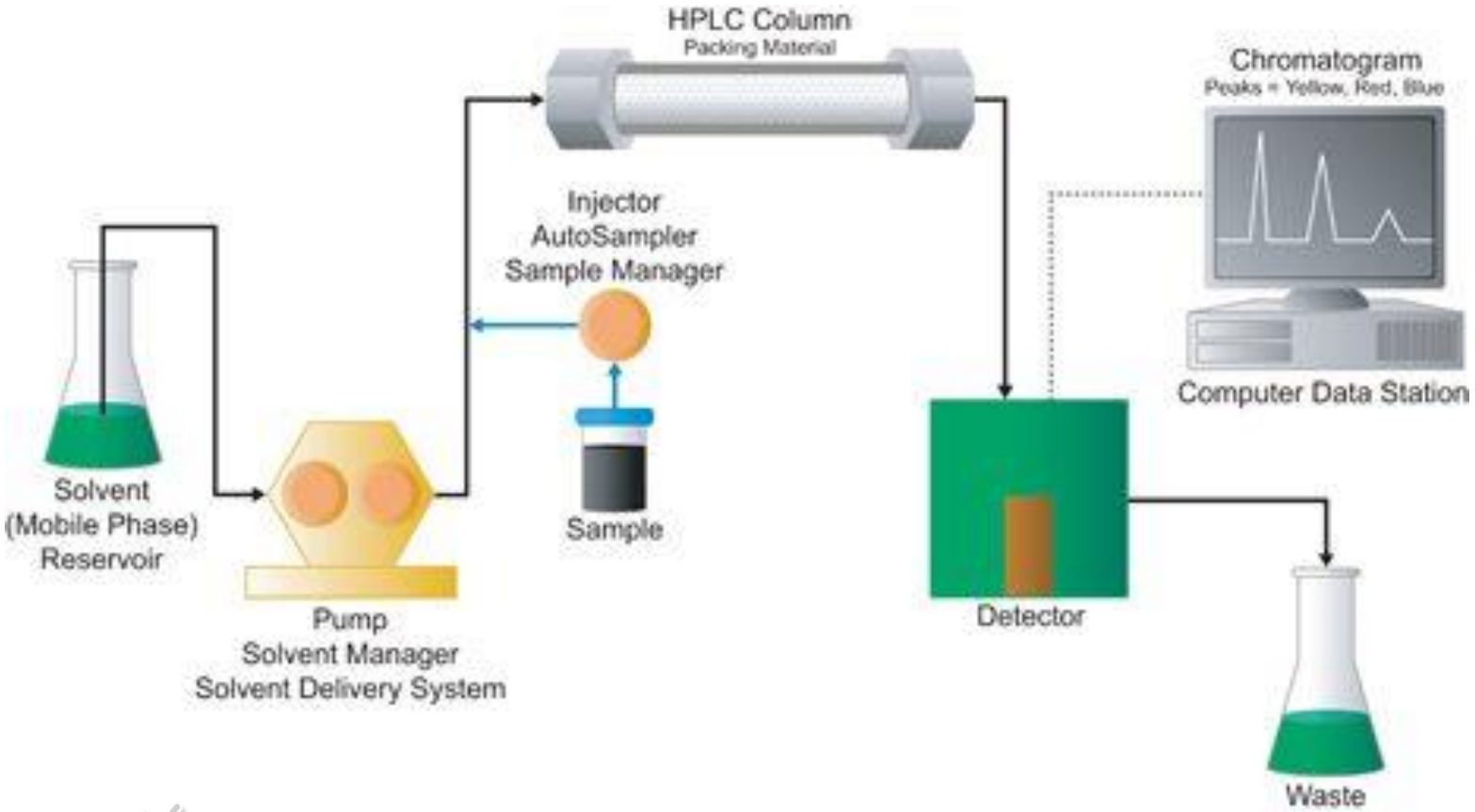
ADVANCES IN FOOD ANALYSIS

LIQUID CHROMATOGRAPHY (LC)

Marco Beccaria, PhD

University of Liege, Belgium
email: mbeccaria@uliege.be

LC Instrument basic schema

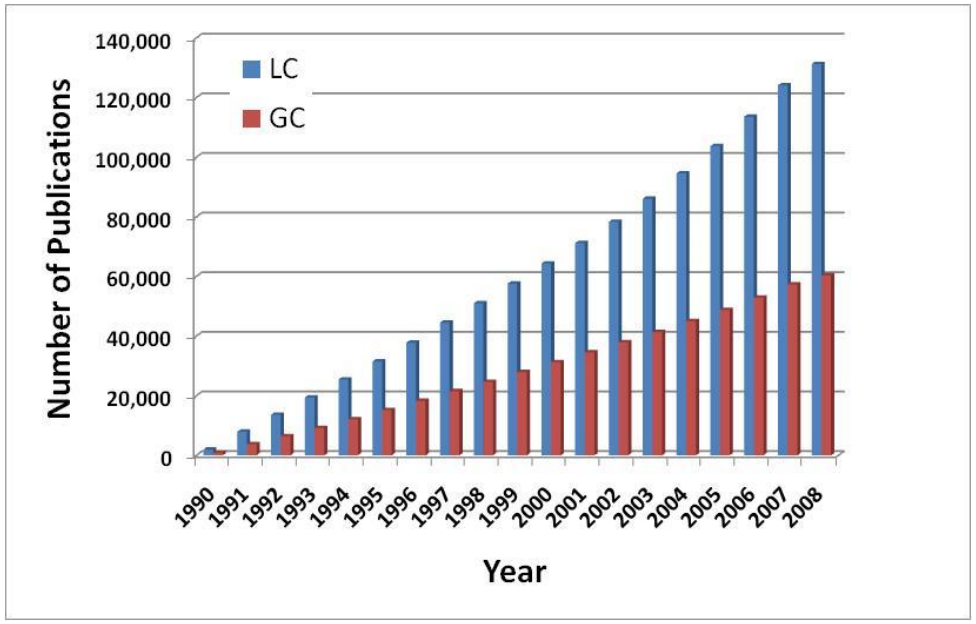


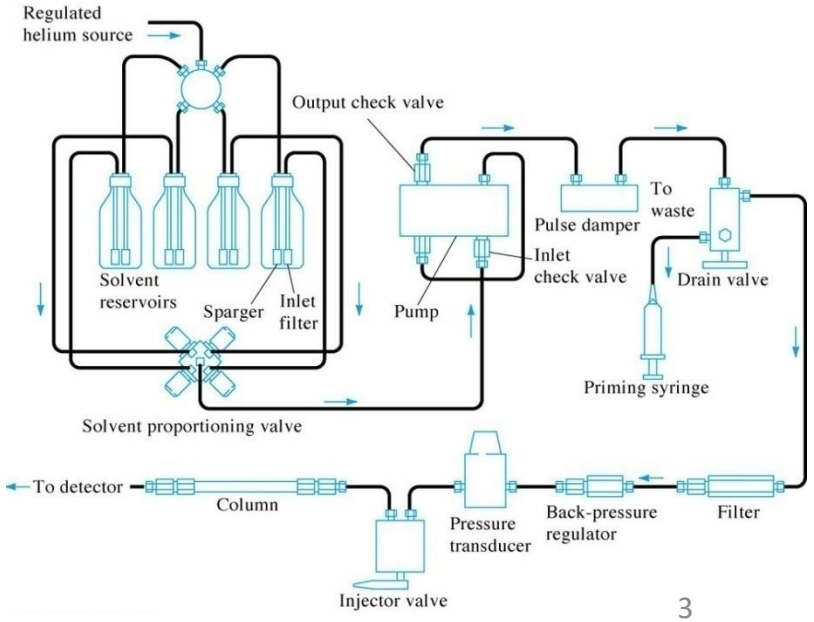
Liquid Chromatography

Liquid Chromatography (LC) is a chromatographic technique in which the mobile phase is a liquid.

LC is a much older technique than GC, but was overshadowed by the rapid development of GC in the 1950's and 1960's.

LC is currently the dominate type of chromatography and is even replacing GC in its more traditional applications.





Advantages of LC compared to GC:

1.) LC can be applied to the separation of any compound that is soluble in a liquid phase.

- ② LC more useful in the separation of biological compounds, synthetic or natural polymers, and inorganic compounds

2.) Liquid mobile phase allows LC to be used at lower temperatures than required by GC

- ② LC better suited than GC for separating compounds that may be thermally labile

3.) Retention of solutes in LC depend on their interaction with both the mobile phase and stationary phase.

- ② GC retention based on volatility and interaction with stationary phase
- ② LC is more flexible in optimizing separations → change either stationary or mobile phase

4.) Most LC detectors are non-destructive

- ② most GC detectors are destructive
- ② LC is better suited for preparative or process-scale separations

Disadvantage of LC compared to GC:

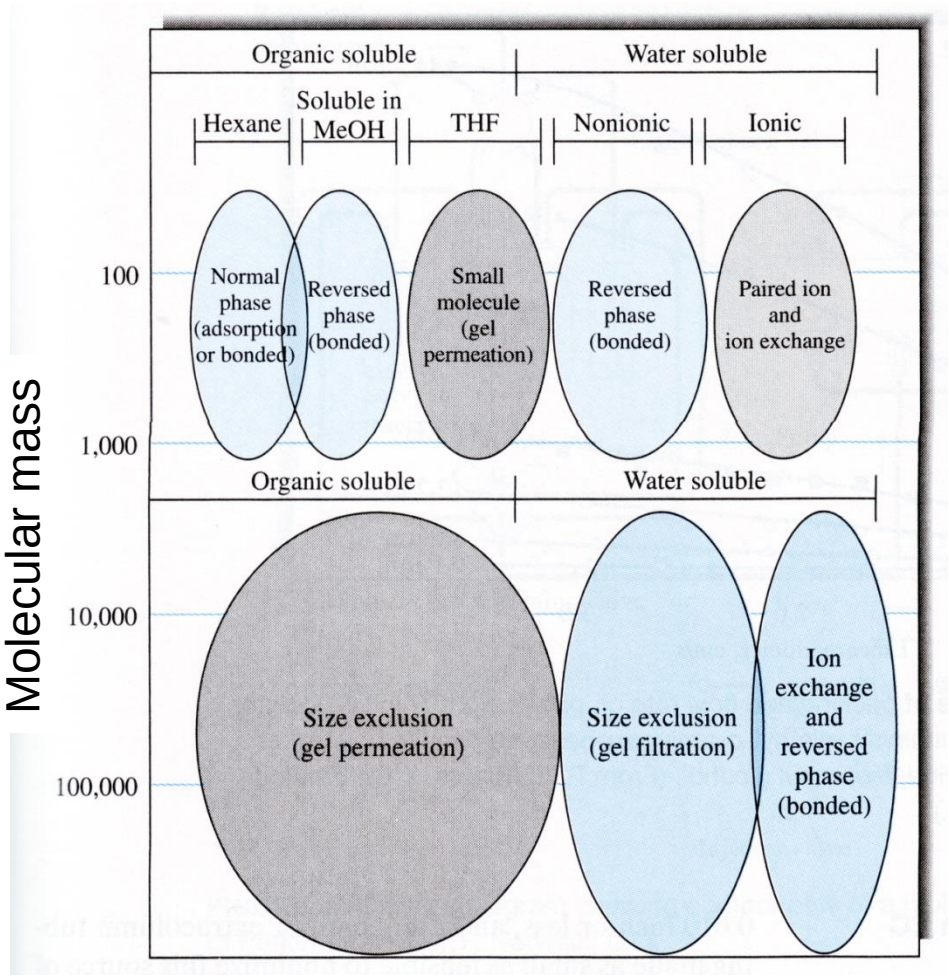
1.) LC is subject to greater peak or band-broadening.

- ② much larger diffusion coefficients of solutes in gases vs. liquids

B.) Low- and High- and Ultra performance Liquid Chromatography:

Many types of liquid chromatography are available, based on different stationary phase and mobile phase combinations.

- each type may be further characterized based on its overall efficiency or *performance*



Low-performance liquid chromatography

– LC methods that use large, non-rigid support material

② particles > 40 μm in diameter

– poor system efficiencies and large plate heights

– such systems have the following characteristics:

② broad peaks

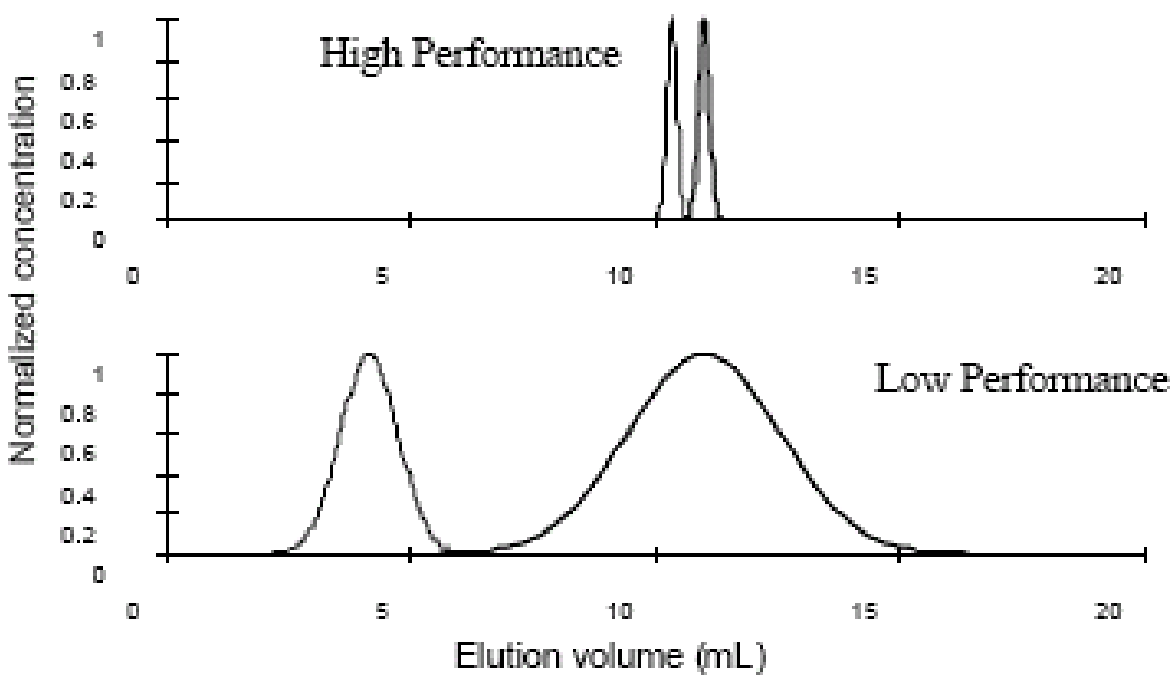
② poor limits of detection

② long separation times

② columns can only tolerate low operating pressures

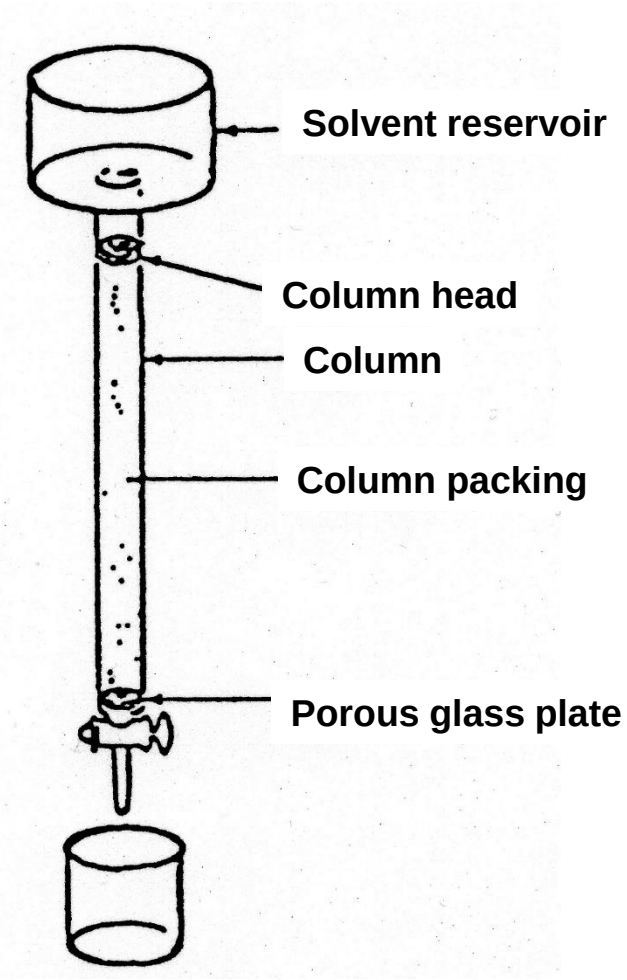
Particle size of LPLC columns is >> 5 μm

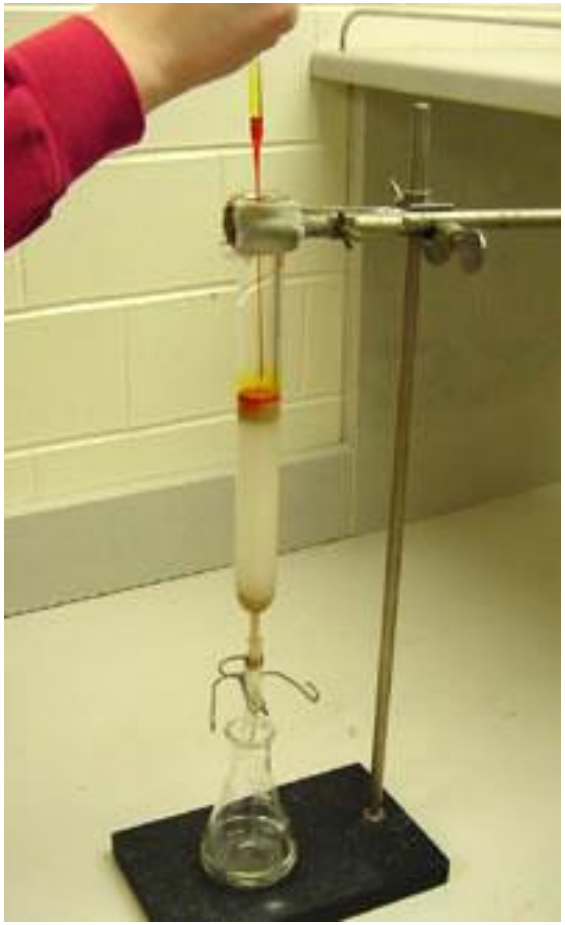
gravity flow or peristaltic pump to apply mobile phase to column



Mainly used in Preparative analysis

Column chromatography – an example of the equipment used in low-performance liquid chromatography





② Sample is usually applied directly to the top of the column.

