Setting up and running a column

PART 1: What you need and theory

PART 2: Using a bellow and packing the column

PART 3: Loading sample to silica

PART 4: Running column

PART 5: TLC analysis and combining fractions

UNIVERSITY OF LIVERPOOL
Chemistry Department

PART 1:
What you need and theory

What you need

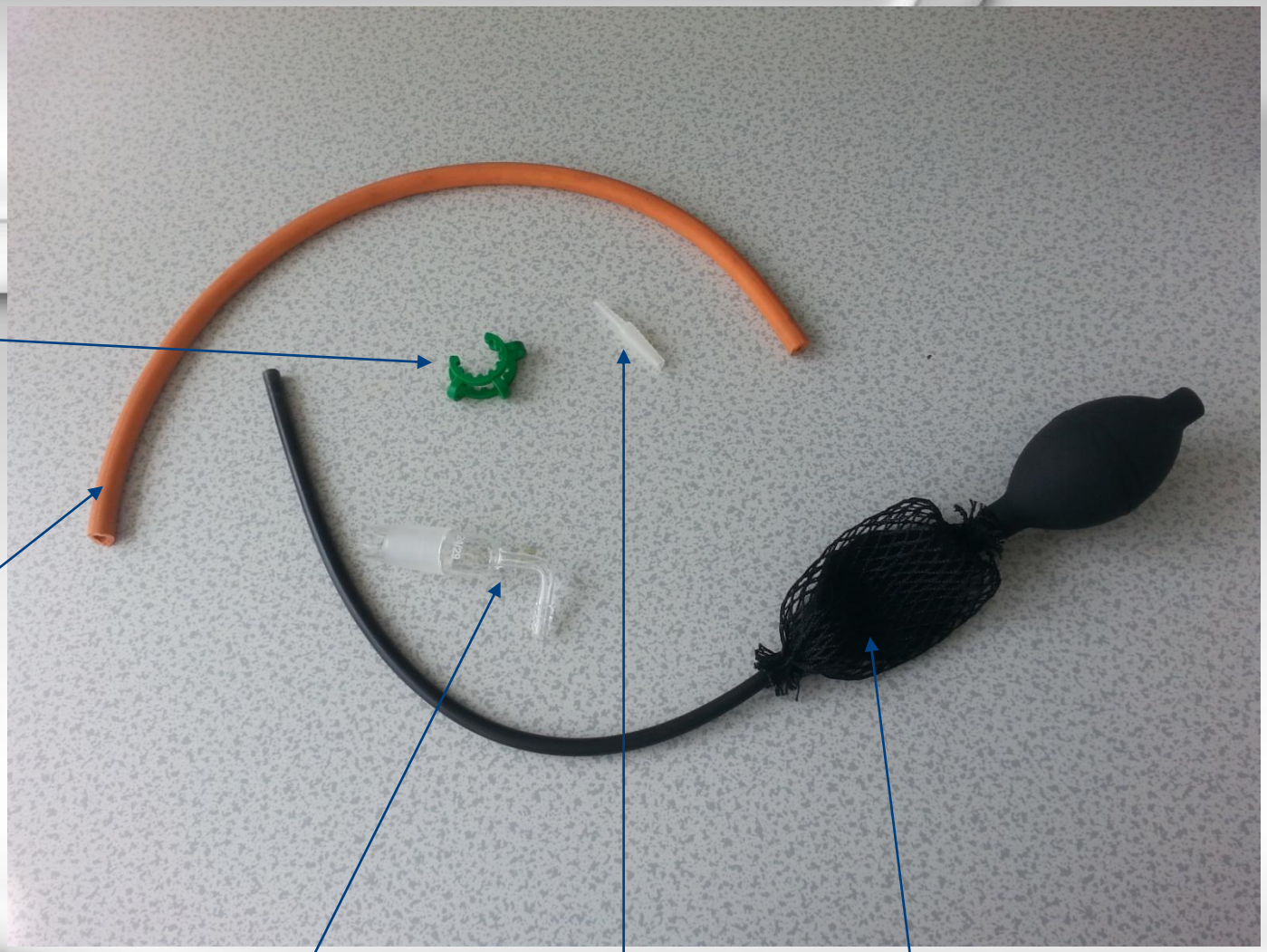
- Column
- Silica Gel (bottle may look different)
- Funnel (for pouring)
- Beaker (useful when packing column)
- Silica in conical (for packing column)
- Your sample/mixture
- Spatula

Other items not on the picture:

- Pasteur pipette/Teat
- TLC plates and related equipment

- Long thin pole to help bung column with cotton wool
- Test tubes and Rack
- Cotton Wool
- Eluent
- Sand (bottle may look different)
- Round bottomed flask (for sample loading)

What you need



Green clip

Small piece of rubber tubing

Cone

Tubing connector

Bellow

Please note:

- Some of these items (tubing, connector and bellow) may be pre-assembled for you

What is the eluent?

In a lot of purification techniques, there is a stationary phase and a mobile phase:

- ***Stationary phase***: The adsorbant material in which the sample being purified binds to.
- ***Mobile phase***: The substance used to unbind the sample to produce the analytes.

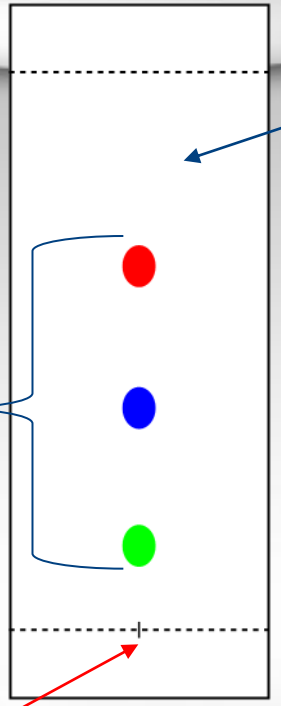
The mobile phase in a column, TLC and GC is referred to as the eluent. It derives from the Latin word for '*washing out*' as the eluent pulls the products through the stationary phase:

- Column/TLC: Eluent is the solvent you are using
- GC: The eluent is the gas being used.

How does a column relate to a TLC?

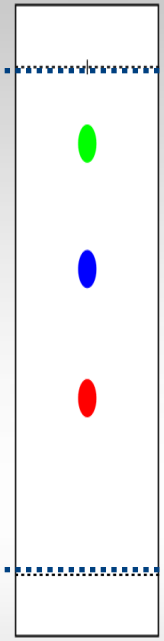
In a column, you load your sample at the top

Silica is the stationary phase in both a TLC and a column



Three products in the mixture

The eluent (mobile phase) in a column moves down, and the products move down also.

