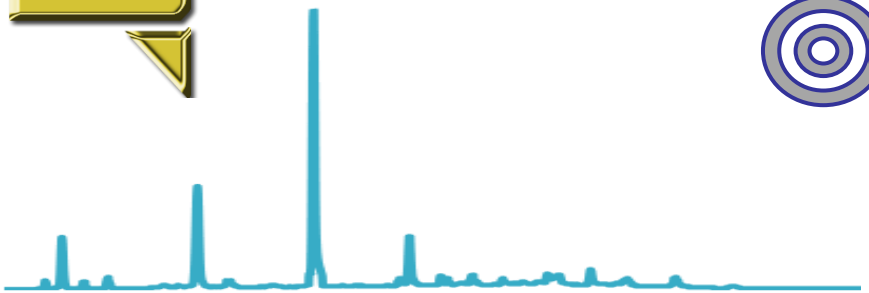
بسم الله الرحمن الرحيم

An Introduction to Chromatographic Separations



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SAUDI CHEMICAL SOCIETY

Outline

- **M**otivation **B**ehind **S**eparation **M**ethods
- **S**eparation **T**echniques **C**lassification
- **C**hromatographic **S**eparation **M**ethods: **A**n **O**verview
- **C**lassification of **C**hromatographic **M**ethods
- **C**hromatography **A**pplications

Why we Need Separation Methods ??????

-For analysis of a substance in presence of other components (matrix).

-To separate the components of a mixture for more advanced use (purification).

Theory of Separation Methods

The goal of an analytical separation is to remove either the analyte or the interferent from the sample matrix.

To achieve a separation there must be at least one significant difference between the chemical or physical properties of the analyte and interferent.

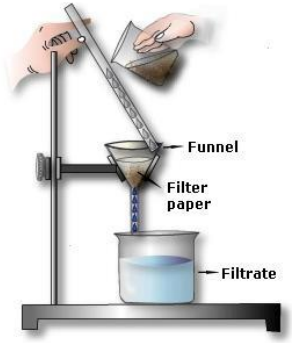
e.g., solubility, volatility, adsorption, boiling point, melting point, ion exchange, molecular size.

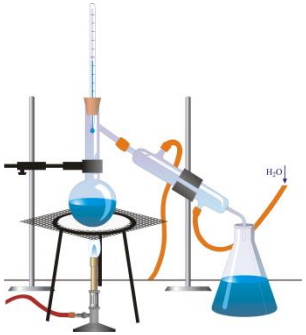
Classifying Separation Techniques

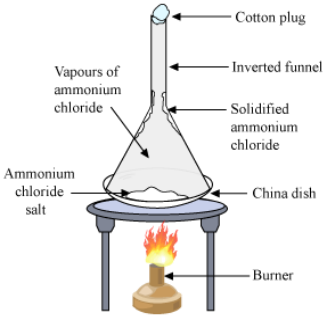
Basis of Separation	Separation Technique
size	filtration dialysis size-exclusion
mass and density	centrifugation
complex formation	masking
change in physical state	distillation sublimation recrystallization
change in chemical state	precipitation ion exchange electrodeposition volatilization
partitioning between phases	extraction chromatography

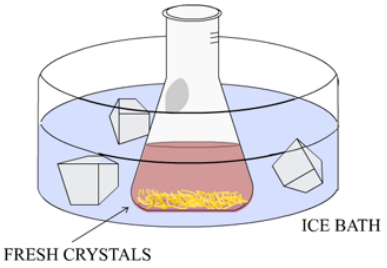
Classifying Separation Techniques

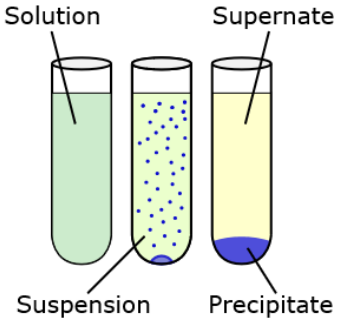
- Traditional Methods of Separation and Purification



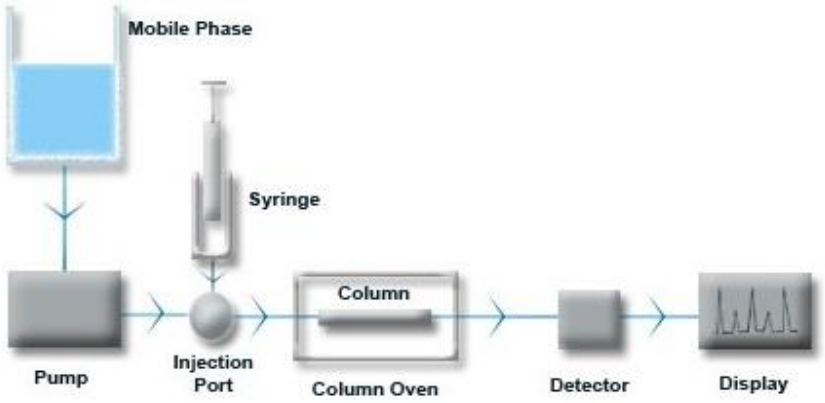


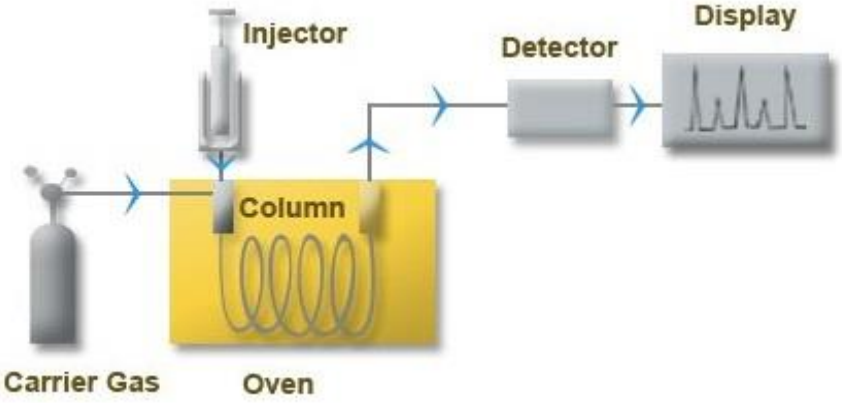






- Instrumental Methods of Separation and Purification





Choice of the Appropriate Technique

The choice of the appropriate method mainly depends on the physico-chemical properties of the **analyte** and of the **matrix** as well as the **objectives** of the overall method.

-Physical state (solid, liquid, gas),
-Chemical structure,
-Functional group,
-Polarity,
-Size and molecular weight,
-Solubility,
-Volatility,
-Charge,
-Stability,
-Detection technique.

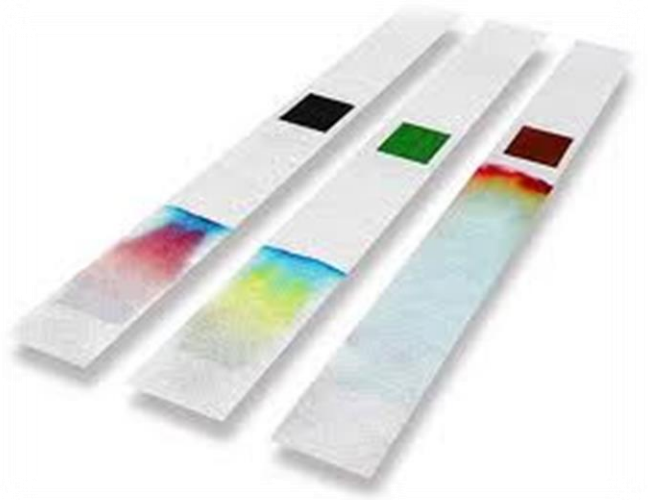
A combination of more than one separation or purification method may have to be used at times.

Definition

According to the IUPAC definition, 1993

"Chromatography is a physical method of separation in which the components to be separated are distributed between two phases; one of which is stationary (**stationary phase**) while the other moves in a definite direction (**mobile phase**)".

Chromatography derives its name from two Greek words as;
(chroma) meaning "**color**",
(graphy) meaning "**writing**".