② Detection is by fraction collection with later analysis of each fraction

advantages:

– simple system requirements

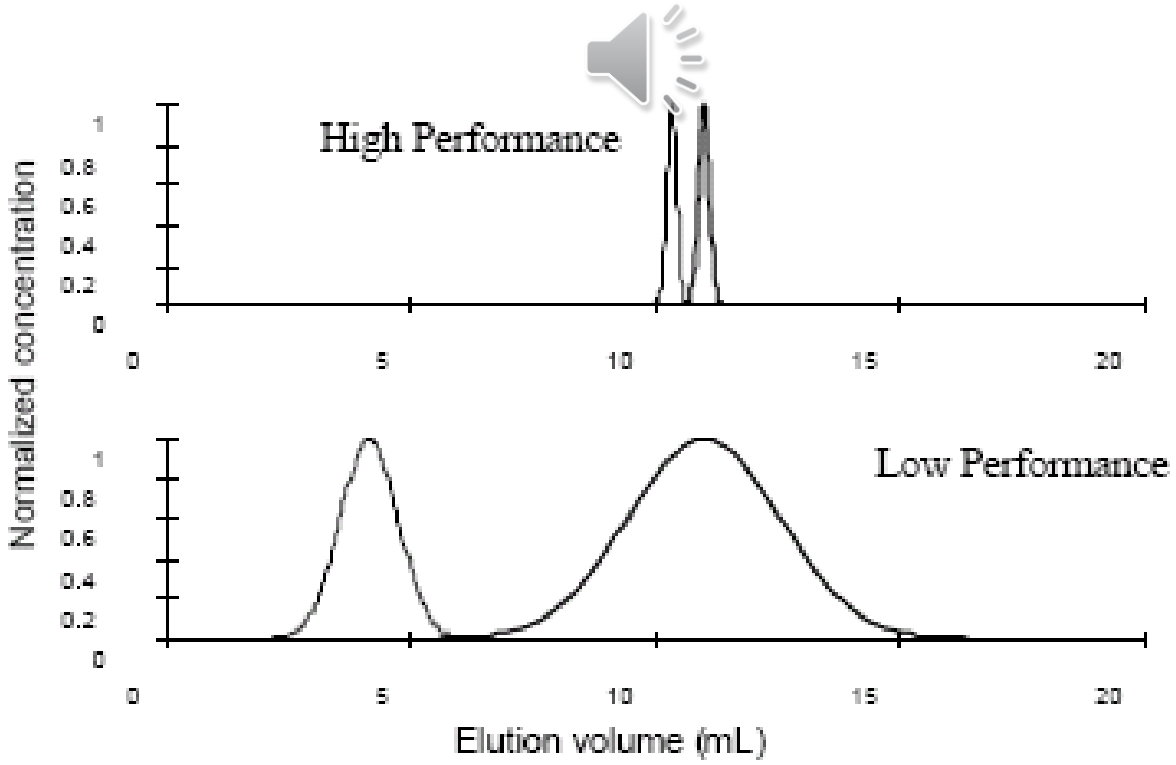
– low cost

– popular in sample purification

– used in the removal of interferences from samples

– used in some analytical applications

② not common due to low efficiency, long analysis times and poor limits of detection



High-performance liquid chromatography (HPLC)

– LC methods that use small, uniform, rigid support material

② particles < 40 μm in diameter

② usually 3-10 μm in practice

– good system efficiencies and small plate heights

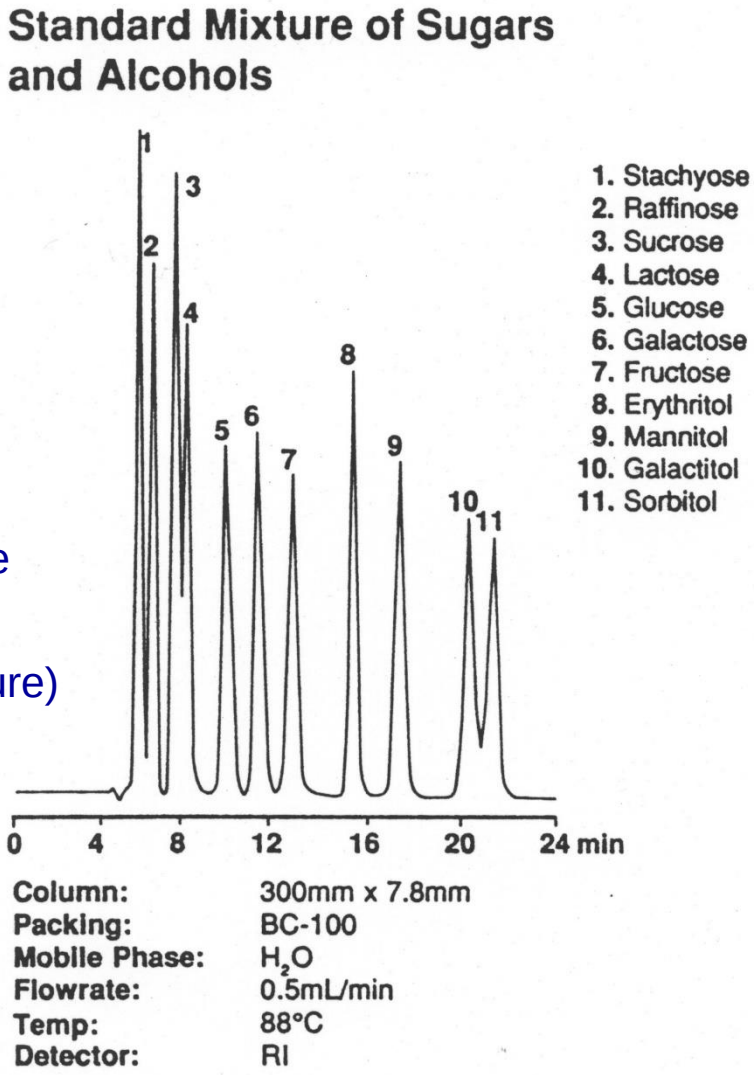
– such systems have the following characteristics:

②narrow peaks

②low limits of detection

②short separation times

②columns and HPLC systems can tolerate high operating pressures and faster flow-rates (up to 440 bar as back-pressure)



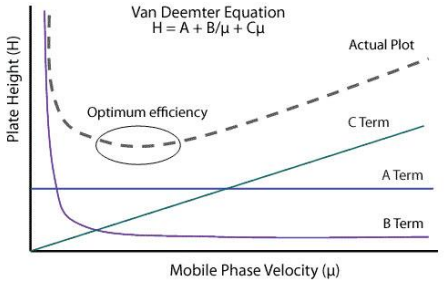
Particle size of HPLC column is in the range from 2 μm up to 5 μm

Ultra-performance liquid chromatography (UPLC or UHPLC)

– LC methods that use very small, uniform, rigid support material

– very good system efficiencies and much smaller plate heights compare to HPLC

– such systems have the following characteristics:



Particle size of UPLC columns is < 2 μm

② narrower peaks (extremely efficient)

② low limits of detection

②very short separation times

②columns can handle ultra operating pressures (up to 1200 bar)

②reducing use of solvents

②very faster flow-rates without loosing efficiency (see Van Demeter equation/graph)

– Disadvantage of UHPLC system

② costs extremely higher

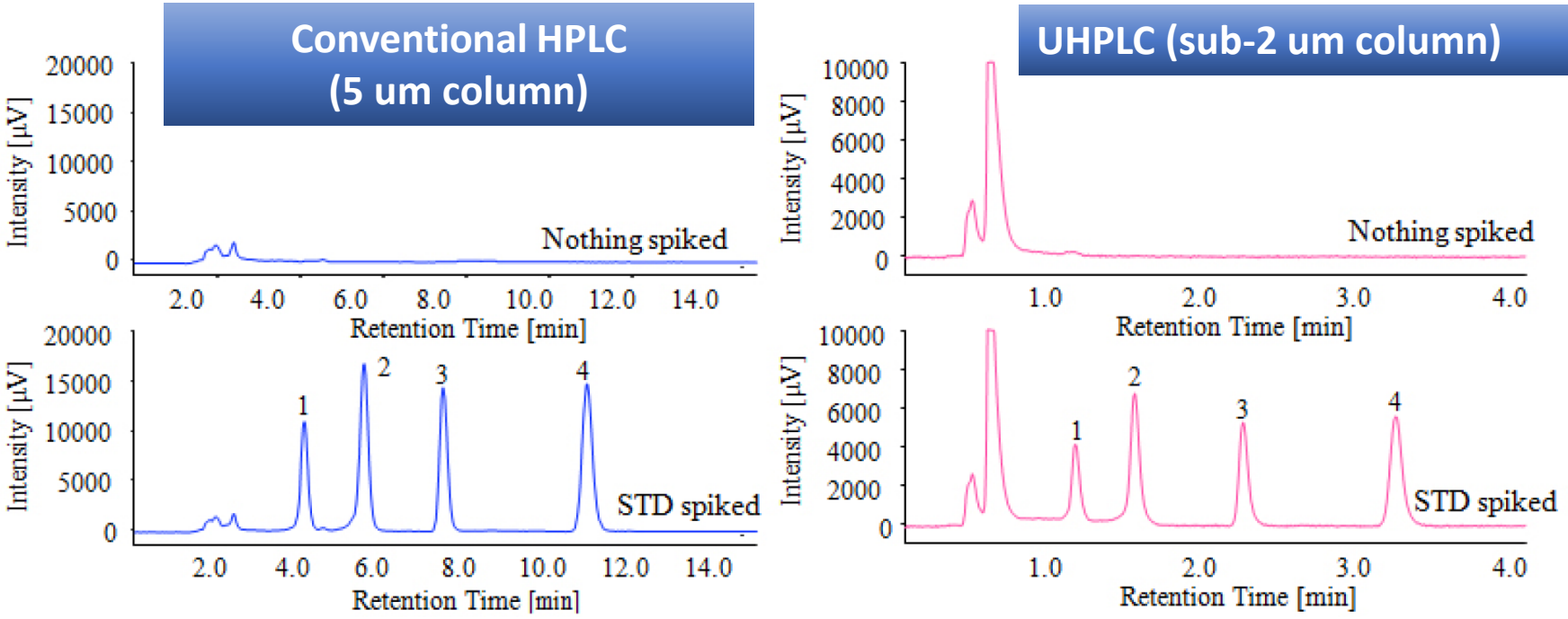
② less sample capacity compared to HPLC columns

② much higher value of back-pressure compared to HPLC columns

② less resistance in the mechanical part and columns due to the high back-pressure value

Ultra-performance liquid chromatography (UPLC or UHPLC)

Aflatoxin Analysis in Food by HPLC-UV and UHPLC-UV

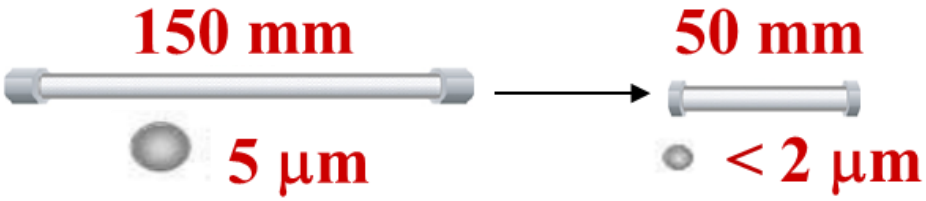


UHPLC analysis > 3 times faster

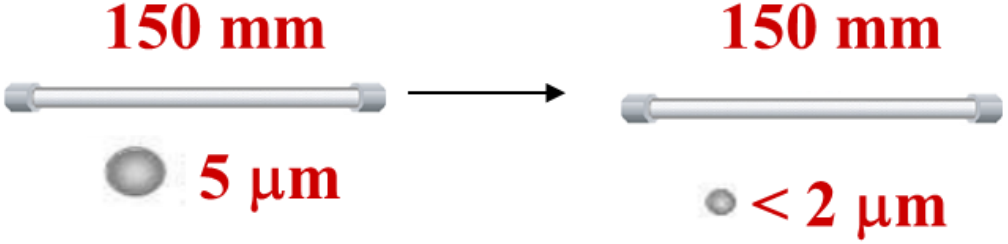
1 = Aflatoxin G1
2 = Aflatoxin B1
3 = Aflatoxin G2
4 = Aflatoxin B2

Method transferring from conventional HPLC to UHPLC

Possible Approaches

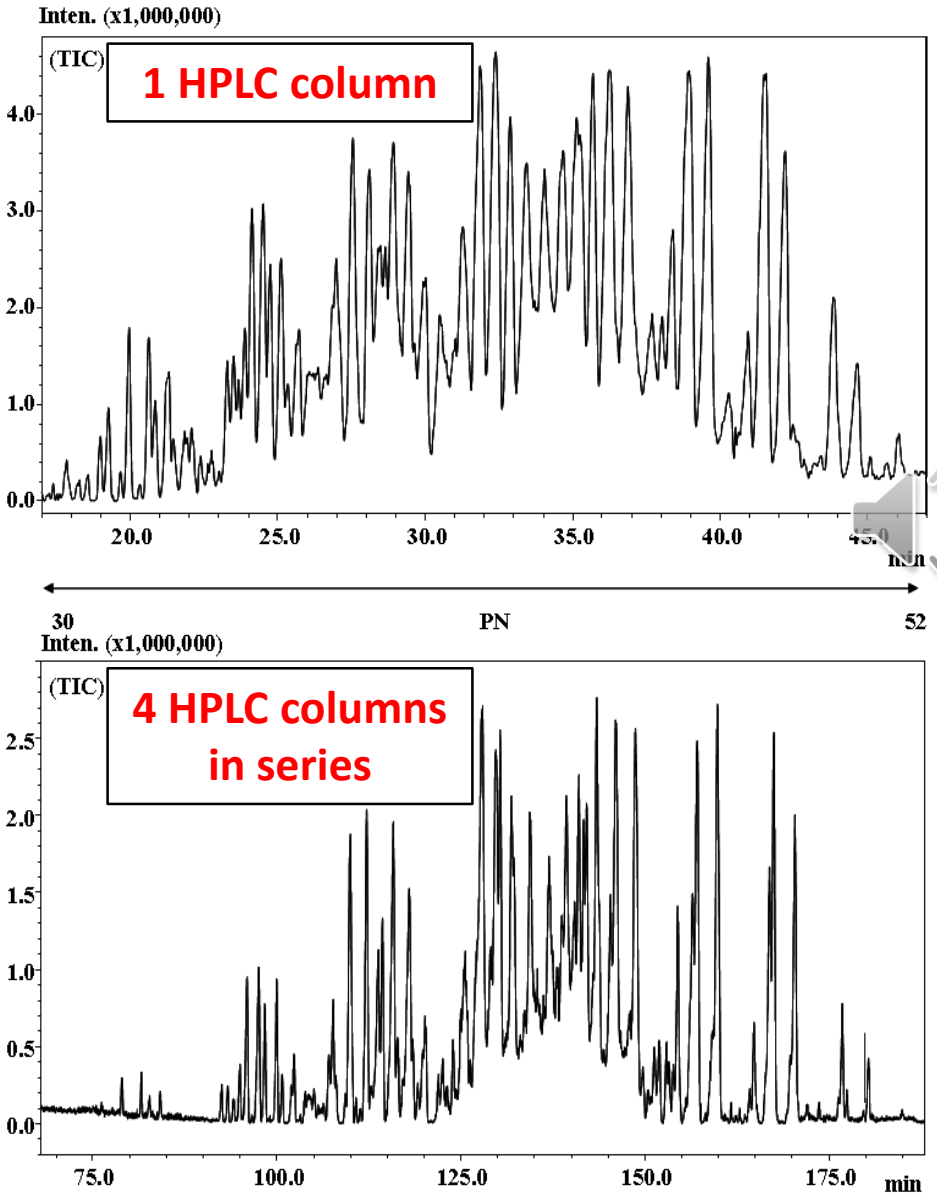


Maintain N constant – Decrease t_R up to 9 fold



Maintain t_R constant – Increase N up to 9 fold

Use of HPLC columns with sub-3 in an UHPLC system to increase the efficiency (N) of the LC method

