A macroscale column is much too big for very small quantities of material \(< 20 \text{ mg}\). Instead, a column can be constructed using a disposable Pasteur pipette.

In order to get good separation, it is ideal if the desired component has an \(R_f\) around 0.35 and is separated from other components by at least 0.2 \(R_f\) units. If the spots to be separated are very close \(< 0.2 \Delta R_f\), it’s best if the middle of the spots has an \(R_f\) of 0.35. An \(R_f\) near 0.35 is ideal because it is slow enough that stationary-mobile phase equilibration can occur, but fast enough to minimize band widening from diffusion.

**Step-by-Step Procedures**

![Image](image1)

**Figure 2.68:** a) TLC plate of purple dye, b) Elution with a pipette column.

The column pictured in this section shows purification of a drop of dilute purple food dye (made from 1 drop red dye, 1 drop blue dye and 15 drops water). The dye is separated as best as possible into its three components: blue, red and pink (as seen in the TLC plate of Figure 2.68a). A 2.5" column of silica gel is used and eluted with a solution made from a 1:3:1 volume ratio of \(6 \text{ M NH}_4\text{OH}:1\)-pentanol:ethanol.

![Image](image2)

**Figure 2.69:** a) Purple dye spotted on the baseline of a TLC plate, b) after elution, c-e) Inserting cotton into the pipette tip.

1. Run a TLC of the sample to be purified (Figures 2.69 a+b) to determine the appropriate solvent for chromatography. The desired component should have an \(R_f\) around 0.35.

**Prepare the dry column**

2. Use a metal rod or hanger (Figure 2.69d) to wedge a bit of cotton or glass wool into the narrow end of a short-stemmed Pasteur pipette. The cotton should be moderately tight so liquid can trickle through, but not solid.
3. Scoop silica or alumina into the wide end of the pipette column (Figure 2.70a), then invert and raise the column so the powder falls to the bottom. Continue to use this scooping method to fill the pipette column to 2 - 2.5 inches high with silica or alumina (this quantity can be altered depending on the amount of sample).

Alternatively, scoop adsorbent into the wide end of a fresh pipette and use it as a funnel to deliver adsorbent through the narrow tip and into the pipette column secured to a ring stand with a three-fingered clamp (Figures 2.70 a–c).

**Safety note:** As silica and alumina are fine powders and lung irritants, be sure to work in a fume hood when handling silica or alumina. Also tap the pipette after scooping to dislodge adsorbent on the outside of the pipette (so it doesn't spill when vertical).

4. Gently clamp the pipette column to a ring stand or latticework using a three-fingered clamp (note: they are fragile!) and tap it to make sure the silica / alumina is settled and the top edge is horizontal.

5. Add approximately \(0.5 \text{ cm}\) of sand atop the silica / alumina layer. If using very fine sand, use another pipette to act as a funnel, as described in step 3. For coarse sand, use a small scooper or the wide end of another pipette to aid in its delivery (Figure 2.70d). A complete pipette column is in Figure 2.70e.

**Wet the column**

6. Position a test tube supported in a small beaker beneath the column. Add a squirt-full of the appropriate eluent (previously determined by TLC in Step 1), gently above the sand layer of the pipette column (Figure 2.71a).

7. Use a pipette bulb (or dropper bulb) to apply gentle air pressure and push the eluent through the column (Figure 2.71b), stopping when the liquid level is in the sand layer. **Throughout the entire elution process, keep this white column section wet with eluent.**

   To apply air pressure using a pipette bulb, create a strong connection between the column and the bulb, and then squeeze the bulb. It is important when releasing the pressure to first break the seal while still keeping your hand clenched (Figure 2.71c) and THEN release your hand (Figure 2.71d). If you release your hand while still connected to the column, the bulb will create suction that can violently pull liquid into the bulb and disrupt the column.

