. The silica sample eluted slightly earlier and with slightly smaller peak widths mainly due to the reduced volume of silica needed to adsorb the sample. Celite adsorbs compounds more weakly than silica, so needs a larger mass to adsorb the same amount of material to create a free-flowing powder; typical adsorption on celite ranges from 10% to 15% sample. Higher loads may allow “crashing” of the sample during purification with high back pressures that may increase run times.

Comparison of Silica and Celite

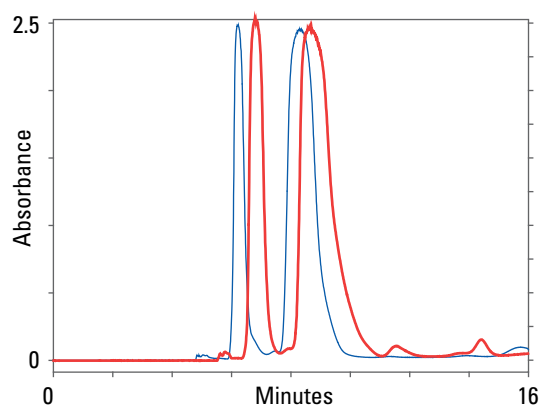


Figure 2: Comparison of loading a sample on Celite and silica Celite red/bold line; silica blue/thin line

Advantages of Adsorbing onto Silica or Celite

Good resolution

Minimal peak broadening

Increased loading possible

Solid load cartridge acts as pre-column

Disadvantages

Extra time required for drying

Solid load cartridge needed

Effects of Air in Empty Solid Load Cartridge

Experimental – Crude sample (6.8 g) was dissolved in 50 mL methanol. This was added to 30 g silica (75–150 μ particle size, 60-3874-091) and dried with a rotary evaporator. The contents were placed in a 65 g solid load cartridge (69-3873-225). Two identical samples were made.

One sample was run normally. The other sample was saturated with hexane after being placed in the solid load cartridge to remove the air.

Results – Figure 3 shows that air has no effect on the purification; both peaks elute at nearly the same time with similar peak shapes.

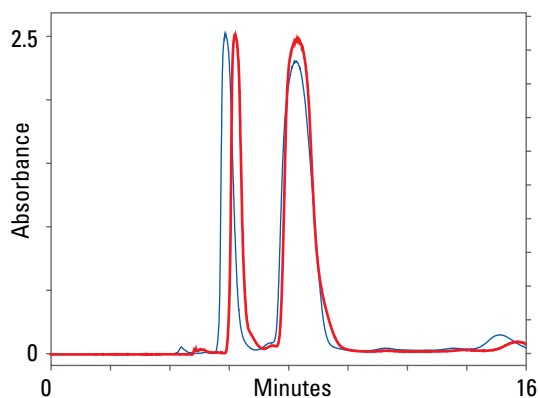


Figure 3: Air in the solid load cartridge has no noticeable effect on the purification. Air not removed red/bold line; air removed blue/thin line

Prepacked Solid Load Cartridge

Prepacked solid load cartridges are convenient to use since the silica is already packed in the solid load cartridge. Pre-packed solid load cartridges can be dried after the sample is loaded.

Dried Sample

Experimental – Crude (6.8 g) sample was dissolved in 60 mL methanol. This was added to a 65 g silica solid load cartridge (69-3873-226) and dried by using the cartridge air purge on the CombiFlash Torrent.

Results – There was baseline separation between the first and second peaks on the flash system. There was slight tailing of the first peak into the second peak, as seen in the HPLC data.

Due to the solvent, the loading was reduced compared to adsorbing the sample on silica (Figure 1). The same amount of sample was adsorbed onto 65 g silica compared to only 30 g needed for the empty solid load cartridge.

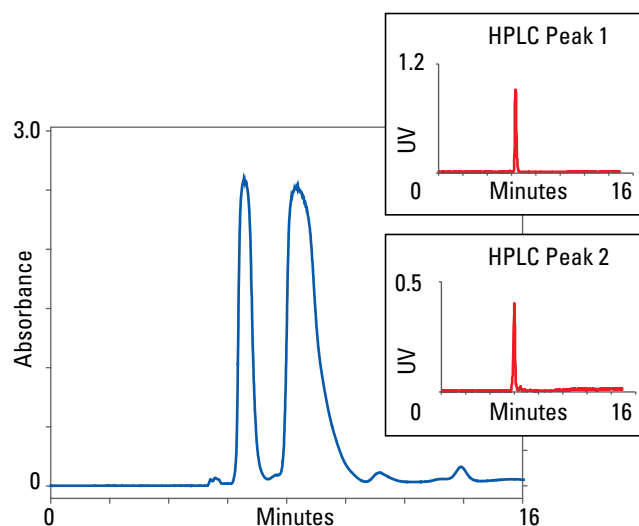


Figure 4: Purification using a prepacked solid load cartridge with drying

Dried Pre-packed Solid Load Cartridge Advantages	Disadvantages
Good resolution	Extra time required for drying
Minimal peak broadening	Solid load cartridge needed
Increased loading possible	
Solid load cartridge acts as pre-column	

Sample Not Dried

Experimental – Crude (6.8 g) sample was dissolved in 60 mL acetone. This was added to a 65 g silica solid load cartridge (69-3873-226) and run without drying. Acetone was used to minimize the solvent used on the cartridge.

Results – There was no longer baseline separation between the first and second peaks on the flash system. The first peak showed fronting due to the acetone carrying small amounts of the mixture ahead of the main peaks. The slight peak mixing is demonstrated in the HPLC traces.

