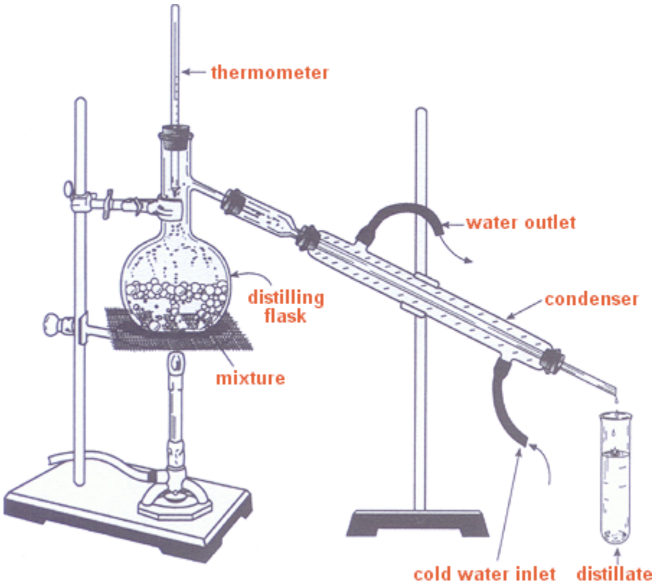
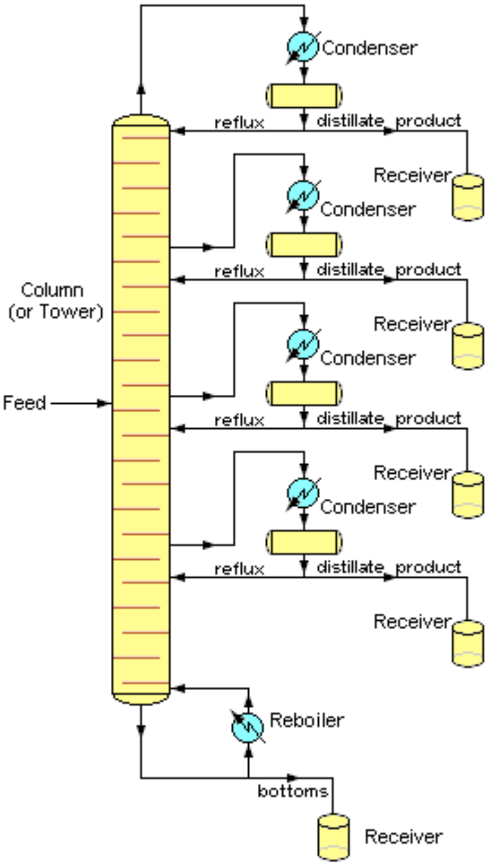
Mixing is easy, but how do we un-mix?

Analytical Separation
- selective precipitation
- selective volatilization (distillation)
- sieving (ultra centrifugation)
- solvent extraction
- chromatography
- electrophoresis

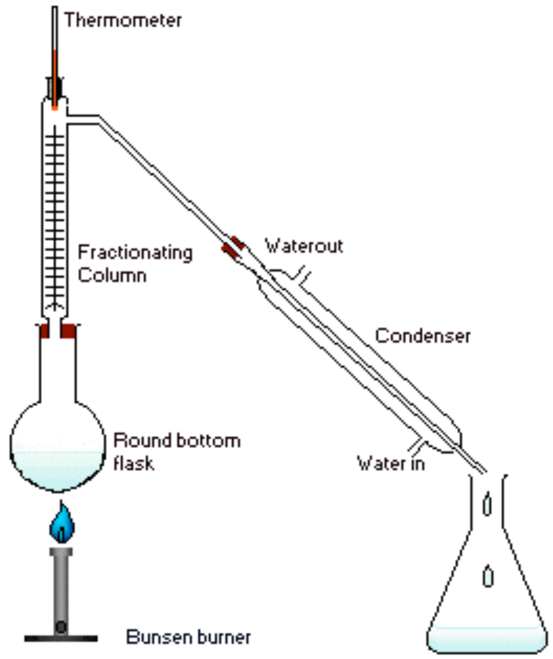


Distillation
- effectively separate a simple mixture based on boiling point

Fractional Distillation
(for more complex mixtures)



