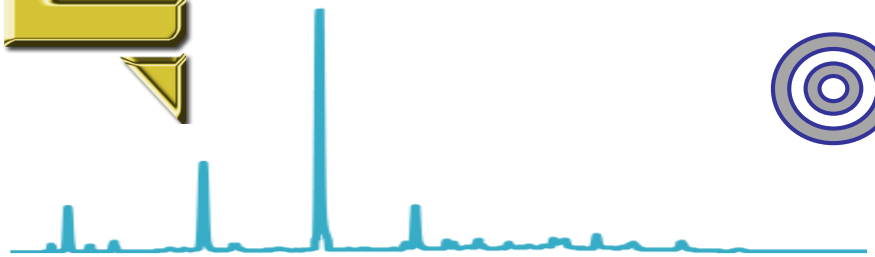
بسم الله الرحمن الرحيم

Chem 458
Introduction to chromatography techniques



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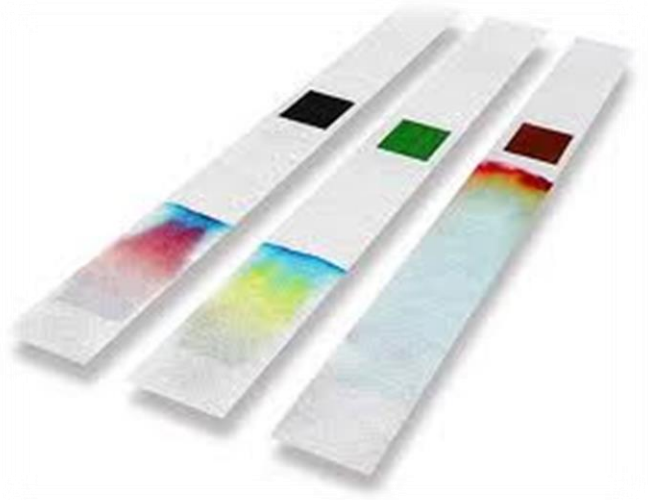
Definition

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**".

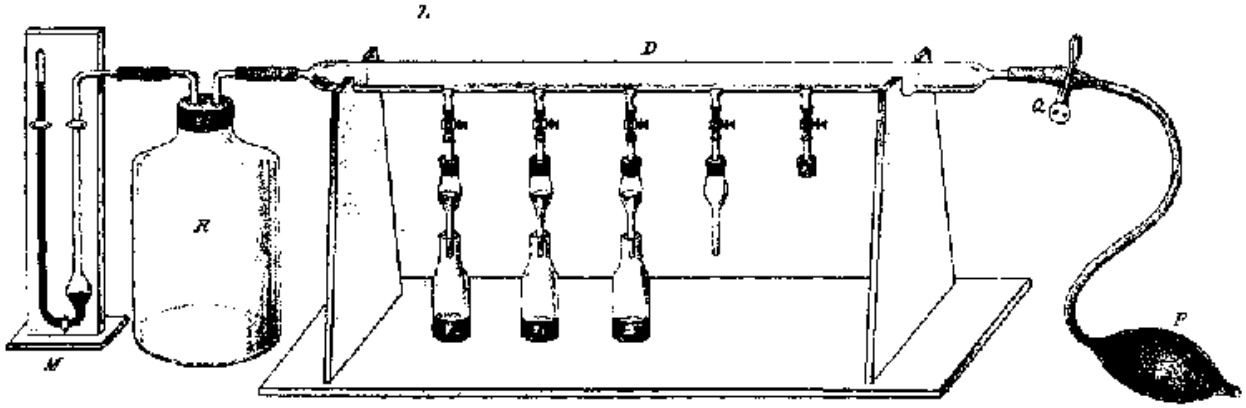


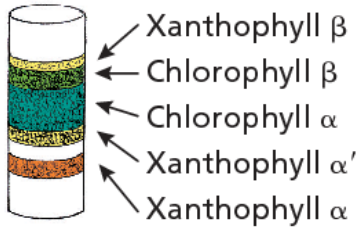
Brief History of Chromatography

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





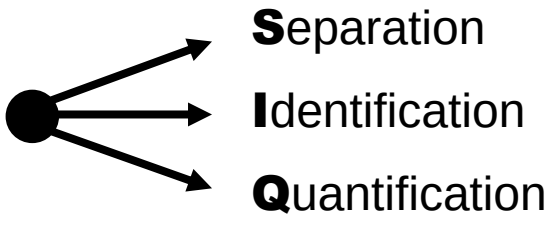
1930:	Classical columns	1970:	HPLC
1940:	Paper chromatography	1980:	SFC
1950:	GC	1990:	CE
1960:	TLC	2000:	CEC

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.

