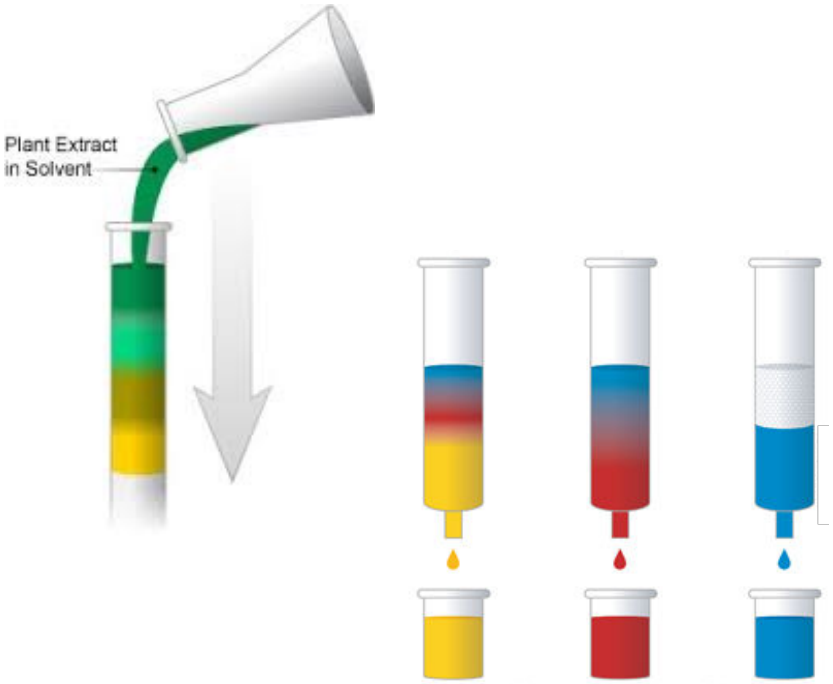
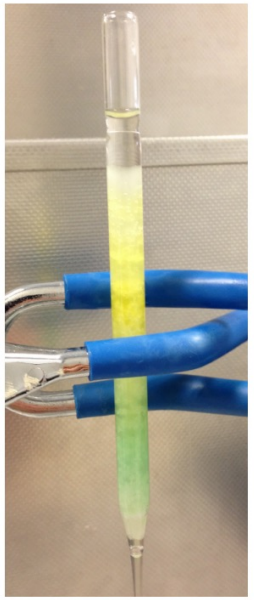
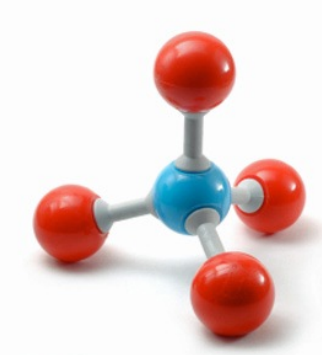
Ex18

Column Chromatography

- Separation Chromatography
  - Elution
  - Eluent Choice & Ratios
  - Sample Analysis with co-TLC
- The Experiment
  - A - Extraction
  - B - Column Setup
  - C - Collecting Fractions
  - D - Analyzing Samples
- For Next Week

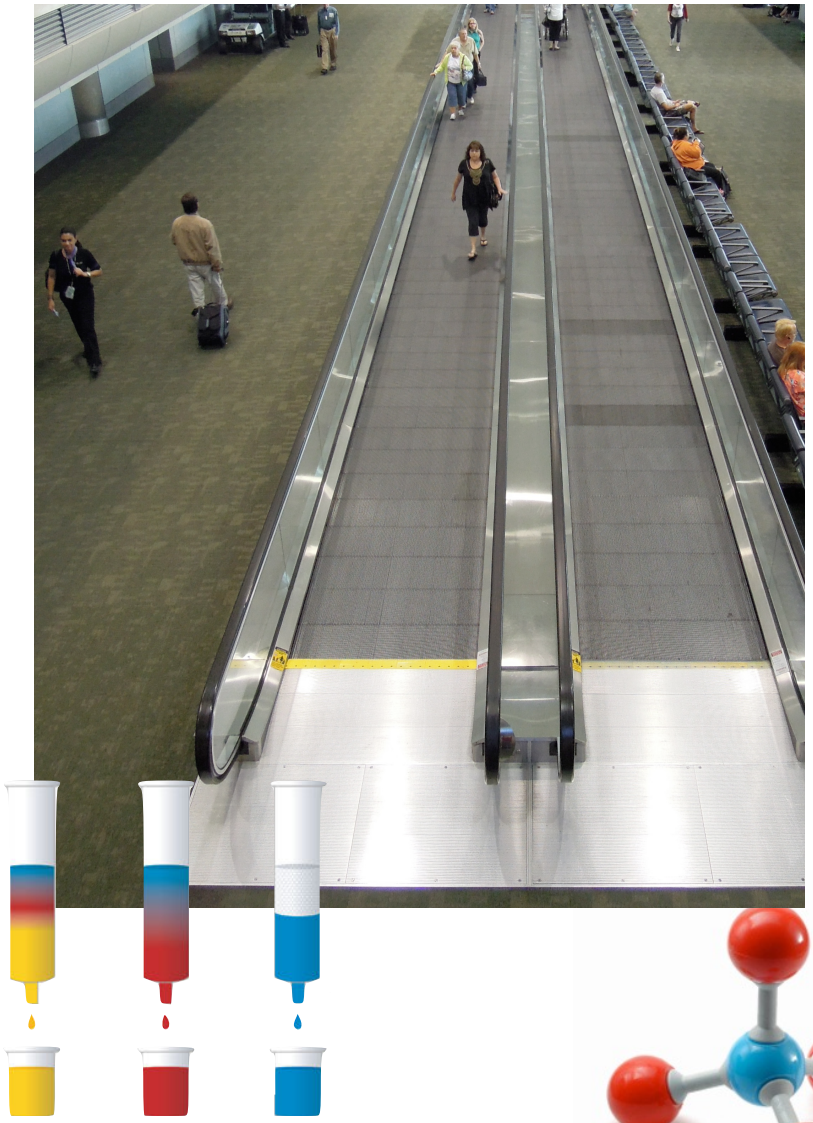






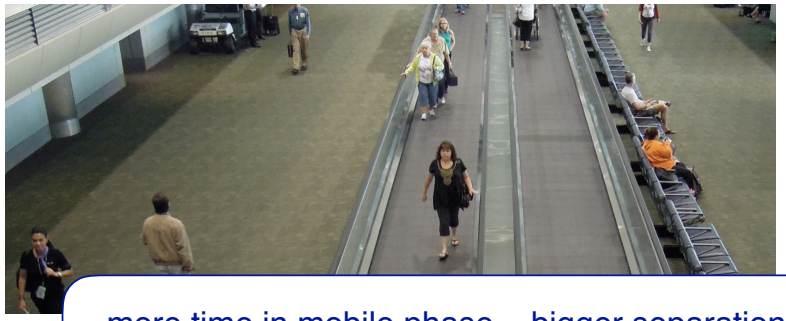
Chromatography

- Chromatography is a technique of separating substances by elution.
  - Substances are loaded onto a stationary phase that binds them.
    - We usually use silica or alumina gel, which reacts with water to produce many OH groups and therefore strongly binds to most substances.
  - A solvent of solvent mixture is chosen that the substances are moderately soluble in.
  - The stationary phase is washed (eluted) in the solvent (the solvent is the mobile phase).
  - The substances being separated are continuously pulled into and fall out of the mobile phase.
    - Think of stepping on and off a moving walkway.
  - The substances that are even slightly more soluble in the mobile phase move faster.
  - Even very slight differences in solubility can result in large separations over time.