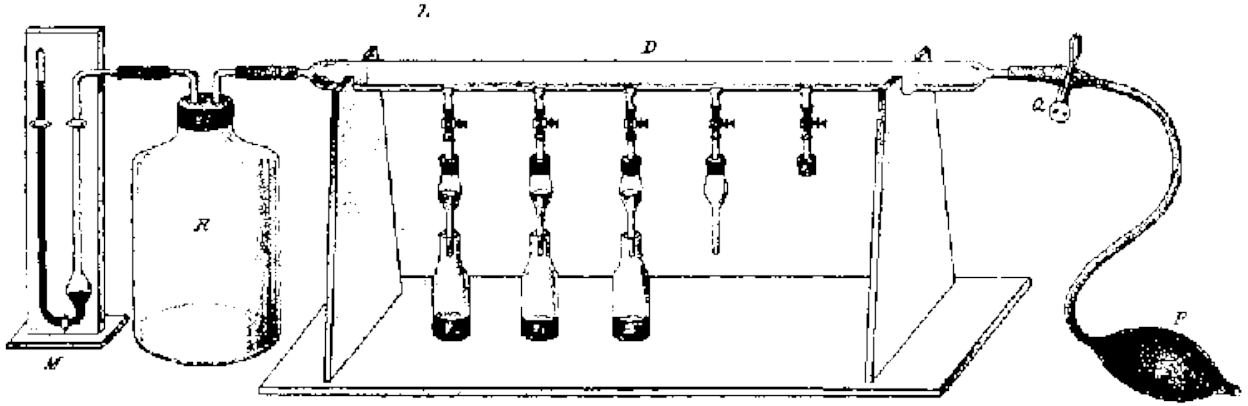


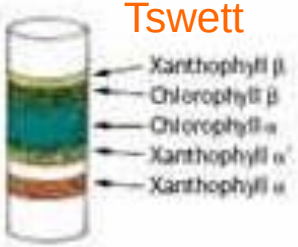
History

1906: Mikhail Tswett, plant pigments (chlorophylls & xanthophylls) separation from leaves through a glass column packed with chalk powders ($CaCO_3$) using petroleum ether as eluent. Tswett is credited as "**father of chromatography**".

Tswett

Tswett's apparatus





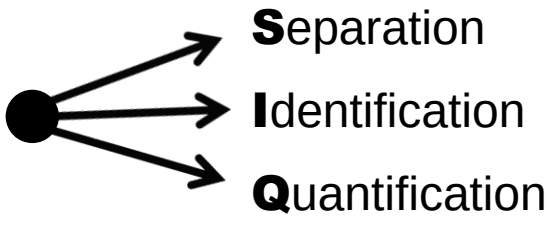
1930:	Classical columns	1970:	HPLC
1940:	Paper chromatography	1980:	SFC
1950:	GC	1990:	CE
1960:	TLC	2000:	CEC

Perhaps more impressive is a list of **twelve Nobel Prize** awards that were based upon work in which chromatography played a vital role. Chromatography is still continuously growing and its fields of application are widening.

Modern Chromatographic Techniques

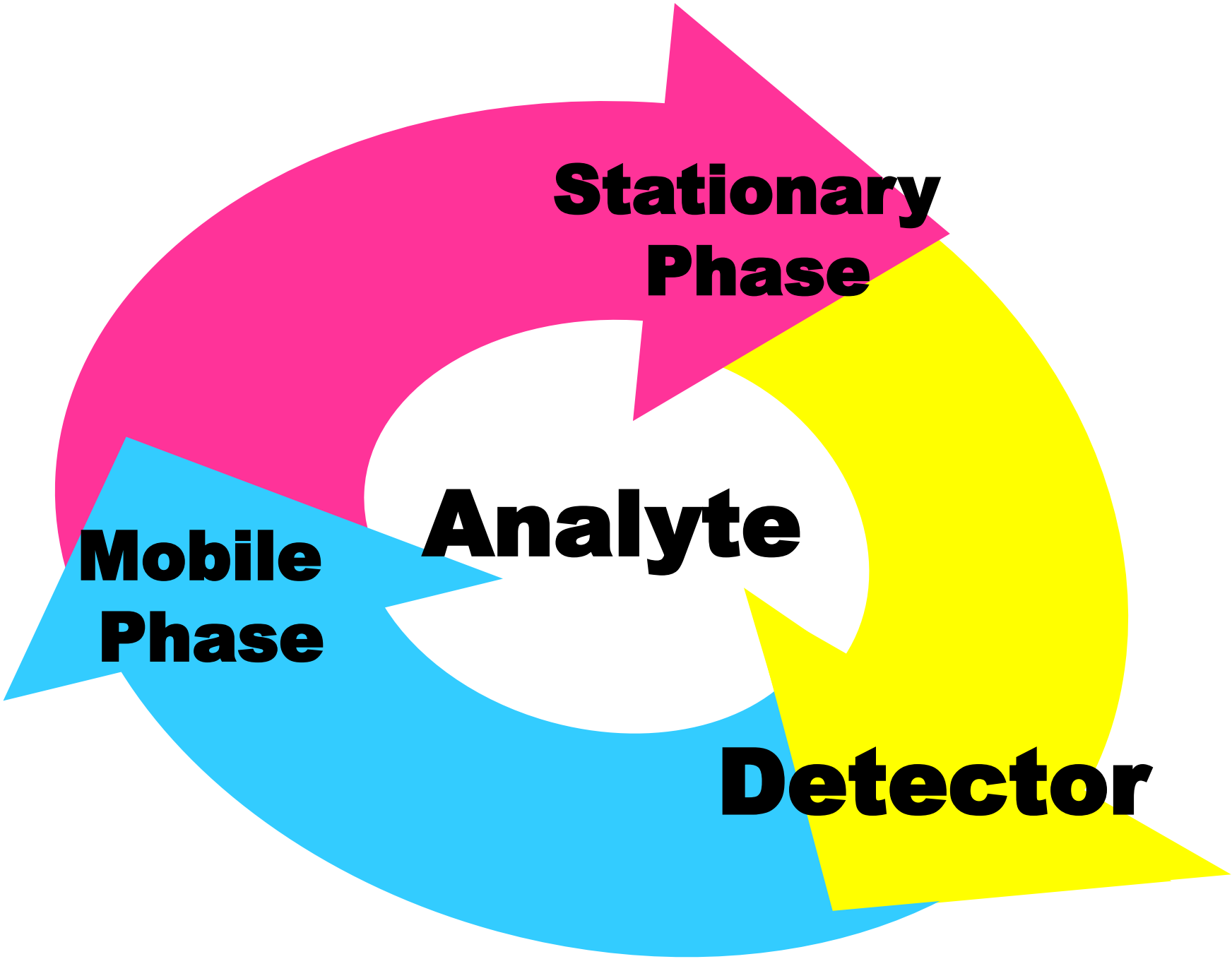
Chromatography is the collective term for a **family** of laboratory techniques for the separation of mixtures.

Modern **Chromatographic methods** have many applications including:



Other applications:

-Preparation of pure substances (purification),
-The study of the kinetics of reactions,
-Testing the purity of a particular substance,
-Structural investigations on the molecular scale,
-Determination of physicochemical constants,
(including stability constants of complexes, enthalpy, entropy & free energy).


Stationary Phase
Analyte
Mobile Phase
Detector

Market size

Chromatography instruments market size
7.06 billion USD in 2015
Expected to reach **9.22** billion USD in 2020
Annual growth rate about **5.5**%

Chromatography resins market size
1.5 billion USD in 2014
(natural, synthetic, inorganic media)
Expected to reach **2.3** billion USD in 2020
Annual growth rate about **7.3**%

HPLC
is the largest product segment in the analytical instruments industry and applications

Classification of Chromatographic Methods

Chromatographic methods can be categorized in several ways:

(1) Based on the physical state of the mobile phase and stationary phase.

Mobile phase could be **gas**, **liquid** or **a supercritical fluid**.
Stationary phase could be **liquid** or **solid**.

(a) Homogeneous techniques:
Have both m.p. and s.p. same physical state (liquid); liquid-liquid chromatography.

(b) Heterogeneous techniques:
Employ different m.p. and s.p. states, e.g., liquid-solid, gas-liquid, gas-solid chromatography.