Chromatographic Techniques Applications

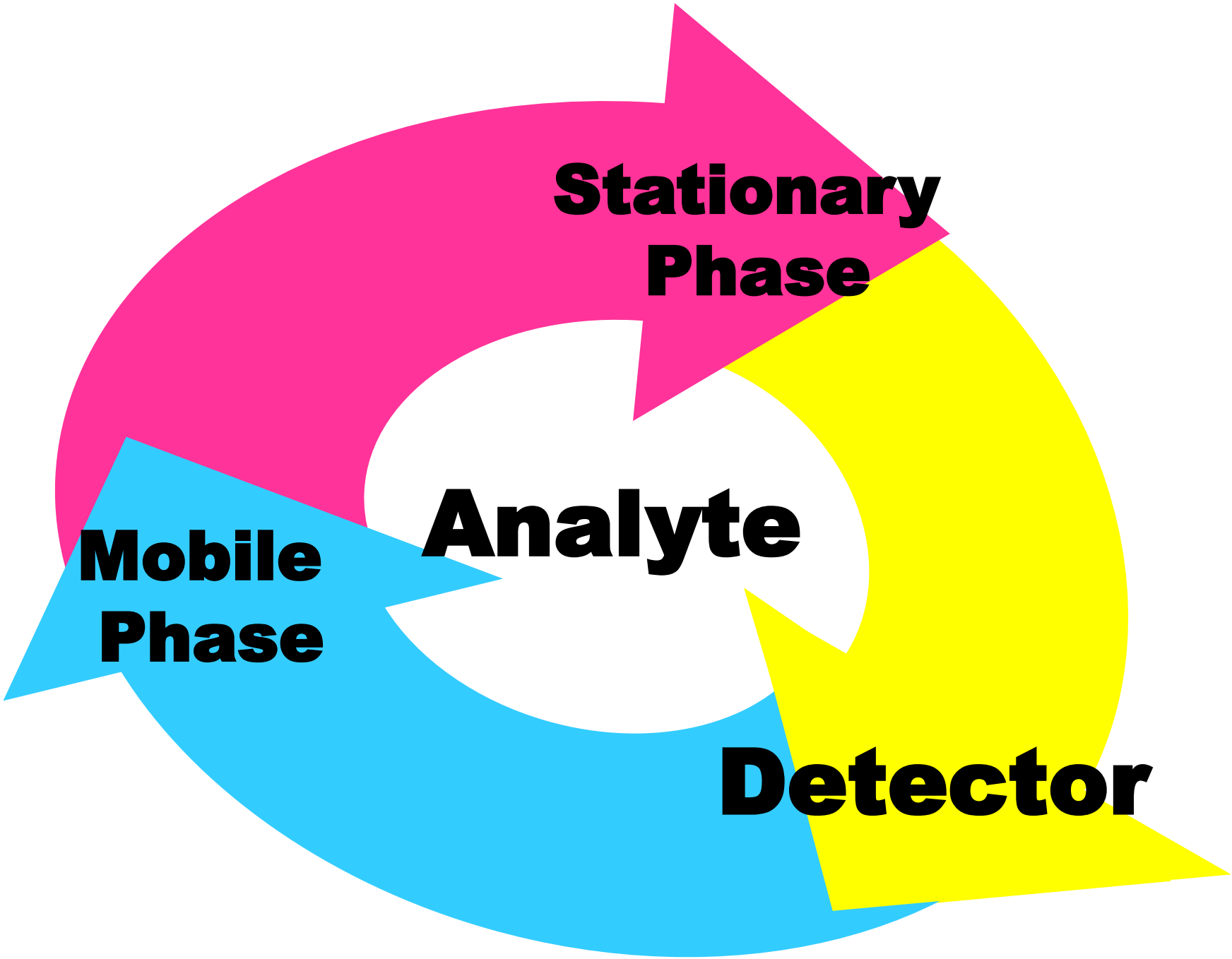
Chromatography is the collective term for a **family** of laboratory techniques for the separation of mixtures.