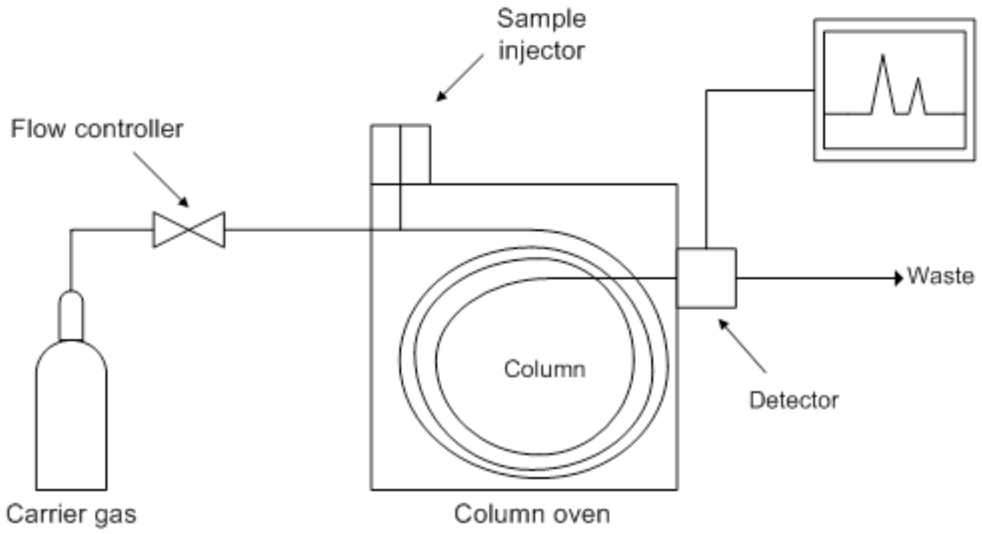
(more fractional distillation)



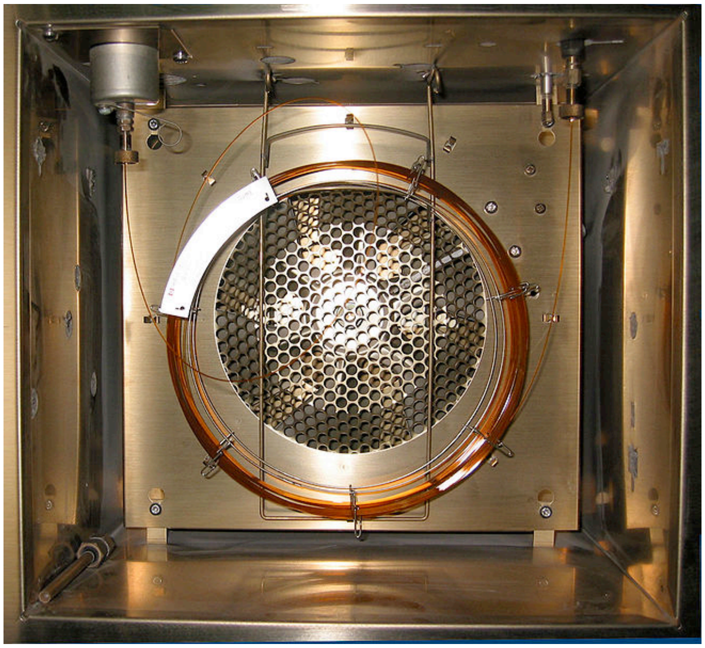
gradually bring up the column temperature

collect the distillates into many small fractions

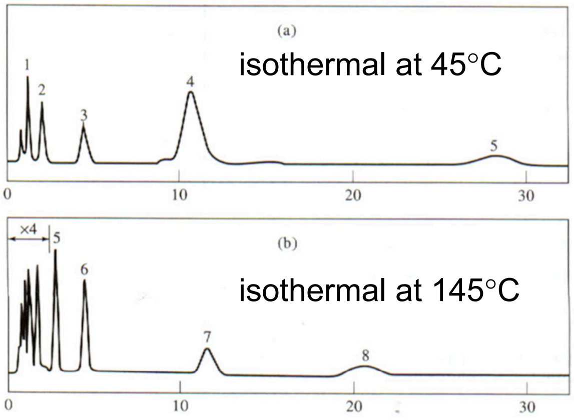
Fractional distillation → Gas chromatography



GC oven



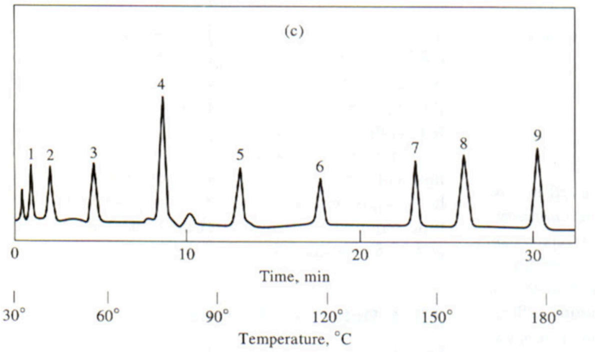
- Temperature is precisely controlled electronically
- The rate at which a sample passes through the column is directly proportional to the temperature of the column. The higher the column temperature, the faster the sample moves through the column.
- Capable of temperature programming during a chromatographic separation