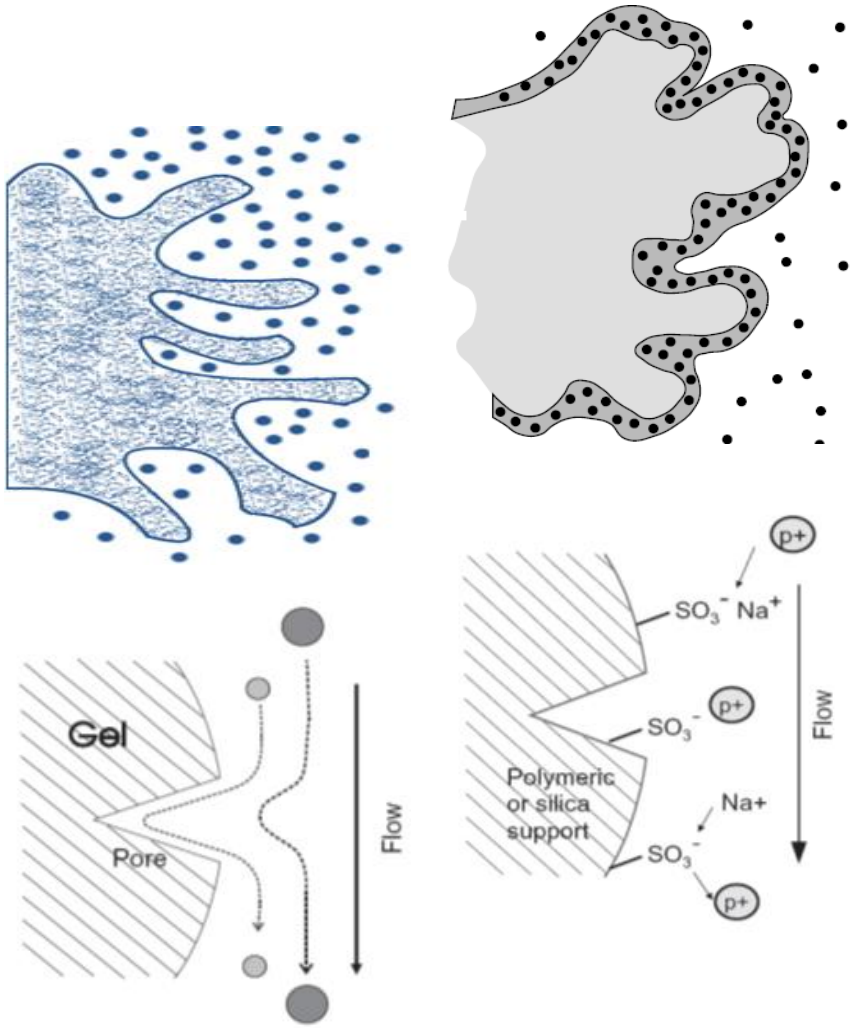
(2) Based on the kind of equilibria involved in the transfer of solutes between phases, principle of separation used (separation mechanism).

(a) Partition chromatography:
Separation based on solubility.
Stationary phase is liquid.

(b) Adsorption chromatography:
Separation based on polarity.
Stationary phase is solid.

(c) Ion exchange chromatography:
Separation based on charge.

(d) Size exclusion chromatography:
Separation based on molecular size.



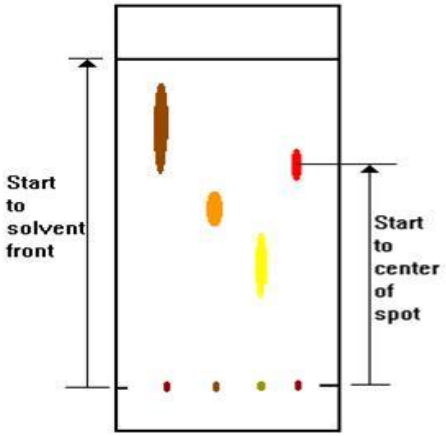
(3) Based on the shape of stationary phase, surface on which the separation to be performed or the way on which the mobile phase pass through the stationary phase.

(a) Planar or plane chromatography:

The stationary phase is placed on a plane surface (on a flat plate or in the interstices of a paper); here, the mobile phase moves through the stationary phase by capillary action or under the influence of gravity.

-Paper chromatography

-TLC



(b) Columnar or column chromatography:

The stationary phase is held in a narrow tube through which the mobile phase is forced under pressure or by gravity.

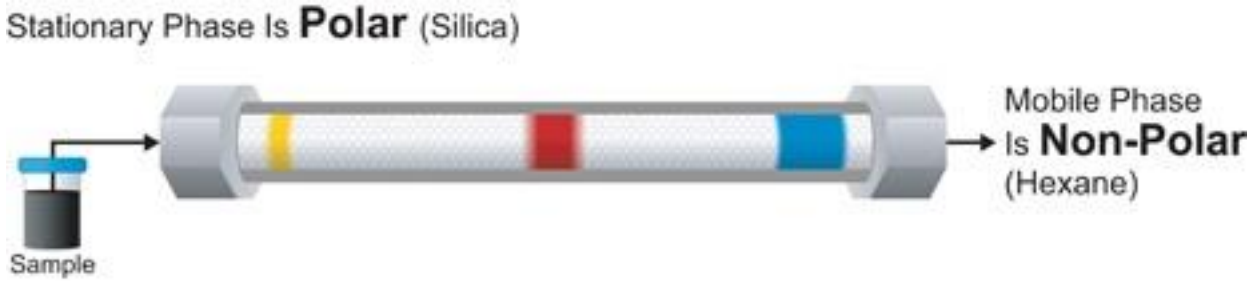
-HPLC

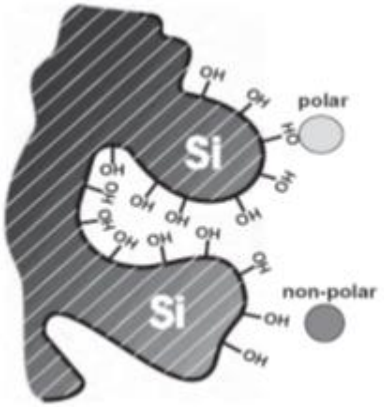
-GC

(4) Based on the chemical nature of stationary phase and mobile phase.

(a) Normal-phase chromatography:

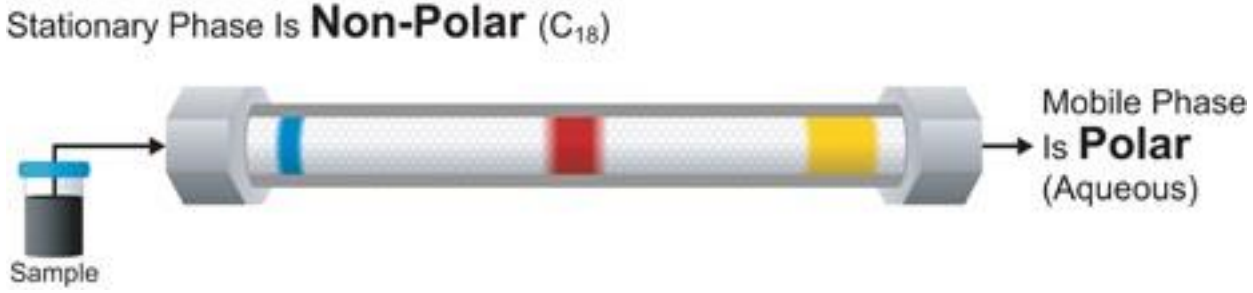
Here the stationary phase is polar in nature and the mobile phase is in non-polar nature.

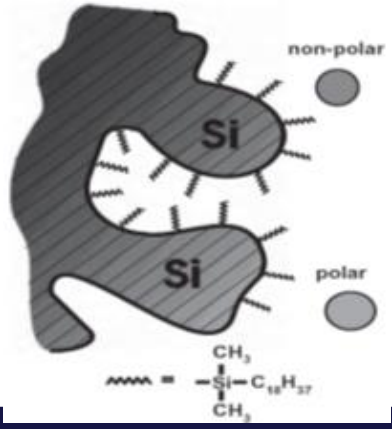




(b) Reverse-phase chromatography:

This is reverse to the above method. The stationary phase is non-polar in nature and the mobile phase is in polar nature.





(5) Based on the purpose of chromatography experiment.

(a) Analytical chromatography:
Used for smaller amounts of materials.
-Qualitative analysis: What is in the sample?
-Quantitative analysis: How much is in the sample?