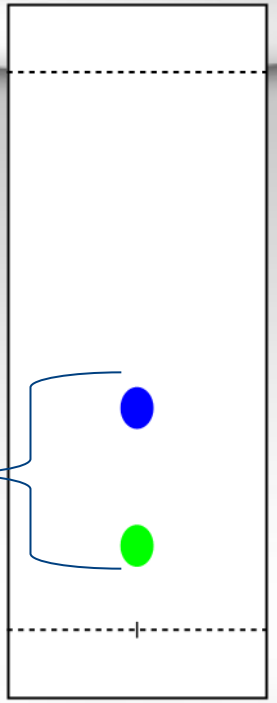


Therefore a column is essentially an upside down TLC, just on a preparative scale where you can collect the samples as they come through the stopcock at the end

In a TLC, you load your sample at the bottom

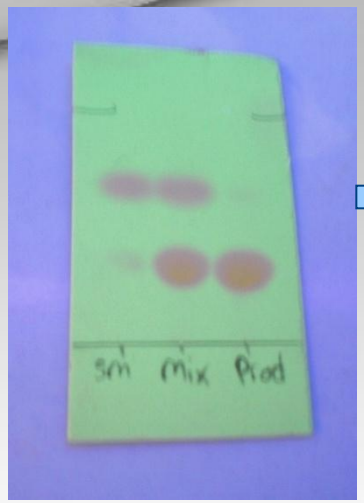
The eluent (mobile phase) in a TLC moves upwards, and the products move upwards also.

How does a column relate to a TLC?



Two products in the mixture

NOTE: TLC analysis should always be completed before a column to determine what eluent system you should be using.



If this is how the TLC looks in 'eluent x', then I would happily use 'eluent x' as the starting eluent for the column.

This was the case for the real TLC and column system shown here. The middle section of the TLC (called 'mix') is the mixture loaded onto the column

NOTE: The assumption has been made that if you are attempting a column you are familiar with how to run a TLC

PART 2a: Packing column

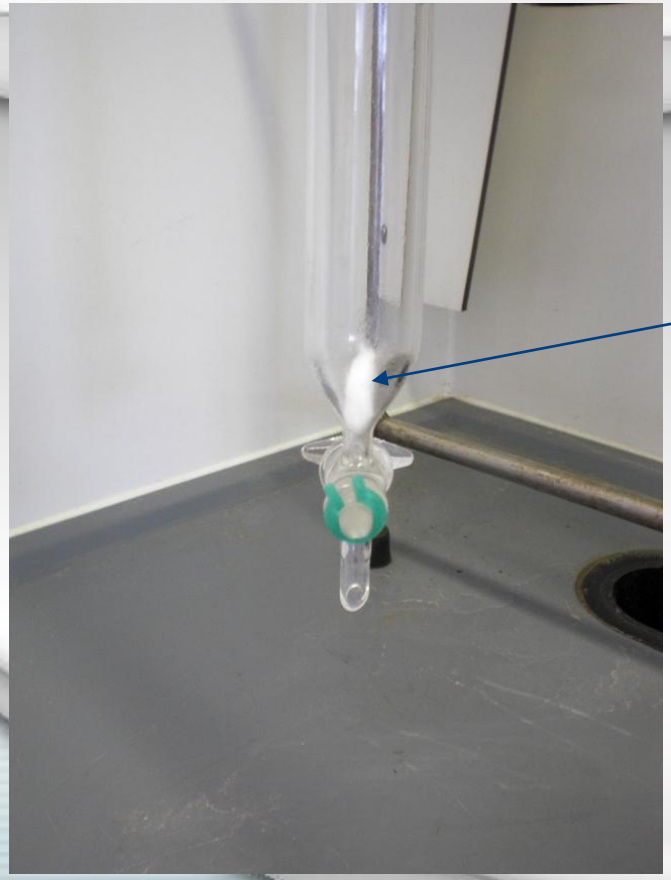
The chromatography column should be completed inside a fume cupboard. It is preferable to complete the packing, loading and running of a column in one day, where possible,

The column. There are some different variations of column. For example, the diameter can vary, the top of the column may not have a quickfit joint, the stopcock could be glass or teflon, and it may also contain a sinter filter towards the bottom.

If the column contains a sinter/filter disc then you can ignore the next few slides.

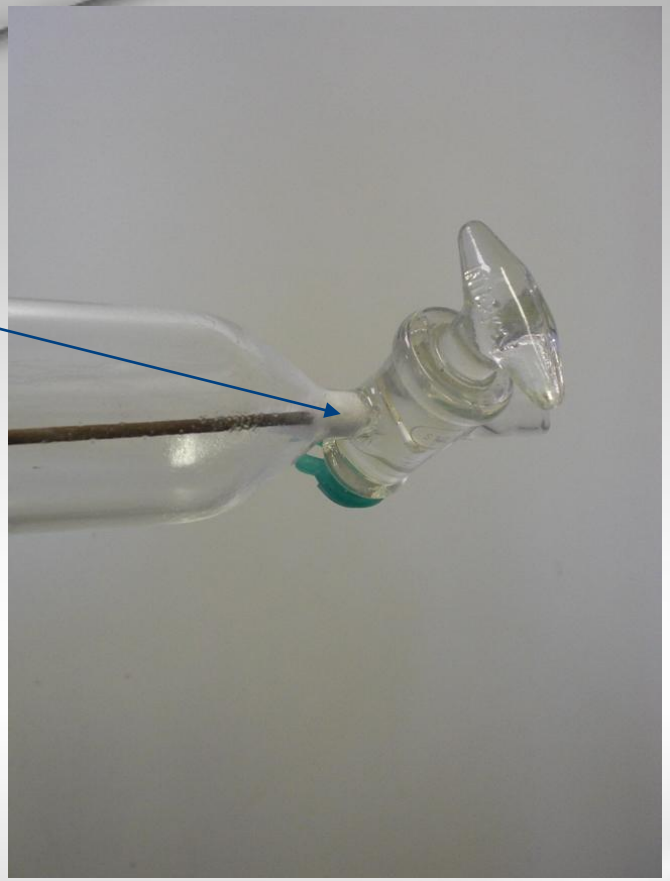
If there is no sinter

To the bottom of the empty column, bung the tap by loosely placing cotton/glass wool.



Use a long thin pole to help bung column with cotton wool.

CARE:
If you do this too tightly, the solvent will pass through too slowly meaning the column will take too long.



If there is no sinter

Place enough sand to that there is a level base. Add a bit of eluent

Take some sand and pour a small portion into the column (using a funnel)

After you have added the sand, pour a small amount of eluent to wash the sides of the flask (sand may have stuck). If sand is not level, tap the sides of the column GENTLY and this should help level the sand.

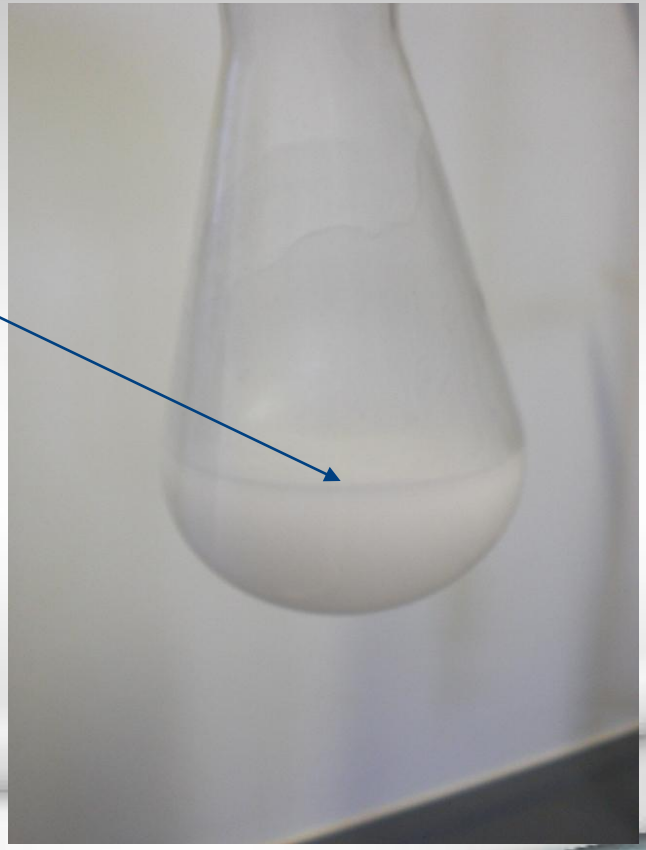
Having a small excess of eluent here is good – when you pour in the silica, it will not disturb the flat sand layer

Create a slurry.