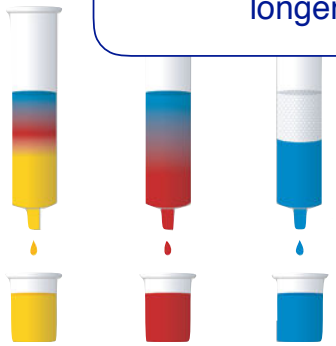
Elution

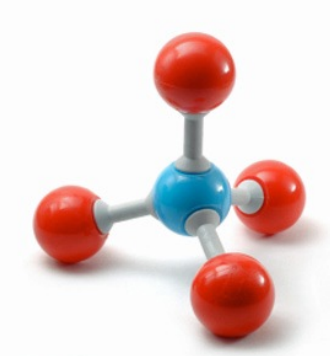
- Because the distance substances move in chromatography depends on their speed.
  - …and because that speed depends on their relative solubility…
- Differences in solubility translate to differences in the ***speed*** at which substances move.
    - Speed is distance over time.
- So we have two controls with which to enhance separation.
  - Their time in the mobile phase (more soluble eluents)
  - The length of the solid phase. (longer solid phase)
- Each can make small differences in solubility produce big separations through chromatography.



more time in mobile phase = bigger separation

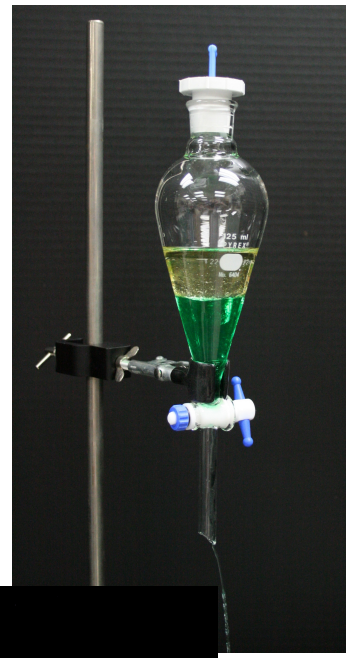
longer race = bigger separation



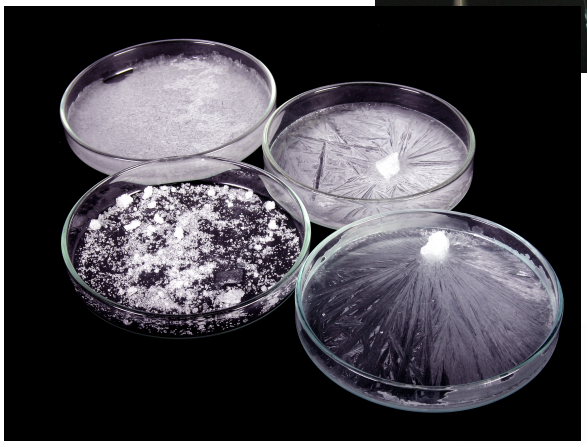


Better Separation

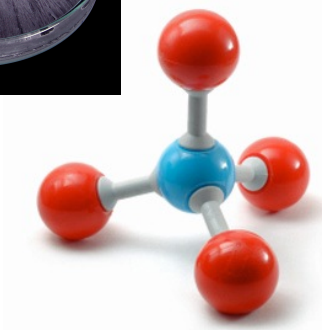
- You use chromatography when two substances have to slight a difference in solubility to be separated by other techniques.
  - If there is a big difference, do an extraction or crystallization.
  - It is lest costly, faster and easier.



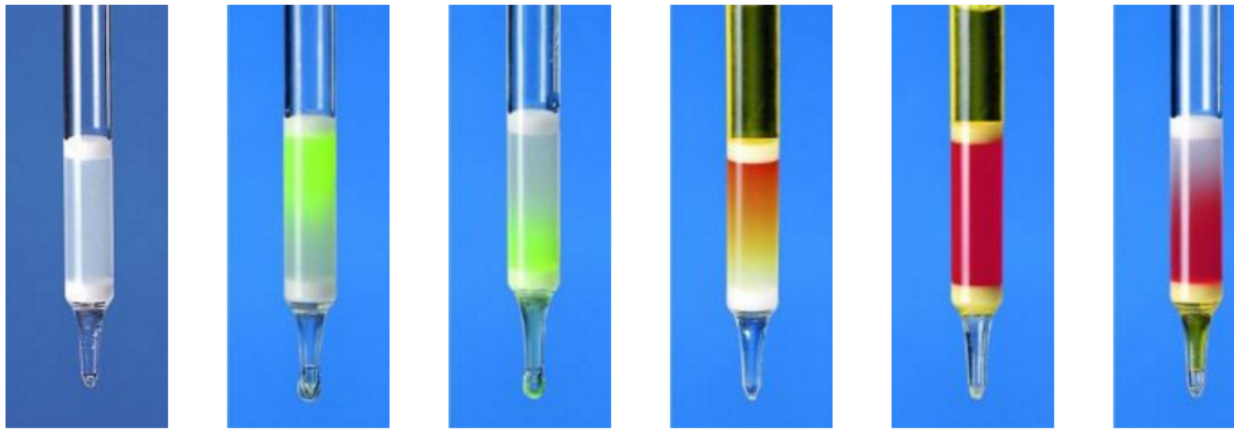
- Chromatography can separate substances with the slightest differences in solubility.
  - … by reducing their time in the mobile phase.
  - … by eluting them over a long enough solid phase.