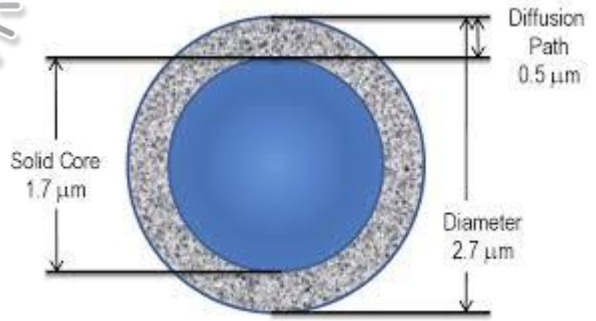


Analysis of a fish oil triacylglycerol fraction by (U)HPLC-APCI(+)-MS

Dugo P & Beccaria M, Journal Chromatogr. A, 2012

P = 225 bar

150 mm L × 4.6 i.d. × 2.7 um *d.p.*

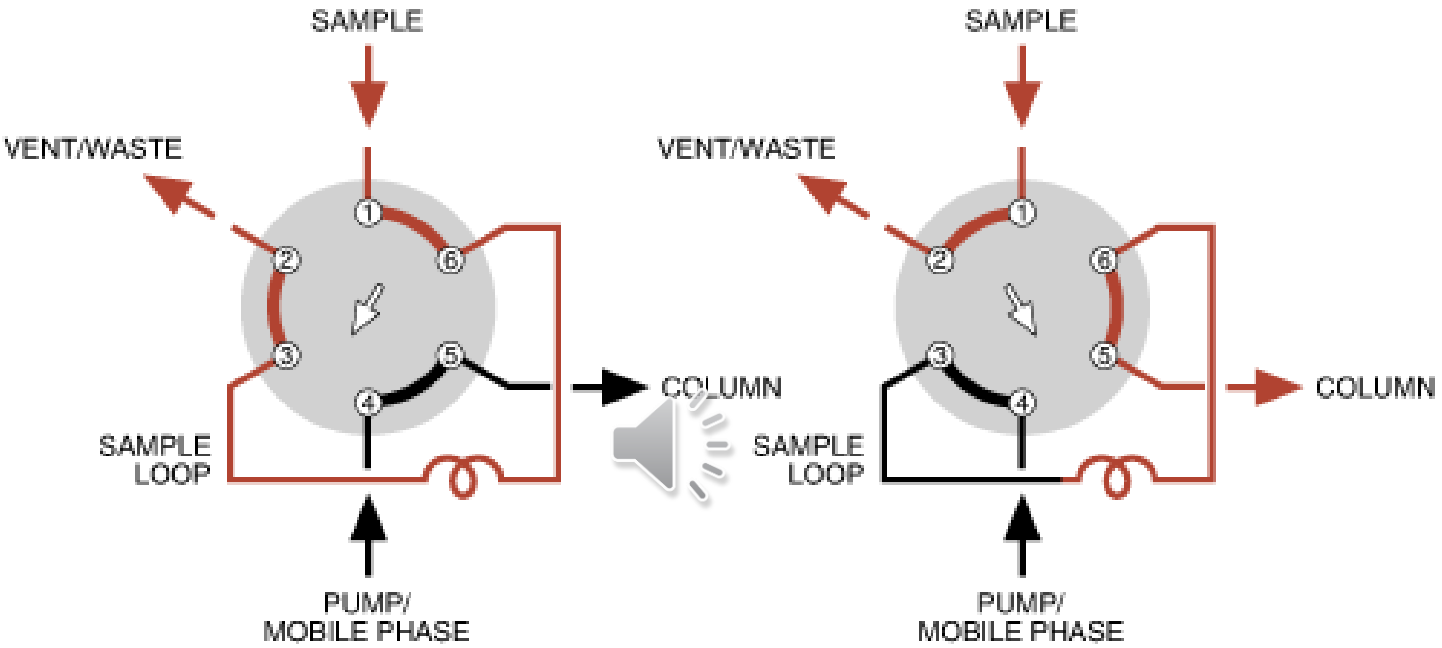


P = 900 bar

UHPLC condition with HPLC columns

600 mm L × 4.6 i.d. × 2.7 um *d.p.*

Injection system in LC



https://www.chromacademy.com/frameset-chromacademy.html?fChannel=0&fCourse=1&fSco=33&fPath=sco33/hplc_3_3_2.asp

Reservoir in LC

Mobile phase reservoir will carry the mobile phase solution and through the pump it will be pumped into the column.
This movement is according to the **gravitational force**.
The mobile phase reservoir is a solvent with different polarities such as water, methanol, and acetonitrile.

These solvents should be highly pure

Degas is done to minimize errors which occur when compounds interact with several gases. If degas or the removal of all the gases, is not done correctly then it may spoil the column.

Watch out for temperature!

Guideline for the optimal flow in HPLC

The flow **(mL/min)** is strictly related to the geometry of the column and the van Deemter curve.

d_c	**150 x 4.6 mm, 5 µm**	**1 mL/min**
	150 x 3 mm, 5 µm	**0.4 mL/min**
	150 x 2.1 mm, 5 µm	**0.2 mL/min**
d_p	**150 x 4.6 mm, 3 µm**	**1.6 mL/min**
	150 x 3 mm, 3 µm	**0.7 mL/min**
	150 x 2.1 mm, 3 µm	**0.3 mL/min**
L	**50 x 4.6 mm, 5 µm**	**1 mL/min**
	100 x 4.6 mm, 5 µm	**1 mL/min**

Elution:

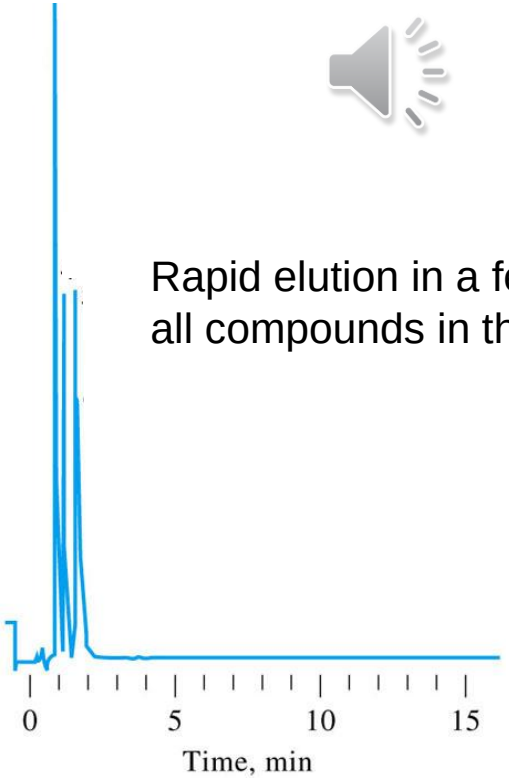
Retention and elution of solutes in LC depends on the interactions of solutes with <u>both</u> the mobile and stationary phases.

- to describe how well solutes are retained on a column with different solvents, the terms *weak mobile phase* and *strong mobile phase* are used.

<u>Strong mobile phase</u>: a solvent that quickly elutes solutes from the column *(i.e.*, small k')

This occurs if the mobile phase is very similar to the stationary phase in its intermolecular interactions with the solutes

- polar solvent would be a strong mobile phase for a column containing a polar stationary phase



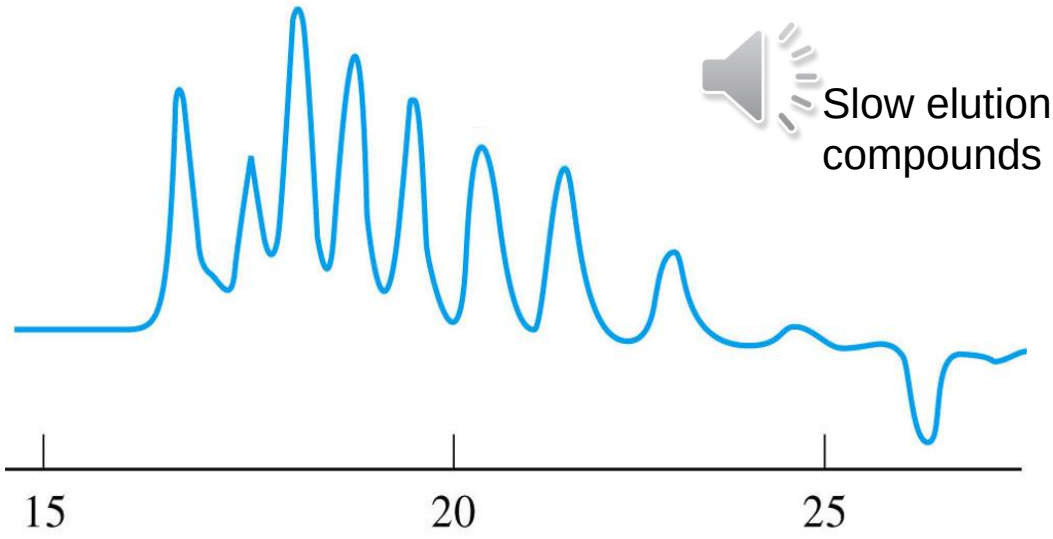
Rapid elution in a few minutes for all compounds in the mixture

Elution:

Weak mobile phase: a solvent that slowly elutes solutes from the column (*i.e.*, high solute retention or large k')

This occurs if the mobile phase is very different from the stationary phase in its intermolecular interactions with the solutes

- a non-polar solvent would be a weak mobile phase for a column containing a polar stationary phase



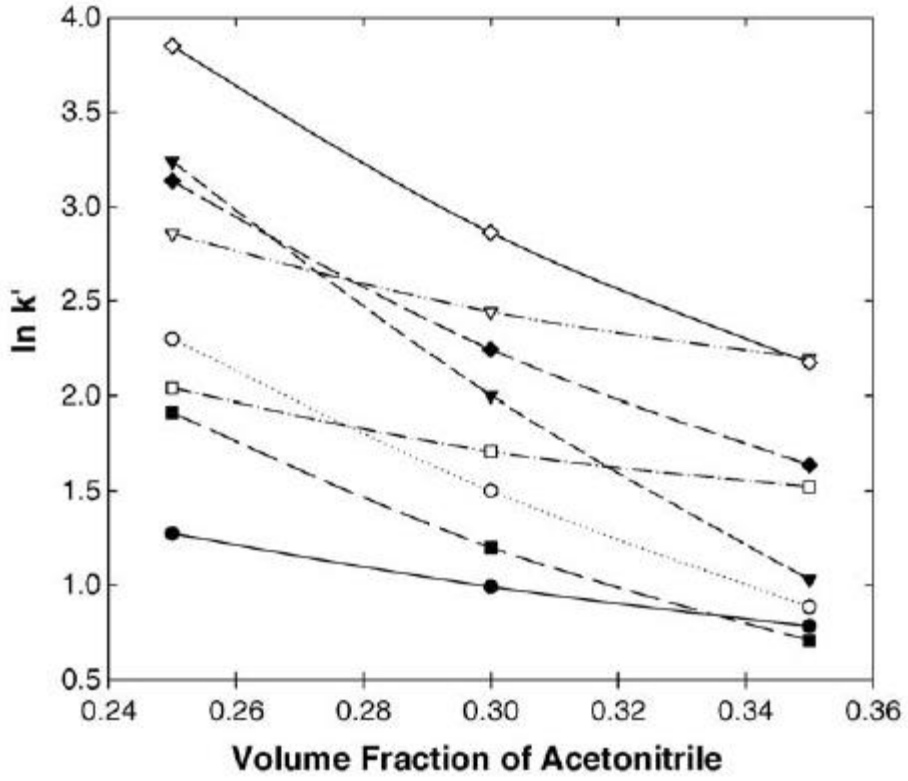
Slow elution (~ 20 minutes) for all compounds in the mixture

Note: whether a solvent is a weak or strong mobile phase depends on the stationary phase being used. Hexane is a weak mobile phase on a polar stationary phase, but a strong mobile phase on a non-polar stationary phase.

Similar to GC, solutes can be eluted from a column by using either a constant column conditions or gradient elution

Isocratic elution: use of a constant mobile phase composition to elute solutes

- ② simple, inexpensive
- ② difficult to elute all solutes with good resolution in a reasonable amount of time → general elution problem



phenol (●), alprenolol (◯), perphenazine (▼), methylbenzoate (▽), 1-(diphenylmethyl)-4-methylpiperazine (■), acetophenone (□), promethazine (♦) and amitriptyline (◊).

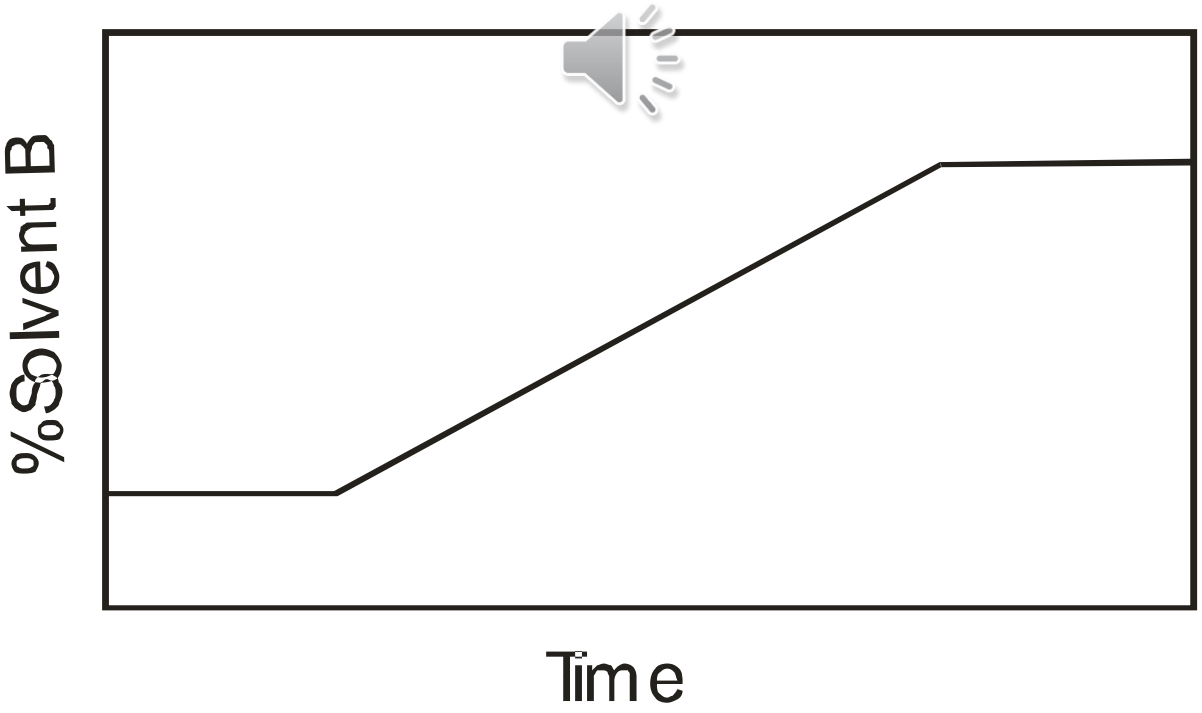
Need to identify solvent composition to obtain optimal separation

Journal of Chromatography A, 1109 (2006) 253-266

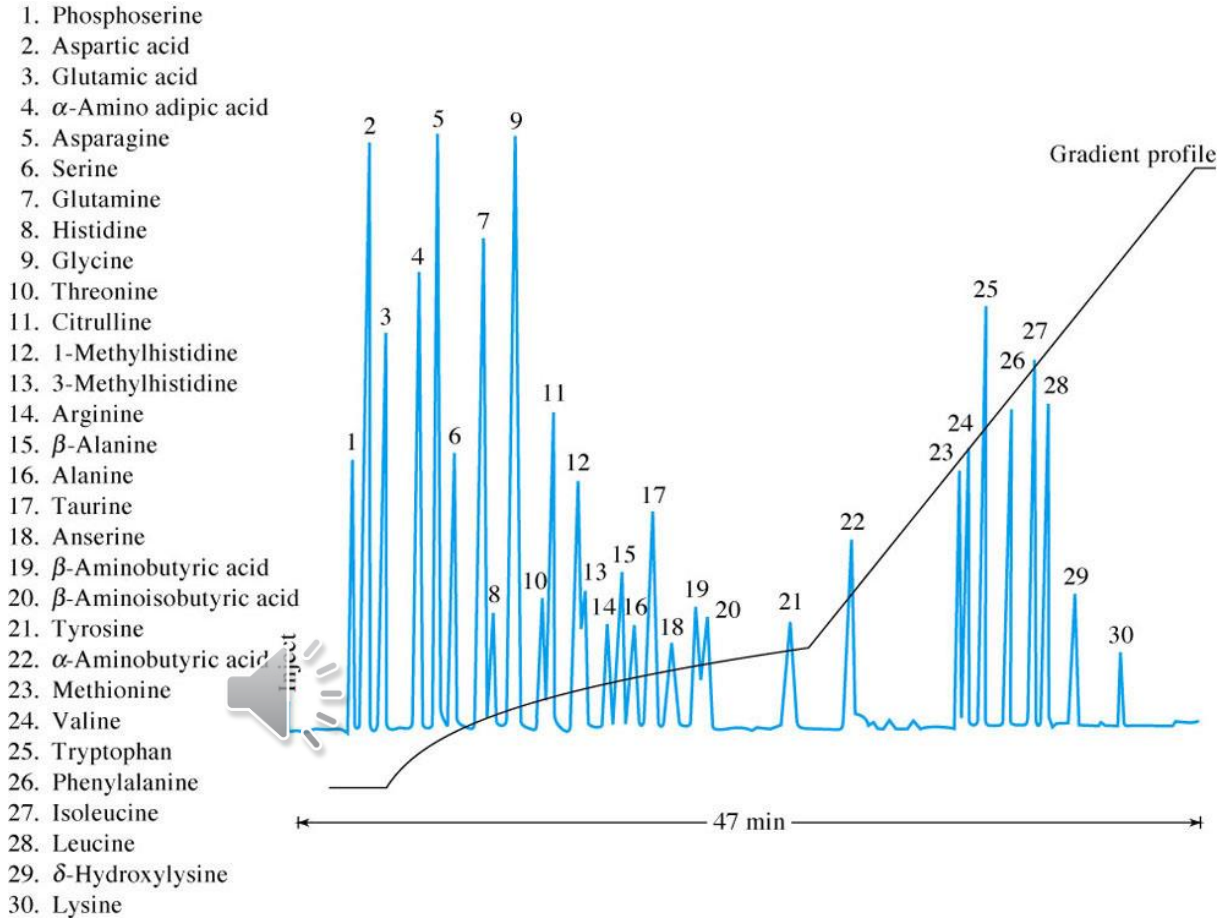
Similar to GC, solutes can be eluted from a column by using either a constant column conditions or gradient elution