isothermal at 45°C

isothermal at 145°C

gradient from 30ºC to 180ºC



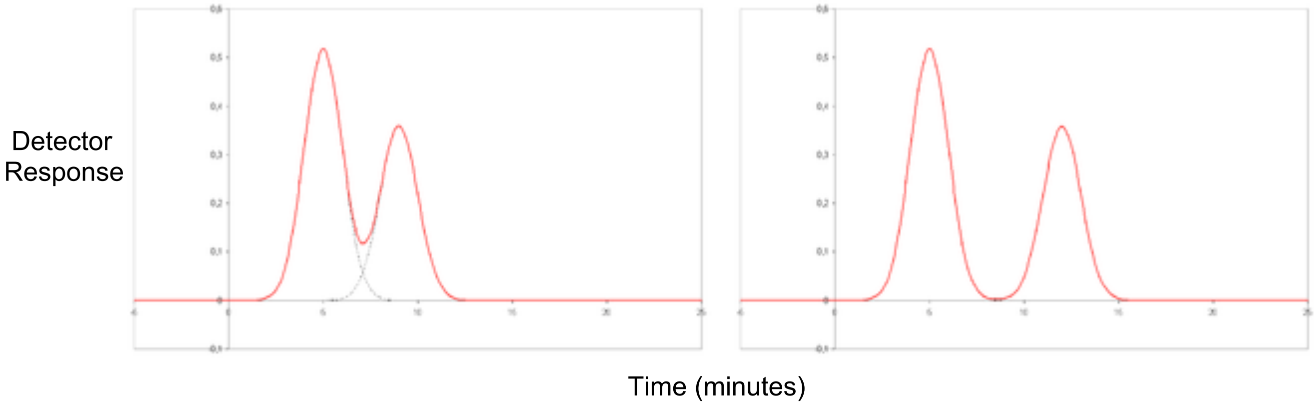
Chromatograms

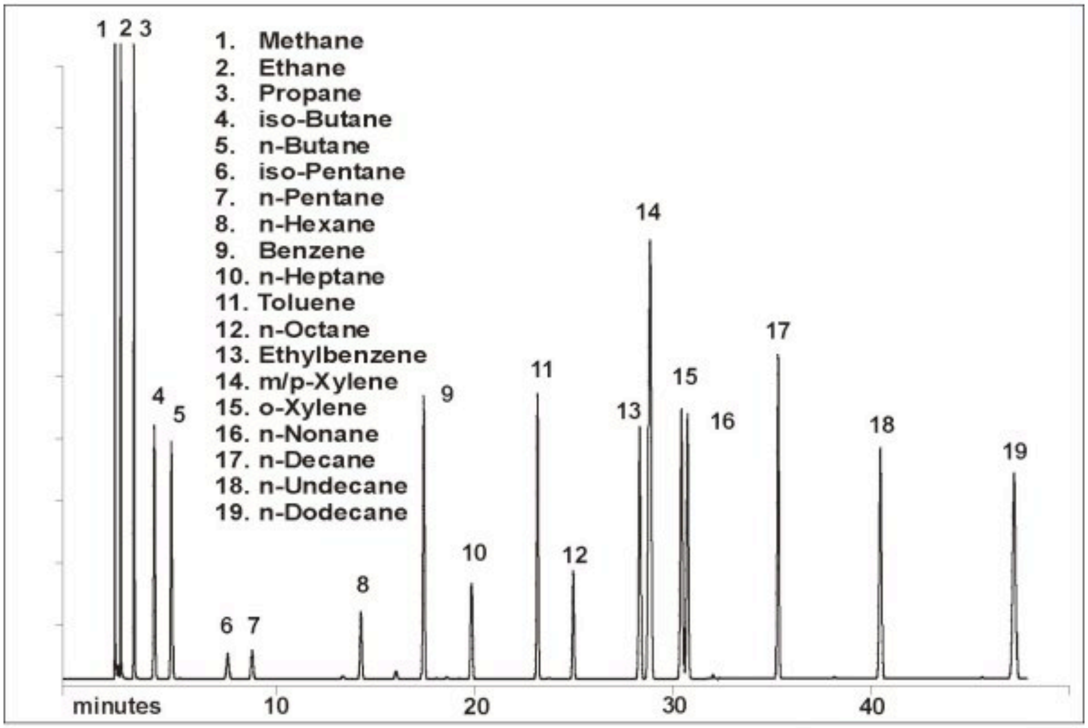
A chromatogram is the visual output of the detector during a run. In the case of an optimal separation, different peaks or patterns on the chromatogram correspond to different components of the separated mixture.

Unresolved components result in an overlapping peak.

Plotted on the x-axis is the retention time and plotted on the y-axis is a signal obtained by a GC detector. For an ideal detector, the signal intensity should correspond to the concentration of the analyte component.

Either peak height or (more commonly) peak area can be used to construct a calibration plot, for quantitative analysis.





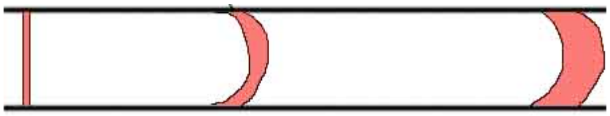
Courtesy of Wasson-ECE

Band Broadening

The physical width of an analyte band usually becomes broader as it travels down the column. (The cause will be discussed in Chem 3372)

You can minimize band broadening, but not likely to eliminate it.