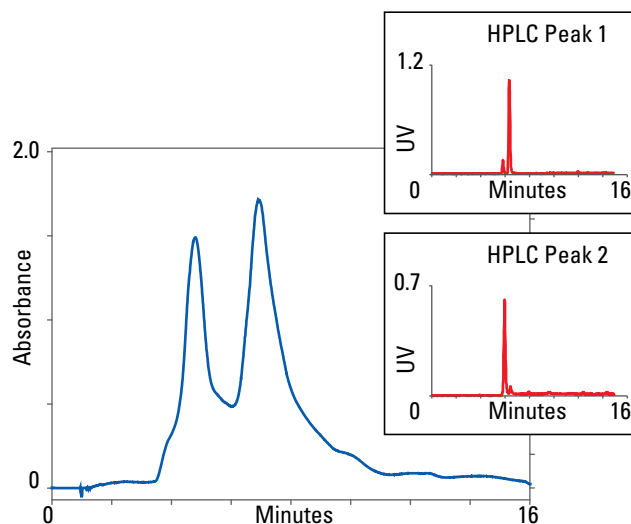


Figure 5: Sample on a solid load cartridge without drying prior to running

The addition of a strong solvent caused the peaks to elute earlier than when the compounds were dried on the silica.

Wet Load Solid Load Cartridge Advantages	Disadvantages
Fair resolution	Solid load cartridge needed
Faster—no drying time	Reduced resolution/loading
	Possibility of compound “crashing”

Wet Loaded Sample - Strong solvent

“Wet loaded” samples are those injected directly onto the column. The compound may be loaded with a very strong solvent, or may be loaded with the “B” solvent used in the gradient. The solvent used to dissolve the sample must be miscible with all solvents used for the purification.

Experimental – Crude sample (6.8 g) was dissolved in 60 mL acetone. Acetone was used to minimize the solvent used to load the compound. The sample was loaded with a Teledyne Isco Sample Load pump

(60-5247-007). The sample was chased with 20 mL acetone. An empty solid load cartridge was used as a funnel to load the pump. If desired, the solid load cartridge used as a funnel could be partially filled with charcoal or another media to act as a pre-column and facilitate purification.

Results – The results were similar to those for the liquid-loaded solid load cartridge. There was not baseline separation between the compounds and there was slight mixing between the compounds.

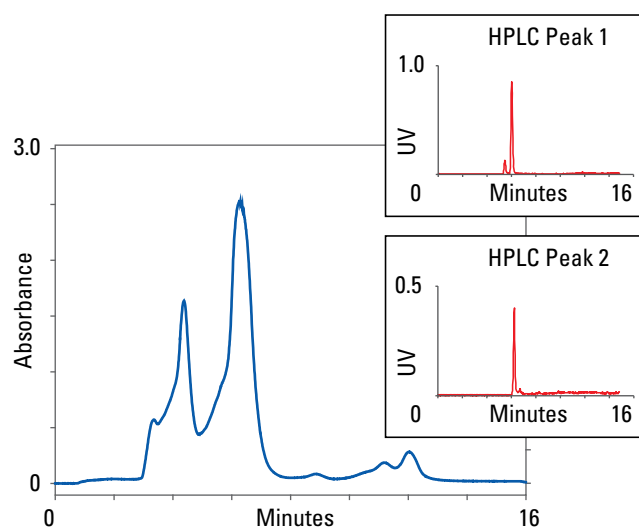


Figure 6: Sample loaded onto column with strong solvent

Wet Loaded Sample - Weaker "B" Solvent

Experimental – Crude sample (6.8 g) was dissolved in 100 mL ethyl acetate (B solvent). The sample was loaded with a Teledyne Isco Sample Load pump (60-5247-007). The mixture was chased with 20 mL ethyl acetate. More solvent was required to dissolve the sample compared to wet loading with strong solvents (Figure 6) because the mixture was less soluble in ethyl acetate. An empty solid load cartridge was used as a funnel to load the pump. No HPLC was run for this example.

Results – The increased solvent required to dissolve the sample, as compared to using acetone, causes the compounds to be mixed. The initial slug of "B" solvent carried some of the sample without it interacting with the column.

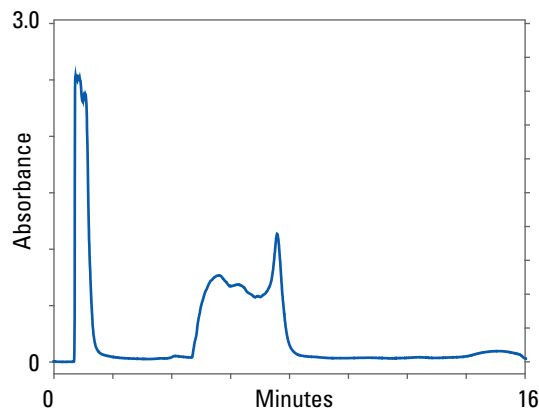


Figure 7: Sample loaded onto column with solvent used for the gradient

Wet Load onto Column Advantages	Disadvantages
Fair resolution	Reduced resolution/loading
Faster—no drying time	Requires good choice of strong solvent
Easiest to load sample	Possibility of compound "crashing"

Conclusion

The use of solid load cartridges provide the best resolution for purifying compounds, although it does require extra steps to prepare the sample. The extra time required to prepare the sample can be recovered from increased loading or improved purity. The air that might be introduced by solid load cartridges during the purification does not affect the chromatography.

Liquid loading allows fast loading of the column but with reduced resolution. The strong solvent tends to wash the compound through the column, reducing interaction with the stationary phase. Liquid loading is useful when there is a great deal of resolution between compounds. The use of a sample load pump allows easier loading.

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