Gradient elution: changing composition of mobile phase with time → *solvent programming*

- going from a weak mobile phase to a strong one.
- weak mobile phase → *solvent A*
- strong mobile phase → *solvent B*
- solvent change can be stepwise, linear or non-linear



Gradient elution of mixture of 30 amino-acids



In choosing a mobile phase for LC, several factors need to be considered

– type of stationary phase used

② determines what will be a strong or weak mobile phase

– solubility of the solutes

– viscosity of the mobile phase

– type of detector used and solvent's background signal

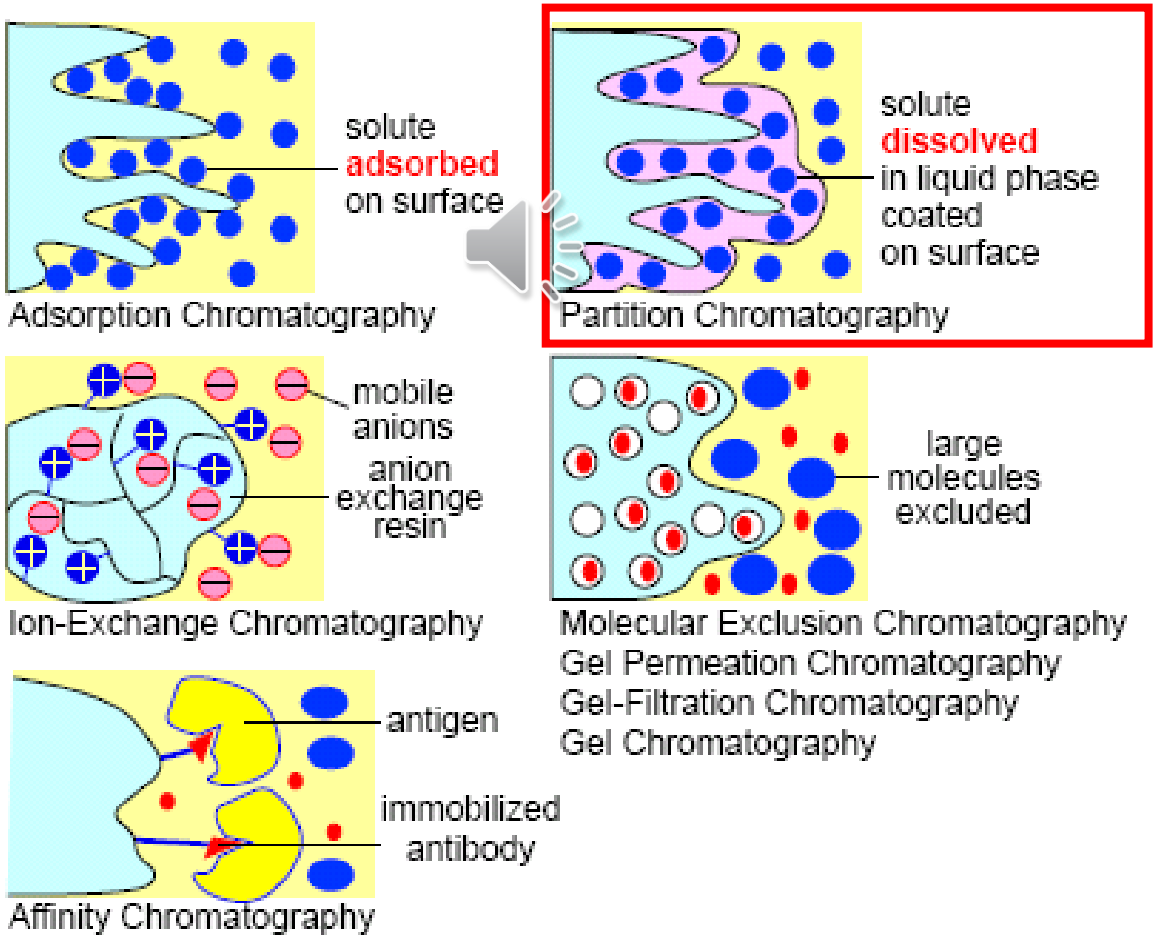
– purity of the solvents

– miscibility of the solvents (for gradient elution)

Types of Liquid Chromatography:

Techniques in LC are classified according to the method of solute separation

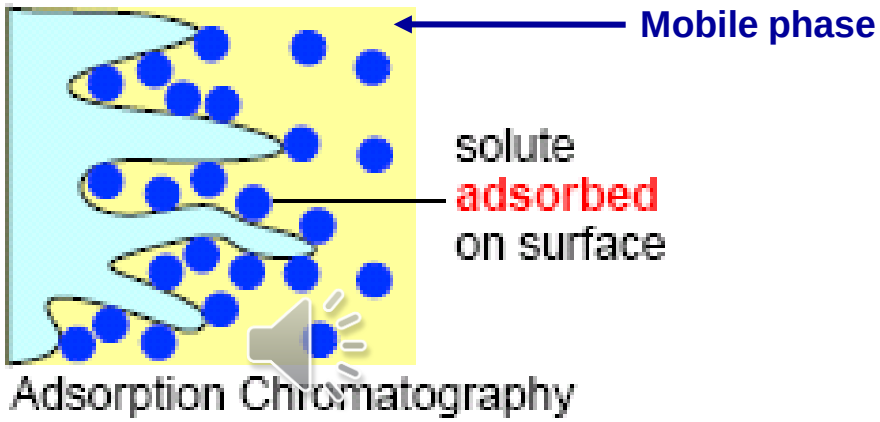
- ② Adsorption chromatography
- ② **Partition chromatography**
- ② Ion-exchange chromatography
- ② Affinity chromatography
- ② Size-exclusion chromatography



1.) Adsorption Chromatography

Separates solutes based on their adsorption to underivatized solid particles.

② similar to gas-solid chromatography in that the same material is used as both the stationary phase and support material



advantages:

– retain and separate some compounds that can not be separated by other methods

② separation of geometrical isomers

disadvantages:

– very strong retention of some solutes

– may cause catalytic changes in solutes

– solid support may have a range of chemical and physical environments → non-symmetrical peaks and variable retention times

Adsorption chromatography stationary phase (or solid support) may be either polar or non-polar

Adsorbent	Surface Type	Application
Silica	Slightly acidic	General Purpose – Basic compounds
Alumina	Slightly basic	General Purpose – Acidic Compounds
Charcoal	Non-polar	Non-polar Compounds
Florisil	Strongly acidic	General purpose – Basic Compounds
Polyamides	Basic	Phenols and Aromatic Nitro Compounds
Others (clay, Kieselguhr, diatomaceous earth, celite, etc.)	Relatively Non-polar	Polar Compounds

For polar supports (silica/alumina), the weak mobile phase is a non-polar solvent (hexane, benzene, etc.) and the strong mobile phase is a polar solvent (water, methanol, etc.)

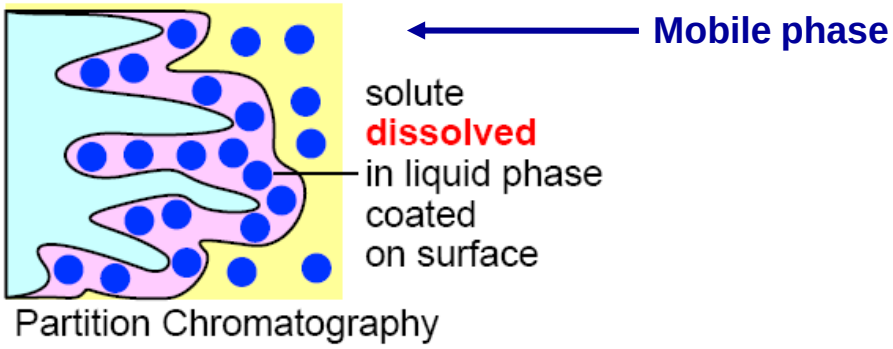
For non-polar supports (charcoal), the weak mobile phase is a polar solvent and the strong mobile phase is a non-polar solvent.

Common applications of Adsorption LC:

- purification of synthetic organic compounds from reaction mixtures
- separation of geometrical isomers (ortho/meta/para, cis/trans, etc)

2.) Partition Chromatography

Separates solutes based on their partitioning between a liquid mobile phase and a liquid stationary phase coated on a solid support.



Partition Chromatography

Support Material – is usually silica, originally involved coating this support with some liquid stationary phase that was not readily soluble in the mobile phase

Two main types of partition chromatography based on the type of stationary phase:

② normal-phase liquid chromatography (polar stationary phase)

② reversed-phase liquid chromatography (non-polar stationary phase)

	Comparison of RPLC & NPLC		
Type	**Stationary phase**	**Weak mobile phase**	**Strong Mobile phase**
RPLC	Non-polar	Polar liquid	More non-polar
NPLC	polar	Non-polar liquid	Polar liquid

Normal Phase liquid Chromatography (NP-LC).

- partition chromatography where the stationary phase is **polar**

② NPLC column strongly retains polar compounds

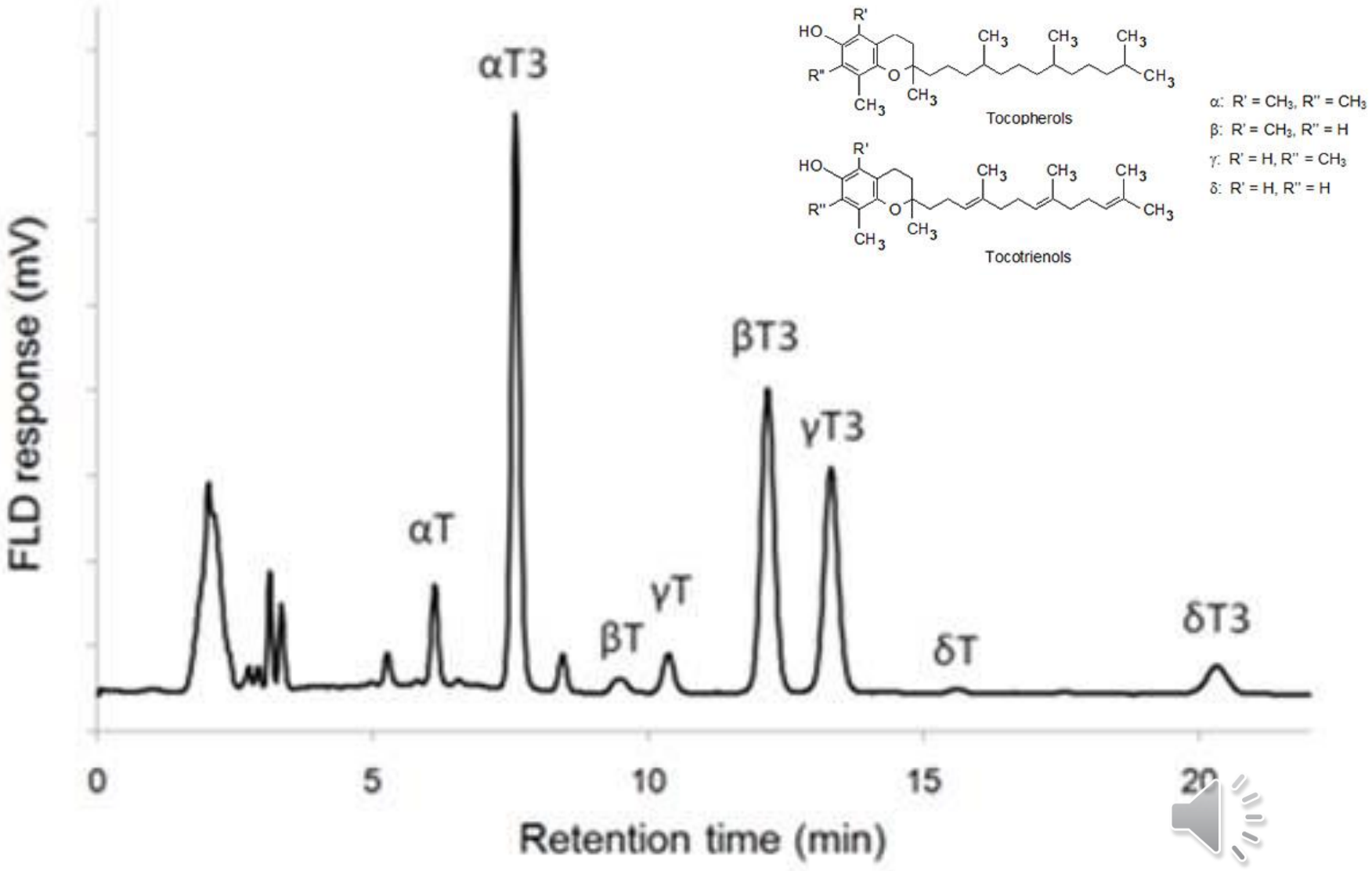
- weak mobile phase is a non-polar liquid: organic solvent
- strong mobile phase is a polar liquid: water or methanol
- stationary phase must have a low miscibility with the mobile phase so the stationary phase is not dissolved from the column

These liquid stationary phases slowly bleed from the column, changing the properties and solute retention time .

Use stationary phases chemically attached to the support

CN	Cyanopropyl	—Si—$CH_2CH_2CH_2CN$
NH2	Aminopropyl	—Si—$CH_2CH_2CH_2NH_2$
PSA	N-propylethylenediamine	—Si—$CH_2CH_2CH_2NHCH_2CH_2NH_2$

Example of NP-LC: tocopherols and tocotrienolsanalysis



NP-HPLC-FLD chromatogram of barley extract.

T: tocopherol, T3: tocotrienol (a silica column and 3% (v/v) of 1,4-dioxane in heptane as eluent)

Reverse Phase liquid Chromatography (RP-LC).

- partition chromatography where the stationary phase is non-polar

② reverse polarity of normal phase LC

② retains non-polar compounds most strongly

- weak mobile phase is a polar liquid: water

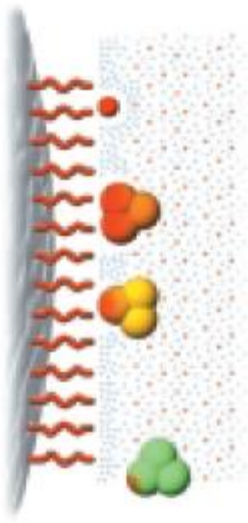
(acetonitrile in the case of Non-Aqueous (NA)RP-LC)

- strong mobile phase is more non-polar liquid: methanol or acetonitrile

- stationary phase must have a low miscibility with the mobile phase so the stationary phase is not dissolved from the column

② examples of liquid RPLC stationary phases:

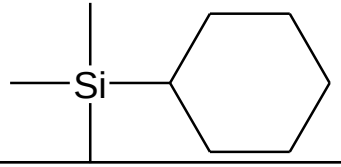
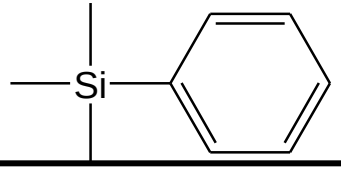
- heptane
- squalene
- hydrocarbon polymers
- dimethylpolysiloxane



Reversed phase

Like NPLC, these liquid stationary phases slowly bleed from the column, changing the properties and solute retention time.

