How to run column chromatography

Column chromatography is a commonly used purification technique in labs of organic chemistry. Done right it can simply and quickly isolate desired compounds from a mixture. But like many aspects of practical chemistry, the quick and efficient setting up and running of a column is something that can take time to master. Here are some instructions to help you set up the column.

1. Introduction

In a typical column (Fig. 1), **the stationary phase**, a solid adsorbent normally silica gel (SiO_2), is placed in a vertical glass column. **The mobile phase**, a liquid, is added to the top of the column and flows down through the column by either gravity or external pressure (flash chromatography). Separation of compounds is achieved through the varying absorption on and interaction between the stationary and mobile phases.

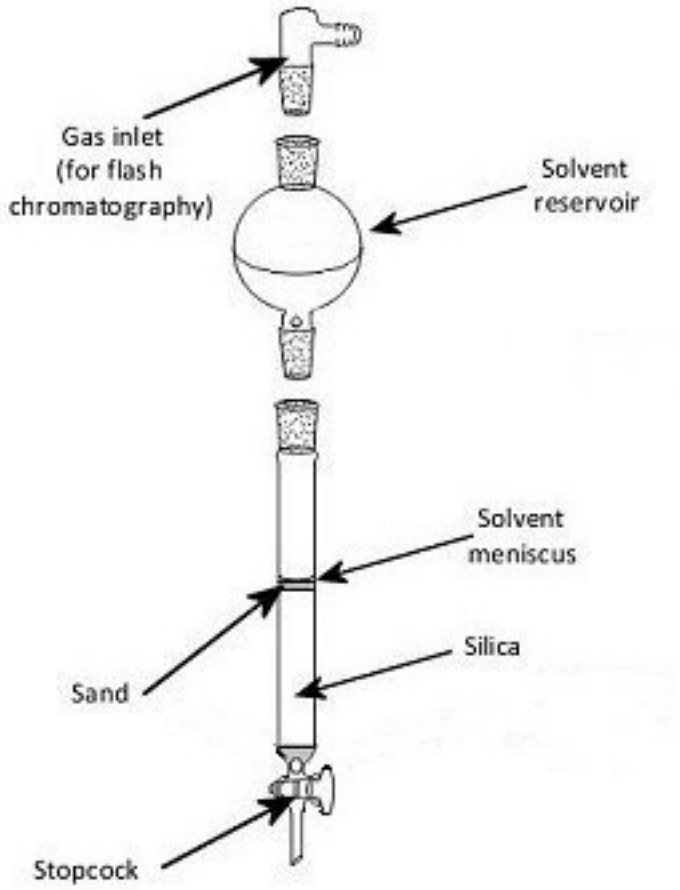


Figure 1. General column set up.

2. **Preparing the Column**

The columns (Fig. 2, **right**) we use are non-fritted and will need to be plugged with either glass wool or cotton wool to prevent loss of the stationary phase out the bottom. Positioning the cotton or glass wool can be awkward at first.

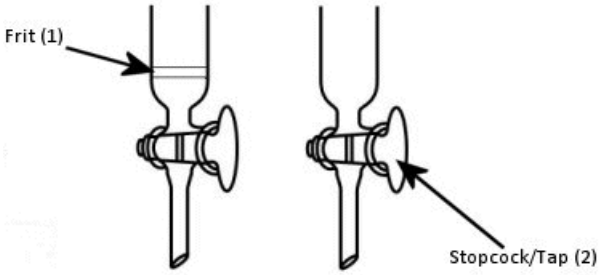


Figure 2. Fritted (left) and non-fritted (right) columns.

The ball of cotton or glass wool should be large enough to plug the bottom of the column, but not so large and densely packed that it restricts solvent flow (Fig. 3). A piece the size of the tip of your little finger should be suitable for most columns.

1. Position the cotton or glass wool ball securely in the narrowest part of the column using a long glass rod or other suitable device.

2. Clamp the column securely and close the tap or stopcock (Fig. 2, **2**).

3. Add a layer of sand until it reaches the main body of the column (approx. 2 cm, Fig. 3). This will give the stationary phase an even base and prevent concentration and streaking of the bands as they come off the column and are collected.