Dissolve 25-35 g of silica gel (that is sufficient for this size column) into your starting eluent. After the next few steps, if you have a silica column path shorter than needed (see in a few slides time), you may need to do this again on a smaller scale to top up your column.

CAUTION: Avoid pouring dry silica into the column. Silica has fine dust particles which can cause irritation.

The 'slurry' never fully dissolves, but it is now easier, to pour into the column without dry silica dust going everywhere



Carefully pour the slurry into the top of the column at a rate not to disturb the sand layer at the bottom. Wash flask containing slurry residue with additional starting eluent and pour that into the column. Allow silica to settle

Pour silica slurry in carefully

Although not shown here, wash flask with additional eluent

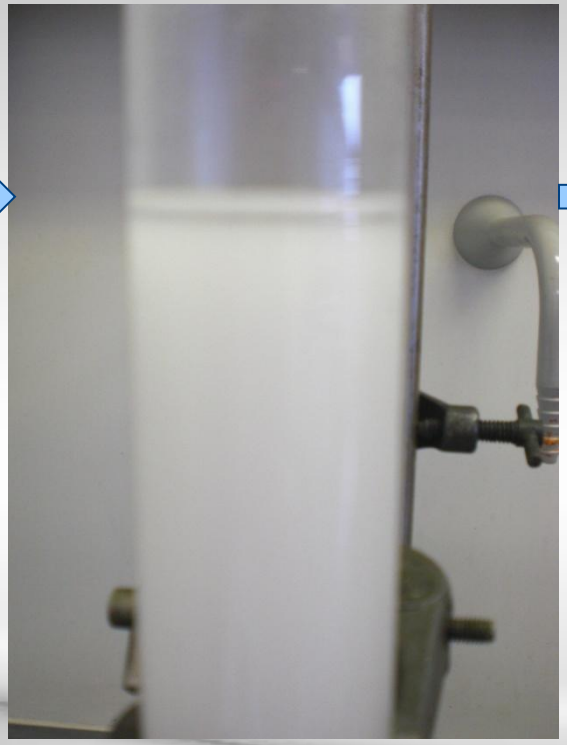
Allow silica to settle. If you can see air bubbles, GENTLY tap the side of the column to help remove the bubbles

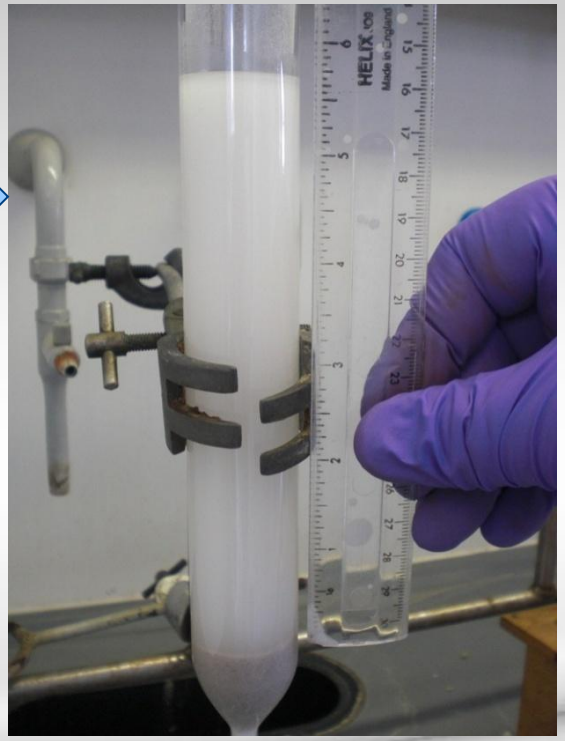
Note: The level of the silica appears to have decreased a little. This occurs as the silica settles and packs itself

Open the stopcock and allow the solvent to run through. Close the tap as the solvent reaches the top of the silica, ensuring it does not go dry. The aim is to get the silica depth at about 6 inches/15 cm. If you are a long way short, you will need to make up a small bit of slurry and top it up

Collect eluent into some sort of flask

The sample is ready to be loaded when the solvent level is close to the top, you have the appropriate path length, and the silica is fully packed (it will appear quite firm).





Solvent collected upto this point can be reused on the column as it is not contaminated

PART 2b:
Using a bellow

To speed up the flow rate inside a column, you can apply a slightly positive pressure on into the column reservoir. This is achieved using a bellow.

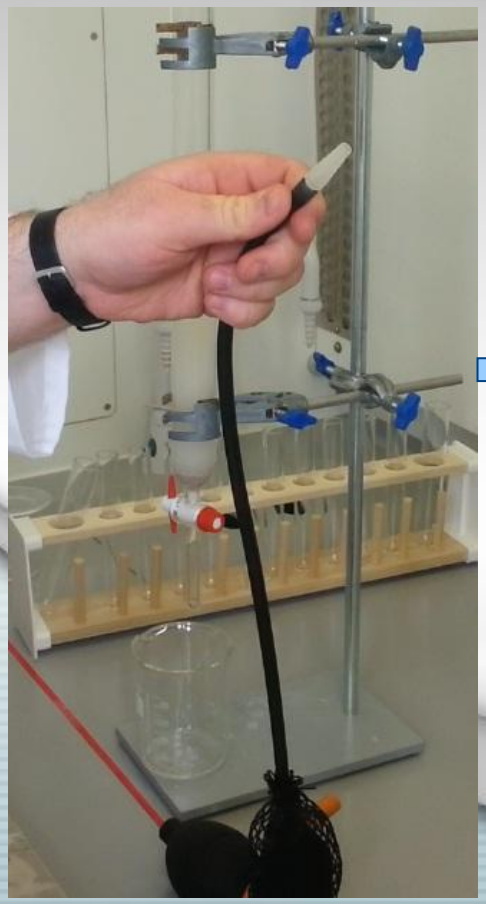
Golden rules and safety when using a bellow:

- If you seal the column with a green clip, the column tap must be open.
- If the column tap needs to be closed, the bellow must be removed.
- Petroleum ether volatility keeps the pressure inside the column quite high, so two squeezes is all that is required.