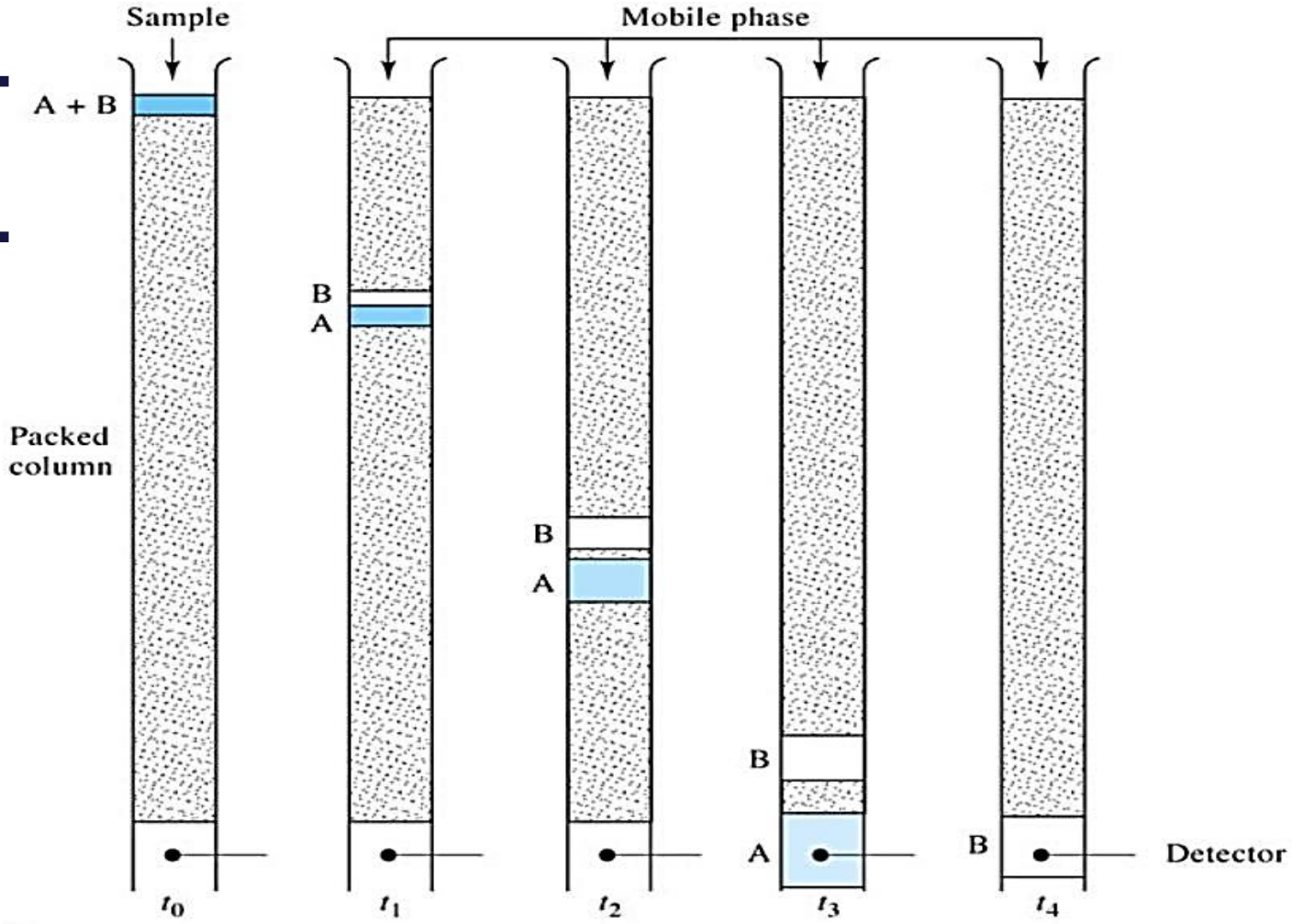
(b) Preparative chromatography:
Used for larger amounts of materials and to separate the components of a mixture for more advanced use (purification and sample preparation).

Classification of Column Chromatographic Methods

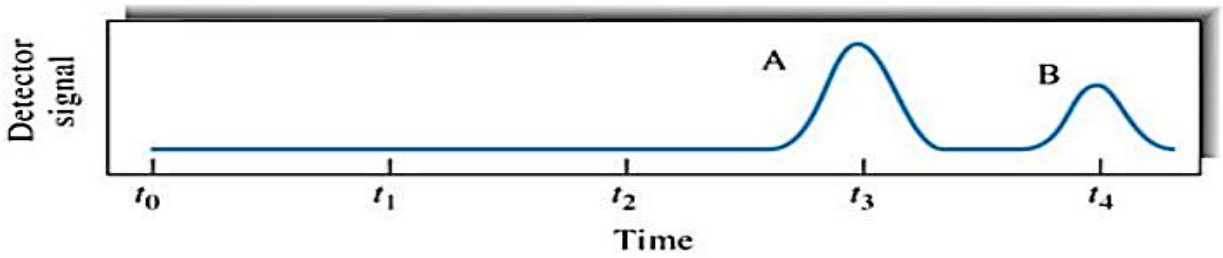
General classification	Specific method	Stationary phase	Type of equilibrium
Liquid chromatography (LC) (m.p.: liquid)	Liquid-liquid, or partition	Liquid adsorbed or bonded on a solid	Partition between immiscible liquids or between liquid and bonded phase
	Liquid-solid, or adsorption	Solid	Adsorption
	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
Gas chromatography (GC) (m.p.: gas)	Gas-liquid	Liquid adsorbed or bonded on a solid	Partition between gas and liquid or between liquid and bonded surface
	Gas-solid	Solid	Adsorption
Supercritical-fluid chromatography (SFC) (m.p.: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical and bonded surface

Elution Chromatography on Columns

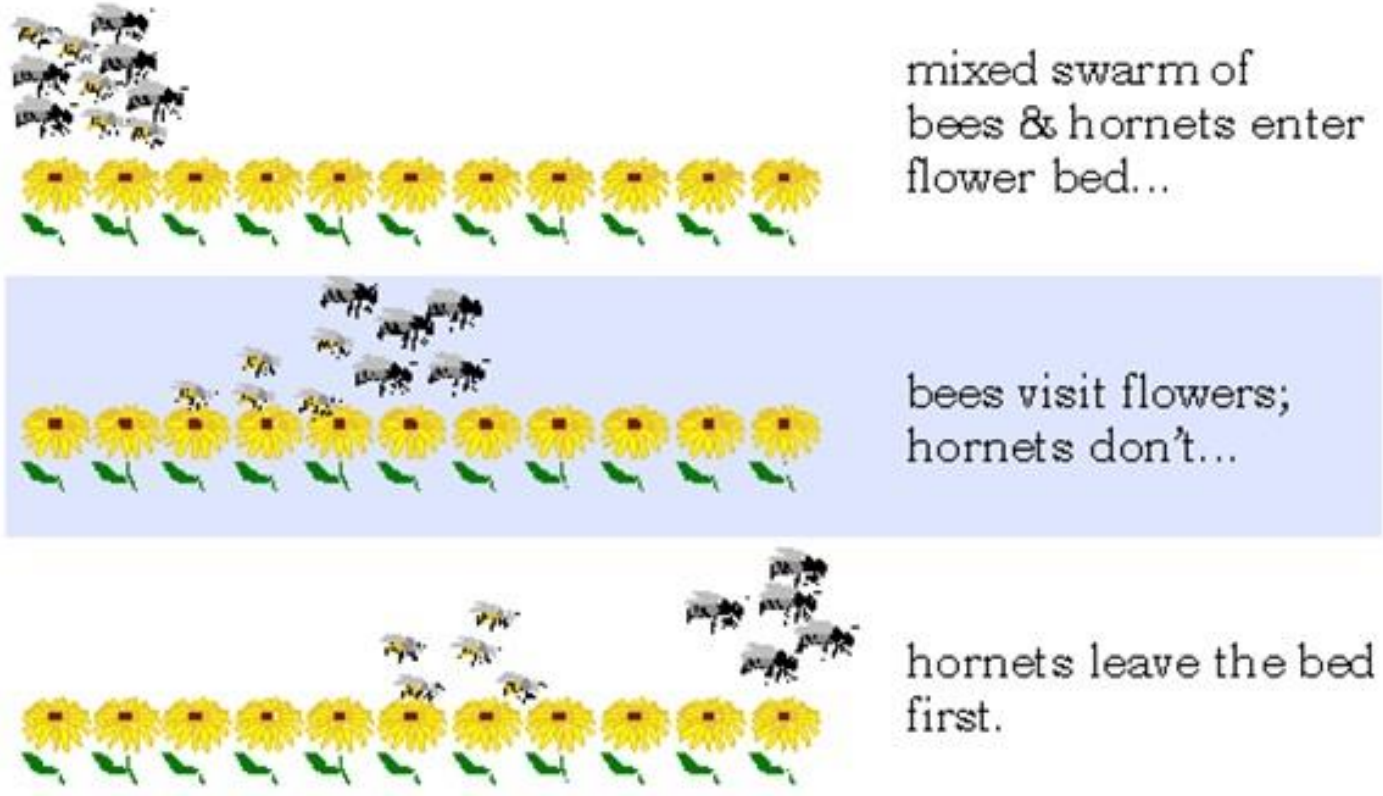
Separation of a mixture of components (A & B)



The output of the signal detector



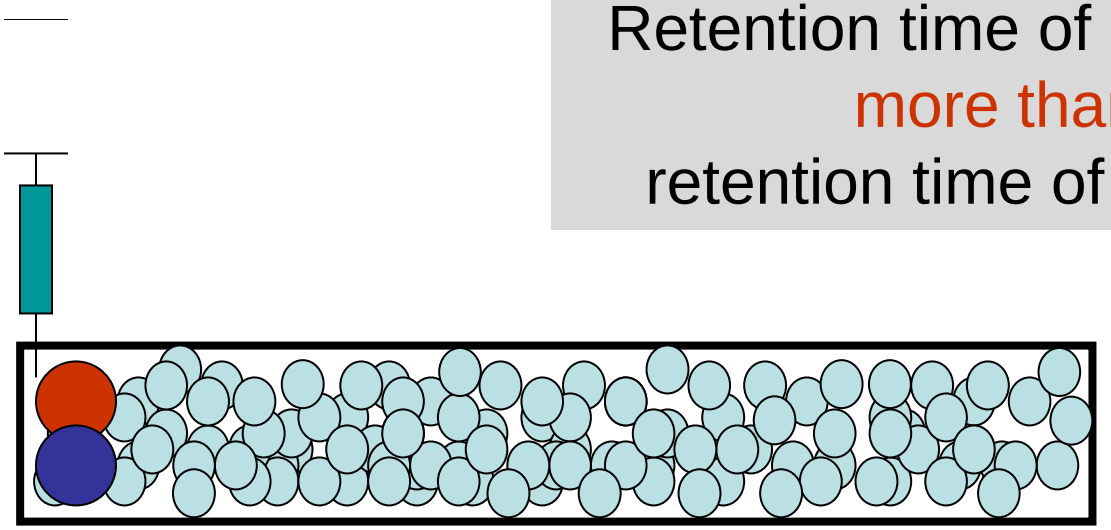
An Analogy for Chromatographic Separation