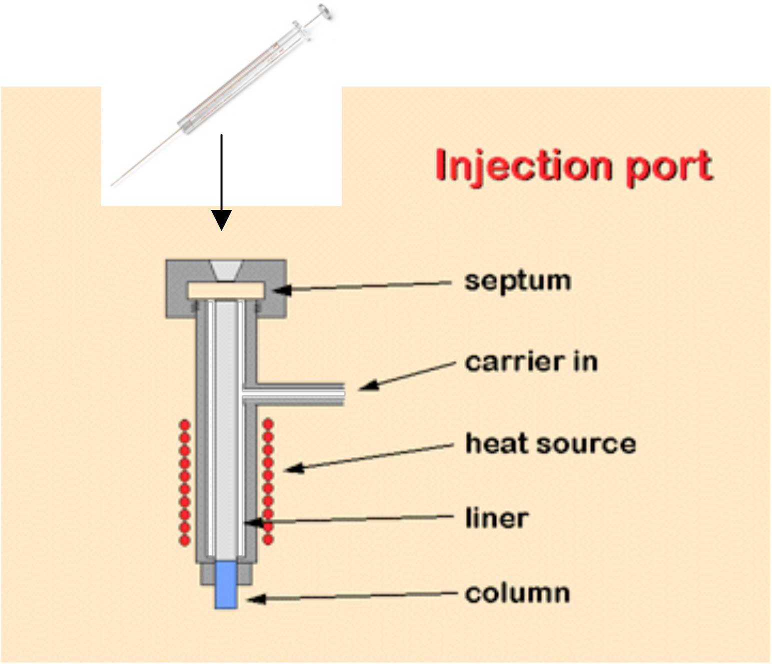
One way to avoid excessively broad bands is to limit the amount of analyte injected.



→ direction of flow

Sample Injection

- introduces a small volume of sample onto the column as a narrow band, without disruption of the carrier gas flow
- a rubber septum is in place to prevent leaks



GC Columns

In GC, the separation (elution) is primarily determined by the boiling points. However, the chemical interaction between the analyte and the column (stationary phase) can also affect elution.

Stationary phase - is the material (solid or liquid) inside the column, to which the analyte interacts with during a separation.

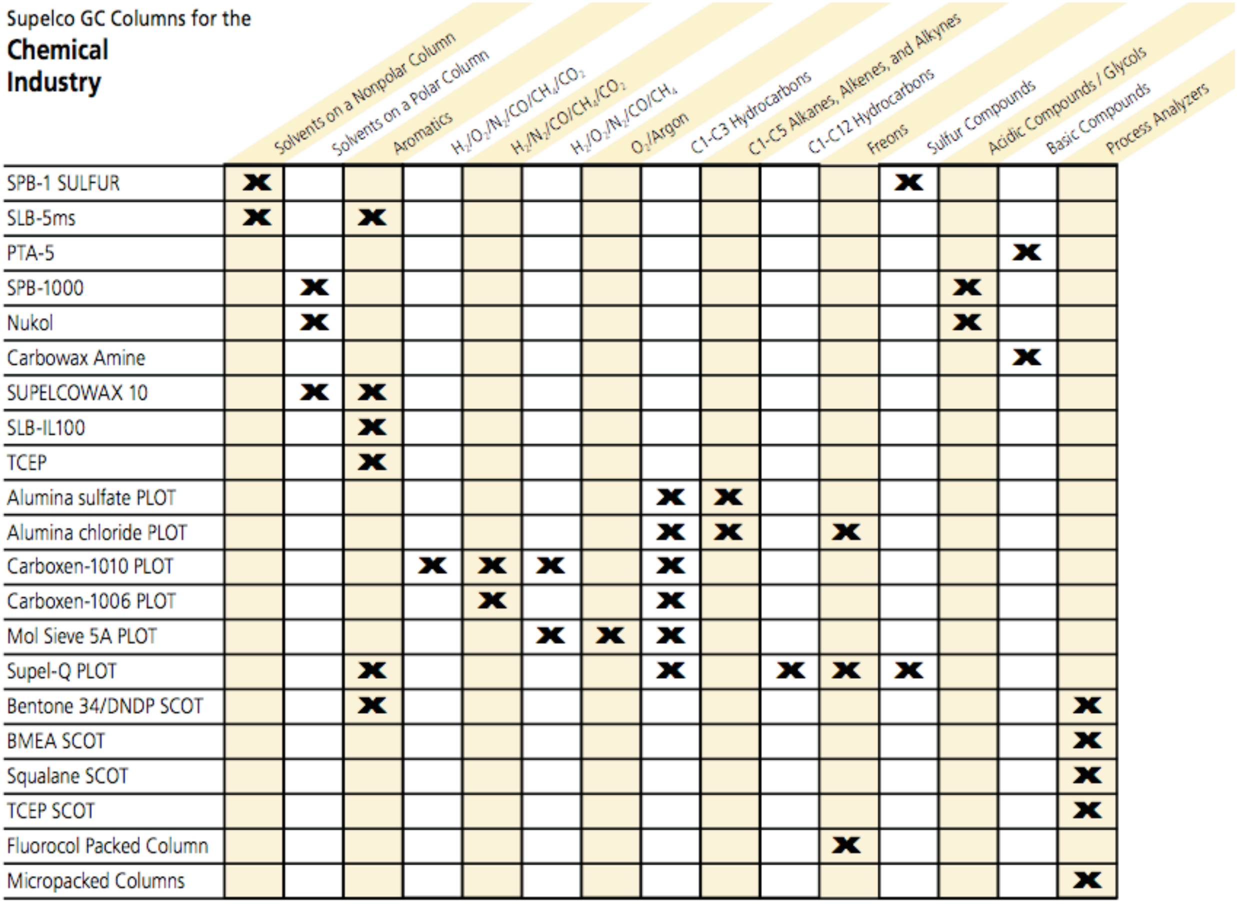
How to choose a column?
- length
- inner diammeter
- chemistry of the column (stationary phase)