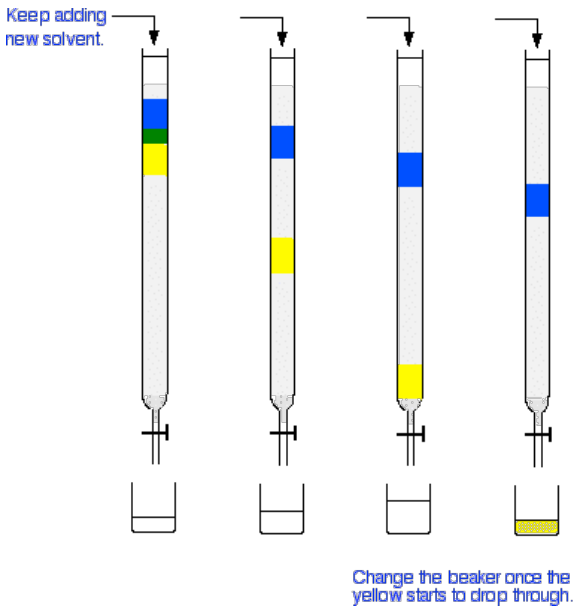
- It is also possible to vary the substance in the solid phase. More aggressive substances are used in gas chromatography and high pressure chromatogrphy to increase retention times.



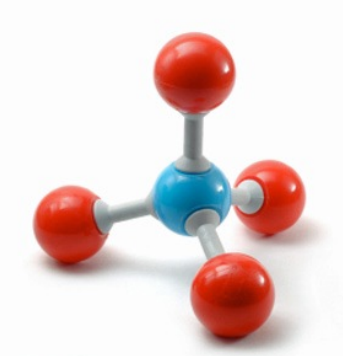
Longer Elution



- Even very small differences in speed, can produce large separations, if you run a long enough race.
- Column Chromatography allows you to run a substance as far you like.
  - Some columns are two stories high.

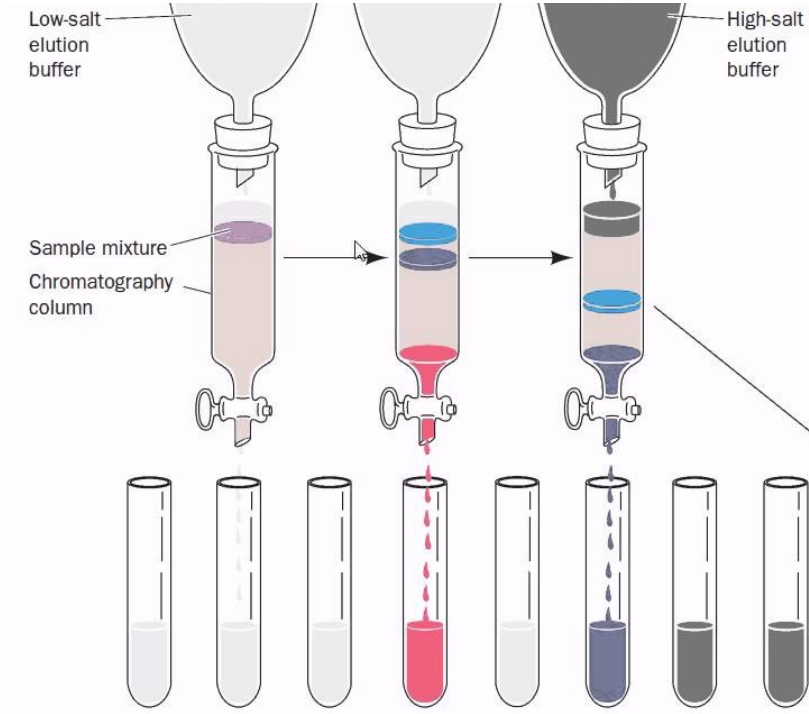


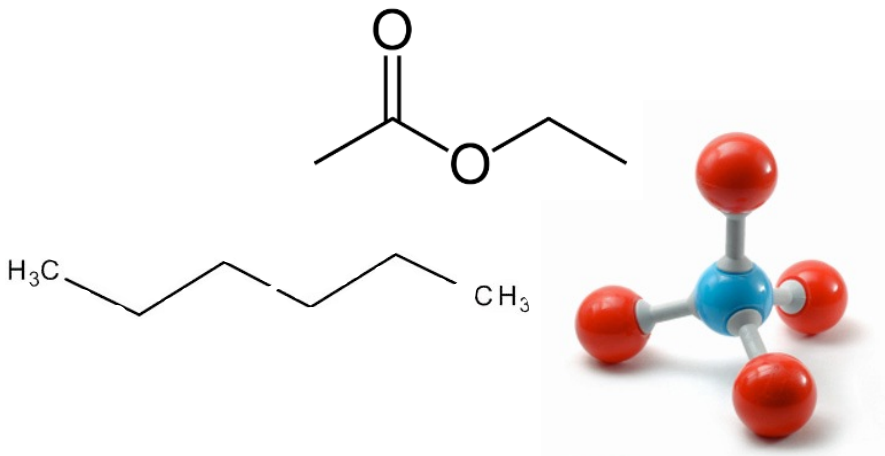
- The process is fundamentally the same as running on a thin layer, but it allows you to separate larger quantities.
- Large enough quantities to be useful as a means of purifying materials.



Elution Mixtures

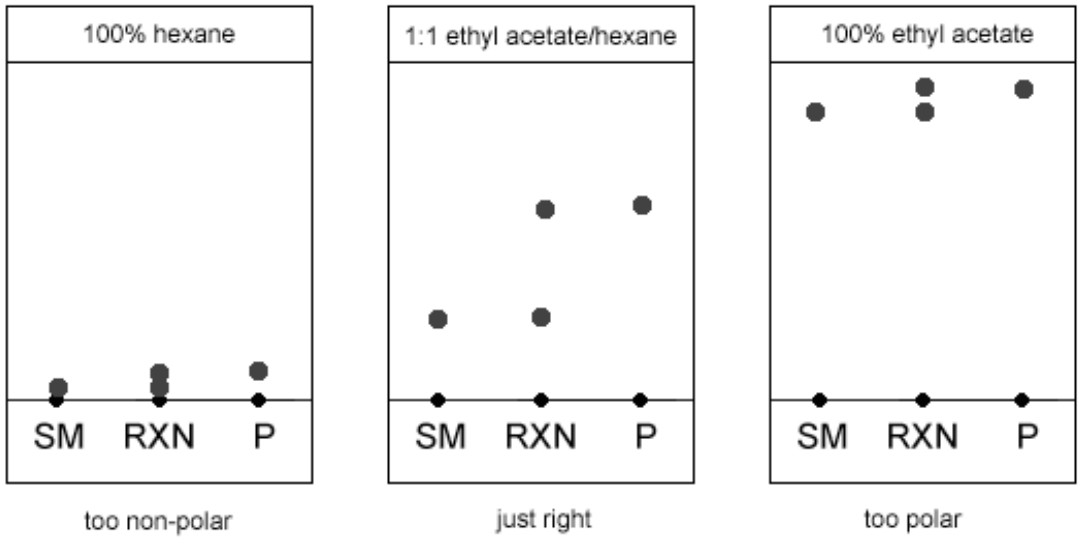
- By tuning the elution mixture you can increase or decrease the time the two substances spend in the mobile phase.
- You can choose a solvent that the substances have medium solubility in.
  - Example ethyl acetate
- You can increase the time the substances spend on the column, and thus separation, by diluting that eluent with a miscible solvent the substances are not soluble in.
  - Example hexane
    - A mixture of 50% hexane, 50% ethyl acetate
- And you can speed things up when needed by switching to a solvent more rich in that more soluble solvent.
  - Example 20% hexane, 80% ethyl acetate