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

Annual growth rate about **5.5**%

Chromatography resins market size
1.5 billion USD in 2014
(natural, synthetic, inorganic media)

Annual growth rate about **7.3**%

HPLC

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

Classification of Chromatographic Methods

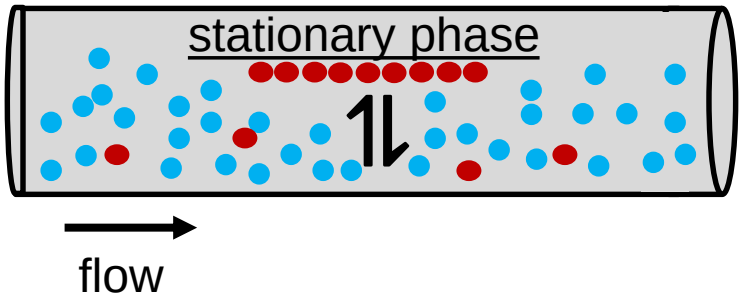
Chromatographic methods can be categorized in several ways:

(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.



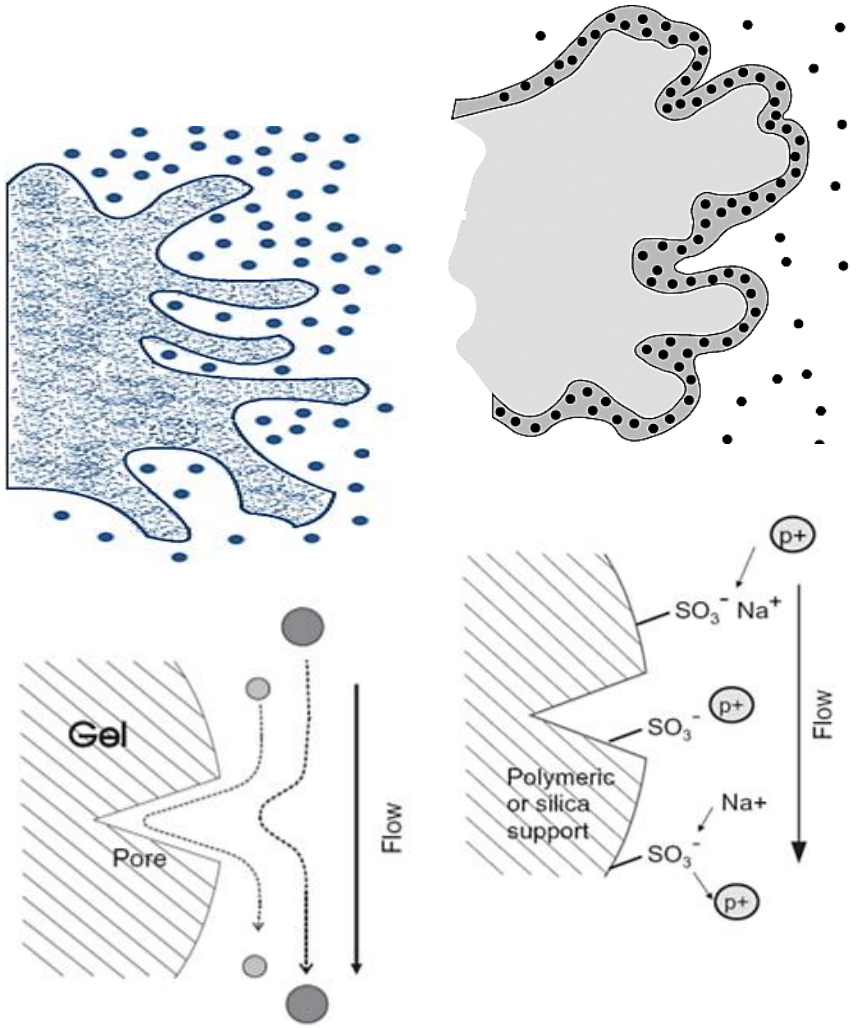
(2) Based on the kind of equilibria involved in the transfer of solutes between phases, principle of separation used (separation mechanism).