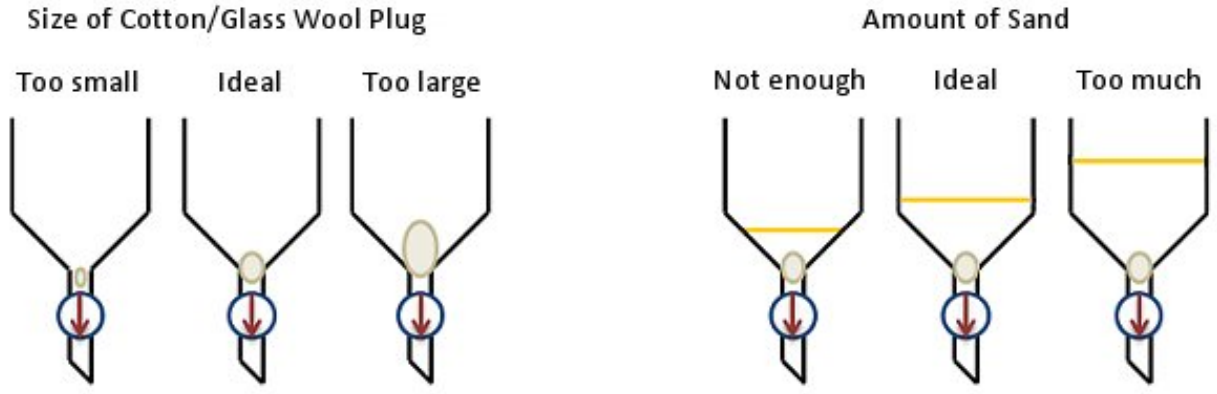
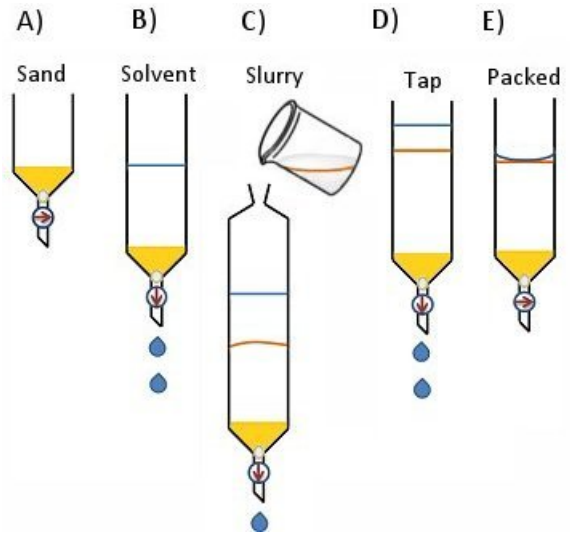


Figure 3. Guidelines for the correct size of cotton or glass wool and sand for non-fritted columns.

3. Filling the Column

There are several methods for preparing columns. We prefer to use the slurry method.

Slurry Method, You will need:
Column prepared as in section 2 above;
2 beakers;
Glass rod;
Funnel suitable for wet solids;
Solvent;
Silica gel;
Pasteur pipette.



Top Tip: Use hand bellows to increase the pressure in the column and to force the silica to compact. Repeat several times, rinsing the sides of the column in between, to ensure complete packing.

Figure 3. The slurry method.

Procedure:

1. Fill the column about one third with solvent (10% EtOAc/Hexanes, Fig. 3, step B).
2. In a beaker, measure out the required amount of silica gel.
3. In a separate flask or beaker, measure solvent approximately one and a half times the volume of silica.
4. Add the silica to the solvent, a little at a time, while swirling. Use a Pasteur pipette or glass rod to mix the slurry.
5. Pour or pipette some of the slurry into the column. Allow the solvent to drain to prevent overflowing (Fig. 3, step C).
6. Tap the column gently to encourage bubbles to rise and the silica to settle (Fig. 3, step D).
7. Continue to transfer the slurry to the column until all the silica gel is added.
8. Rinse the inside of the column by pipetting solvent down the inside edge.
9. Drain the solvent until the solvent level is just even with the surface of the stationary phase (Fig. 3, step E).

10. Add a 3 mm layer of sand on top of the silica gel to prevent it from moving during solvent addition.

You are now ready to load your column with your reaction mixture and isolate the desired Schiff base.

1. Dissolve your reaction mixture in 1-2 mL of dichloromethane (DCM);

2. Use a pipette to transfer the DCM solution of your product mixture to the column;

3. Let the solvent run just slight below the surface of the silica gel (or sand);

4. First use 50 mL of 10% EtOAc/hexanes to elute the column and start collect the fractions in test tubes that are fitted in a rack;

5. Then increase the solvent polarity to 20% EtOAc/hexanes and continue to elute the column until TLC shows no more product is coming down from the column.

6. Combine the fractions of the test tubes that show product spots.

7. Evaporate the solvents and transfer the product to a vial and submit to your TA.

4. Emptying the Column

Remember! Silica dust is hazardous to inhale. Always handle silica in a fumehood and wear suitable protective clothing. Try to minimize raising dust during handling.

Once you have your products isolated, all that remains is to empty and clean the column ready for next time. To speed up the process, elute all of the solvent using compressed air and allow air to flow through the column for approximately 15 min. This will give dry, free-flowing silica that is easy to pour into the silica waste container. Alternatively, elute all the solvent and secure the column upside down over a large beaker and allow to dry overnight in a fume hood.

Cleaning the column by rinsing with water and acetone is usually sufficient.