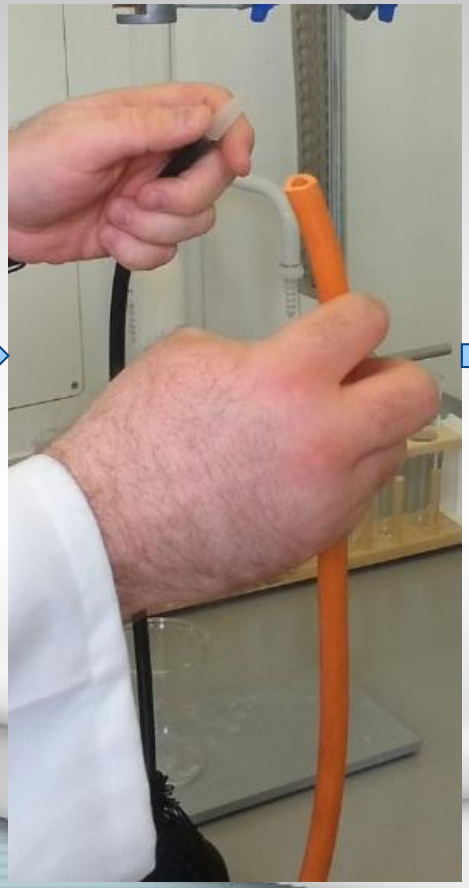
Using a bellow.

Setting up the below is shown below – this may already been done for you.

Connect the bellow to the tubing connector



Connect a piece of tubing to the other side of the tubing connector



Add a cone to the other end of the tubing

Using a bellow.

Place the bellow onto the top of the column and seal with a green clip. The tap must be open when a green clip is attached

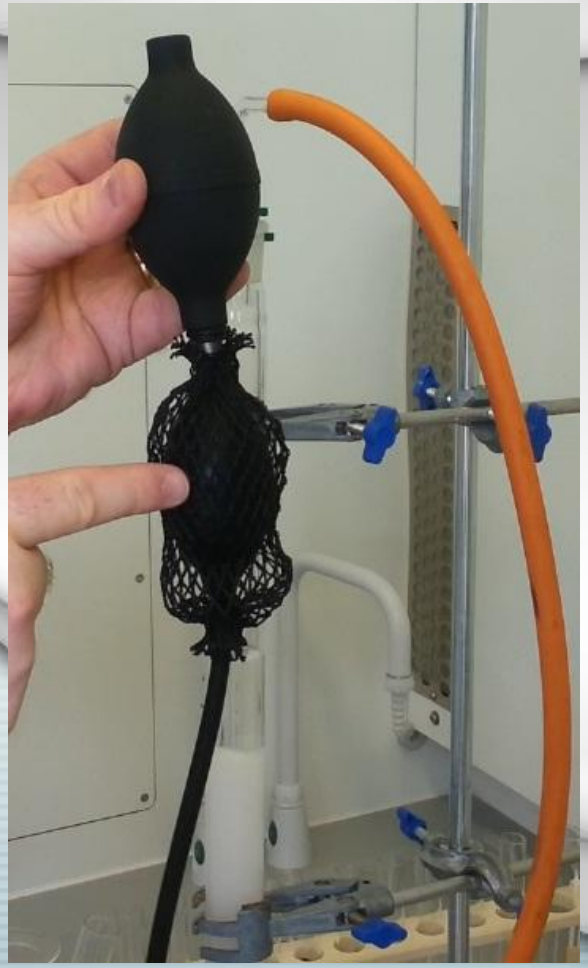
Petroleum ether has a high volatility so a natural pressure will build up in the column…

…You are working with pressures greater than atmospheric, using glass to hold the pressure…

…if you over pressurise the column, it could smash.

Using a bellow.

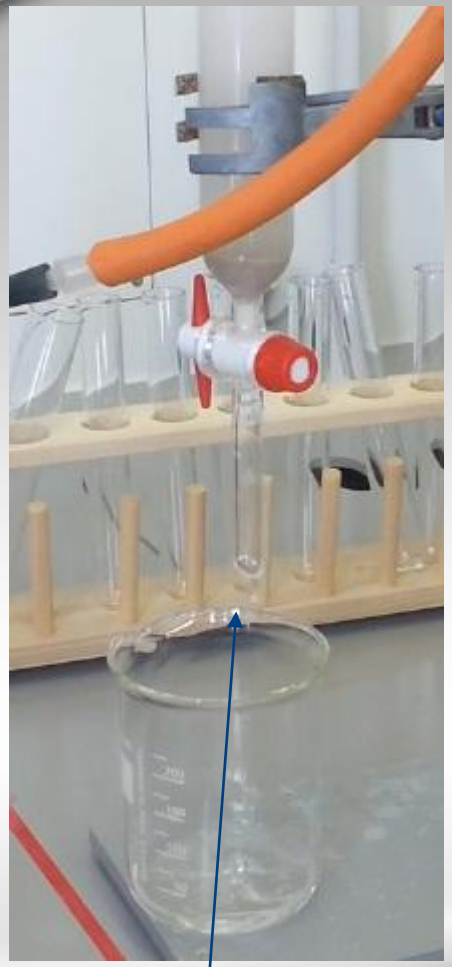
With the clip in place, **and the tap open**, squeeze the top part of the bellow a maximum of 3 times – you will see the lower bulb expand. Only once the lower bulb deflates, and the flow rate drops again should you squeeze the top part of the bellow again.



The pressure inside the column will increase when the bellow is squeezed and the flow rate (right) will increase until the pressure in the lower bulb drops and the flow rate drops.

...You are working with pressures greater than atmospheric, using glass to hold the pressure...

...if you over pressurise the column, it could smash.

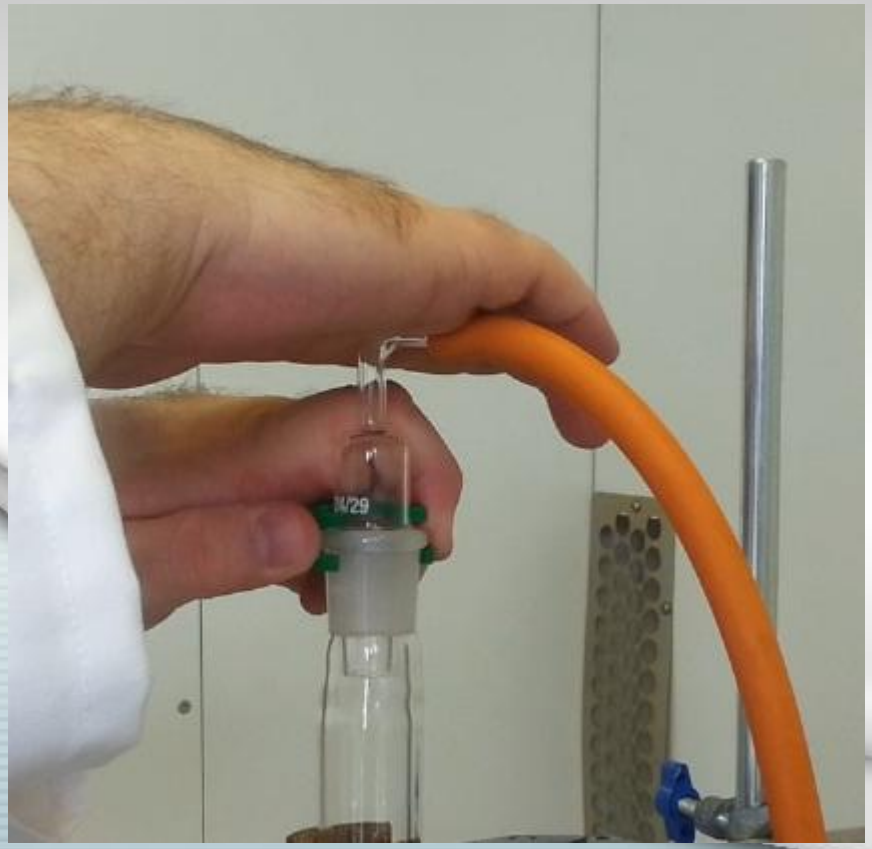


Increased flow rate

Using a bellow.