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

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

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

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



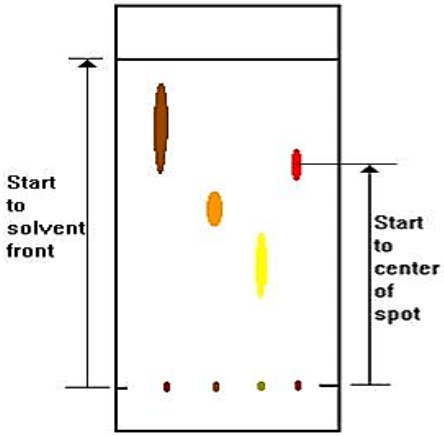
(3) Based on the shape of stationary phase, surface on which the separation to be performed or the way on which the mobile phase pass through the stationary phase.

(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.

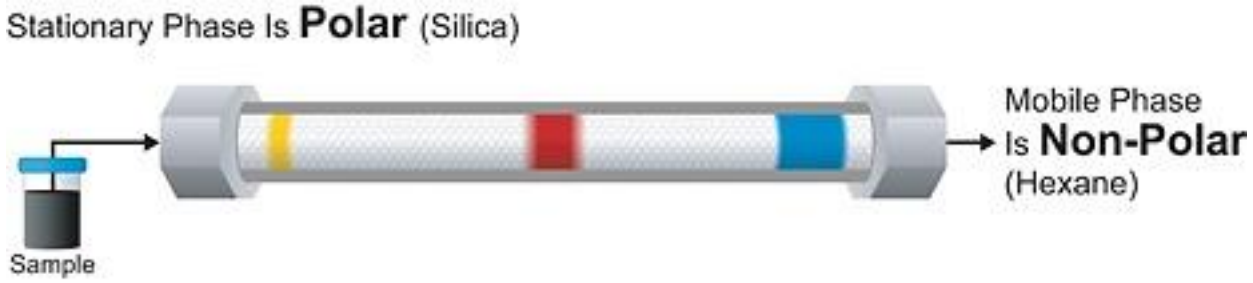
-HPLC

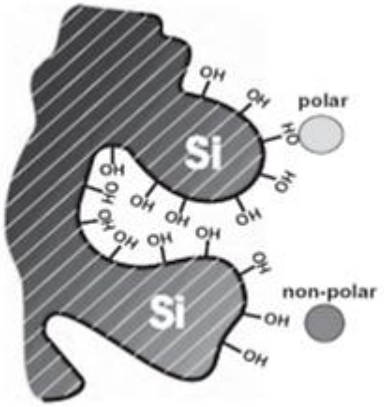
-GC

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

(a) Normal-phase chromatography:

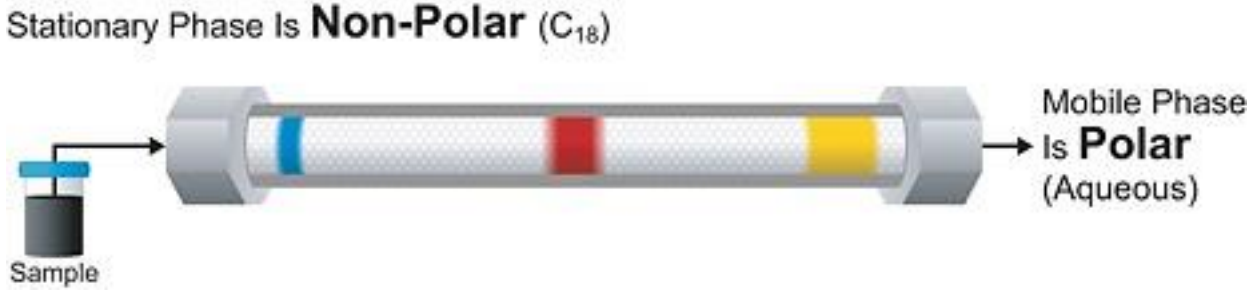
Here the stationary phase is polar in nature and the mobile phase is in non-polar nature.