Like dissolve like (like attract like)
Non-polar stationary phases best for non-polar analytes
Polar stationary phases best for polar analytes

Like dissolve like (like attract like)

Retention time of glucose is more than retention time of fructose



polar **SP**

Fructose

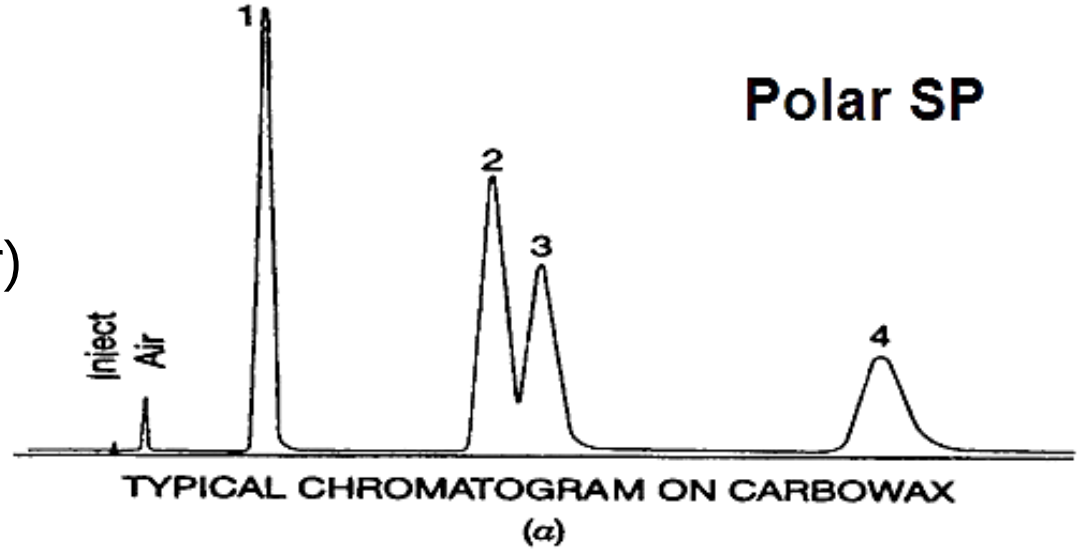
Glucose

SP

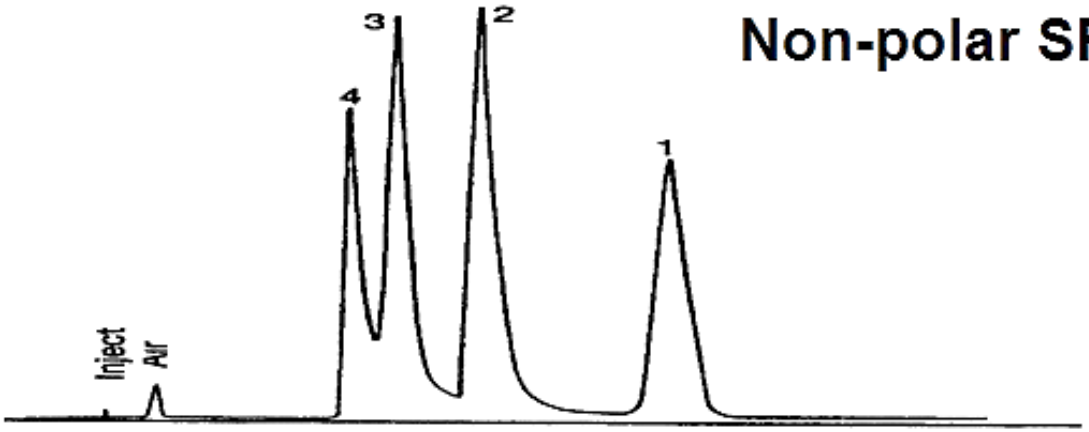
Glucose is more polar than **fructose** and is more attracted to SP and therefore travels slower through column.

Effect of Stationary Phase on Retention

(**1**) n-heptane (less polar)
(**2**) tetrahydrofuran
(**3**) 2-butanone
(**4**) n-propanol (more polar)

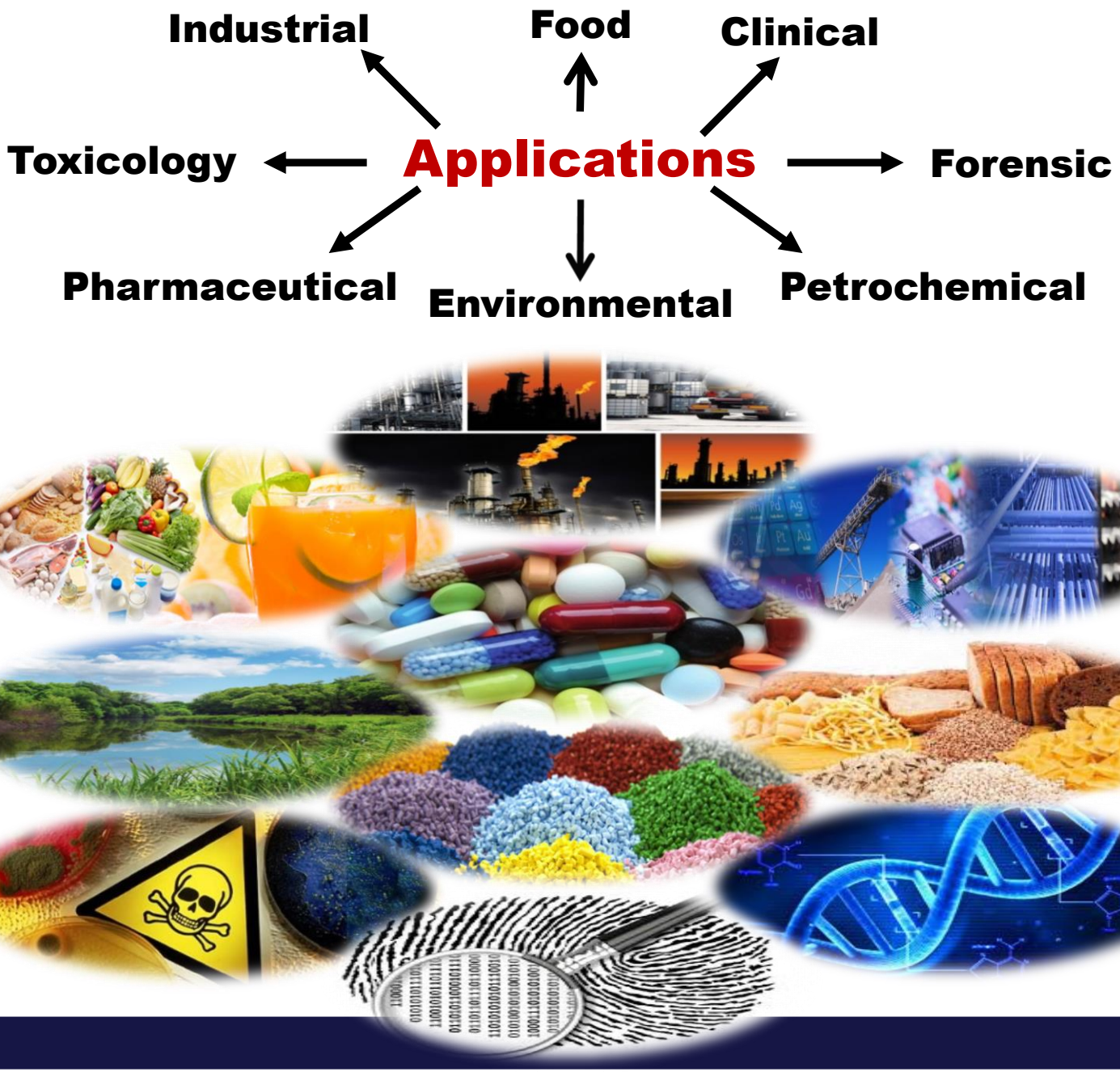


TYPICAL CHROMATOGRAM ON CARBOWAX
(*a*)