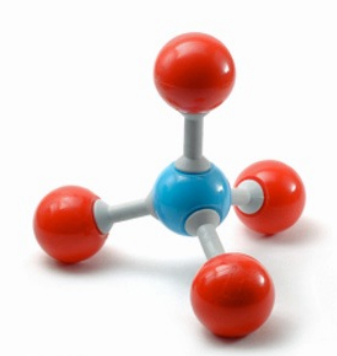




co-TLC Analysis

- Column Chromatography is often monitored with TLC.
- You can often use TLC to determine optimal elution mixtures for separation.
- And monitor the fractions you take off the column the same way.

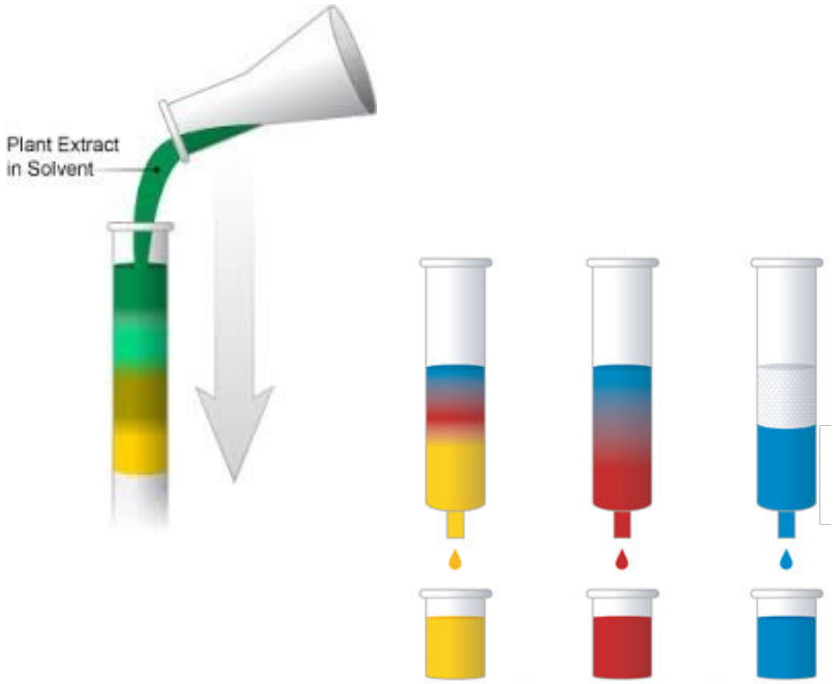


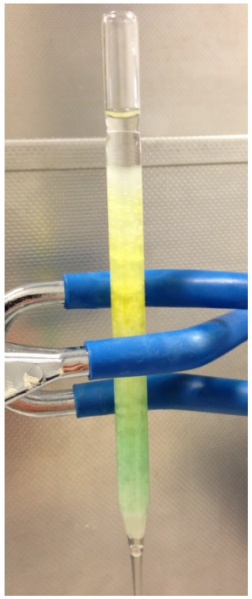


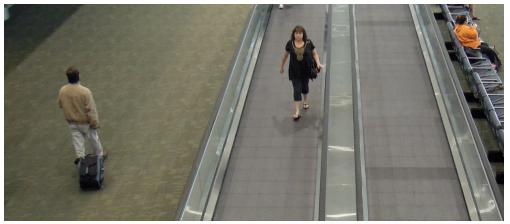
Ex18

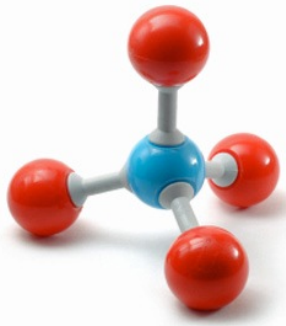
Column Chromatography

- Separation Chromatography
  - Elution
  - Eluent Choice & Ratios
  - Sample Analysis with co-TLC
- The Experiment
  - A - Extraction
  - B - Column Setup
  - C - Collecting Fractions
  - D - Analyzing Samples
- For Next Week