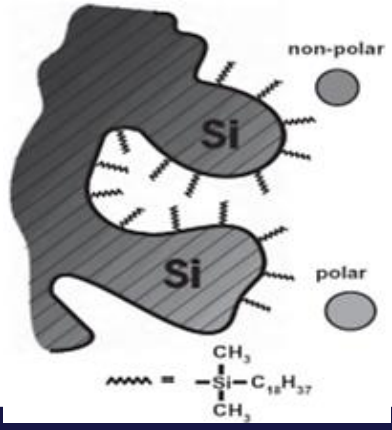




(b) Reverse-phase chromatography:

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?

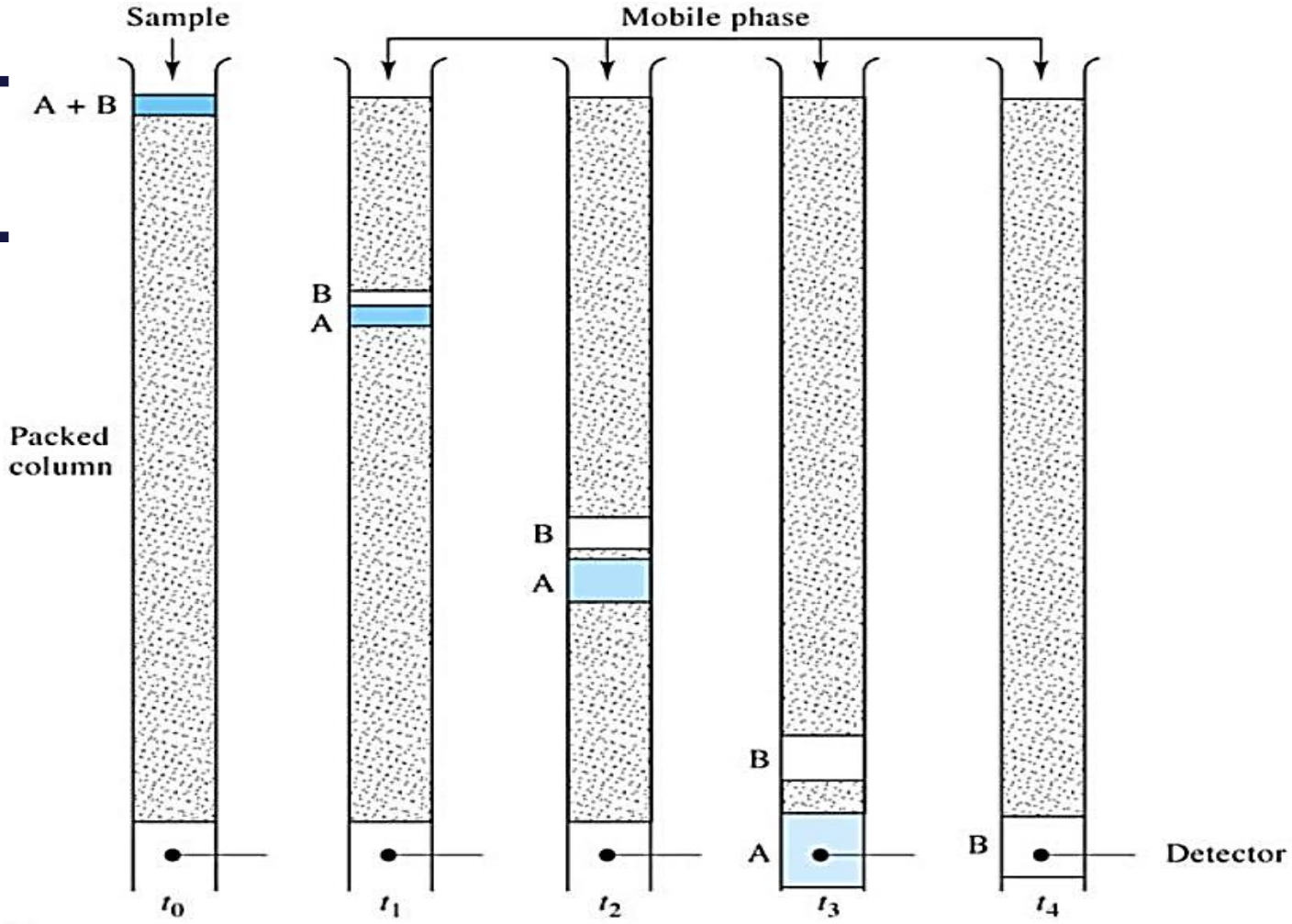
(b) Preparative chromatography:
Used for larger amounts of materials and to separate the components of a mixture for more advanced use (purification and sample preparation).

