SOP: Flash-chromatography (manual)

1. Choose eluent, columns size and volumes of fractions to be collected

Guidelines are found in for instance: Still, W. C. *et al. J. Org. Chem.* **1978**, *43*, 2923-2925.

2. Packing the column

1. Examine the columns to be used and make sure that there are no cracks in the glass so the column will not break when pressure is applied and that the stop-cock and caps fits the columns so there will be no leaks during elution.
2. Place a small "plug" of glass-wool in the bottom of the column and put teflon tape around the threads of the column. Close lower opening. The flash column is placed in a hood where there is access to N_2-gass.
3. The column is filled with stationary phase [normally silica-gel (corn size 40-63 μm)] until a few cm below the lower opening of the column. Knock gently on the side of the column, using for instance a hose, to pack the column properly and when the level of the gel sinks, fill with more. The surface of the silica must be linear.
4. Fill the column with the eluent. Be careful in the beginning so that the surface is not destroyed.
5. The stopcock in the bottom of the column is opened and a suitable Erlenmeyer flask is put underneath. The pressure regulator on top of the column is connected to the N_2-cylinder. By using N_2-gass, the eluent will now be forced through the column and pack it utterly. Run eluent through the column by applying a steady N_2-pressure until all gel is evenly wet with the eluent and no part of the column is hot. When the column is properly packed, the gel will look more transparent than before. When the column is ready, the remaining eluent is pushed through the column until the liquid level is *just above* the gel surface. Release the pressure and close the stopcock at the bottom of the column.

3. Application of the sample; alternative A - as solution

1. Dissolve the sample to be purified in some eluent. A suitable concentration is about 20-25%.
2. The sample-solution is applied on top of the gel using a pipette. Use the lower opening on the side of the column and be very gentle so you don't ruin the linear gel surface.
3. Open the stopcock at the bottom of the column gently applying N_2-gass again so that the sample is pushed into the gel. N*B! The upper part of the gel must not run dry*.
4. Release some over pressure and close the stopcock at the bottom of the column.

4. Application of the sample; alternative B -absorbed to stationary phase

1. Dissolve the sample in a suitable solvent in a suitable sized round bottom flask
2. Add some stationary phase (normally silika)
3. Evaporate the solvent (see separate SOP for rotavapor)
4. Apply the dry residue on top of the packed column

5. Eluting the column

1. The column is filled with the eluent. Use the eluent you used for packing the column once more. Again, be very gentle in the beginning so that the surface is not stirred up. It can pay off to fill the first few ml through the lower opening on the side of the column just above the gel surface.
2. A suitable beaker is put under the column, the stopcock is opened and N_2-pressure is applied so that the liquid volume decreases with a rate of about 5 cm pr. minute.

3. Collect fractions with the recommended volume (see *Choose eluent, columns size and volumes of fractions to be collected*.).
4. Elute the column until the liquid is a few cm above the gel surface. Release the pressure and close the stopcock. Check the fractions as described under 6. If all the wanted products have departed the column, the elution can be stopped. If not, the column is filled with more eluent, and the elution is continued.

6. Examining fractions and evaporation of pure fractions

1. All the collected fractions are checked on TLC.
2. Fractions pure on TLC with regard to the wanted product, are combined.
3. The solution is filtrated to remove silica particles etc.
4. The solution is evaporated in a weighed round bottom flask (see separate SOP for rotavapors).