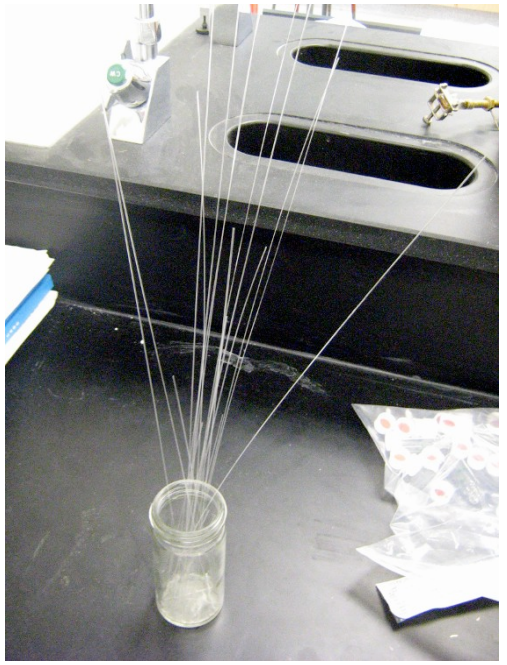


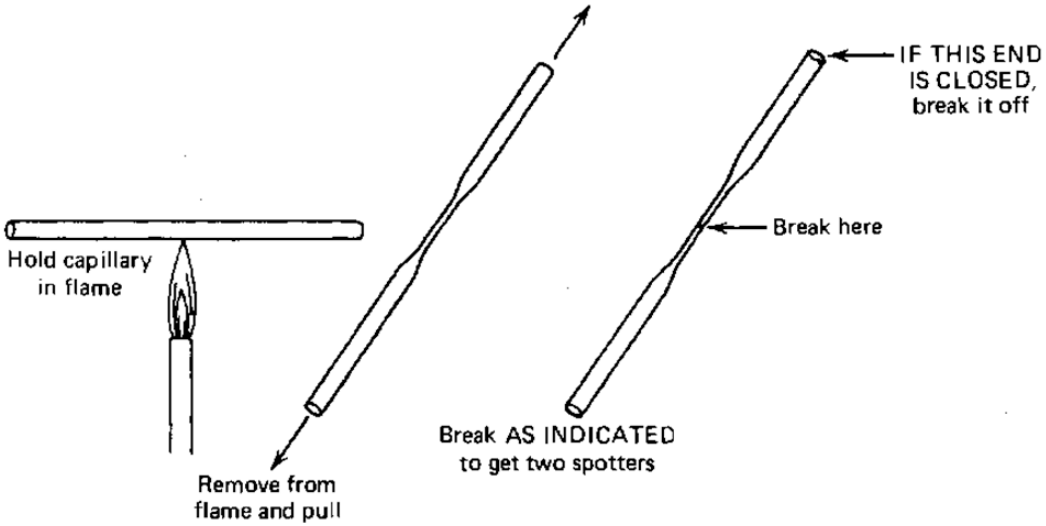


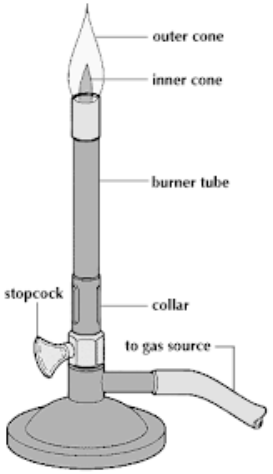


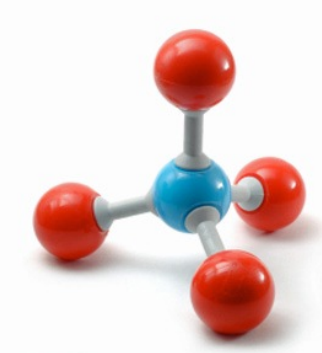
Column Chromatography

- Prepare Capillaries
  - If you are out, make 20 micro capillaries.
    - Heat capillary or disposable pipet at tip of burner flame for 30-90 seconds
      - Time needed will vary.
      - Look for glass to get jelly like.
    - Remove from flame before pulling
    - Pull as soon as removed from flame
    - Let cool, break into two



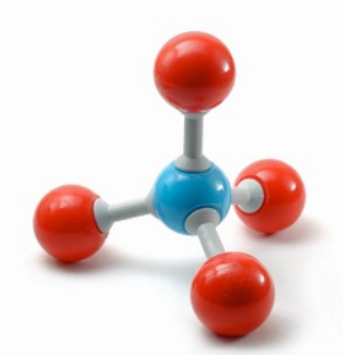






Column Chromatography

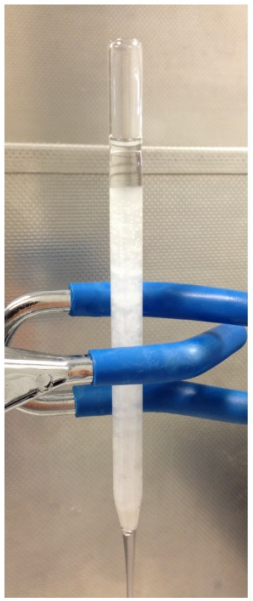
- Prepare Spinach
  - Weight 0.5 grams spinach
    - Leaves only, avoid large veins and stalks
  - Cut into small pieces
  - Add 1.0 mL COLD Acetone
  - Grind with mortar and pestle into liquid
  - Add slightly (0.5 mL) more acetone if needed to produce liquid
  - Pipette into centrifuge tube
  - Chase with 1 mL cold acetone
  - Centrifuge mixture

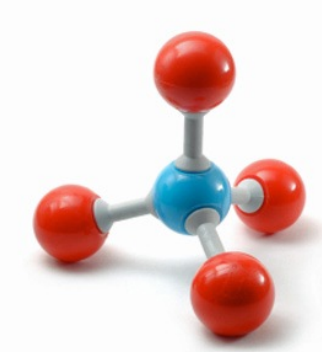


Column Chromatography

- Extract Spinach
  - Transfer liquid to a new centrifuge tube with cap.
  - Add 2.0 mL hexanes and shake capped tube.
  - Then, add 2.0 mL deionized water and shake tube.
    - Vent tube frequently by opening cap.
  - Centrifuge to break emulsion
  - Remove bottom aqueous layer
- Dry Hexane Layer
  - Clamp a pasteur pippete with lab stand to create filtration column
  - Wedge a small compressed ball of cotton in end
  - Carefully add 0.5 g anhydrous Na_2SO_4
    - (about half full)
  - Add clean dry test tube or small erelenymber flask under filtration column
  - Transfer hexane through column by pipette
  - Chase with 0.5 mL Hexane

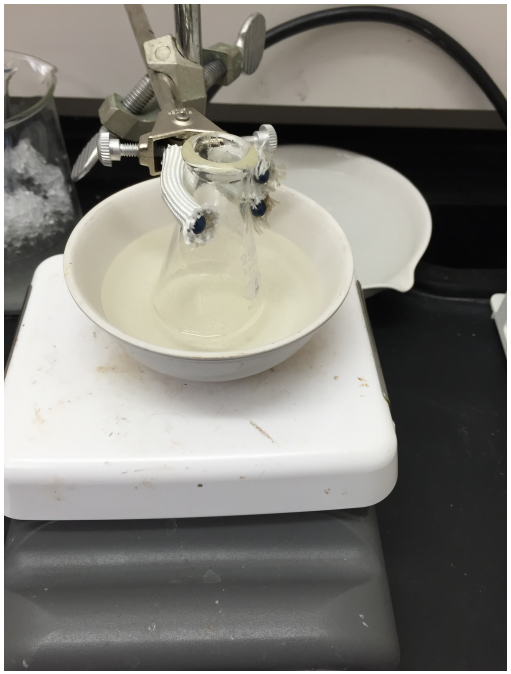
Use the folded weigh paper to more easily add Na_2SO_4





Column Chromatography

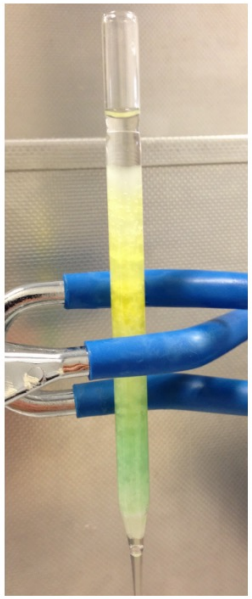
- Concentrate Extracts
  - Reduce extracts with hot water bath (40-60˚ C) to small concentrated sample
  - Collect extracts in minimal amount of hexane
  - Stopper flask and place in dark drawer, until you are ready to run column.