Removing the cone from the top of the column should be done with care. Place one hand over the top of the cone and remove the green clip with the other. Using the same hand that removed the green clip, carefully pull the cone out of the column

Remember: If there is still any pressure inside the column, the cone may jump out and smash (trust me, it'll happen!)



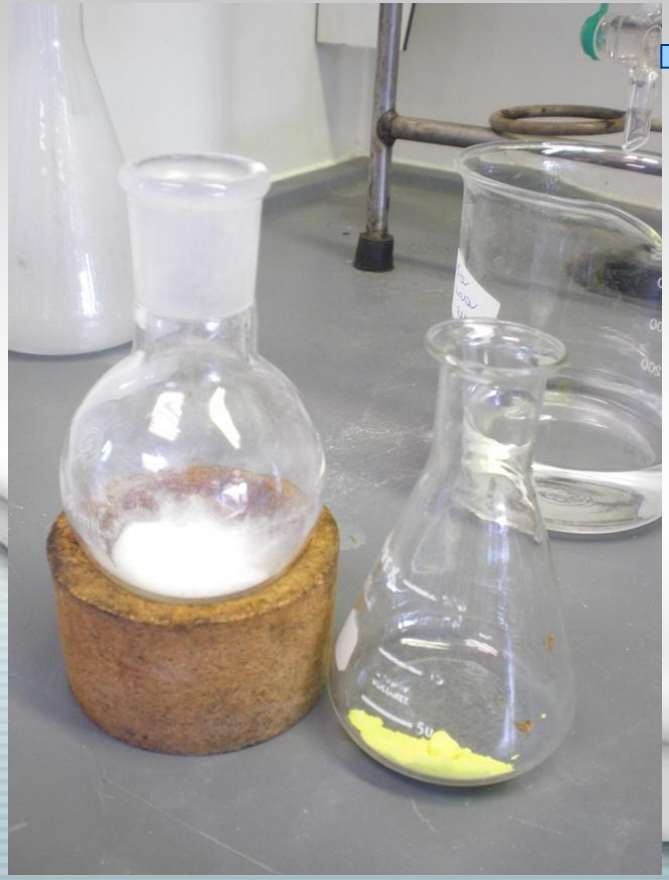
PART 3:
Loading sample to silica

The chromatography column should be completed inside a fume cupboard. It is preferable to complete the packing, loading and running of a column in one day, where possible,

If the sample is not soluble in the starting eluent, it must be pre-adsorbed onto silica to help loading onto the column.

Take a small amount of silica in a round bottom flask and add your sample to it (the volume of silica and sample should be roughly the same).

Create a slurry using a low boiling & volatile solvent eluent (in a similar fashion to earlier).



If the sample is not soluble in the starting eluent, do not use this for pre-loading your sample to silica. You need something the sample dissolves in (ideally a solvent you will use later in the column).

Place your slurry on a rotary evaporator and remove all traces of solvent. It is imperative that the sample is completely dry

Splash guard

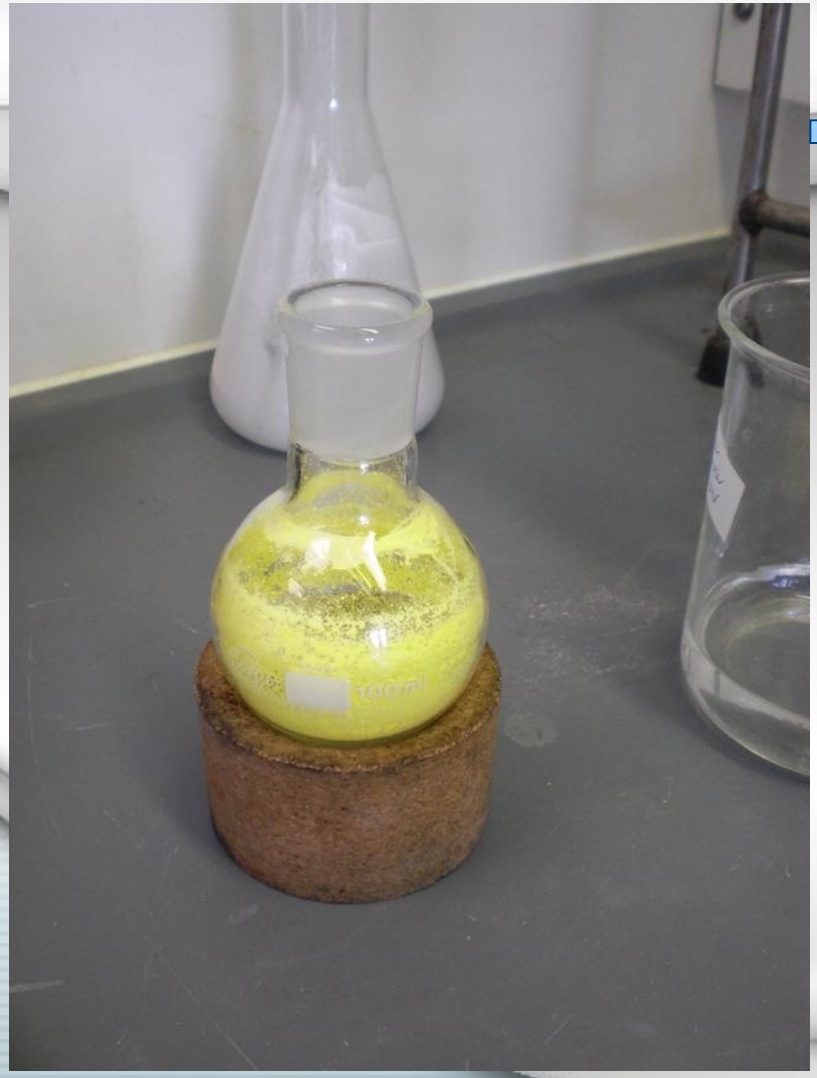
It is important that the sample is dry for one main reason:

- If you use a solvent that is more polar than the starting eluent for preloading, any traces of this at the start of the column will ruin your column.

The whole point of a column is to gradually increase the solvent polarity to separate materials – if there is a more polar solvent present straight away, little separation will be observed

Take care when removing solvent from a slurry on a rotary evaporator. As it approaches dryness, the silica can have a tendency to 'bump'. Although not shown here, it can be beneficial to place some cotton wool in the end of the splash guard. If the sample does 'bump', the cotton wool will prevent loss of product into the splash guard.

Scratch the sample/silica mix off the sides of the flask using a spatula.