Phase Polarity

This is the single most important characteristic in selecting a capillary column because it dictates selectivity, or the ability of the column to separate sample components.

Phase selection is based on the general chemical principle that “likes dissolves like”. A non-polar column is best for the analyses of non-polar compounds. Polar columns most effectively separate polar compounds.

(Example of a column selection catalogue)

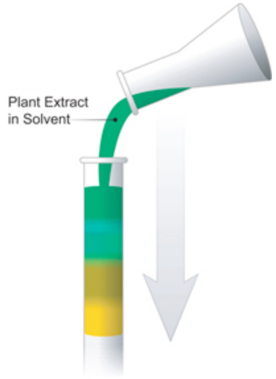


Supelco GC Columns for the **Chemical Industry**

	Solvents on a Nonpolar Column	Solvents on a Polar Column	Aromatics	$H_2/O_2/N_2/CO/CH_4/CO_2$	$H_2/N_2/CO/CH_4/CO_2$	$H_2/O_2/N_2/CO/CH_4$	O_2/Argon	C1-C3 Hydrocarbons	C1-C5 Alkanes, Alkenes, and Alkynes	C1-C12 Hydrocarbons	Freons	Sulfur Compounds	Acidic Compounds / Glycols	Basic Compounds	Process Analyzers
SPB-1 SULFUR	X											X			
SLB-5ms	X		X												
PTA-5														X	
SPB-1000		X											X		
Nukol		X											X		
Carbowax Amine														X	
SUPELCOWAX 10		X	X												
SLB-IL100			X												
TCEP			X												
Alumina sulfate PLOT								X	X						
Alumina chloride PLOT								X	X		X				
Carboxen-1010 PLOT				X	X	X		X							
Carboxen-1006 PLOT					X			X							
Mol Sieve 5A PLOT						X	X	X							
Supel-Q PLOT			X					X		X	X	X			
Bentone 34/DNDP SCOT			X												X
BMEA SCOT															X
Squalane SCOT															X
TCEP SCOT															X
Fluorocol Packed Column											X				
Micropacked Columns															X

Liquid Chromatography

~ performing a series of liquid-liquid extraction

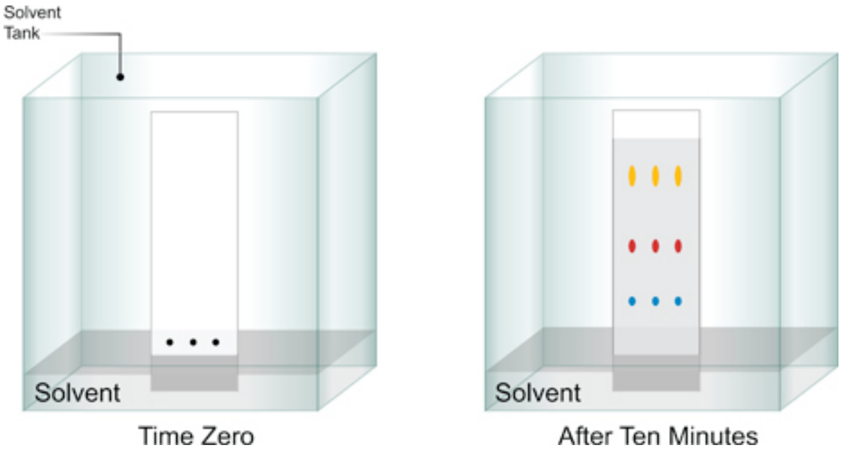


- discovered in early 1900s by Russian botanist, Tswett. He poured extract of plant leaves down a column of powdered chalk, and observed a separation of plant pigments.

All chromatographic techniques flow the mixture, that is to be separated, through a material that retains some components more than others

Thin-layer chromatography is an example of liquid chromatography

TLC is a simple, quick, and inexpensive procedure that gives a quick answer as to how many components are in a mixture.