Use stationary phases chemically attached to the support, C_8 and C_{18} are most common

C18	Octadecyl	—Si—$C_{18}H_{37}$
C8	Octyl	—Si—C_8H_{17}
C2	Ethyl	—Si—C_2H_5
CH	Cyclohexyl	—Si— 
PH	Phenyl	—Si— 

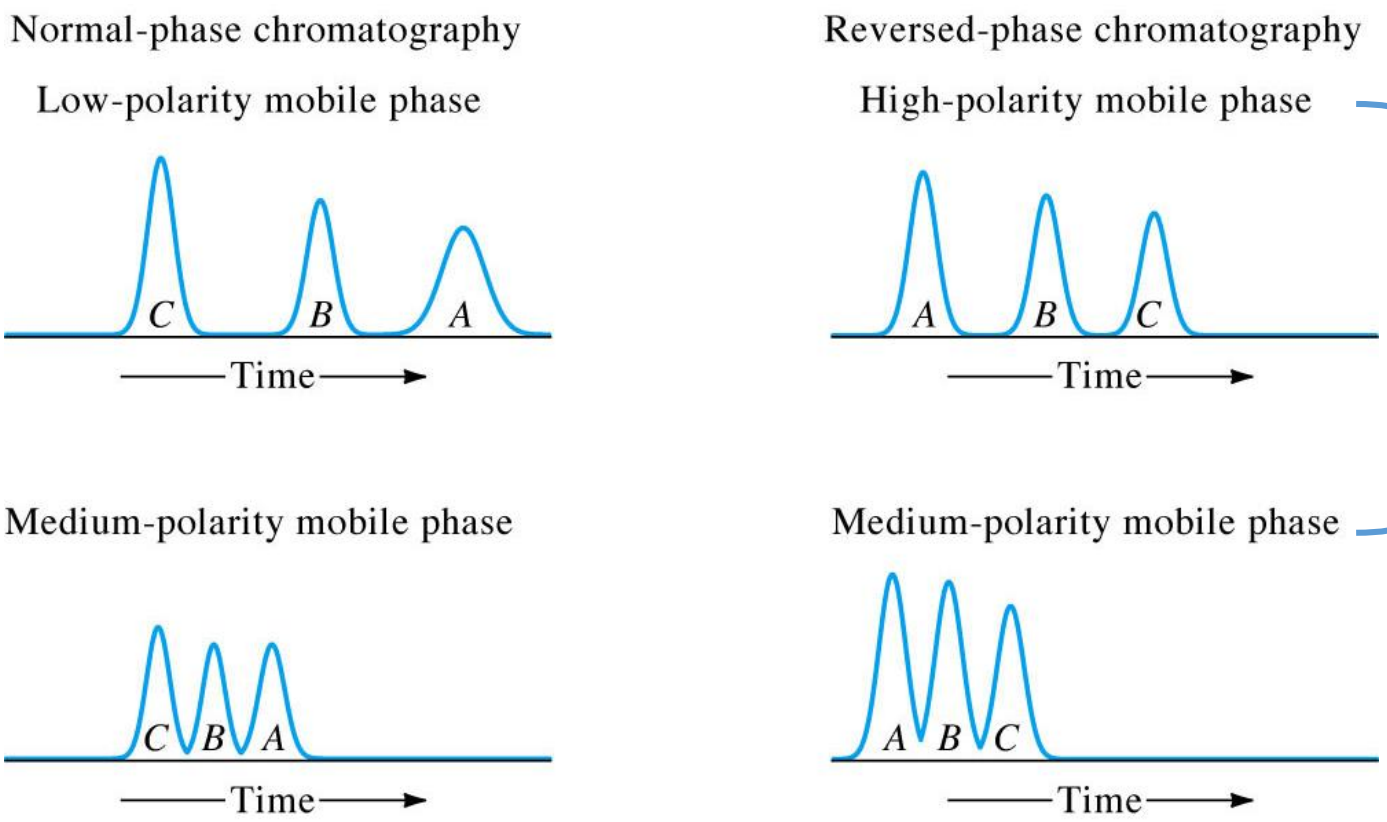
Common applications of RPLC:

- most popular type of liquid chromatography

② separation of a wide variety of non-polar **and** polar solutes

- popularity → weak mobile phase is a polar solvent (*e.g.*, water)

② ideal for the separation of solutes in aqueous-based samples, such as biological compounds



Solute polarities: $A > B > C$

Even if it is not always in this way!

In RP-LC the composition of the mobile phase can affect the expected elution order of the analytes

Common applications of RP-LC:

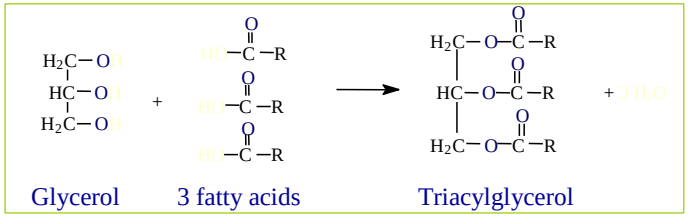
- purification of biological and organic compounds present in aqueous solutions
- pharmaceutical analysis (drug quantitation and quality control) (mainly in target mode)
- protein & peptide mapping
- analysis of soil and water samples
- clinical analysis of blood and urine samples
- **food analysis (mainly in untargeted mode)**

NARP-LC

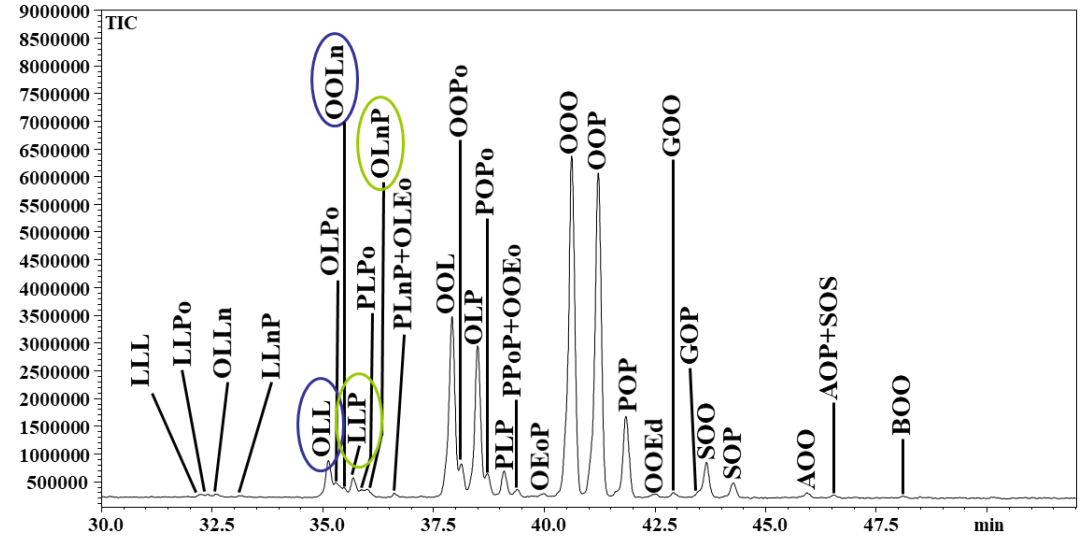
Analysis of medium- and non- polar molecules (e.g. lipids)

Elution is based on

Partition Number (PN) = CN – 2*DBN



***NARP-LC-APCI(+)-MS of TAG fraction of EVOO sample**



TAGs C54:5; PN (44) and DB (5)

C18:1C18:2C18:2 **(OLL)** < C18:1C18:1C18:3 **(OOLn)**

TAGs C52:4; PN (44) and DB (4)

C18:2C18:2C16:0 **(LLP)** < C18:1C18:3C16:0 **(OLnP)**

**Beccaria et al, Food Chemistry, 2016*

Hydrophilic interaction liquid chromatography (HILIC)

- a sort of partition chromatography, where the stationary phase is polar

② Aqueous Normal Phase (ANP) LC

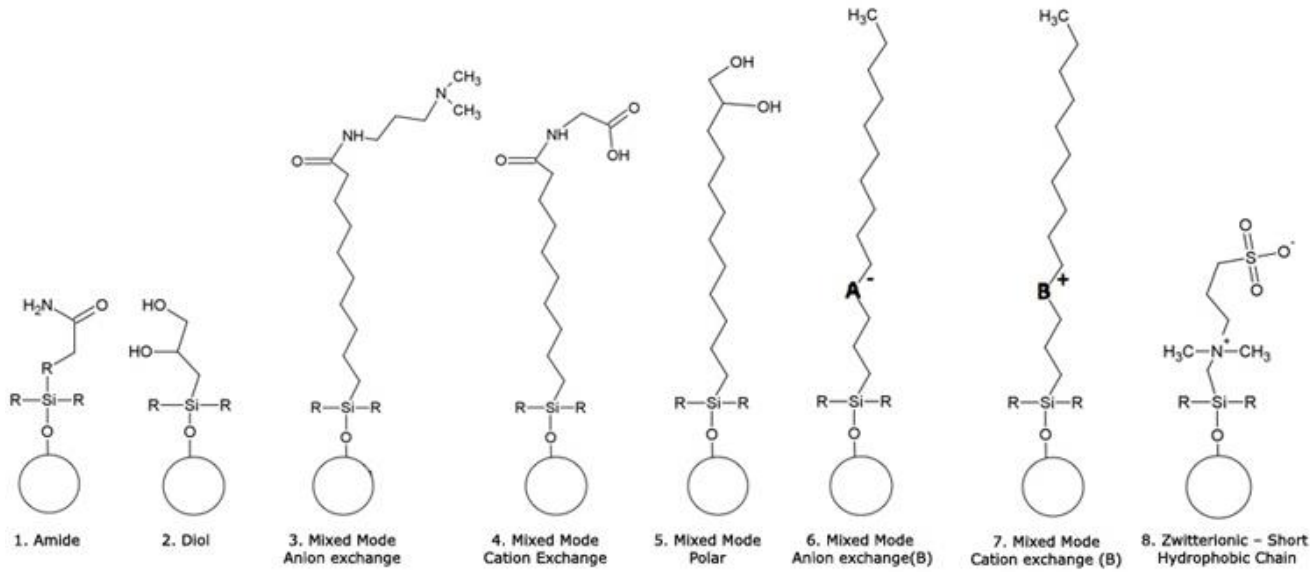
② retains polar compounds most strongly

- Using it instead of RP chromatography when analytes are not being retained on a column

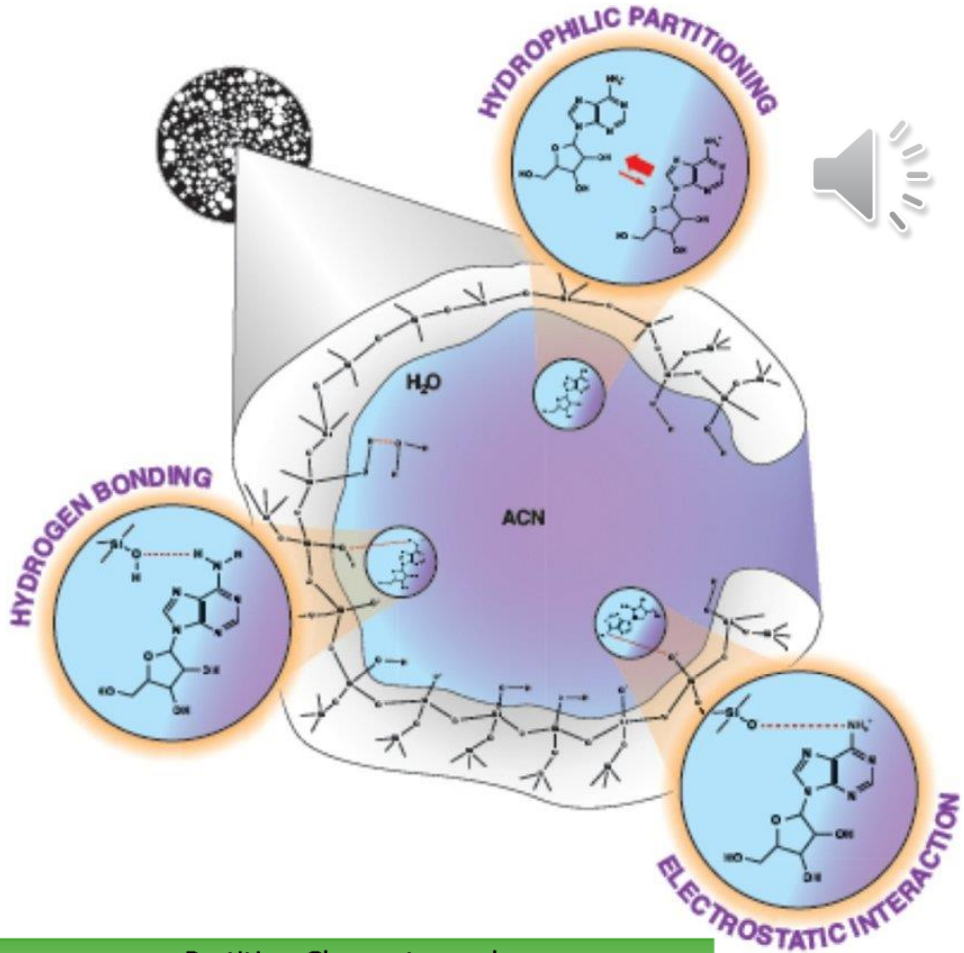
- weak mobile phase is the semi-polar organic solvent: mainly acetonitrile

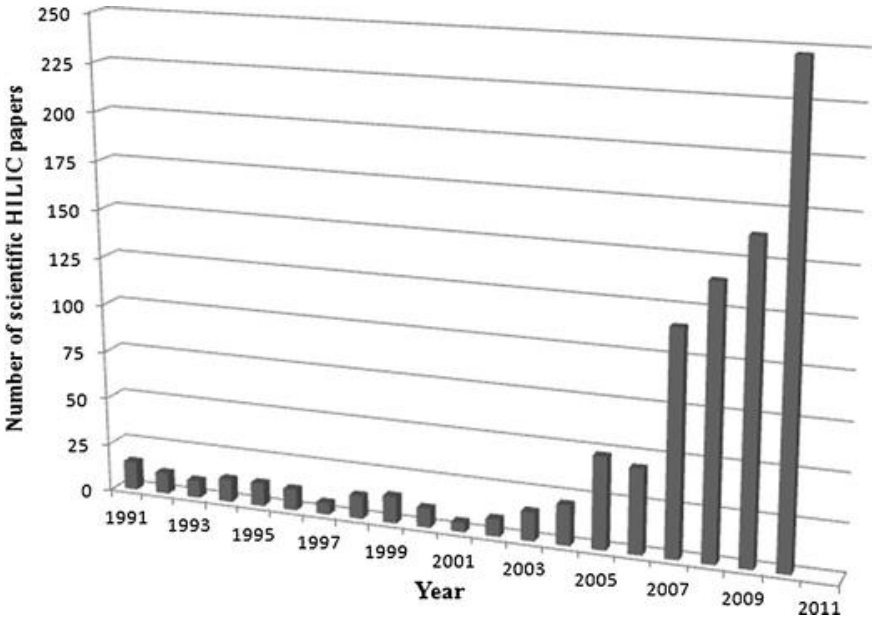
- strong mobile phase is the polar inorganic solvent: water

- Several types of stationary phases available



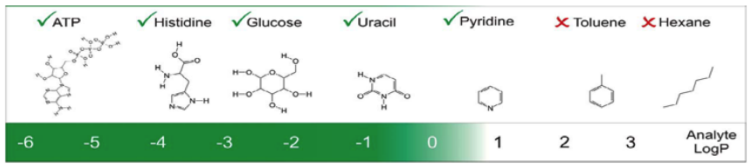
Hydrophilic interaction liquid chromatography (HILIC)