8. Add more eluent if needed, and use bulb pressure until the entire column is saturated with eluent (Figure 2.71e), and the eluent level is in the sand layer.
Add the sample

9. Use a pipette to add the entire sample to the sand layer. If the sample is a liquid, add it directly. If it is a solid, dissolve it in the smallest amount of solvent possible, preferably the eluent. If the solid is not soluble in the eluent, use the minimum amount of dichloromethane. Position the pipette tip near the sand layer and add the sample carefully, trying not to splash compound onto the sides (Figure 2.72a).

10. Rinse the original container with a little solvent and add the rinsing to the column using the same pipette (in order to rinse the pipette as well).

11. Apply pressure with the bulb to force the sample just past the sand layer (Figures 2.72 b+c).

12. Add more eluent (approximately 0.5 cm high) to rinse the sides of the column (Figure 2.72d). Again use bulb pressure to force the dye onto the adsorbent (Figure 2.72e), and then fill the pipette above the sand layer as high as possible with eluent.

Elute the Column and Collect Fractions

13. Apply gentle bulb pressure to begin eluting the sample through the column (Figure 2.73a), refilling the pipette whenever the solvent level nears the sand layer.

14. Immediately begin collecting the liquid eluting beneath the column into an empty test tube. Change the test tube for a fresh one periodically (Figure 2.73c), based on your judgment or your instructor's guidance (perhaps when a small test tube fills to about 1 cm high).

These different tubes are called "fractions". The goal of a column is to collect small enough fractions that most (or some) fractions contain pure material. If the separation of the mixture is difficult (if the \(\Delta R_f\) of the components is low), it may be best to collect even smaller fractions (e.g. 0.5 cm high).

15. Keep the test tubes in order on a test tube rack (Figure 2.73d).
Find and Concentrate the Desired Component

16. In finding the desired component in the test tube fractions, it is helpful to understand the relationship between $R_f$ and elution order in column chromatography.

In column chromatography, the sample is deposited on the top of the column and eluted down, while in thin layer chromatography the sample is spotted on the bottom of the plate and eluted up. Therefore, a column can be thought of like an upside-down TLC plate. A compound with a higher $R_f$ runs “faster,” meaning it will end up higher on a TLC plate, and will be collected first with a column.

In the pipette column pictured in this section, the pink component had the highest $R_f$ on the TLC plate (Figure 2.74a), and was collected first from the column (Figure 2.74b). A purple fraction was also collected (Figure 2.74c), due to incomplete separation of the red and blue components.

17. Use TLC as described in the section on macroscale columns to determine which tubes contain the desired component.

18. Combine the pure fractions into an appropriately sized round-bottomed flask using a funnel, rinse each tube with a small amount of eluent (or other solvent if solubility is an issue), and add the rinsing to the flask. Remove the solvent on the rotary evaporator.

19. To clean up the pipette column, use bulb pressure to force the excess liquid out of the pipette column, and dispose of the semi-dry column (silica gel and all) in the broken glass container.
Wedge a bit of cotton into the bottom of a pipette.

Use a scooping method to fill silica or alumina to 2-2.5 inches high.

Add a 0.5 cm layer of sand.

Add eluent to the column and apply pressure with a pipette bulb to force eluent through the column to completely wet it.

Remember to break the seal before letting go of the pipette bulb, or suction will ruin the column.

Refill the column as necessary.
Adjust the eluent level to the sand layer, and then delicately add the sample.

Use pressure to push the eluent down onto the silica/alumina layer.

Rinse with one portion of eluent and push the solvent onto the column.

Fill the pipette with eluent and apply pressure to elute the column.

Collect liquid into test tubes.

**Always keep the white column section wet** (refill whenever the eluent level nears the sand layer).

Switch test tubes periodically (perhaps when they are \(1 \text{ cm}\) high in small test tubes) to collect different fractions.

Keep fractions organized in a test tube rack in the order they are eluted.

Use TLC to determine the purity of the fractions, and combine appropriate fractions.

Remove the solvent with the rotary evaporator.


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