***Stationary phase*: surface of TLC plate**

- A sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent; usually silica gel (SiO_2) or Alumina (Al_2O_3)

***Mobile phase*: solvent**

- The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column.

- Polar solvents can more effectively compete with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also better solvate the polar constituents.

- Proper choice of an eluting solvent is crucial to the successful application of column chromatography as a separation technique:
  A highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will result. If a solvent is not polar enough, no compounds will elute from the column.

Separation:

- As the solvent moves past the spot that was applied, an equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution.

- Compounds interact with the silica or alumina largely due to polar interactions.

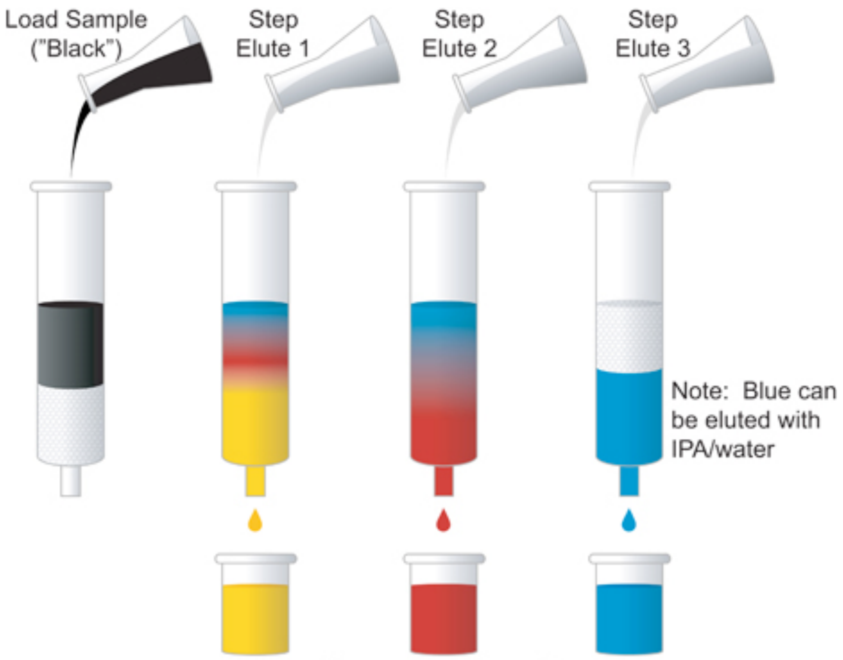
- To achieve separation, the components should differ in solubility (determined by mobile phase) and/or in the strength of their adsorption to the adsorbent (determined by stationary phase).

Visualizing the Spots

- If the compounds are colored, they are easy to see with the naked eye. If not, a UV lamp is used

Column Liquid Chromatography

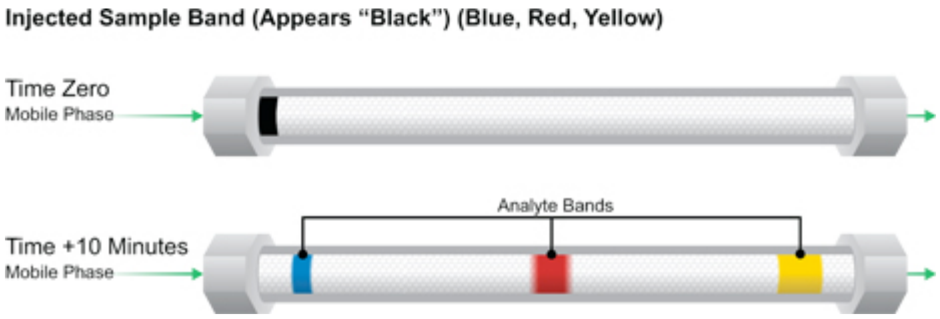
The sample passes through a column containing chromatographic packing material particles [stationary phase]. Solvent [mobile phase] flows through the column. The sample is loaded onto the head of the column and the solvent stream carries the sample through the column. As in Tswett's experiment, the compounds in the sample are then separated by traveling at different individual speeds.



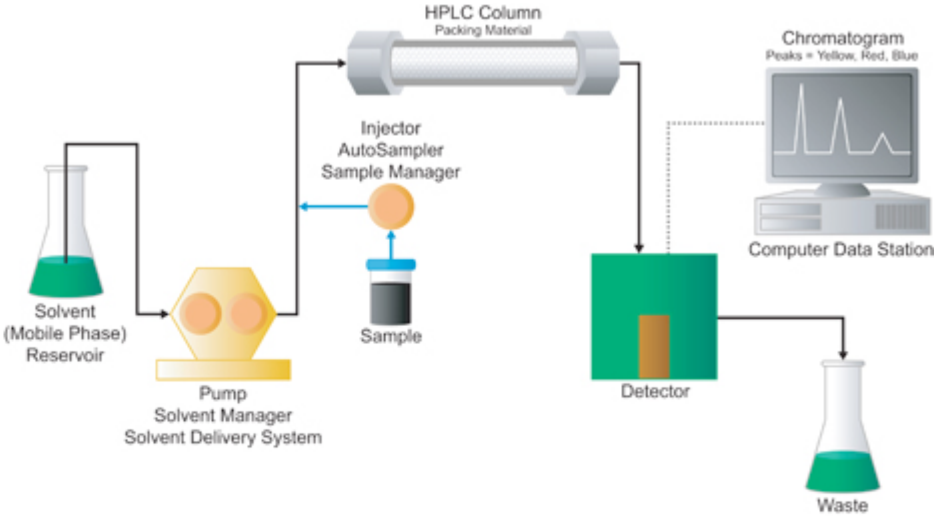
One cartridge can separate all three dyes