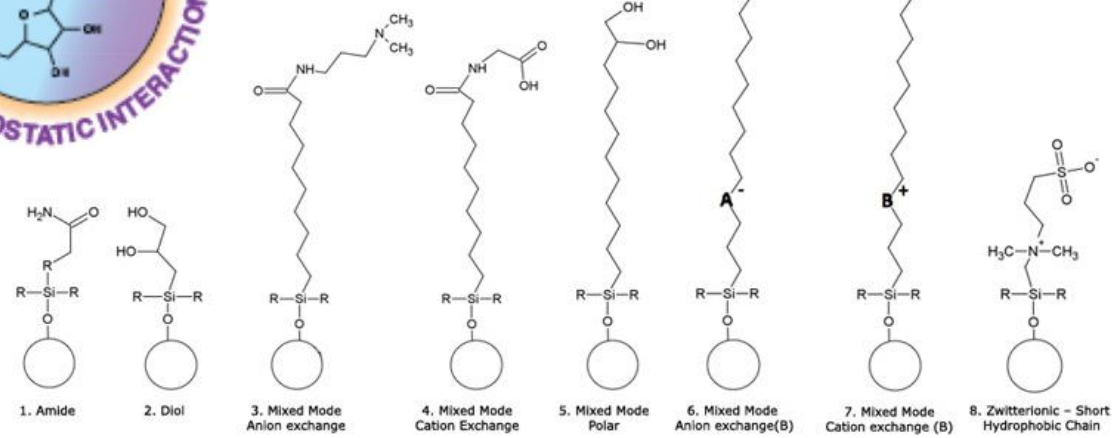


Partition Chromatography

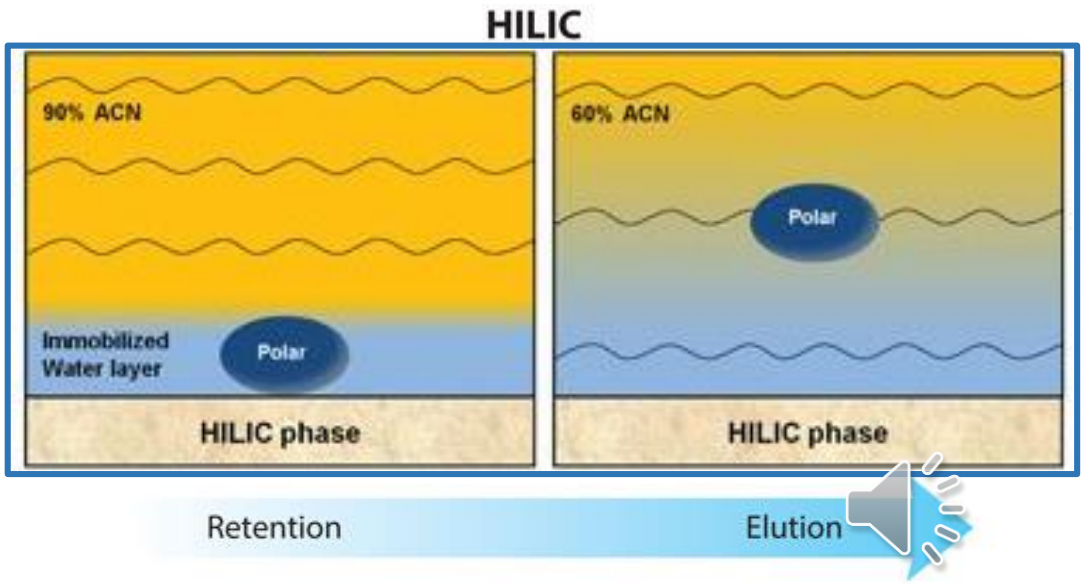
HILIC	RPC
MeCN >> H_2O	H_2O >> MeCN



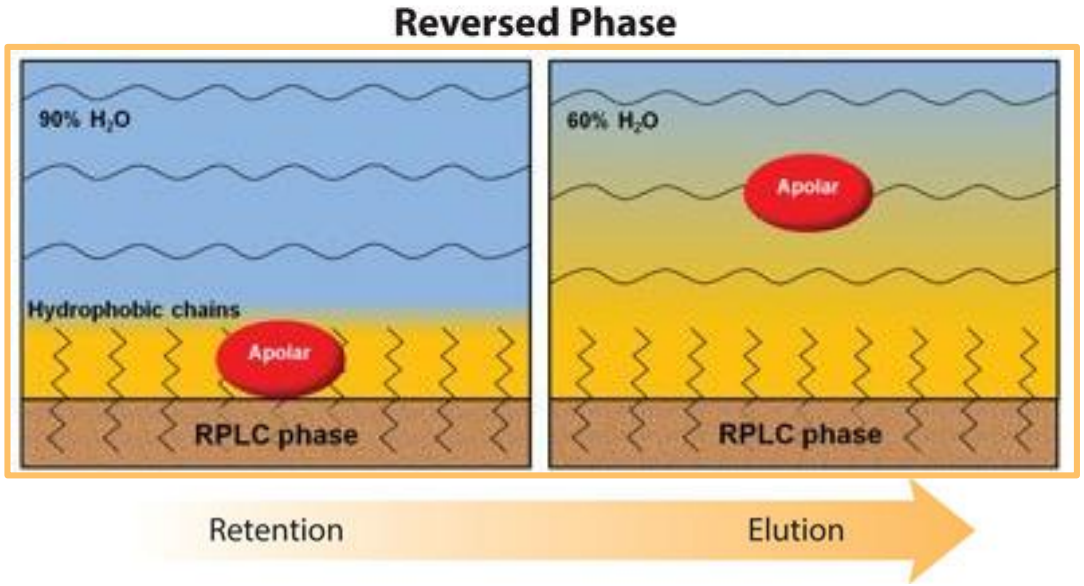
LogP = (octanol-water partitioning)



HILIC vs RP



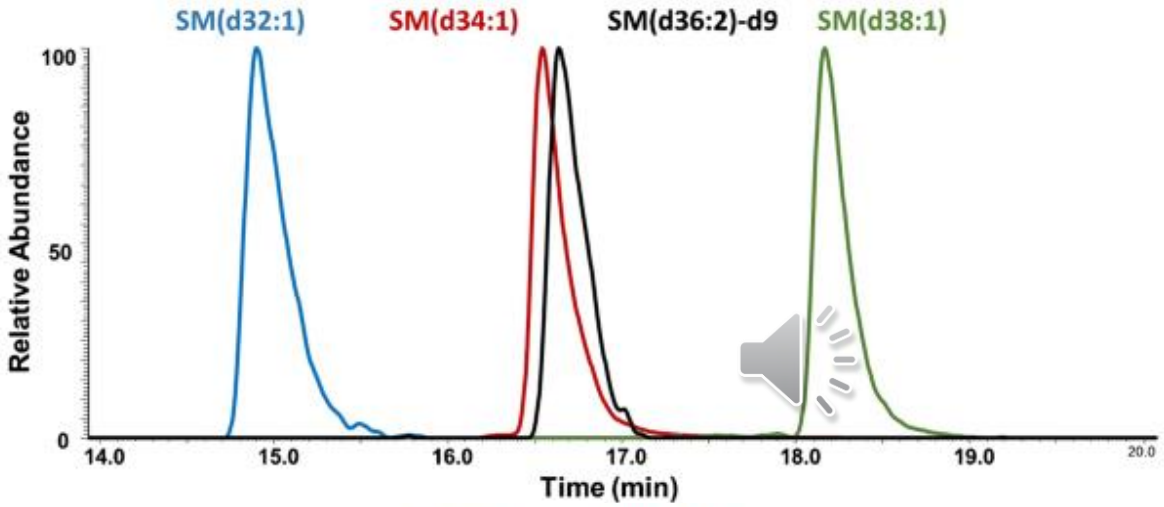
Scheme of HILIC partitioning of a general polar analyte into the water layer adsorbed on the hydrophilic phase surface at high acetonitrile content in the mobile phase, and its elution with the increase of the water content in the mobile phase.



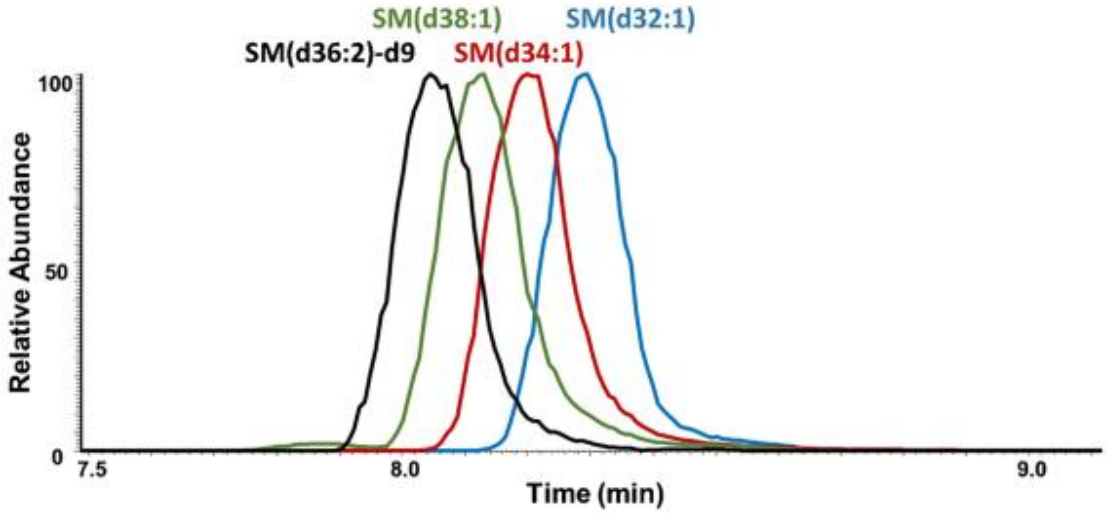
Scheme of RPLC partitioning of a general apolar analyte into the hydrophobic environment near the phase surface at high water content in the mobile phase, and its elution with the increase of the organic content in the mobile phase.

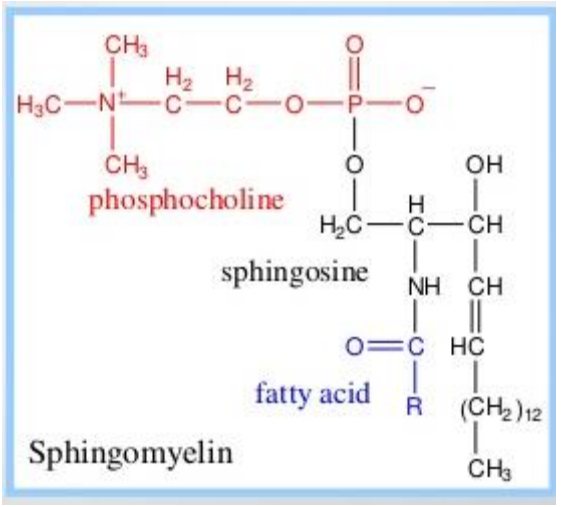
HILIC vs RP
Example of sphingomyelin (phospholipid) separation

RPLC



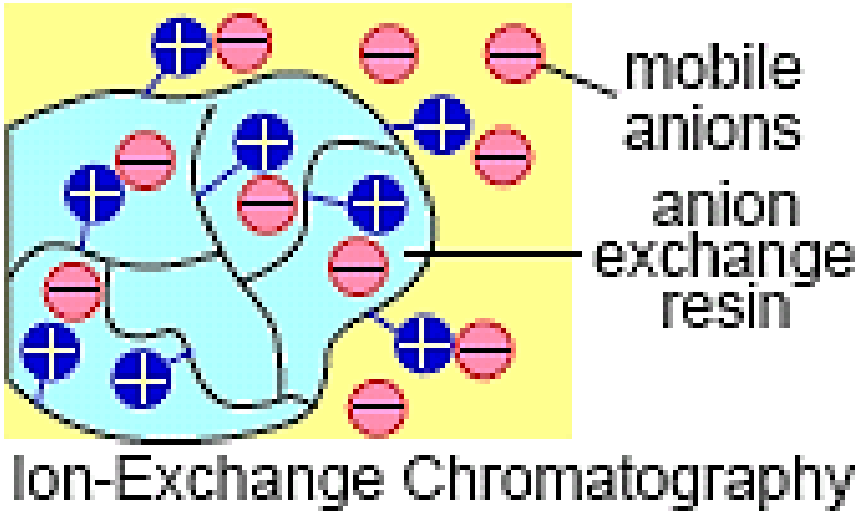
HILIC





3.) Ion-exchange Chromatography (IEC)

Separates solutes by their adsorption onto a support containing fixed charges on its surface. A high concentration of a competing ion is often added to the mobile phase to elute the analytes from the column



https://www.labtube.tv/video/Principles-of-Ion-exchange-chromatography-120352

$$xRSO_3^-H^+ + M^{x+} \leftrightarrow (RSO_3^-)_xM^{x+} + xH^+$$

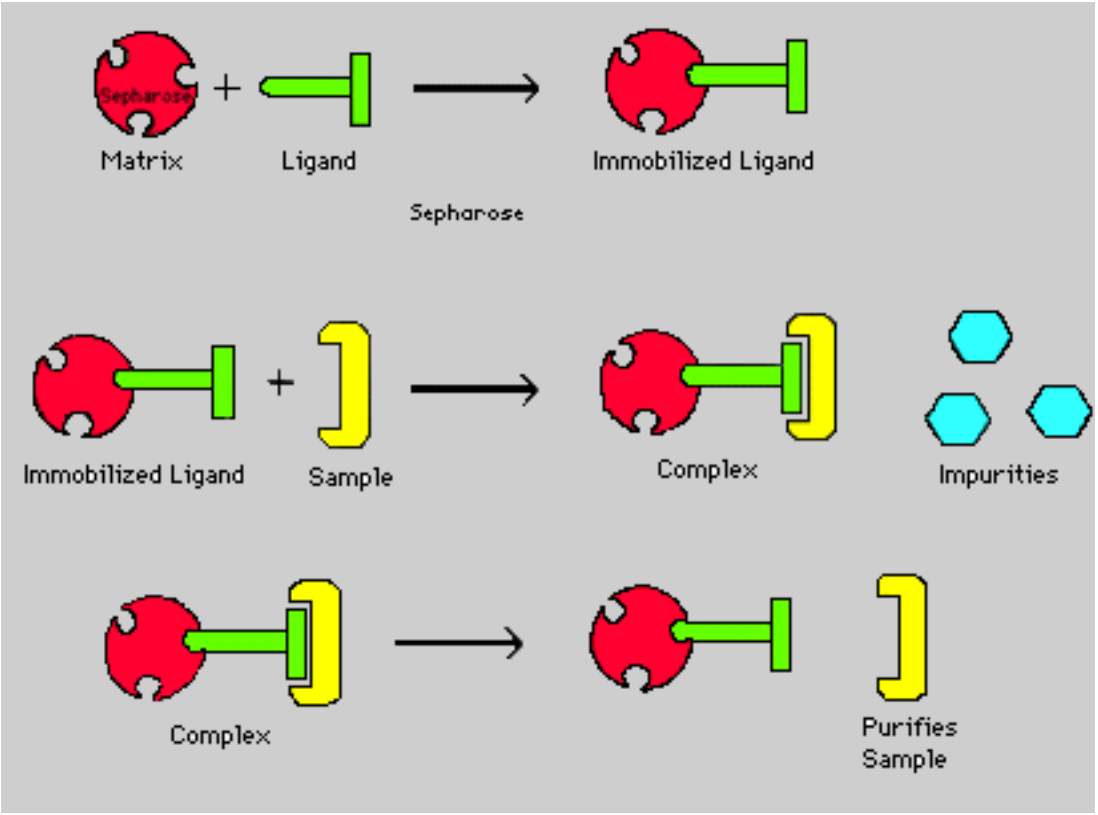
Cation exchange

$$xRN(CH_3)_3OH^- + A^{x-} \leftrightarrow [RH(CH_3)_3^+]_xA^{x-} + xOH^-$$

Anion exchange

4.) Affinity Chromatography (AC)

Separates based on the use of immobilized biological molecules (and related compounds) as the stationary phase



Based on the selective, reversible interactions that characterize most biological systems

- binding of an enzyme with its substrate or a hormone with its receptor
- immobilize one of a pair of interacting molecules onto a solid support
- immobilized molecule on column is referred to as the *affinity ligand*

Two Main Types of Affinity Ligands Used in AC:

High-specificity ligands – compounds which bind to only one or a few very closely related molecules

Affinity Ligand	Retained Compounds
Antibodies	Antigens
Antigens	Antibodies
Inhibitors/Substrates	Enzymes
Nucleic Acids	Complimentary Nucleic acids

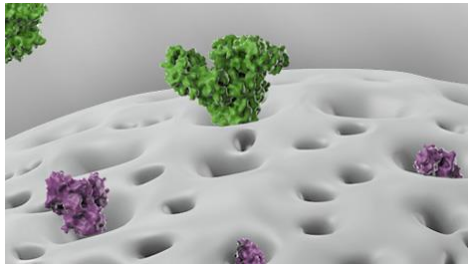
General or group specific ligands – molecules which bind to a family or class of related molecules

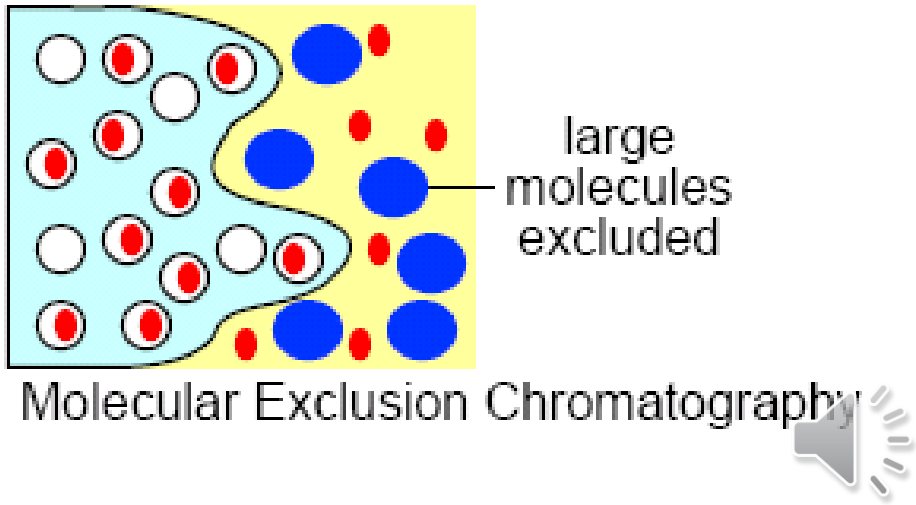
Affinity Ligand	Retained Compounds
Lectins	Glycoproteins, carbohydrates, membrane proteins
Triazine dyes	NADH- or NADPH Dependent Enzymes
Phenylboronic acid	*Cis*-Diol Containing Compounds
Protein A/Protein G	Antibodies
Metal Chelates	Metal-Binding Proteins & Peptides

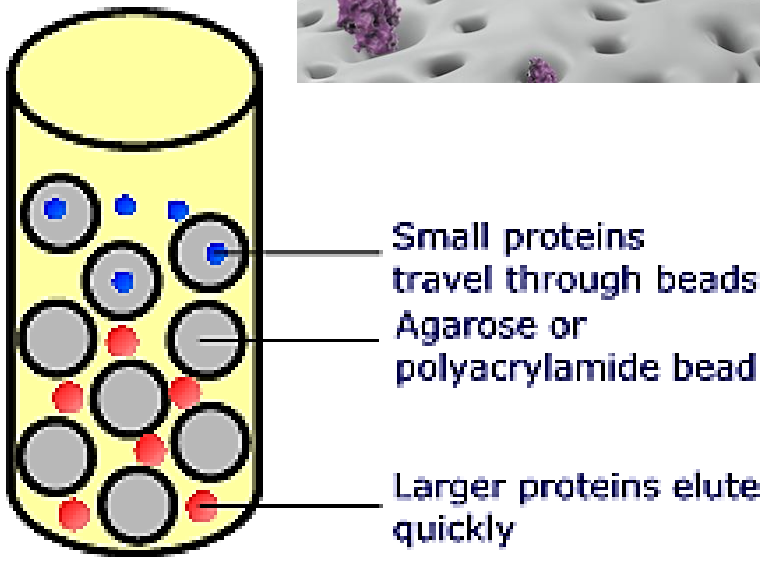
***Note:* the affinity ligand does not necessarily have to be of biological origin**