Classification of Column Chromatographic Methods

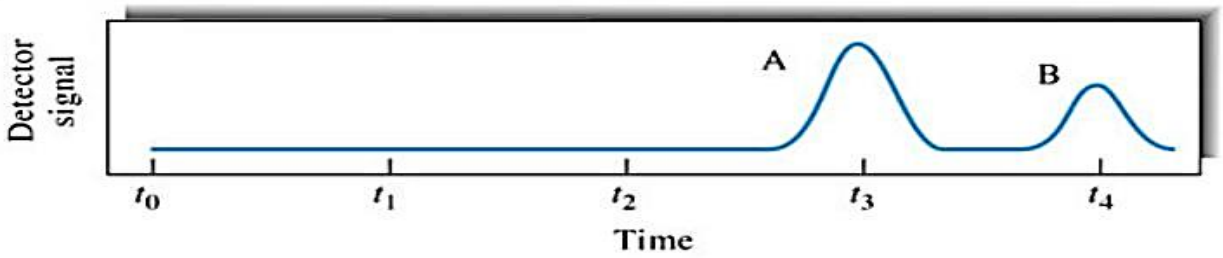
General classification	Specific method	Stationary phase	Type of equilibrium
Liquid chromatography (LC) (m.p.: liquid)	Liquid-liquid, or partition	Liquid adsorbed or bonded on a solid	Partition between immiscible liquids or between liquid and bonded phase
	Liquid-solid, or adsorption	Solid	Adsorption
	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

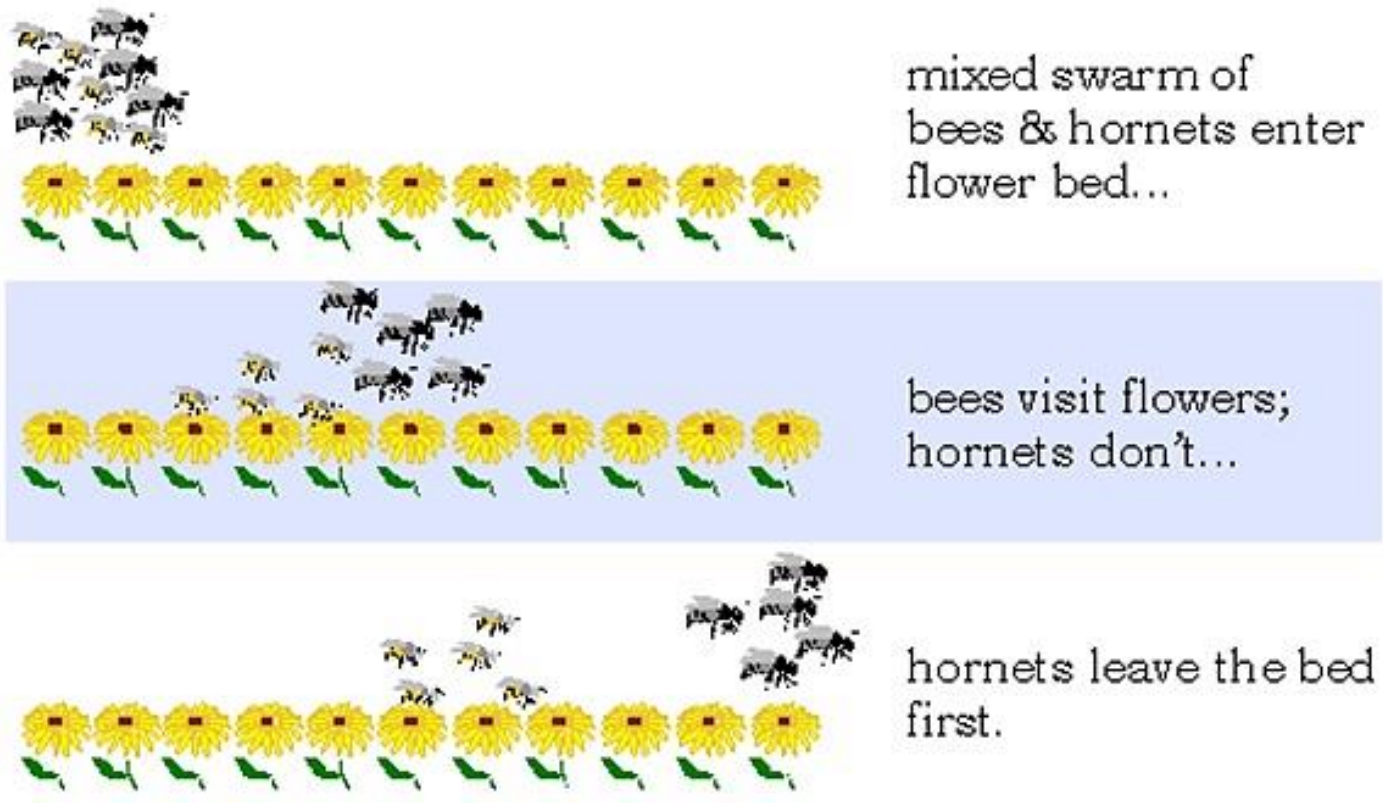
Separation of a mixture of components (A & B)