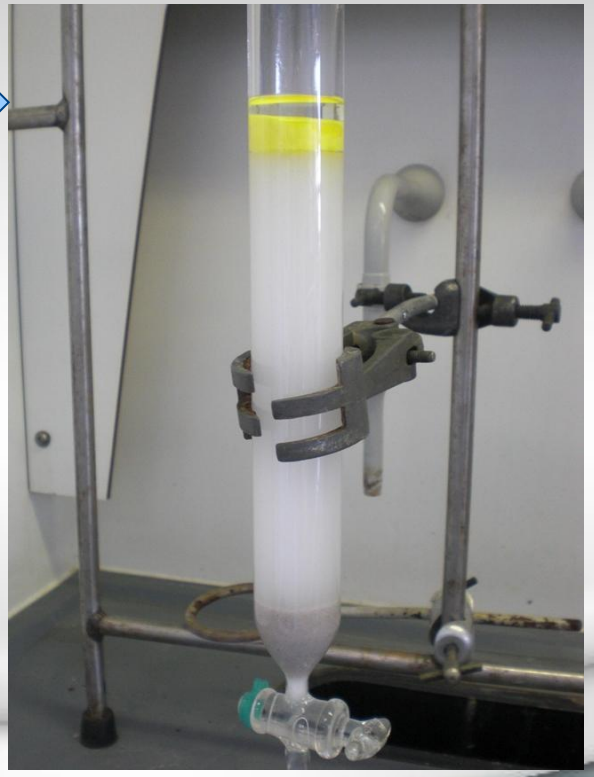
Carefully pour the mixture onto the top of the column. The aim is that the combined sample/silica mix is not much more than 1-2cm in depth

Using a few millilitres of starting eluent in a pasteur pipette, rinse the sides of the column to ensure that all of your sample/silica mixture is on top of the silica bed. Adjust the amount of starting eluent so that the amount of solvent is approximately level to the top of your sample.

The aim is that the combined sample/silica mix is not much more than 1-2cm in depth

Add a small amount of staring eluent to wash the sides of the column

At this point, you can now add a protective layer of sand



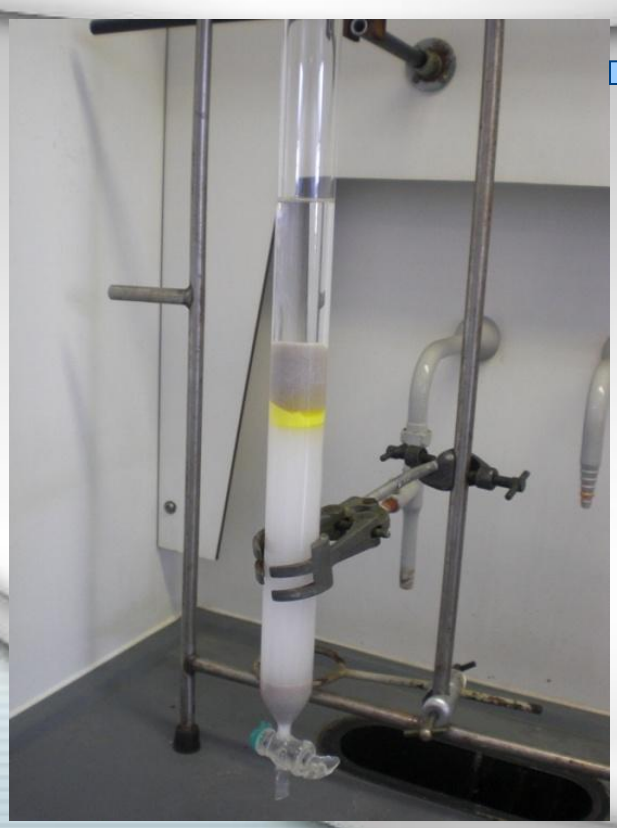
Protect the top of the silica by adding a small quantity of sand (to a depth of about 1-3 cm). Add a few more millilitres of starting eluent from a pasteur pipette to wash sand from the side of the column. Then top up the reservoir and the column is ready to run.

PART 4:
Running column

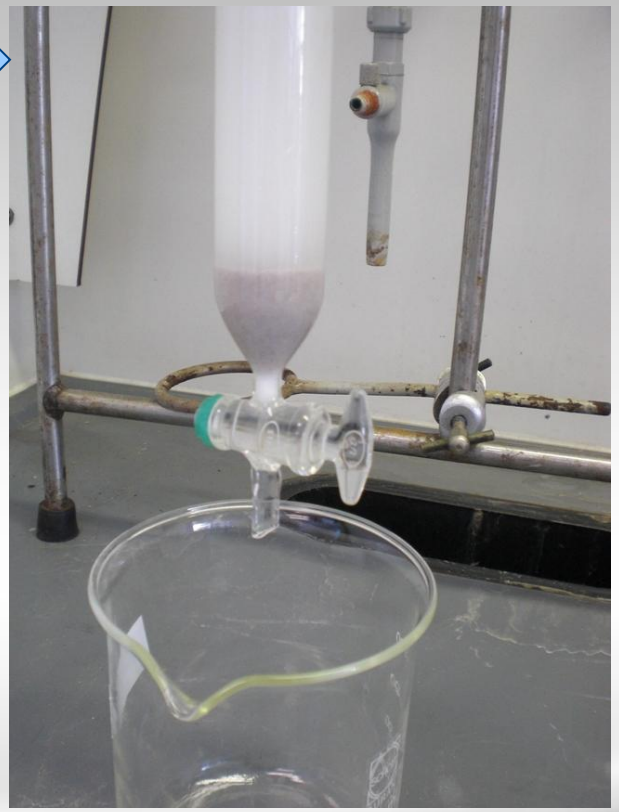
The chromatography column should be completed inside a fume cupboard. It is preferable to complete the packing, loading and running of a column in one day, where possible,

After topping up the reservoir with starting eluent, open the tap and start collecting fractions. You can use a bellow (see earlier) to help with this.

Note: Do not let the reservoir empty. You can allow the solvent level to reach the top of the sand, but try and avoid the level dropping any further, as this can introduce air bubbles into the column.

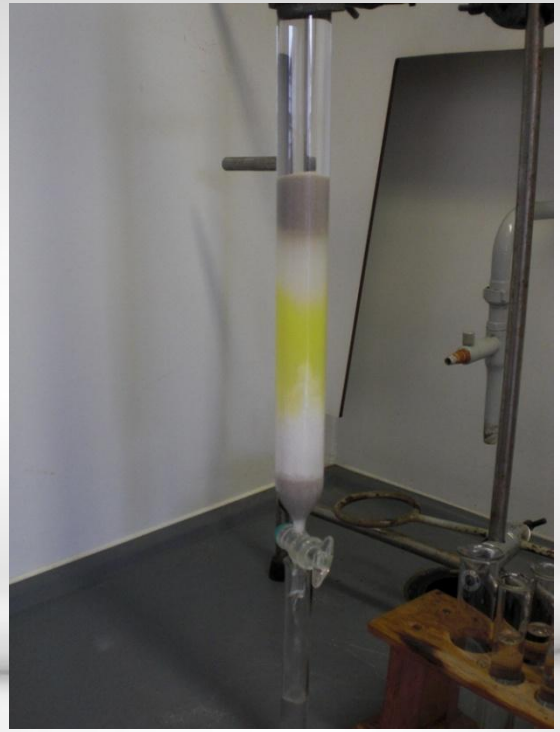


Remember that the sample can only run through the column at the speed the eluent moves. Whilst it is ok to collect a small amount in the beaker, you should quickly switch to test tubes (next slides)



Collect fractions of about 15 mL (test tubes shown are sufficient). You will need to perform TLC analysis (later) on the fractions to see where your products are.

