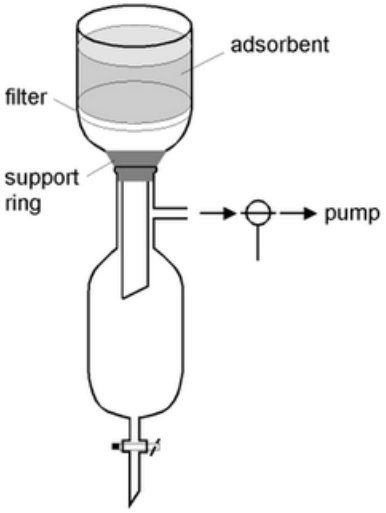
A Short guide to Dry Column Vacuum Chromatography (DCVC)

Christian Tortzen

29th August 2008

DCVC is an old method that has most recently been reported with an improved procedure by Daniel Sejer Pedersen.[1] This guide is an elaboration of this methodology with some improvements in sample loading and choice of solvent gradient. Using this type of column chromatography it is possible to routinely separate with a better than TLC resolution. The method is very scalable and has been used to separate more than 150 g of compound per column. Generally it is faster, uses less solvent, less silica and has better resolution than Flash Chromatography.



Silica: Merck Silica Gel 60 - 0,015-0,040 mm - Merck number 1.15111.1000 - for 1 kg.

Equipment: Cylindrical sintered glass funnel - at least 10 cm high. A separating funnel, and a glass joint connecting these two with a sidearm to apply vacuum. Vacuum from a membrane pump or water-pressure/aspirator pump is suitable.

[1] D. S. Pedersen, C. Rosenbohm, Synthesis **2001**, (16), 2431-2434

Solvents: All solvents used in Flash Chromatography can be used. Low boiling solvents (ether or DCM) can cause problems when purifying very crystalline compounds, as the cooling from evaporation of solvent can cause the compounds to crystallise in the sintered glass. Very polar solvents can also be used, including MeOH and water. Because the column is packed dry even abrupt changes in polarity will not cause the silica to crack as in Flash Chromatography

Length of column: The resolution of the columns seems to have a maximum around 7 cm of packed column. Longer columns will not lead to better separation. Typically 4-7 cm is used.

Loading: The rule of thumb is around 500 mg of mixture per cm^2 column area for standard separations. For difficult separations a lower loading can desirable. The column should be chosen with a diameter accordingly.

Fraction volume: With the vacuum applied there should be at least 2 cm of solvent above the the surface of the silica, when the first drops leave the column. There is no upper limit for the fraction size. The size of a the test tubes used for collecting the fractions is a practical choice.

Packing: Without proper packing this method will not work at all. The columns are packed by filling the column with dry silica. The correct amount of silica can be hard to determine before the column is packed, but when filling in the silica, slightly less than double the height of the packed column should be used (8-9 cm loose silica = 5 cm packed). The silica has a very small particle size and is very "powdery" so it should be handled inside a fume-hood. After adding the silica the top of the column is covered with para-film, and the column can be removed from the fume-hood. The actual packing consists of three steps:

First the column is tapped repeatedly onto a table/book/stack of papers until the silica is tightly packed. This can take several minutes. The sound from the tapping of the column will change from a hollow sound to a more "solid" sound. When properly packed it should be possible to turn the column upside down without the silica loosening. If this fails the column has to be re-packed. It is also extremely important that the silica is packed as horizontal as possible. It is quite normal that the silica will pack unevenly at first. This can be solved by knocking carefully at the side of the collumn near the top until the silica at the top loosens, and then repeat the packing/tapping.

When the silica is solid and evenly packed, the parafilm is removed and the column is placed onto the separating funnel and the vacuum is applied. A filter paper is added to the top of the column to protect the silica. The silica is then compressed from the top side of the column with something suitable(for example the piston of a syringe). Special care should be taken to press down along the sides of the column to prevent channels forming along the edges.

With the vacuum still on, a fraction of the apolar eluent component (typically heptane) is added. This step can be used to determine the correct fraction size for the chosen size of column. As the eluent passes down through the column, it is checked that the solvent front is straight and horizontal. If this is not the case the column should be repacked. Immediately after the last solvent has gone into the silica, the column is tapped again from the top side. The column is now packed and ready.