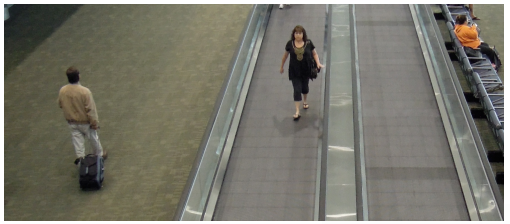


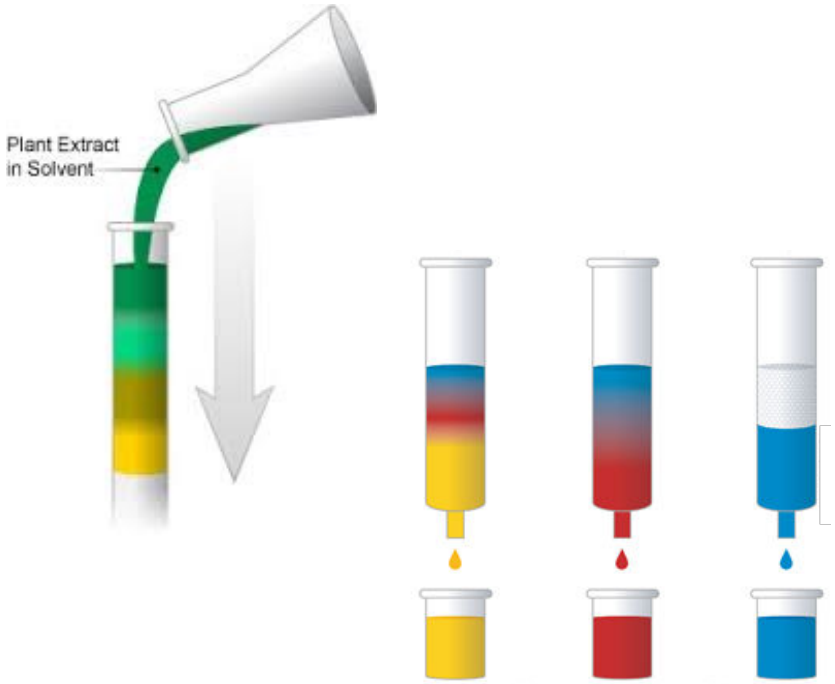
Ex18

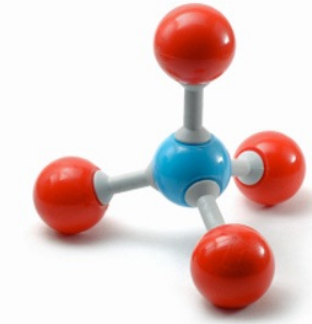
Column Chromatography

- Separation Chromatography
  - Elution
  - Eluent Choice & Ratios
  - Sample Analysis with co-TLC
- The Experiment
  - A - Extraction
  - B - Column Setup
  - C - Collecting Fractions
  - D - Analyzing Samples
- For Next Week





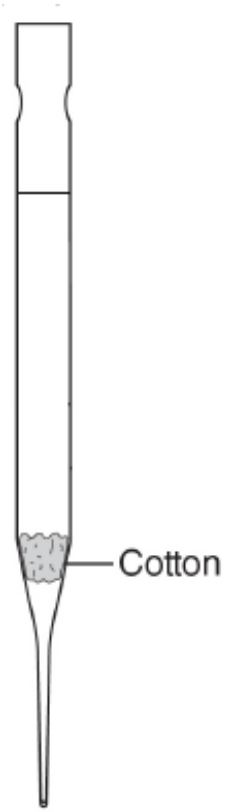


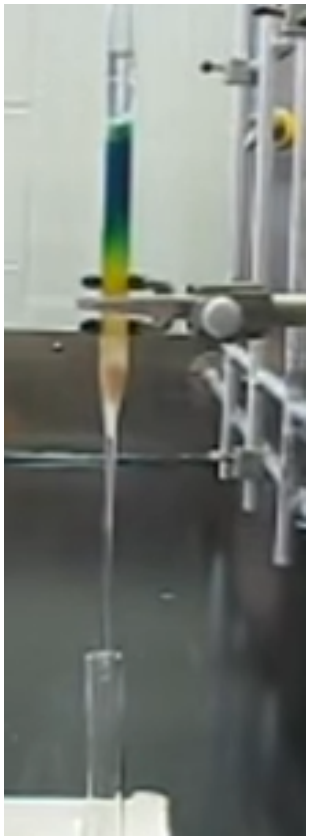


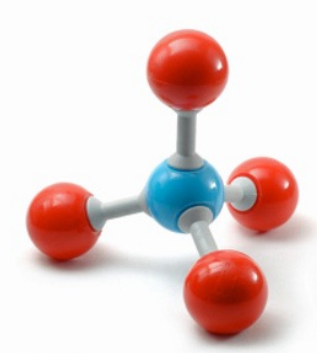
Column Chromatography

- Prepare Elution Mixtures
  - 10.0 mL Hexane
  - 6.0 mL 70% Hex, 30% Acetone
  - 6.0 mL Acetone
  - 6.0 mL 80% Acetone, 20% MeOH

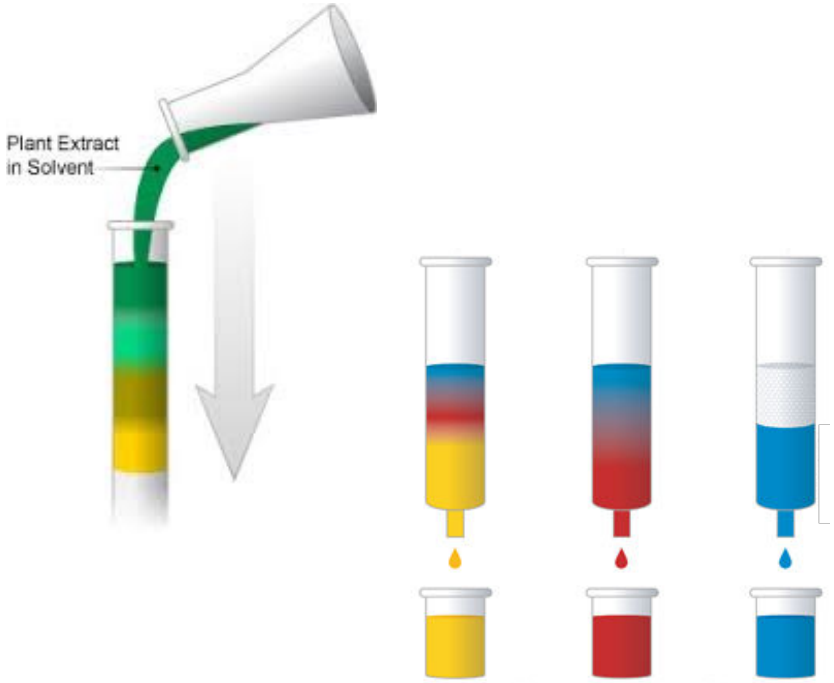
- Prepare the Column
  - Pack end of pipette with Cotton
  - Ad 1.25 g Alumina Gel
    - Tap column to let gel settle
    - Use folded weighing paper to add gel
  - Clamp Column to lab jack
  - Position first test tube under column
  - Charge column with hexane
    - Slowly add about 3.0 mL of hexane to column, let drain
    - Use pipette bulb if it drains too slowly
    - Keep top of column wet (don't let run dry)