Note: In the example here, we have used a sample which is yellow to the eye, so you can nicely see the progress of the material down the column. This is not usually the case, so TLC analysis is the main way to determine column progress.



Increasing solvent polarity

In the example shown, the first product to elute is a white solid, which is colourless in solution (left test tube). The second product to elute is a yellow solid/solution (right test tube).

By doing TLC analysis, it was determined when the first product was eluted. After all the first spot was eluted, the eluent polarity was increased to elute the second product. You will probably need to do something similar with your compound.

Note: Do not change the polarity too dramatically, too quickly. Instead, do it gradually.

In this example, the starting eluent was petroleum ether, and the increased polarity eluent was 10% ethyl acetate in petroleum ether. This level of increase was ok in one go.

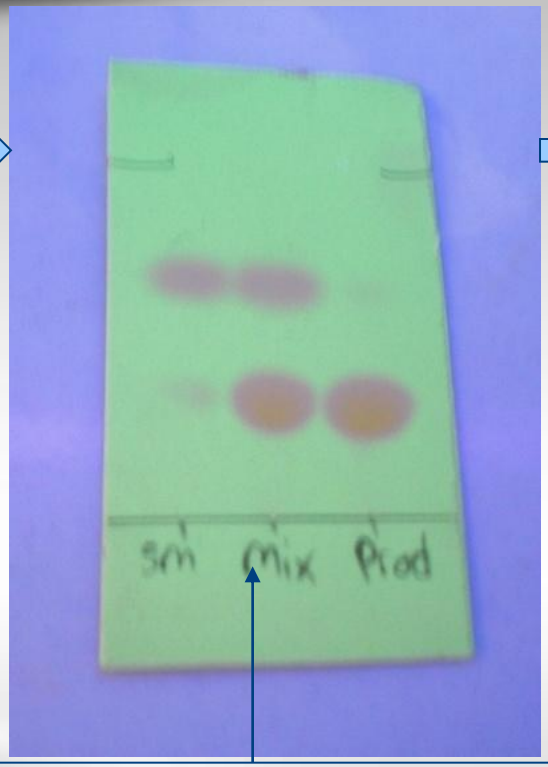
PART 5:
TLC analysis and combining fractions

The chromatography column should be completed inside a fume cupboard. It is preferable to complete the packing, loading and running of a column in one day, where possible,

For the example given here, this is what the TLC looked like under various conditions. This was completed prior to the column to check that the eluent system wass sufficient.

Under natural light

Under UV



Under natural light with products highlighted using pencil

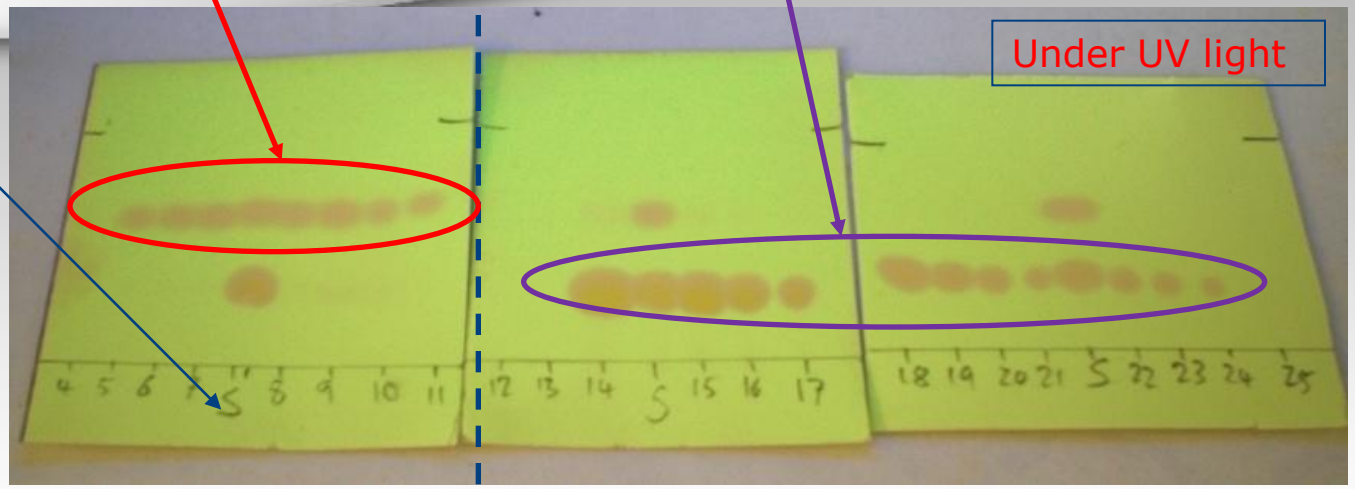
The middle section of the TLC (called 'mix') is the mixture loaded onto the column

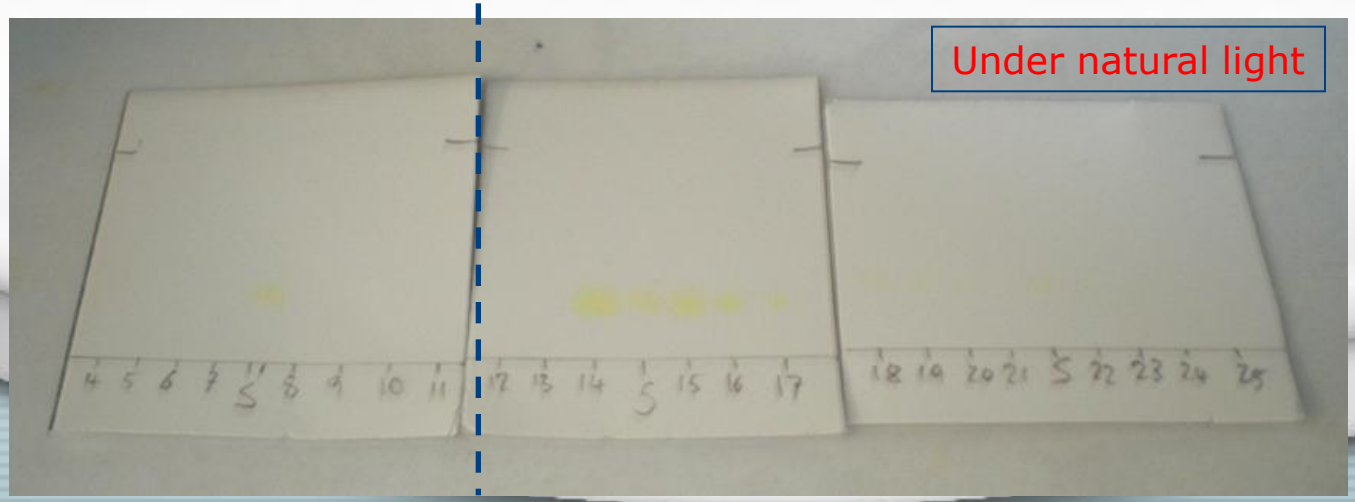
A great result! This is the aim of any column as the top spot has been successfully separated from the bottom spot. The number of 'empty' fractions does not matter

Fractions 5 – 11 could be combined in a round bottom flask and taken to dryness to give you a pure sample of the top spot.