High Performance Liquid Chromatography (HPLC)

High pressure solvent (mobile phase) delivery system and allows the use of more <u>uniform</u>, tighter stationary phase packing in column - minimized band broadening.



HPLC Instrument:



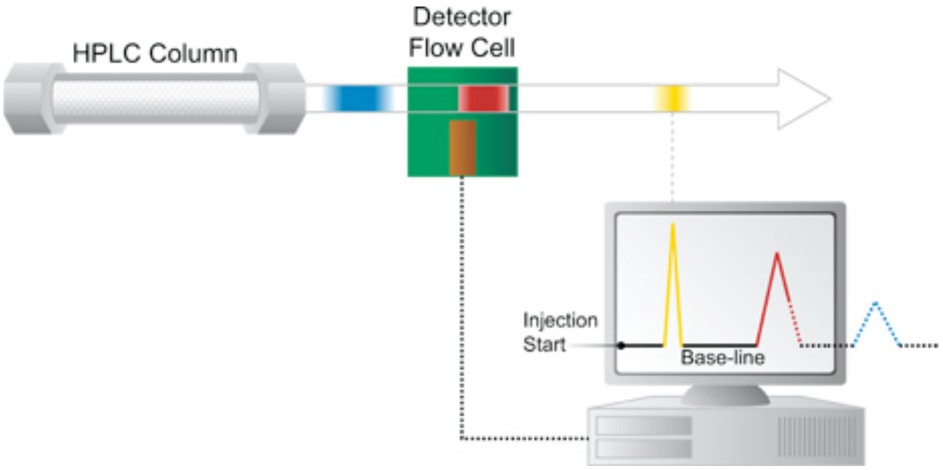
Pump - pump mobile phase at high pressure, allows a mixture of solvents at variable ratio to be delivered

Injector - introduction of sample at minimum disruption to the continuous solvent flow

Column - facilitates separation

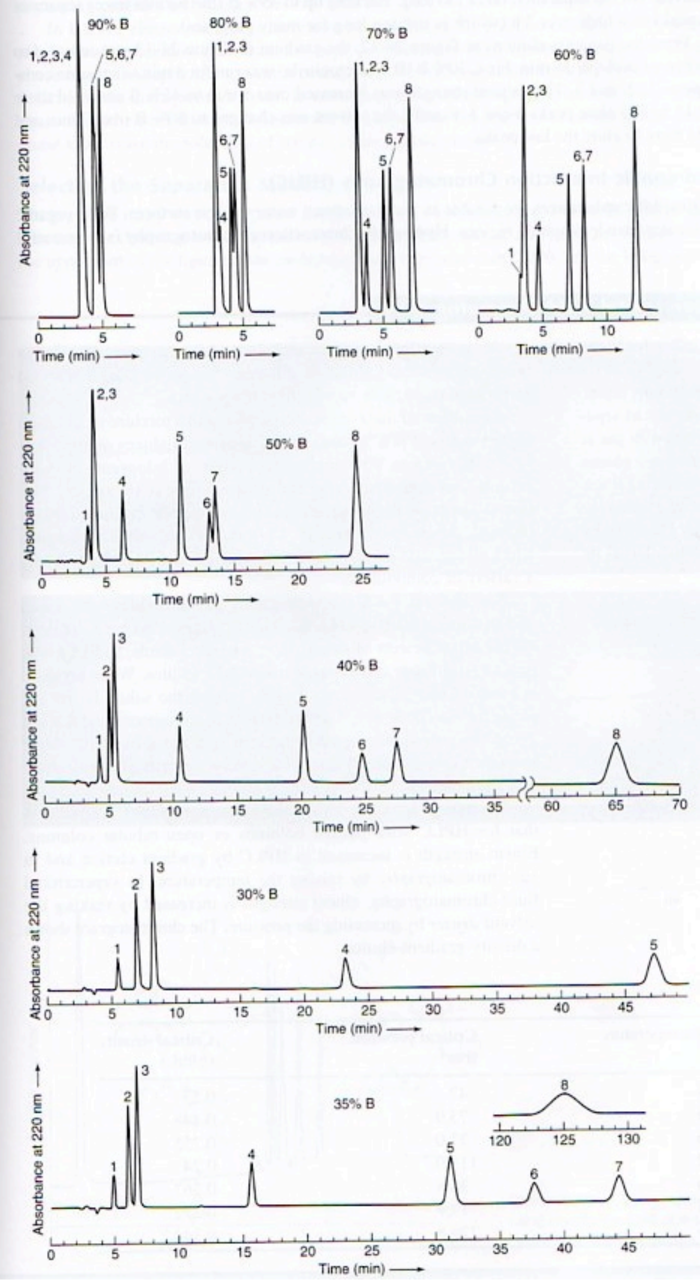
Detector - quantify (peak height) and/or identify (absorption profile) the separated components