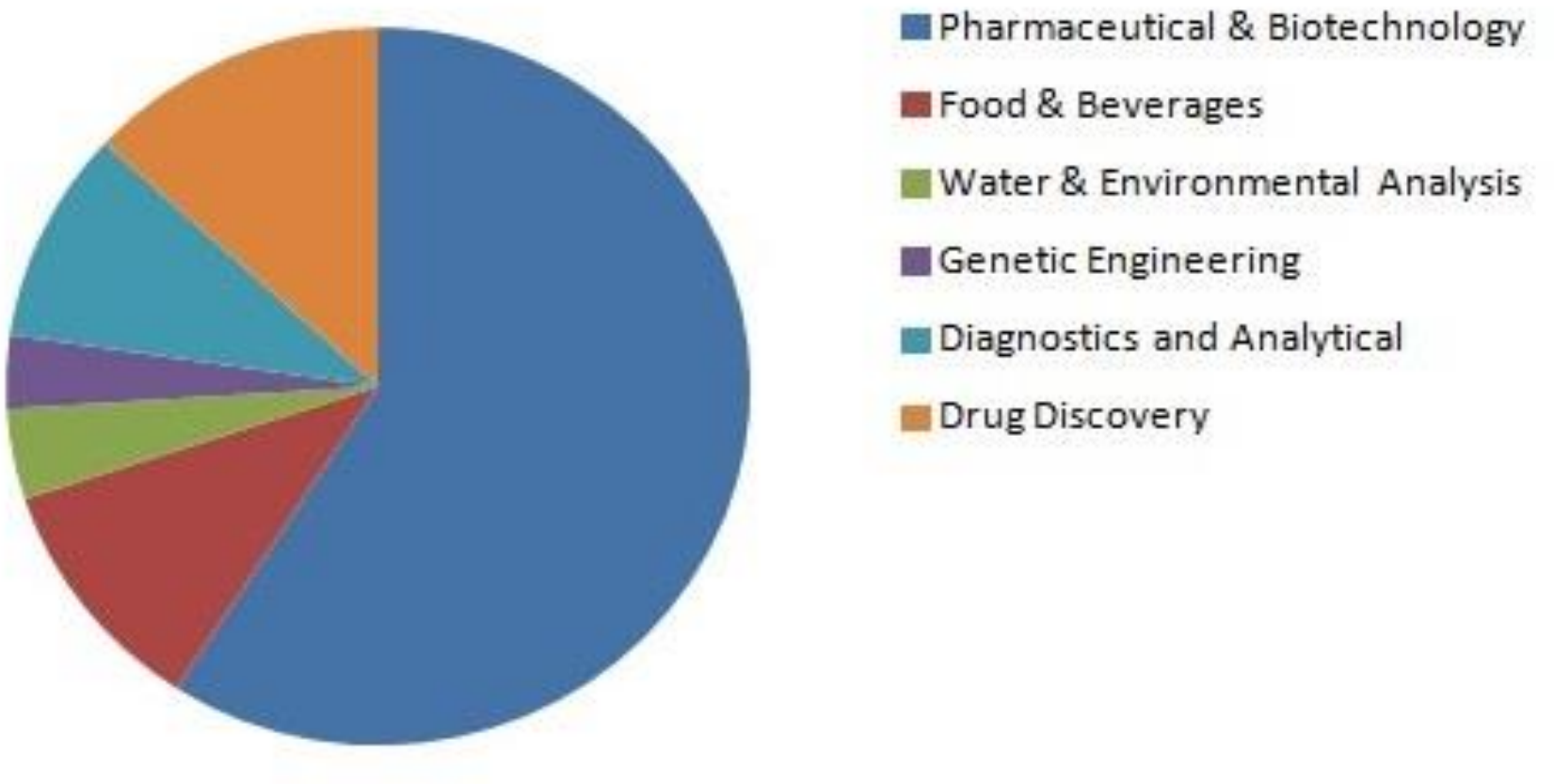


TYPICAL CHROMATOGRAM ON DC 200
(*b*)

Application Areas

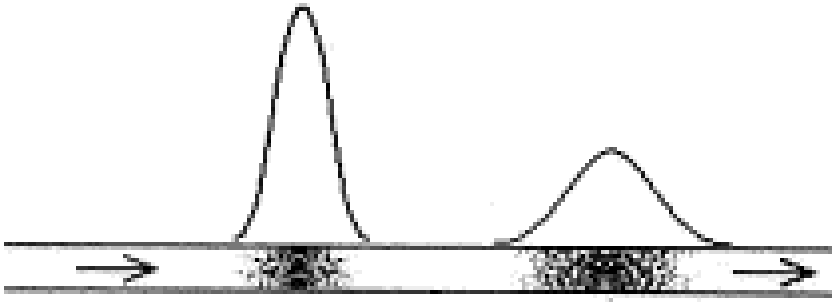


Application Segments

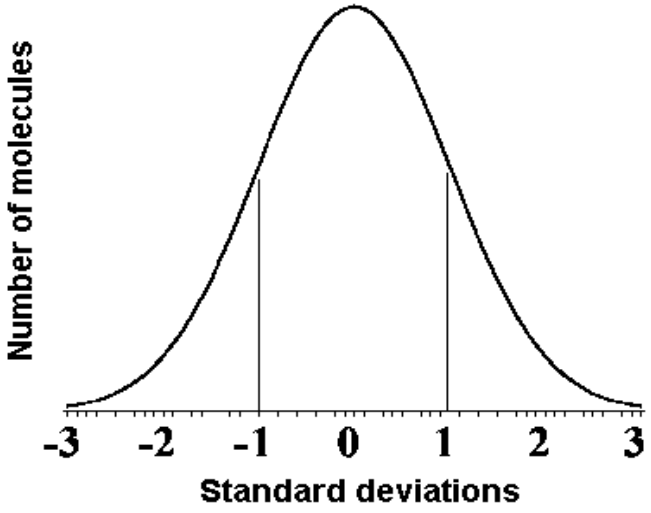


Peak Shape

The recorder will give **peak shape** exactly like the **mass distribution peak**.



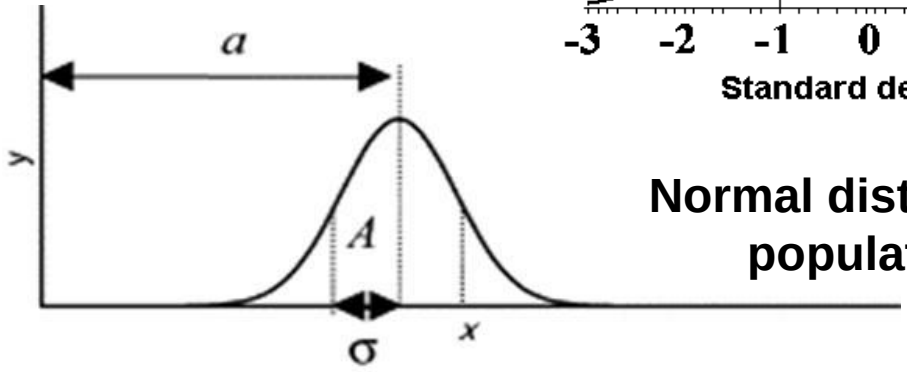
The mass distribution is symmetrical throughout but the sample zone is expanding while moving, the detector will record a steeper peak front and a prolonged peak tail. The zone breadth is directly related to residence time on the column and inversely related to the velocity at which the mobile phase flows.