The output of the signal detector



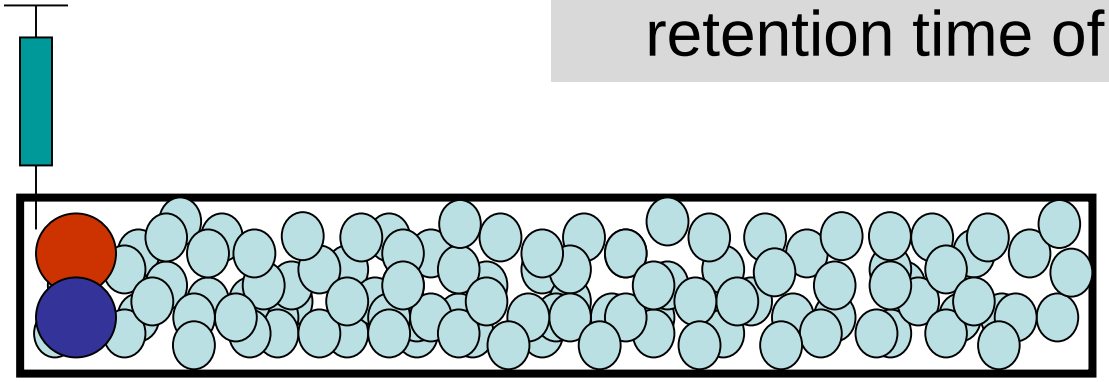
An Analogy for Chromatographic Separation