All components of the sample should eventually be eluted off the column and detected by an online detector:



Retention is significantly determined by the mobile phase:

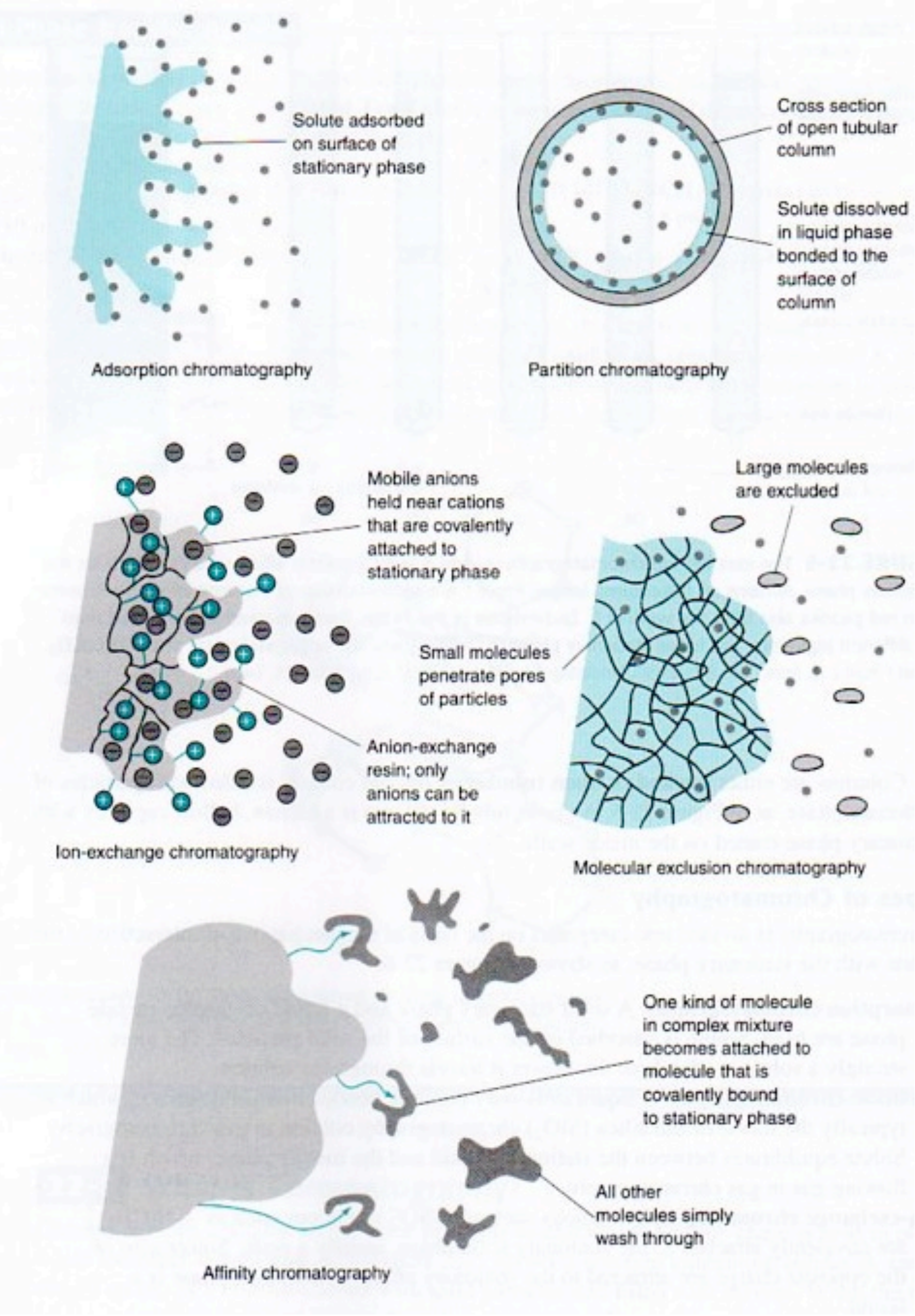
10% water, 90% acetonitrile
stronger eluent



65% water, 35% acetonitrile
weaker eluent

Instead of a change in temperature (as in GC), the mobile phase composition is varied during gradient elution in LC.

LC Stationary Phases / Separation Mode



Adsorption chromatography

Partition chromatography

Ion-exchange chromatography

Molecular exclusion chromatography

Affinity chromatography