Basic Gaussian curve:

(A,a,σ are independent)

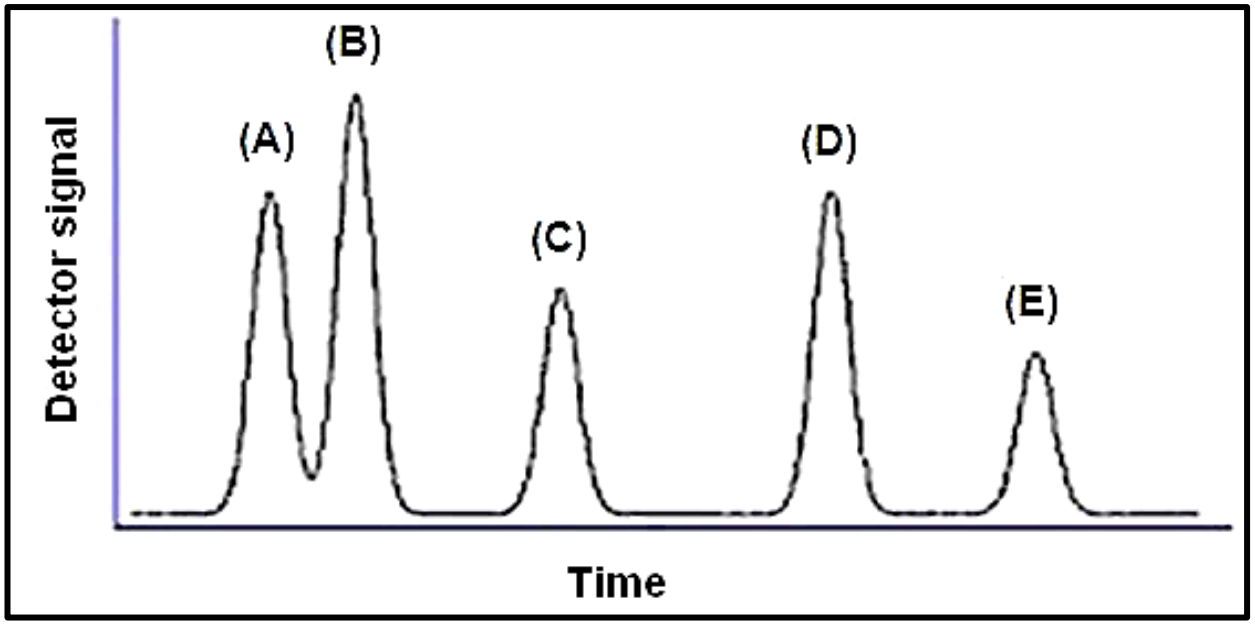
$$y(x) = \frac{A}{\sqrt{2\pi}\sigma} e^{-[(x-a)/(\sqrt{2}\sigma)]^2}$$



Normal distribution population

Chromatograms

If a detector is placed at the end of the column and its signal is plotted as function of time, a series of peaks is obtained. Such a plot, called a **chromatogram**. The chromatogram can be used to provide information on separation process.

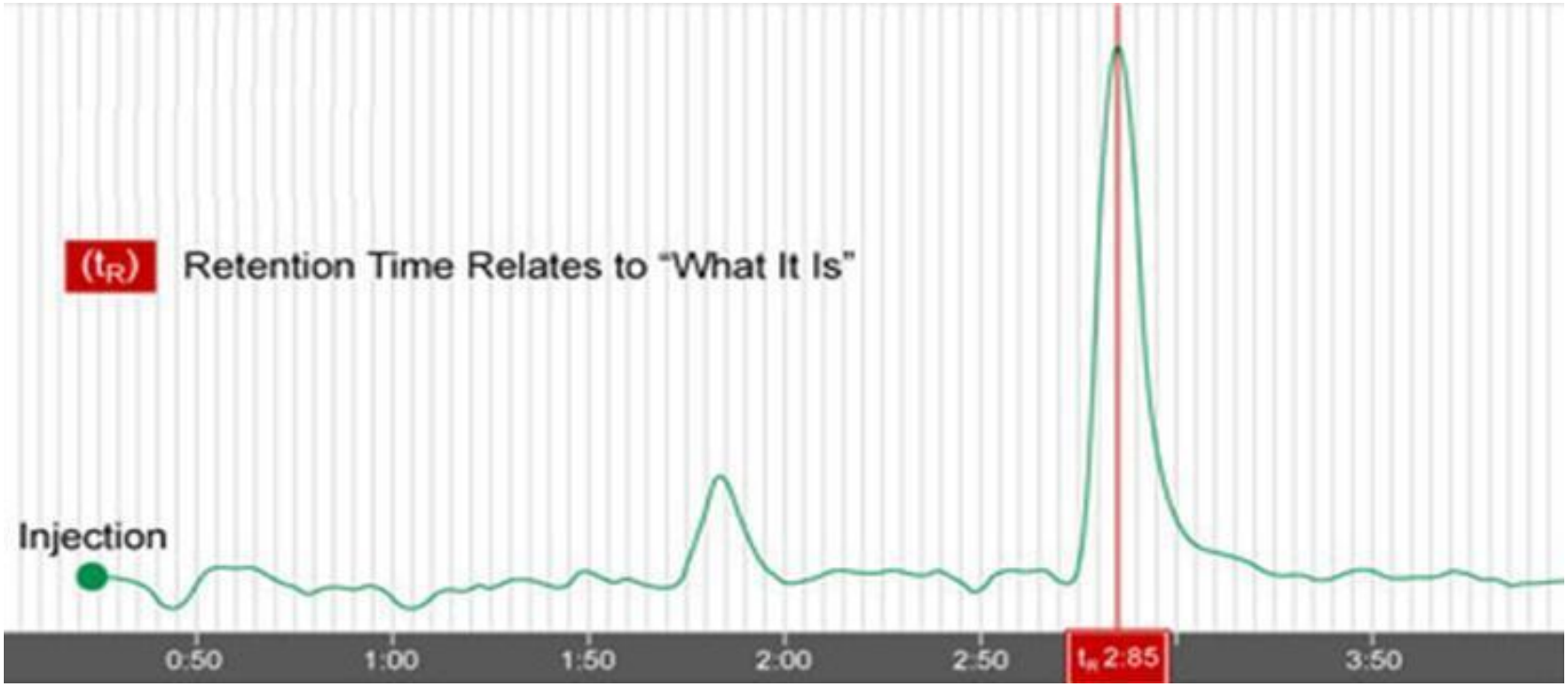


Typical chromatogram of detector response as a function of retention time.

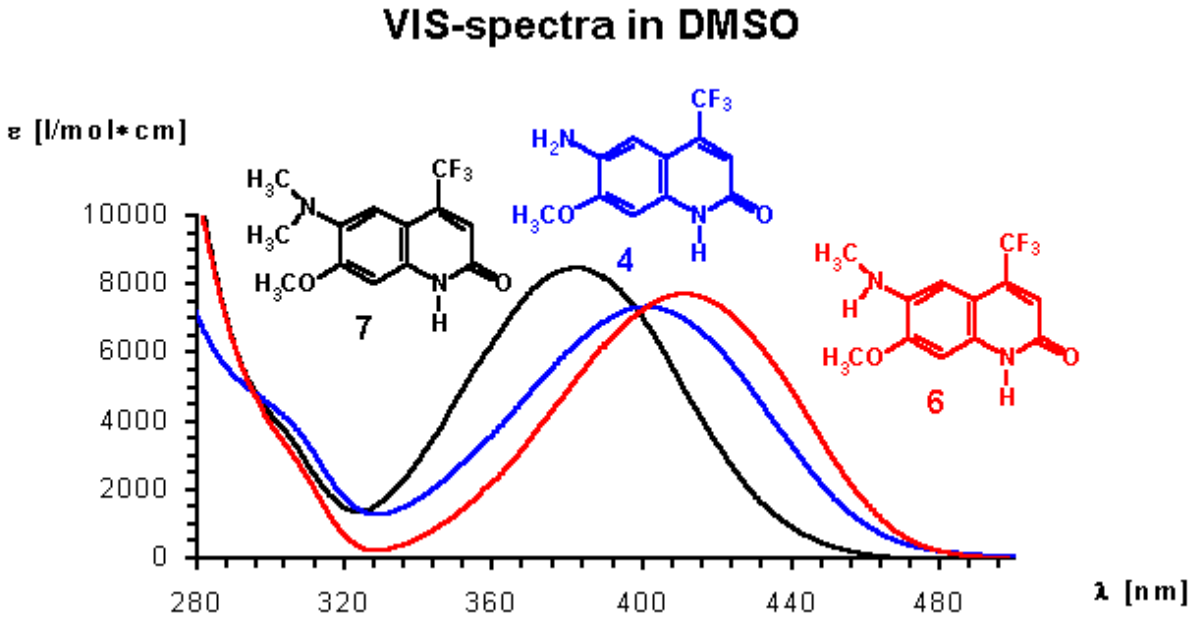
A chromatogram is useful for both **qualitative** and **quantitative** analysis;
-Every peak represents one component.
-The positions of peaks on the time axis may serve to identify the components of the sample.
-The peaks heights or areas provide a quantitative measure of the amount of each component.
-Species E is more strongly retained; thus, E lags during the migration.