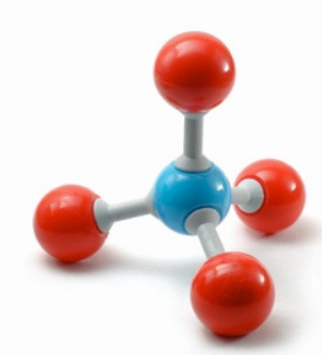




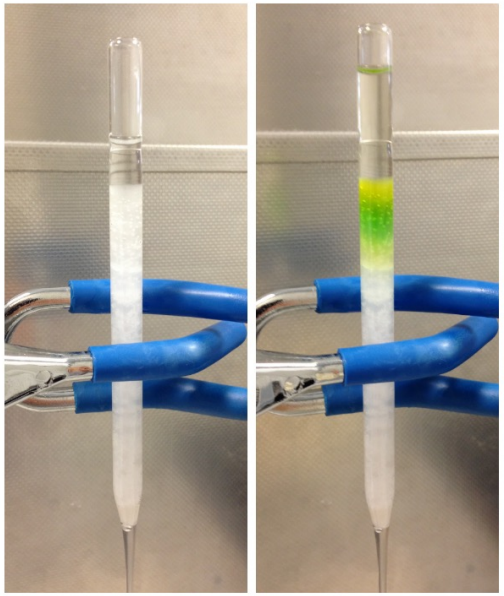


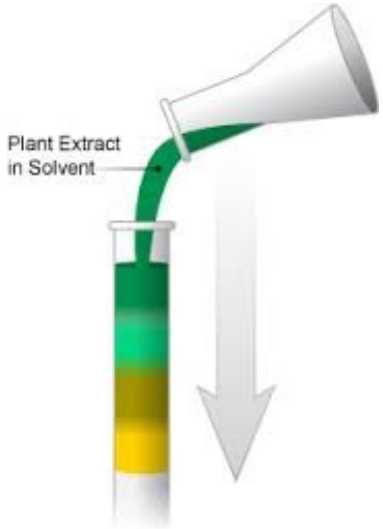
- Run Column
  - Using a remarked pipette, at 0.25 mL of your extract to the top of the column
  - Let the extract settle to the to of the alumina gel.
  - After it settles add your first elution mixture (hexane).
  - As the solvent runs through the column, the mixture will separate into at least two bands.
  - Collect the first (yellow) band.
  - Once the solvent is running clear, use your second eluent mixture (70% hexane, 30% acetone).
  - Collect the second band.
  - If needed, increase the solvent eluent strength by using your third or fourth eluent

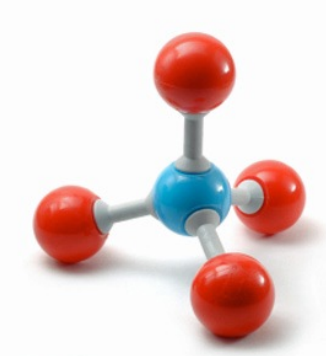




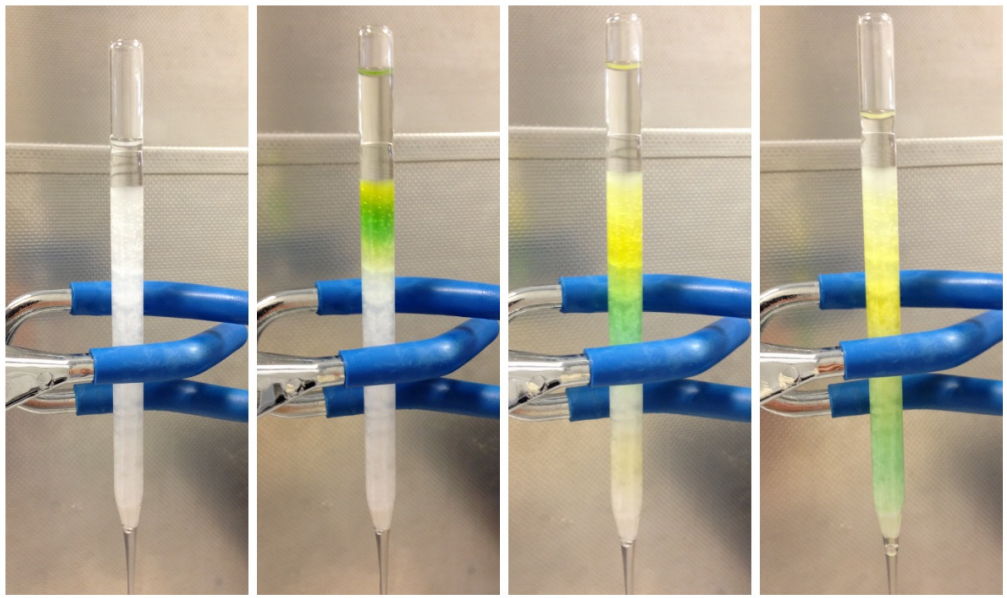
- Run Column
  - Using a remarked pipette, at 0.25 mL of your extract to the top of the column
  - Let the extract settle to the to of the alumina gel.
  - After it settles add your first elution mixture (hexane).
  - As the solvent runs through the column, the mixture will separate into at least two bands.
  - Collect the first (yellow) band.
  - Once the solvent is running clear, use your second eluent mixture (70% hexane, 30% acetone).
  - Collect the second band.
  - If needed, increase the solvent eluent strength by using your third or fourth eluent