Application of compound mixture: This can be done in two ways. The most foolproof way is adsorbtion of the mixture onto celite. This is easily done by dissolving the mixture in a volatile solvent, adding enough celite, and evaporating to dryness on a rotary-evaporator. Celite should be used, not silica. Dry-loading with silica is counter-productive as it will result in a broad band of mixture needing to get separated. Celite does not "hold back" the compounds, but still has a very large surface area which results in a narrow band on the silica as soon as the compound is soluble in the eluent.

The other, and faster, way of applying the compound is to dissolve the mixture of compounds in as little as possible of the polar eluent-component. The column is then filled to the brim with the apolar component(with the vacuum on), and the mixture is quickly added below the surface of the apolar solvent. After the eluent has passed through the column another portion of the apolar eluent-component is added before the gradient elution starts.

Choice of eluent system: The eluent system is chosen as normal. Ethylacetate/Heptane works very well and will be used as an example. If an eluent system can give TLC-separation below R_f=0,3 the mixture will separate on the column if done correctly. The separation on TLC doesn't have to be complete in one run of the TLC plate for the column to give separation. If the eluent system doesn't yield satisfactory separation another two-component system is tried until TLC separation is achieved. Other good eluent systems include THF/Heptane, MeCN/Toluene and MeOH/DCM - All these eluent-systems will give different separations, not only based on polarity but on distance between spots and sometimes order for spots on the plate. If separation is still not achieved on TLC three-component systems can be tried.

Elution: The elution of the column is done with a planned gradient. It is very important that the vacuum is on when each fraction is added. Each fraction poured onto the column should have a higher polarity than the last. These types of columns can be run isocraticly, but with a huge loss of resolution. The gradient is chosen before the elution starts and each fraction is mixed to the desired polarity before adding it to the column. After the fraction has passed through the column (when the column only drips slowly) the vacuum is removed and the separating funnel is emptied into a test tube. The separating funnel should then be washed with a few ml's of the polar eluent component to ensure no contamination of the next fraction takes place. Then the vacuum is applied again and the next fraction is added. With experience, the time used for elution of all fractions should be between 20 and 40 minutes.

Choice of Gradient: The rule of thumb for choosing a good gradient is: The number of fractions added to the column, when the polarity of the eluent would give a R_f=0,3 on TLC, should be between 5 and 10. This means that an eluent mixture which gives R_f=0,3 should be found, or guesstimated. When found, the polarity is divided by 5 or 10 according to how well the spots separate.

Example: If the wanted compound has an R_f=0,3 in 25% EtOAc/Heptane and separates nicely a good gradient would be 5% increase in each fraction(25%/5). If the compounds doesn't separate nicely, a gradient of 2,5% increase in each fraction should be chosen(25%/10). The first fraction should always be pure apolar component. In this example the polarity of the fractions would then be: pure heptane, 2,5% EtOAc/Heptane, 5% EtOAc/Heptane, 7,5%, 10%, etc. This is continued until the wanted compounds has been eluted. This normally happens, when the gradient is chosen like this, around the same polarity of the eluent that would give a R_f=0,3-0,4

on TLC. It should never be necessary to run more than 20-25 fractions, and most often 10-15 is enough. Typically one compound elutes in 1-2 fractions.

In very difficult separations the gradient can be slowed down around the polarity where the wanted compound elutes. For example if the compound is known (or suspected) to elute at 25% EtOAc/Heptane a gradient of 5%, 10%, 15%, 17,5%, 20%, 21%, 22%, 23% etc. could be used to increase separation. This will not actually increase the resolution in the separation, but will spread out the compounds in a larger number of fractions, minimizing the amount compound in mixture fractions. Normally this is not needed to achieve perfect separation.

After the elution of compounds has ended the column should be eluted with a couple of fractions of the polar component, to ensure that no compound is caught on the column. The columns can be re-used if they are flushed through several times with the polar eluent component, followed by several fractions of the apolar eluent component.

Cleaning: The columns are easily cleaned by pushing out the silica with compressed air or nitrogen. This too should be done inside a fume-hood.