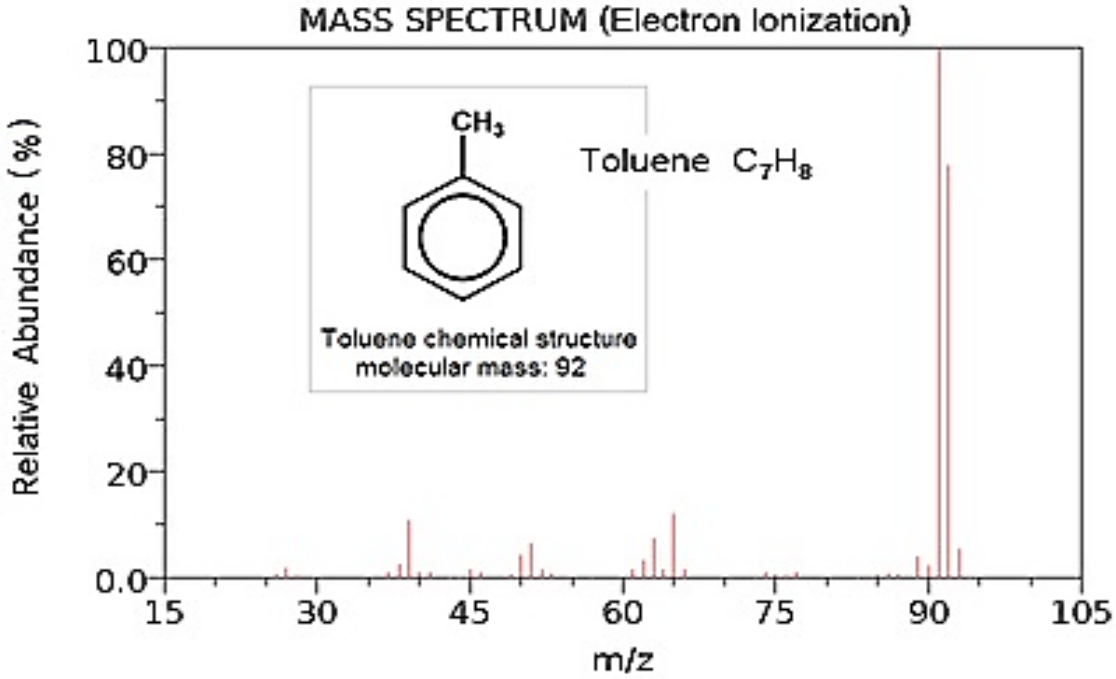
Qualitative analysis

-Qualitative analysis is the identification of the mixture constituents separated by chromatography.
-It is generally based on comparison of reference standards to the unknown chromatogram peaks.
-Components having the same retention time are assumed to be the same.
-It is a widely used tool for recognizing the presence or absence of components of mixtures containing a limited number of possible species whose identities are known and to check the purity of a specific substance.



UV-spectra (diode array detector, DAD)

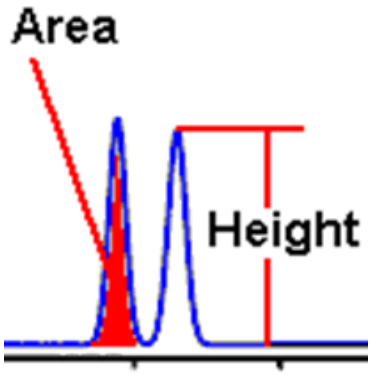


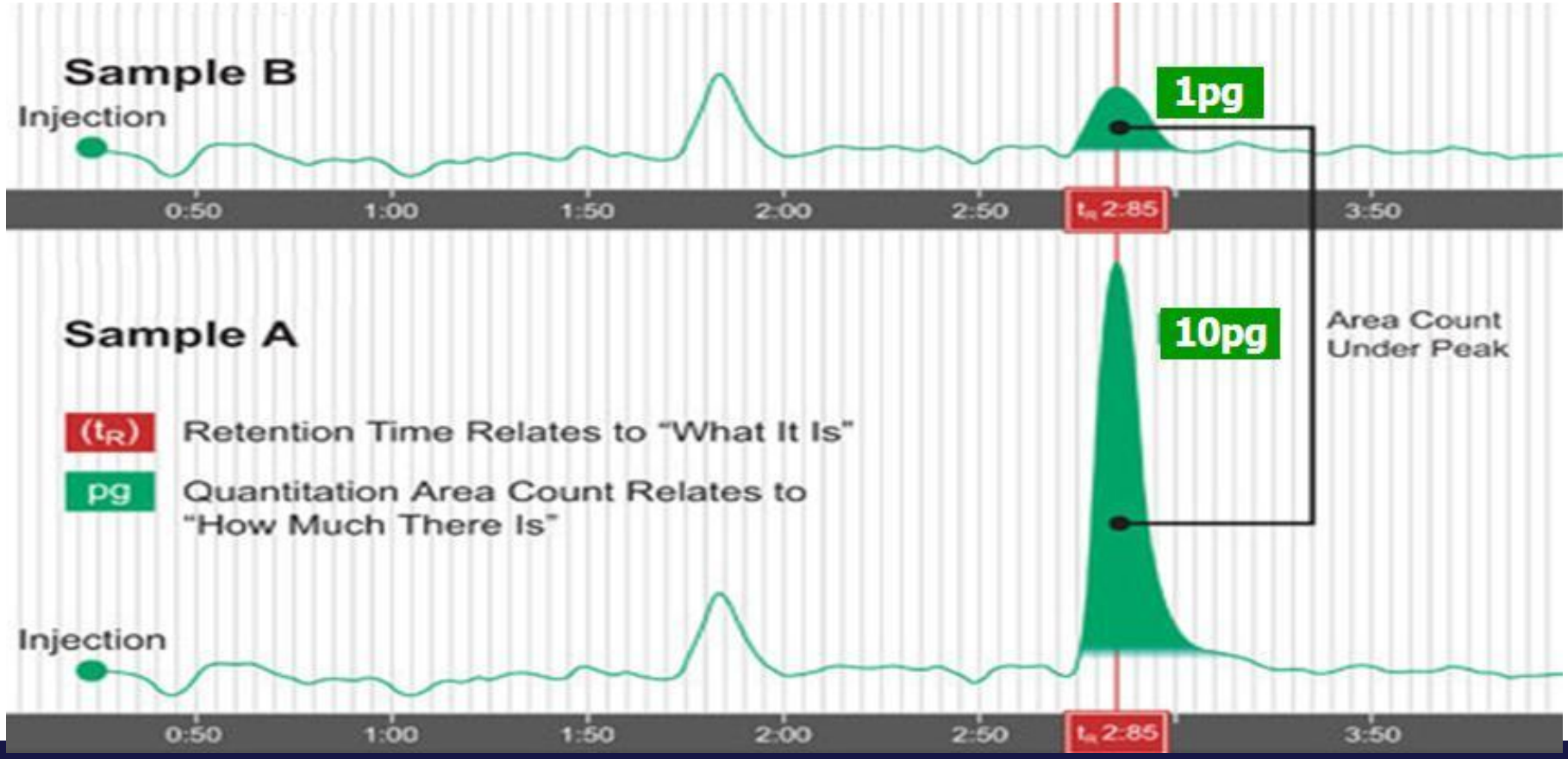


Mass spectra (mass spectrometric detector, MS)

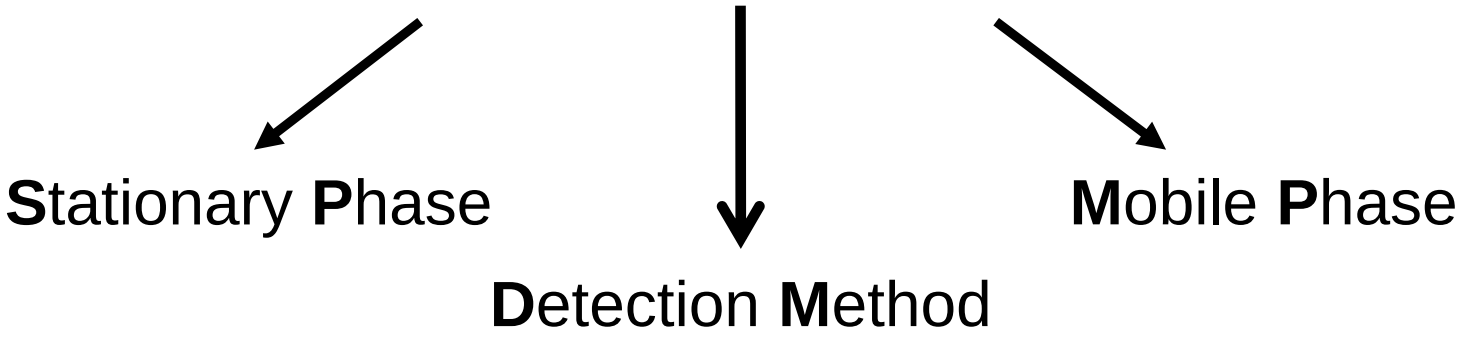
Quantitative analysis

-Quantitative analysis is the determination the concentration of each constituent in the separated mixture.
-Quantitative column chromatography is based upon a comparison of either **height** or **area** of the analyte peak with that of standards.
-If conditions are properly controlled, these parameters vary linearly with concentration.





Selection of an **appropriate** separation **method**



The goal in chromatography is the **highest possible resolution in the shortest possible elapsed time**. Unfortunately, these goals tend to be incompatible and cannot both be optimized under the same conditions, consequently, a compromise between the two is usually necessary.

Chromatographic Methods are Compromised Techniques


Thank You!