

Sample loading techniques for large scale flash chromatography

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Abstract

There are a variety of methods available to the chemist to load samples on to flash chromatography columns. Due to the size of the column and volume of solvent, methods that work well on smaller scales may not provide adequate performance at larger scales. The advantages and disadvantages of liquid loading and solid loading (by adsorbing the sample onto silica or other materials) is discussed with example chromatograms for comparison of the techniques.

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) 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 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.

Experimental

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.

For each run, 6.8 g of crude 3-(2-nitrophenyl amino) propionitrile was used. The mixture was purified on a 330 g RediSep® Rf silica column (PN 69-2203-330) with a CombiFlash® Torrent system (PN 68-5240-003). A standard PeakTrak® software gradient method and flow rate (200 mL/min) for that column was used. Solvent A (starting solvent) was hexane; Solvent B was ethyl acetate. Peaks were collected at 254 nm. Fractions were collected into 25 mm tubes with a Foxy R2 fraction collector (PN 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 system using a C18 column (Restek, 250 × 4.6 mm, 5 μ , 1.00 mL/min, 50 to 100% MeOH in water over 15 min).

Further experimental conditions are listed with each example.

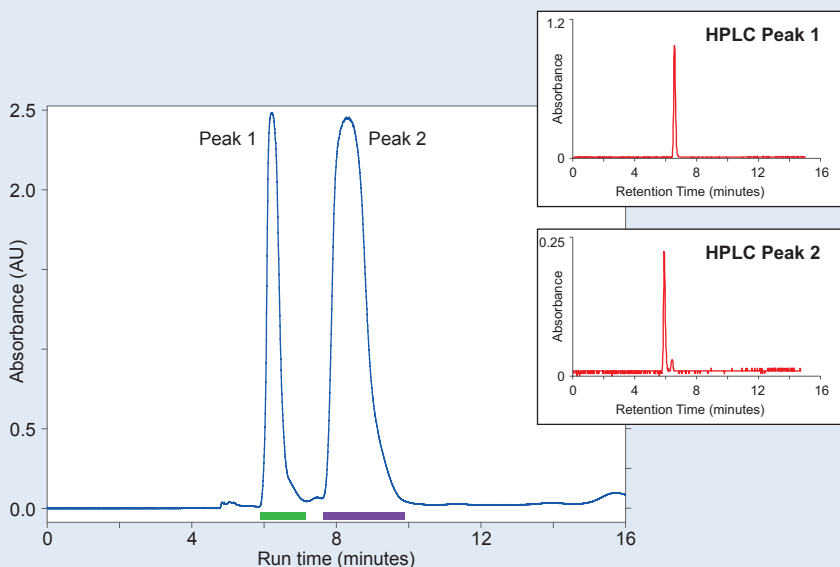
Examples and Results

Example 1: Sample adsorbed on silica with empty solid load cartridge

Experimental: Crude sample (6.8 g) was dissolved in 50 mL methanol. This was added to 30 g silica (PN 60-3874-09) and dried with a rotary evaporator. The contents were placed in a 65 g solid load cartridge (PN 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.



Advantages of Adsorbing onto Silica:

- Good resolution
- Minimal peak broadening
- Increased loading possible
- Solid load cartridge acts as pre-column
- No possibility of “crashing” — sample already out of solution

Disadvantages:

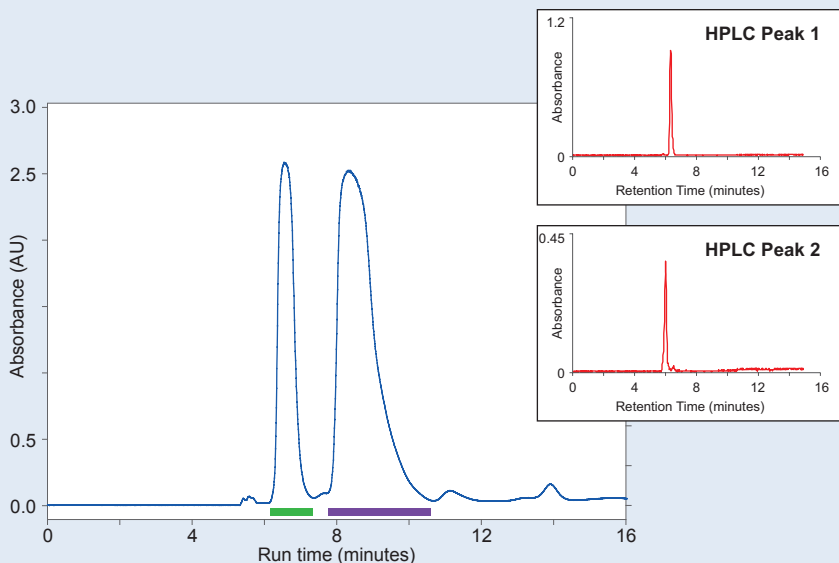
- Extra time required for drying
- Solid load cartridge needed