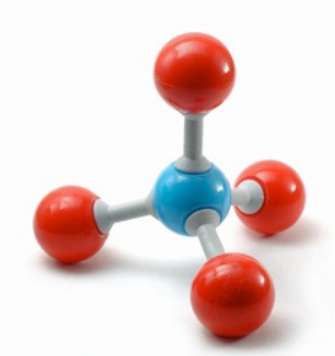






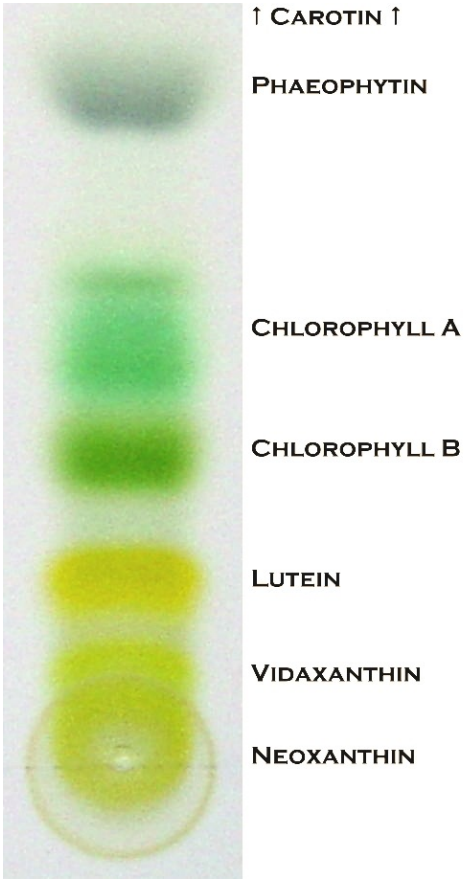
- Run Column
  - Using a remarked pipette, at 0.25 mL of your extract to the top of the column
  - Let the extract settle to the to of the alumina gel.
  - After it settles add your first elution mixture (hexane).
  - As the solvent runs through the column, the mixture will separate into at least two bands.
  - Collect the first (yellow) band.
  - Once the solvent is running clear, use your second eluent mixture (70% hexane, 30% acetone).
  - Collect the second band.
  - If needed, increase the solvent eluent strength by using your third or fourth eluent

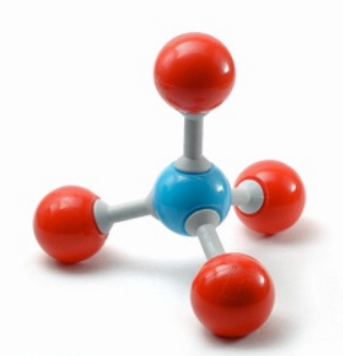




Column Chromatography

- Analysis
  - Spot a TLC plate with three tracks.
    - Your extract
    - Your yellow band
    - Your green band
  - Develop the TLC plate
    - Elution 70% hex, 30% Acetone
  - Based on the relative Rf's and colors reported in your lab description identify the substances in each band.

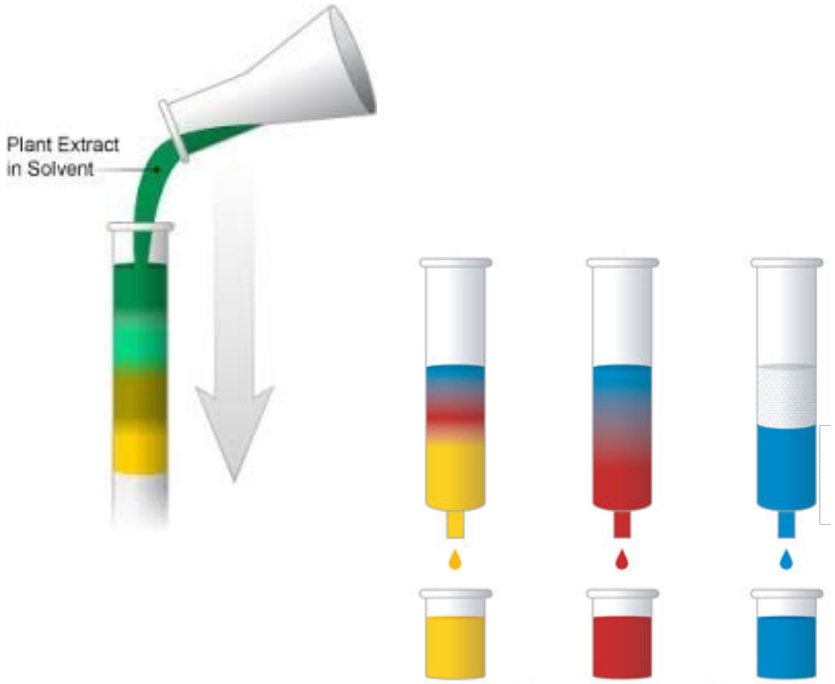


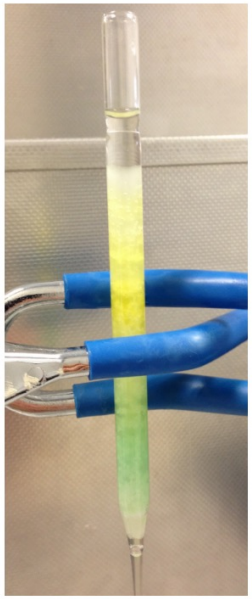


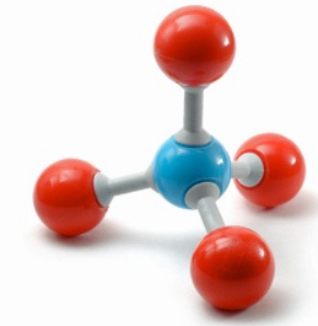
Ex18

Column Chromatography

- Separation Chromatography
  - Elution
  - Eluent Choice & Ratios
  - Sample Analysis with co-TLC
- The Experiment
  - A - Extraction
  - B - Column Setup
  - C - Collecting Fractions
  - D - Analyzing Samples
- For Next Week

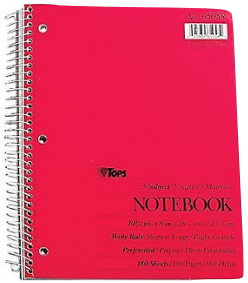


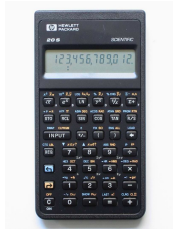


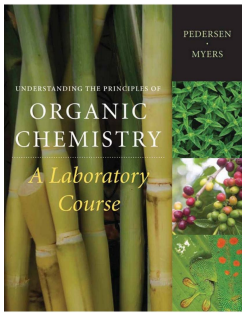


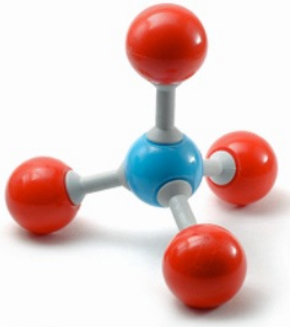
Next Meeting

- For next Meeting:
  - Midterm Exam
  - Questions will be based on the topics lists.
  - Should take less than an hour, but you are welcome to use the full lab period.









Questions?