Fractions 14 – 24 could be combined in a round bottom flask and taken to dryness to give a pure sample of the bottom spot

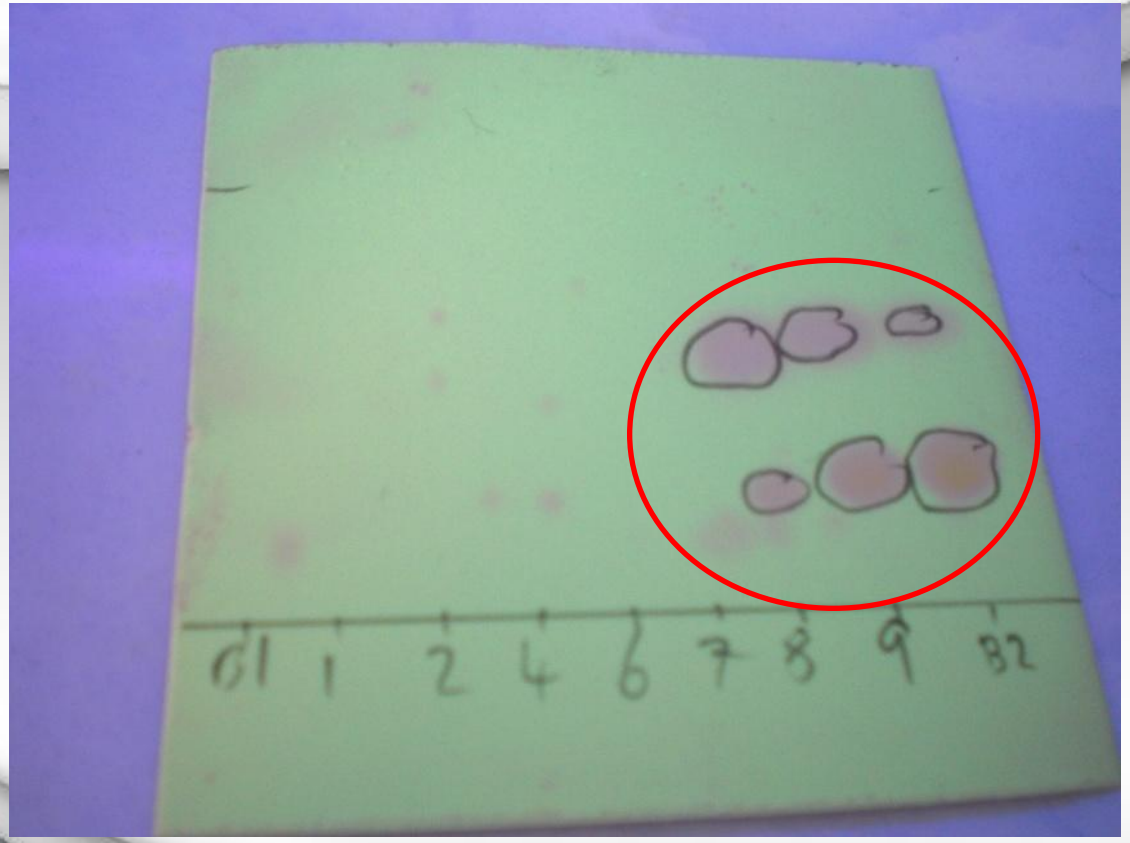
The 'S' here is just a reference spot of the starting material. It shows that the spots eluting from the column correlate to what was there at the start





Note: Every fraction has been TLC'ed here for demonstration purposes. It is acceptable to TLC every other fraction until you determine where your sample is. You can then go back and do another TLC, if required

A terrible result! Your spots have co-eluted. No separation has been achieved so in this situation you should remove all your solvent and start again…



Both spots have eluted together so the column has not worked

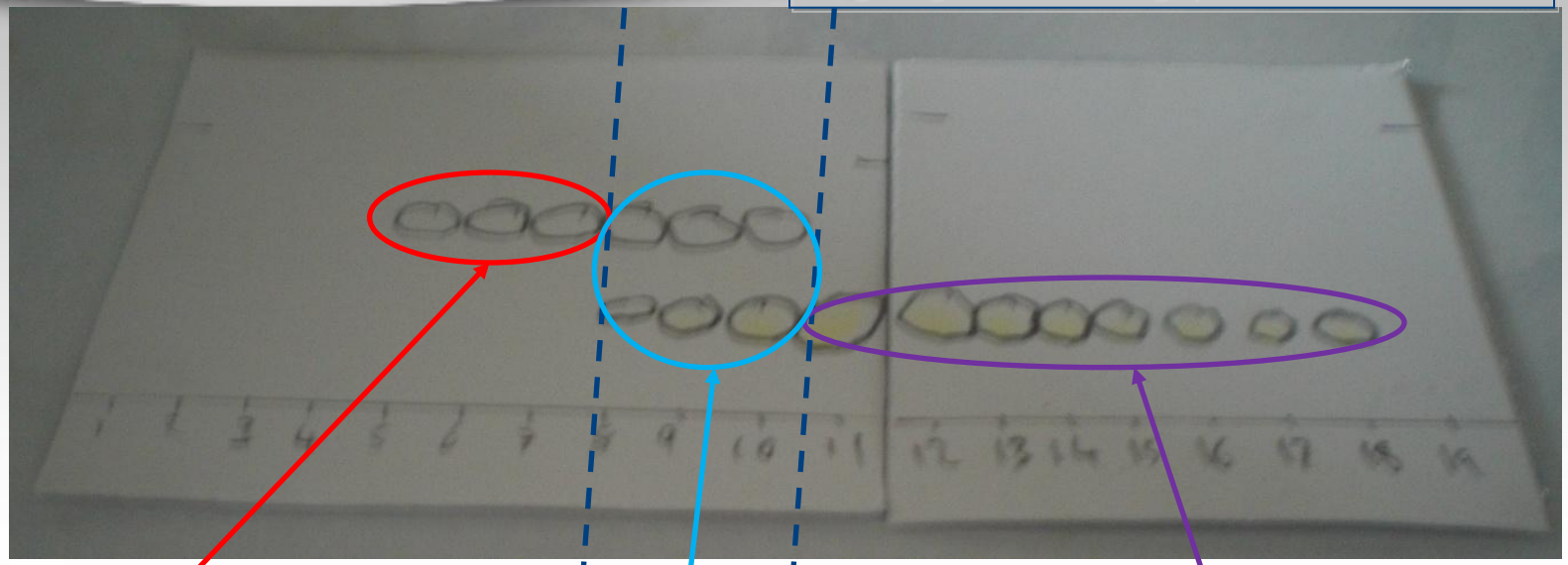
Nor a good or terrible result! The column has resulted in some pure top spot, some pure bottom spot, but with a mixture in the middle. On this occasion, you should collect 3 fractions:

Fraction A: Top spot

Fraction B: Mixture

Fraction C: Bottom spot

Under natural light with products highlighted using pencil



Fractions 5 – 7 could be combined and taken to dryness to give you a pure sample of the top spot.

Fractions 8 – 10 could be combined to give you a mixture. ***THEN CONSIDER RE-COLUMNING THIS SECTION ONLY***

Fractions 14 – 24 could be combined and taken to dryness to give a pure sample of the bottom spot.