5.) Size Exclusion Chromatography (SEC)

separates molecules according to differences in their size







SEC is based on the use of a support material that has a certain range of pore sizes

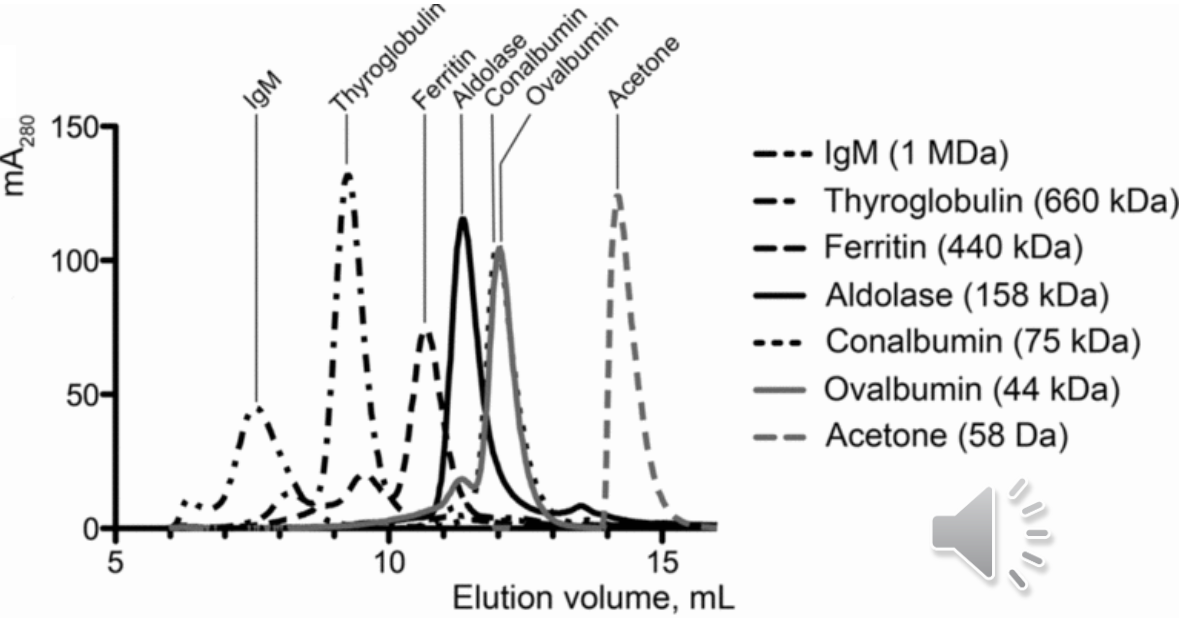
- as solute travels through the support, small molecules can enter the pores while large molecules can not
- since the larger molecules sample a smaller volume of the column, they elute before the smaller molecules.
- separation based on size or molecular weight

SEC does not have a “weak” or “strong” mobile phase since retention is based only on size/shape of the analyte and the pore distribution of the support.

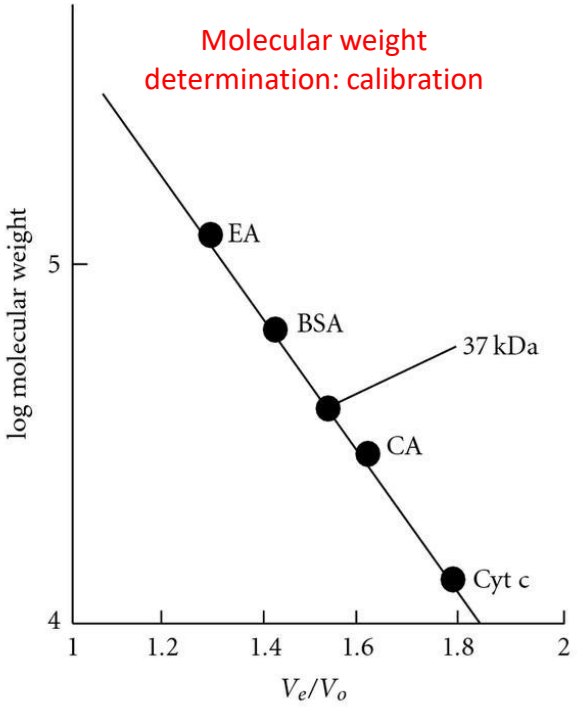
- *gel filtration chromatography*: if an aqueous mobile phase is used
- *gel permeation chromatography*: if an organic mobile phase is used (usually tetrahydrofuran)

Common applications of SEC:

- Separation of Biological Molecules (e.g., proteins from peptides)
- Separation/analysis of organic polymers
- molecular-weight determination



Molecular weight determination: calibration



E.) LC Detectors (except MS):

Common types of LC Detectors

② Refractive Index Detector, Evaporative Light Scattering Detector (ELSD)

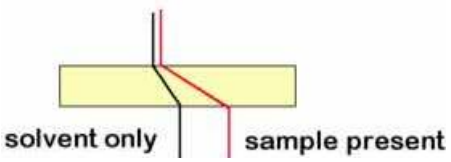
② Conductivity Detector② UV/Vis Absorbance Detector② Electrochemical Detector

② Fluorescence Detector

As in GC, the choice of detector will depend on the analyte and how the LC method is being used (*i.e.*, analytical or preparative scale)

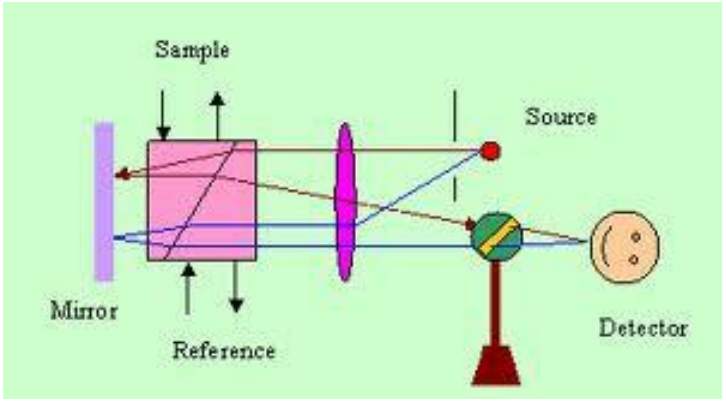
Detector	Specificity	Sensitivity	Notes
Refractive Index	Good	Poor	Any component that differs in refractive index from the eluate can be detected, despite its low sensitivity. Cannot be used to perform gradient analysis.
Evaporative Light Scattering Detector (ELSD)	Excellent	Good	The mobile phase enters the detector, is evaporated in a heated device and the remaining solute is finally detected by the way it scatters light. The intensity of the light scattered from solid suspended particles depends on their particle size.
UV/Vis	Moderate	Good	A wide variety of substances can be detected that absorb light from 190 to 900 nm. Sensitivity depends strongly on the component.
Fluorescence	Moderate	Excellent	Components emitting fluorescence can be detected selectively with high sensitivity. This is often used for pre-column and post-column derivatization.
Conductivity	Moderate	Good	Ionized components are detected. This detector is used mainly for ion chromatography.

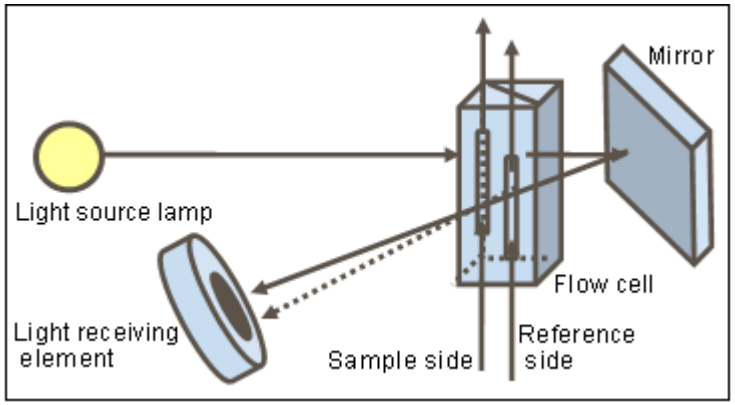
1.) Refractive Index Detector (RI)



Measures the overall ability of the mobile phase and its solutes to refract or bend light.

② one of the few universal detectors available for LC





Process:

– light from source passes through flow-cells containing either sample stream or a reference stream

– when refractive index is the same between the two cells, no bending of light occurs at the interface between the flow-cells

② maximum amount of light reaches the detector

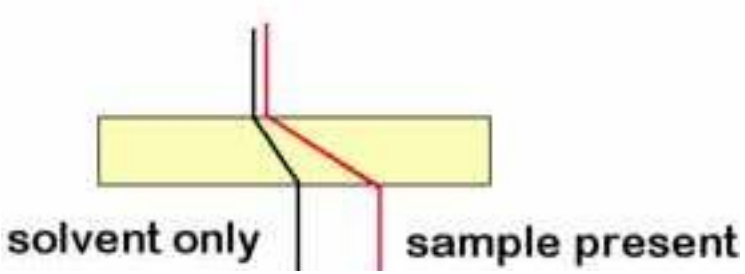
– as solute elutes, refractive index changes between reference and sample cell

② light is bent as it passes through flow cell interface

② amount of light reaching detector is decreased

Linear response of the detector: no calibration is required

1.) Refractive Index Detector (RI)



advantages:

– non-destructive and universal detector

② applicable to the detection of any solute in LC

– applicable to preliminary LC work where the nature and properties of the solute are unknown

② provided concentration is high enough for detection

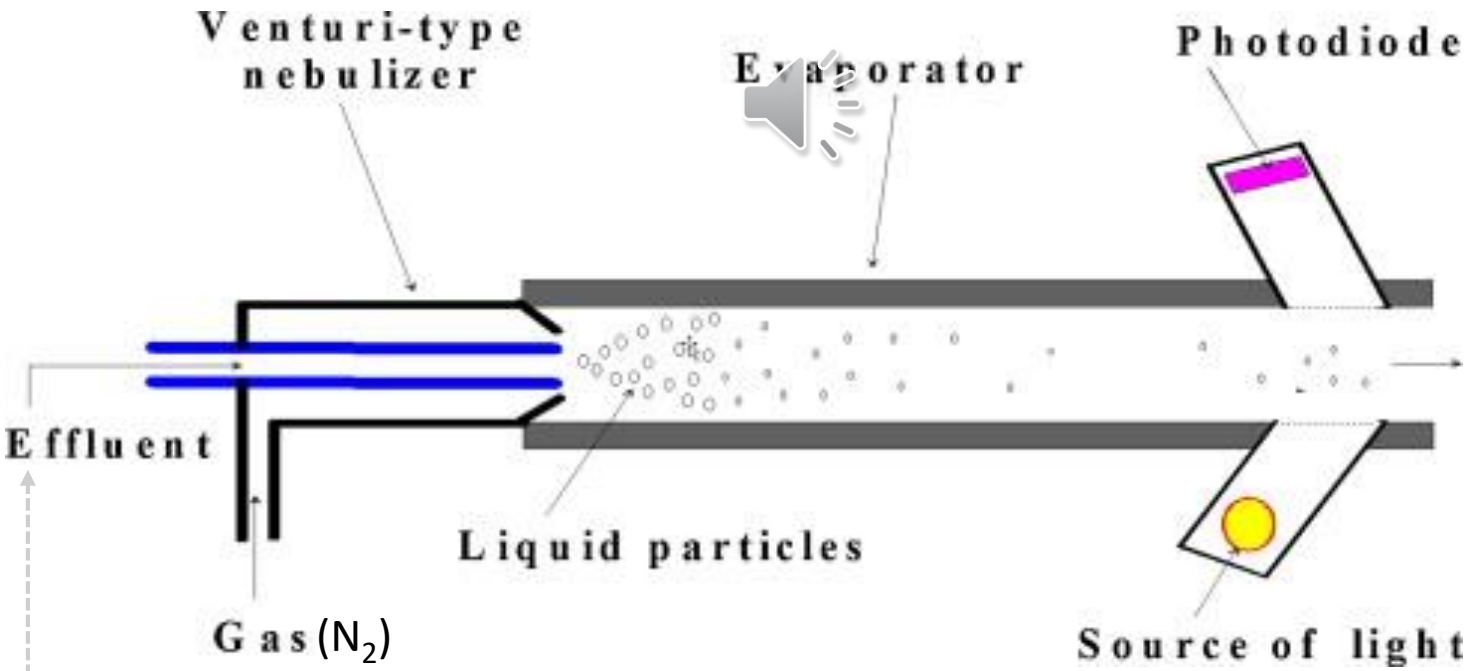
disadvantages:

– high limits of detection (10^{-6} to 10^{-5} M)

– difficult to use with gradient elution

2.) Evaportive Light Scattering Detector (ELSD)

- ELSD is able to detect all compound which are less volatile than the mobile phase, i.e. non volatile and semi-volatile compounds.



2.) Evaportive Light Scattering Detector (ELSD)

Applications:

- The ELSD is an universal detector, detecting semi- and non-volatile analytes.

Particular useful for:

Carbohydrates, Lipids and Fatty Acids, Steroids, Polymers & Surfactants, Pharmaceuticals

- limits of detection

about 200 picograms

- Fully Gradient Compatible

Handles any combination of volatile mobile phases and gradients

- Accepts a wide range of mobile phase flows

from 1 µL/min. to 5.0 mL/min (with some adjustment)

3.) UV/Vis Absorbance Detector

Measures the ability of solutes to absorb light at a particular wavelength(s) in the ultraviolet (UV) or visible (Vis) wavelength range.

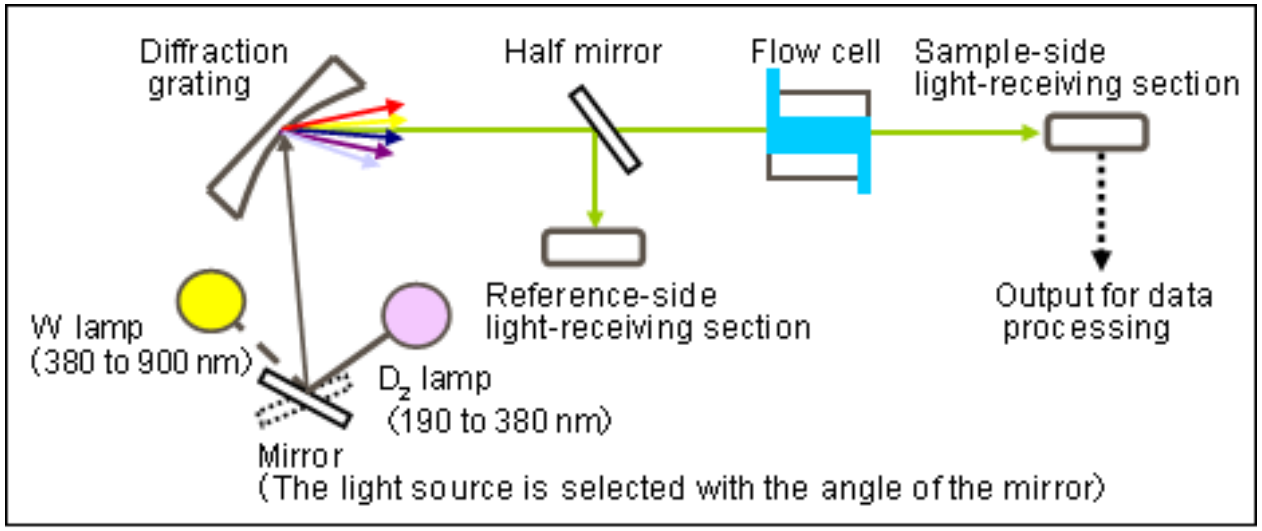
② most common type of LC detector

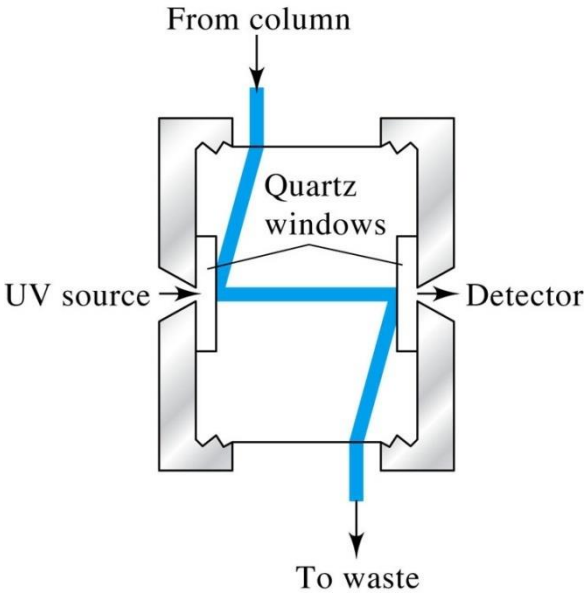
Three Common types of UV/Vis Absorbance Detectors

② Fixed wavelength detectors

② Variable wavelength detectors

② Photodiode array detectors (DAD)





3.) UV/Vis Absorbance Detector

Fixed Wavelength Detector absorbance of only one given wavelength is monitored by the system at all times (usually 254 nm)

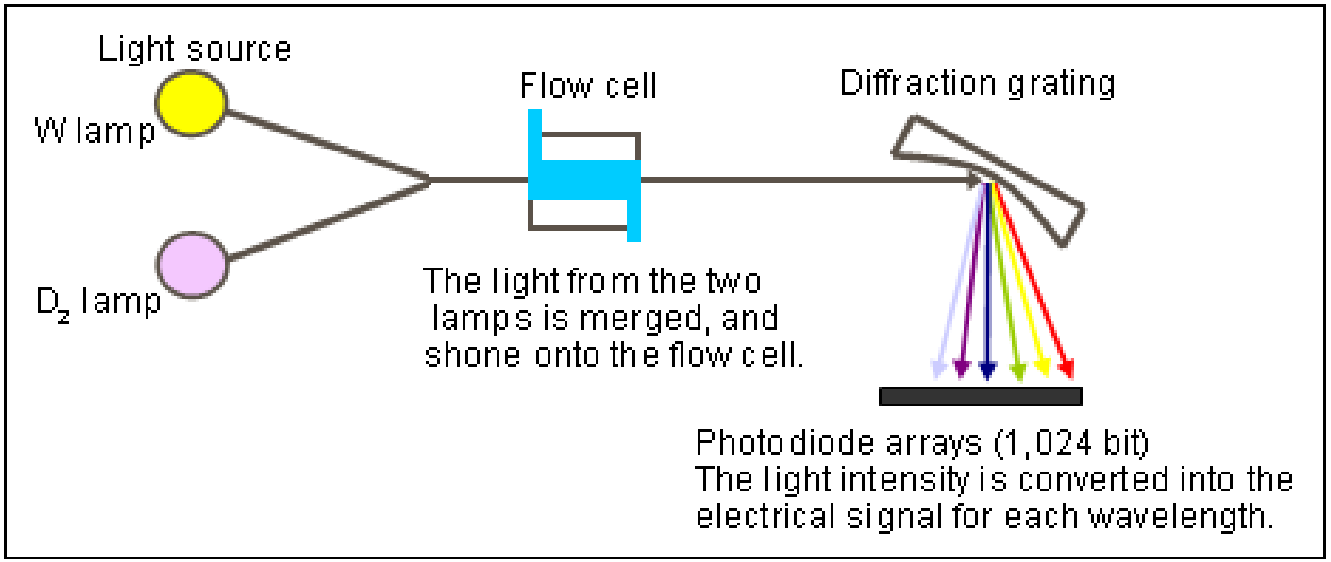
- ② simplest and cheapest of the UV/Vis detectors
- ② limited in flexibility
- ② limited in types of compounds that can be monitored

Variable Wavelength Detector a single wavelength is monitored at any given time, but any wavelength in a wide spectral range can be selected

- ② wavelengths vary from 190-900 nm.
- ② more expensive, requires more advanced optics
- ② more versatile, used for a wider range of compounds

Photodiode Array Detector operates by *simultaneously* monitoring absorbance of solutes at several different wavelengths.