Example 2: Sample dried on silica with pre-packed solid load cartridge

Experimental: Crude sample (6.8 g) was dissolved in 60 mL methanol. This was added to a 65 g silica solid load cartridge (PN 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 in *Example 1* above. The same amount of sample was adsorbed onto 65 g silica compared to only 30 g needed for *Example 1*.



Dried Pre-packed Solid Load Cartridge Advantages:

- Good resolution
- Minimal peak broadening
- Increased loading possible
- Solid load cartridge acts as pre-column
- Sample cannot “crash”

Disadvantages:

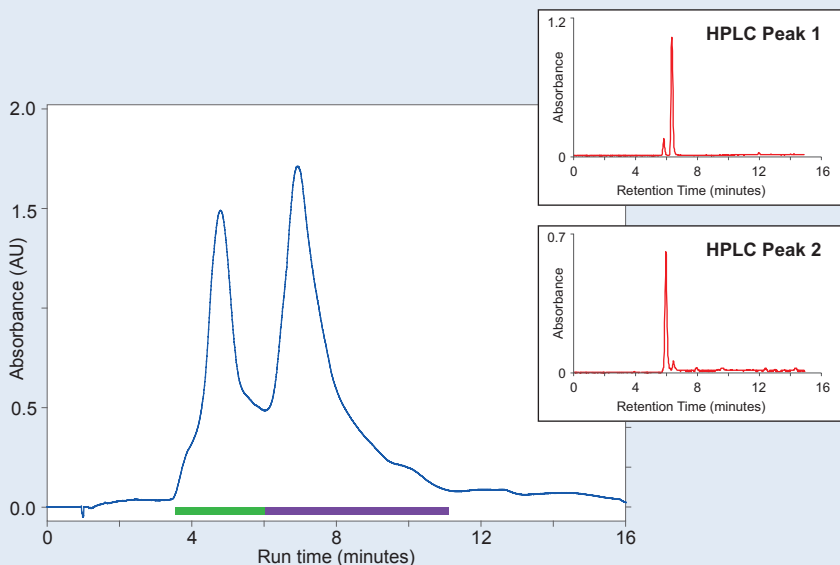
- Extra time required for drying
- Solid load cartridge needed

Example 3: Sample wet loaded onto pre-packed solid load cartridge

Experimental: Crude sample (6.8 g) was dissolved in 60 mL acetone. This was added to a 65 g silica solid load cartridge (PN 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 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:

- Fair resolution
- Faster—no drying time

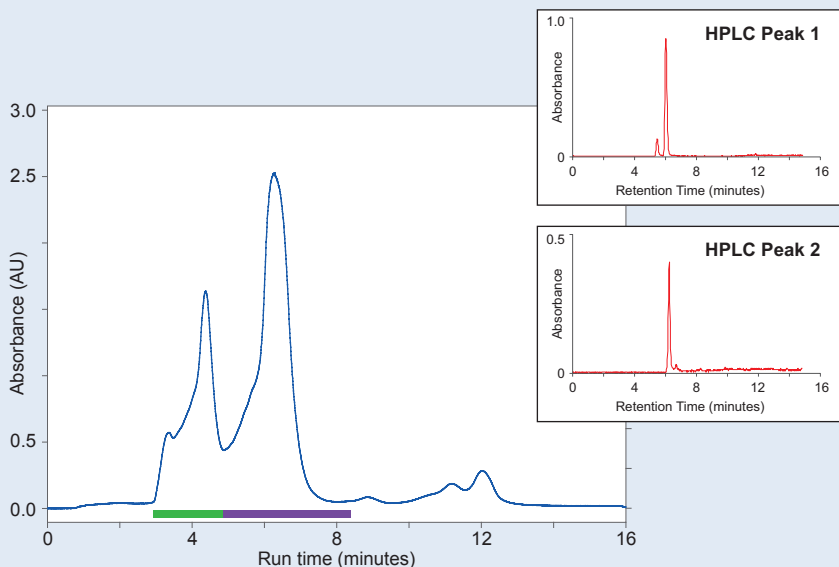
Disadvantages:

- Solid load cartridge needed
- Reduced resolution/loading
- Possibility of compound “crashing” upon contact with “A” solvent

Example 4: Sample wet loaded onto column with strong solvent

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 (PN 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.

Results: The results were similar to those for *Example 3*. There was not baseline separation between the compounds and there was slight mixing between the compounds. 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.



Wet Load onto Column, Strong Solvent

Advantages:

- Fair resolution
- Faster—no drying time
- Easiest to load sample

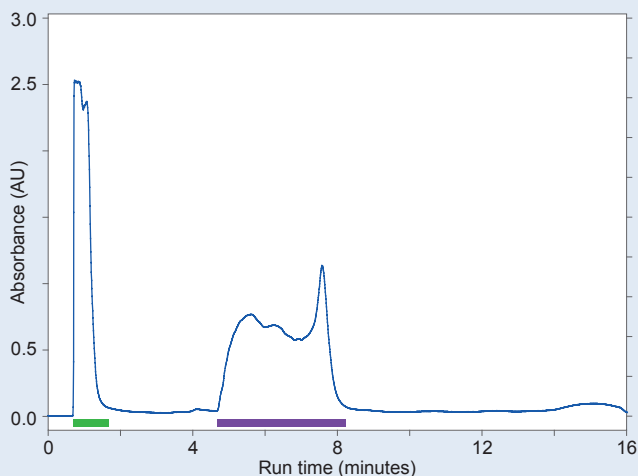
Disadvantages:

- Reduced resolution/loading
- Requires good choice of strong solvent
- Possibility of compound “crashing”

Example 5: Sample wet loaded onto column with “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 (PN 60-5247-007). The mixture was chased with 20 mL ethyl acetate. More solvent was required to dissolve the sample compared to *Example 4* 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. 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.



Wet Load onto Column, “B” Solvent

Advantages:

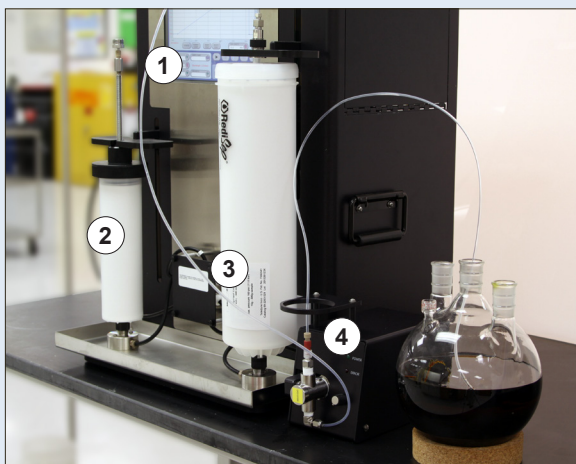
- Faster— no drying time
- Easiest to load sample

Disadvantages:

- Lowest resolution/loading capacity
- Possibility of compound “crashing”

Teledyne Isco's
CombiFlash Torrent
Large-scale flash
chromatography system
is shown with the Sample
Load Pump option.

1. CombiFlash Torrent
2. Solid Load Cartridge
3. RediSep Flash Column
4. Sample Load Pump



Conclusion

The use of solid load cartridges provide the best resolution for purifying compounds, although it does require an extra drying step to prepare the sample. The extra time required to prepare the sample can be recovered from increased loading or improved purity. Solid load cartridges can be used with C18 columns with the use of C18 solid load cartridges or celite.

Liquid loading allows fast sample loading 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. A Sample Load Pump allows easier loading, especially with C18 columns with their high backpressure.

Part Numbers are Teledyne Isco catalog numbers, unless otherwise stated.

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