- ② uses a series or an array of several detector cells within the instrument, with each responding to changes in absorbance at different wavelengths.
- ② entire spectrum of a compound can be taken in a minimum amount of time
- ② useful in detecting the presence of poorly resolved peaks or peak contaminants

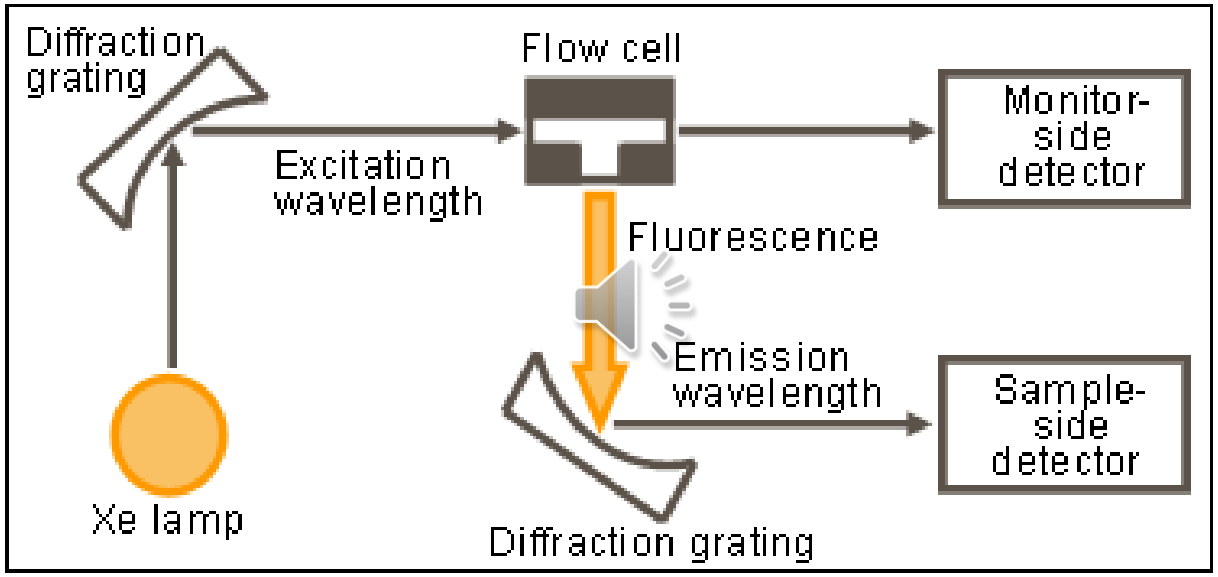


Applications:

- UV/Vis absorbance detectors can be used to detect any compound that absorbs at the wavelength being monitored
- Common wavelengths:
  - ② 254 nm for unsaturated organic compounds
  - ② 260 nm for nucleic acids
  - ② 280 or 215 nm for proteins or peptides
- Absorbance detectors can be used with gradient elution
  - ② wavelength being monitored is above the cutoff range of the solvents being used in the mobile phase
- limits of detection for fixed and variable UV/Vis absorbance detectors are ~ 10^{-8} M
- limits of detection for photodiode array detectors are ~ 10^{-7} M

4.) Fluorescence Detector

A selective LC detector that measures the ability of eluting solutes to fluoresce at a given set of excitation and emission wavelengths



4.) Fluorescence Detector

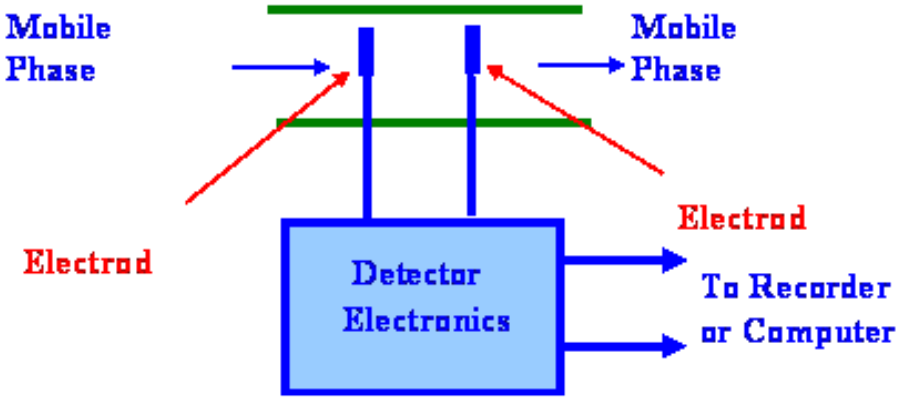
Applications:

- Fluorescence can be used to selectively detect any compound that absorbs and emits light at the chosen set of excitation and emission wavelengths
  - ② Relatively few compounds undergo fluorescence
  - ② high selectivity, low background signal
- limits of detection for a fluorescence detector are ~ 10^{-10} M
- Typical applications
  - ② drugs
  - ② food additives
  - ② environmental pollutants
  - ② any compound that can be converted to a fluorescent derivative: alcohols, amines, amino acids and proteins
- Can be used with gradient elution
  - ② requires extremely pure mobile phases
  - ② trace impurities can affect background signal or quench the fluorescence of solutes

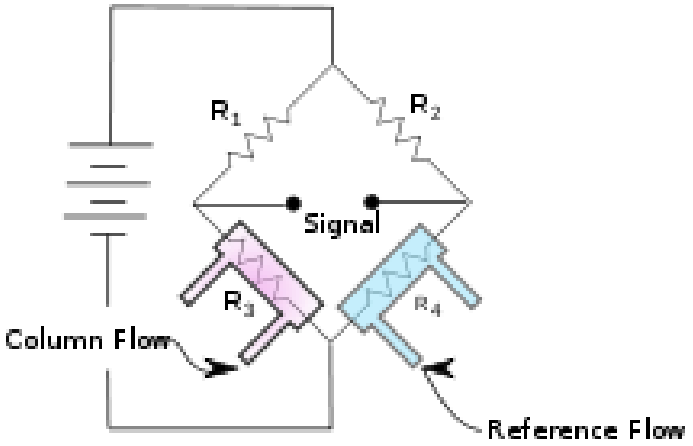
5.) Conductivity Detector

Used in analytical applications of ion-exchange chromatography for the detection of ionic compounds

- ② detector measures the ability of the mobile phase to conduct a current when placed in a flow-cell between two electrodes
- ② current conducted within the cell will depend on the number and types of ions present in the mobile phase



Two electrodes placed in mobile phase each corresponding to one arm of a Wheatstone Bridge



Typical Wheatstone Bridge

When ions flow into the sensor cell, the impedance between the electrodes changes producing an "out of balance" signal

5.) Conductivity Detector

Applications:

- can be used to detect any compound that is ionic or weakly ionic
  - ② high selectivity, low background signal

- limits of detection for a conductivity detector are ~ 10^{-6} M

- Typical applications
  - ② food components
  - ② industrial samples
  - ② environmental samples

- Can be used with gradient elution

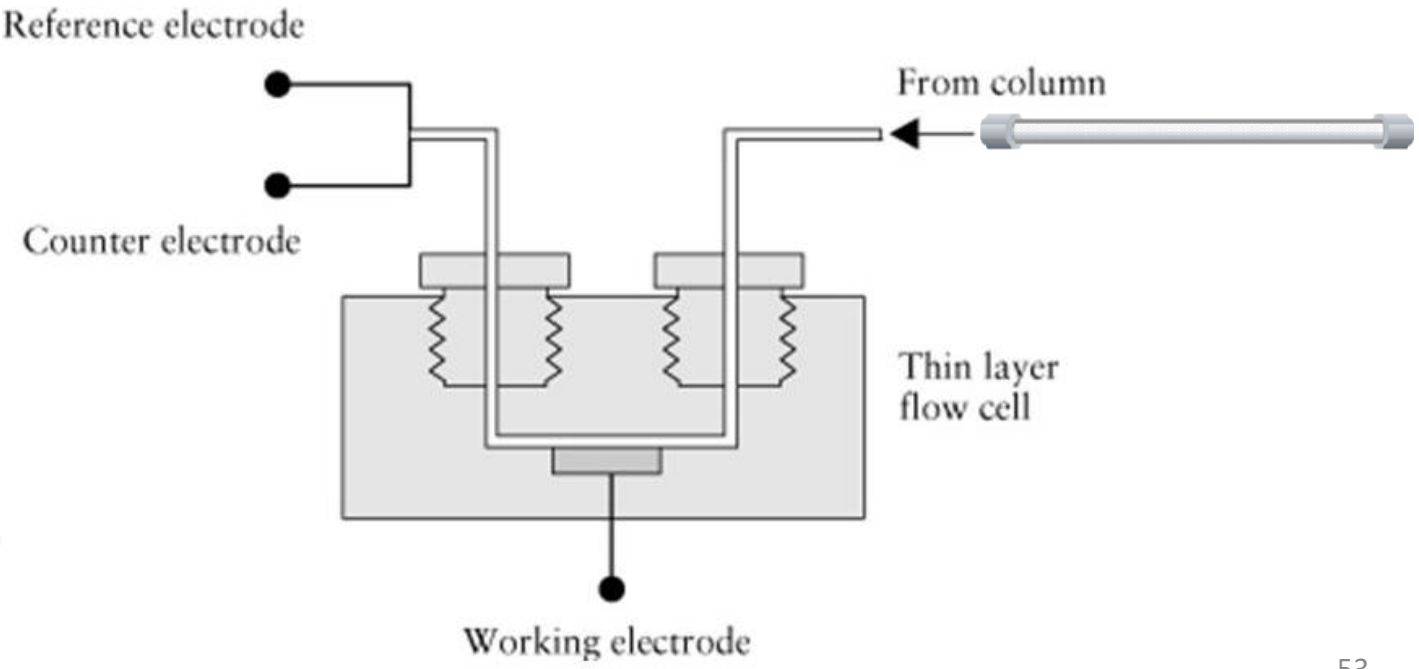
② constant ionic strength and pH of mobile phase

② background conductance of the mobile phase is sufficiently low

6.) Electrochemical Detector

Used to monitor any compound in the mobile phase that can undergo an oxidation or reduction

- ② electrochemical detection in liquid chromatography is sometimes referred to as LC/EC
- ② generally includes two or more electrodes which monitor the current that is produced by the oxidation or reduction of eluting compounds at a fixed potential
- ② generally electrical output is an electron flow generated by a reaction that takes place at the surface of the electrodes.



6.) Electrochemical Detector

Applications:

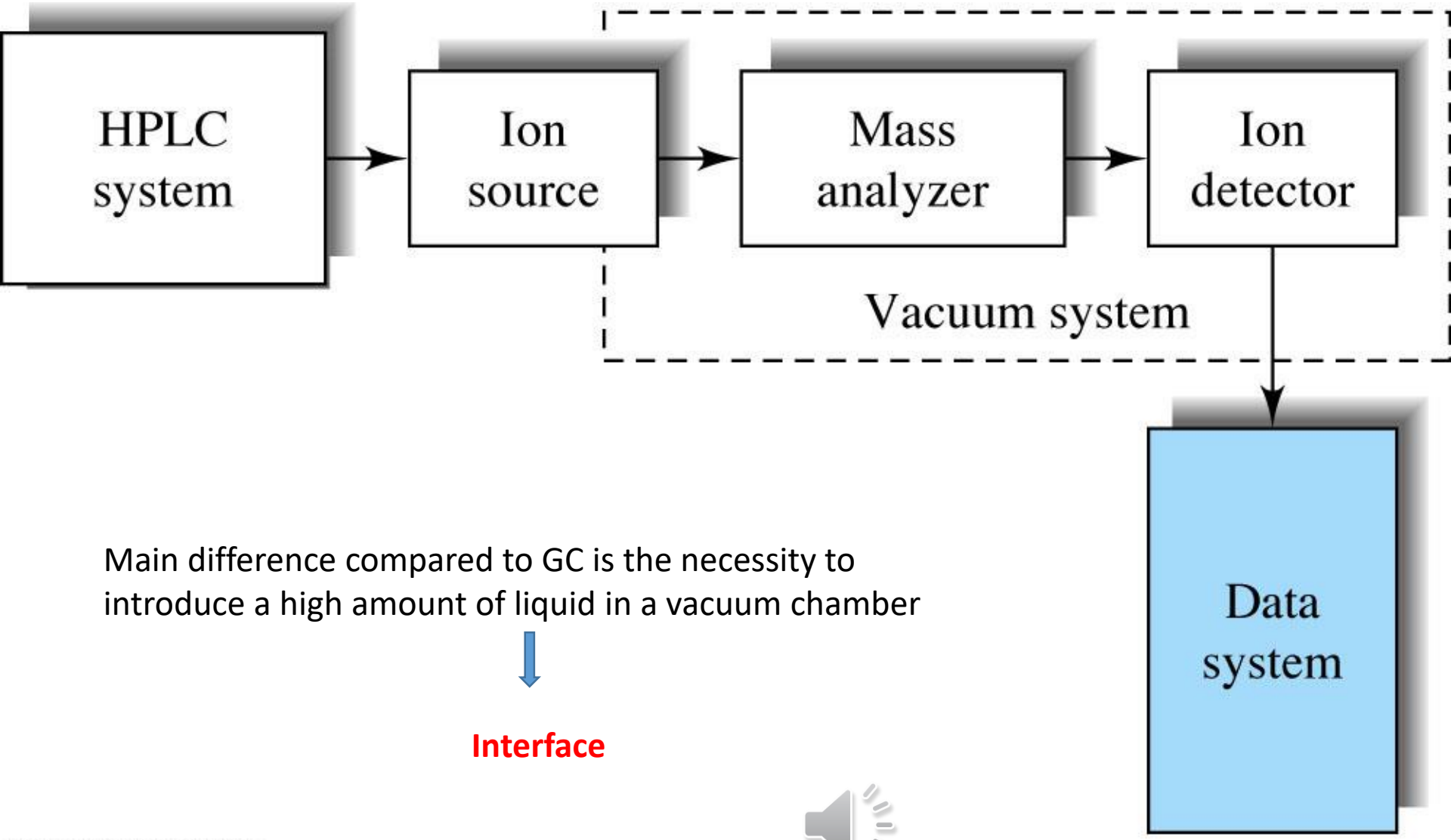
- can be used to detect any solute that can undergo oxidation or reduction
  - ② detectors can be made specific for a given compound or class of compounds by properly choosing the conditions at the electrodes
  - ② high selectivity, low background signal

- limits of detection for a electrochemical detector are ~ 10^{-11} M
  - ② due to extreme accuracy with which chemical measurements, especially current measurements, can be made

- compounds that can be detected by reduction
  - ② aldehydes
  - ② ketones
  - ② esters
  - ② unsaturated compounds

- compounds that can be detected by oxidation
  - ② phenols
  - ② mercaptans (RSH)
  - ② aromatic amines
  - ② dihydroxy compounds

Mass spectrometry



Main difference compared to GC is the necessity to introduce a high amount of liquid in a vacuum chamber

Interface