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

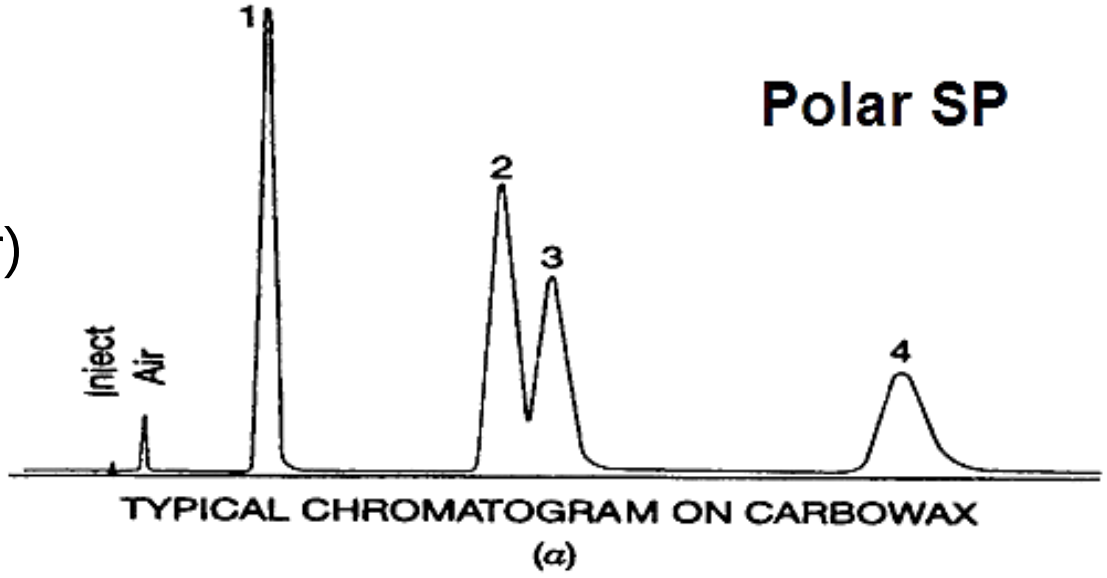
Glucose

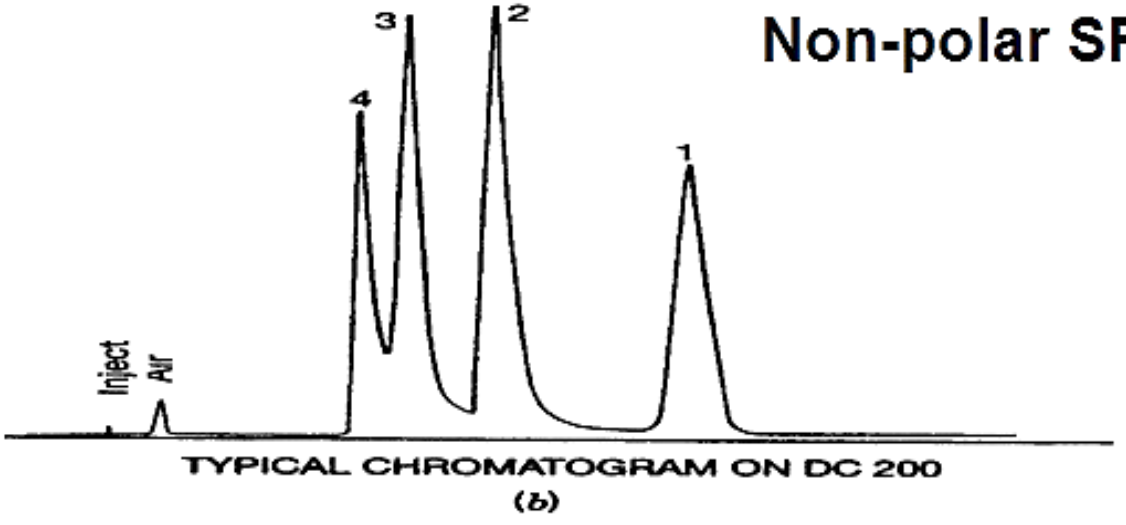
SP

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

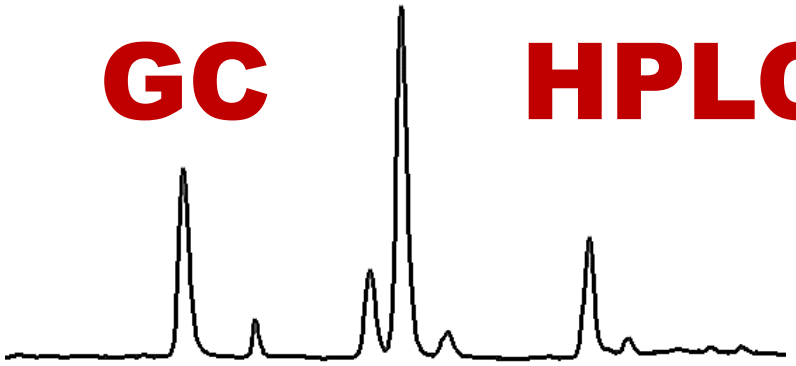
Effect of Stationary Phase on Retention

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

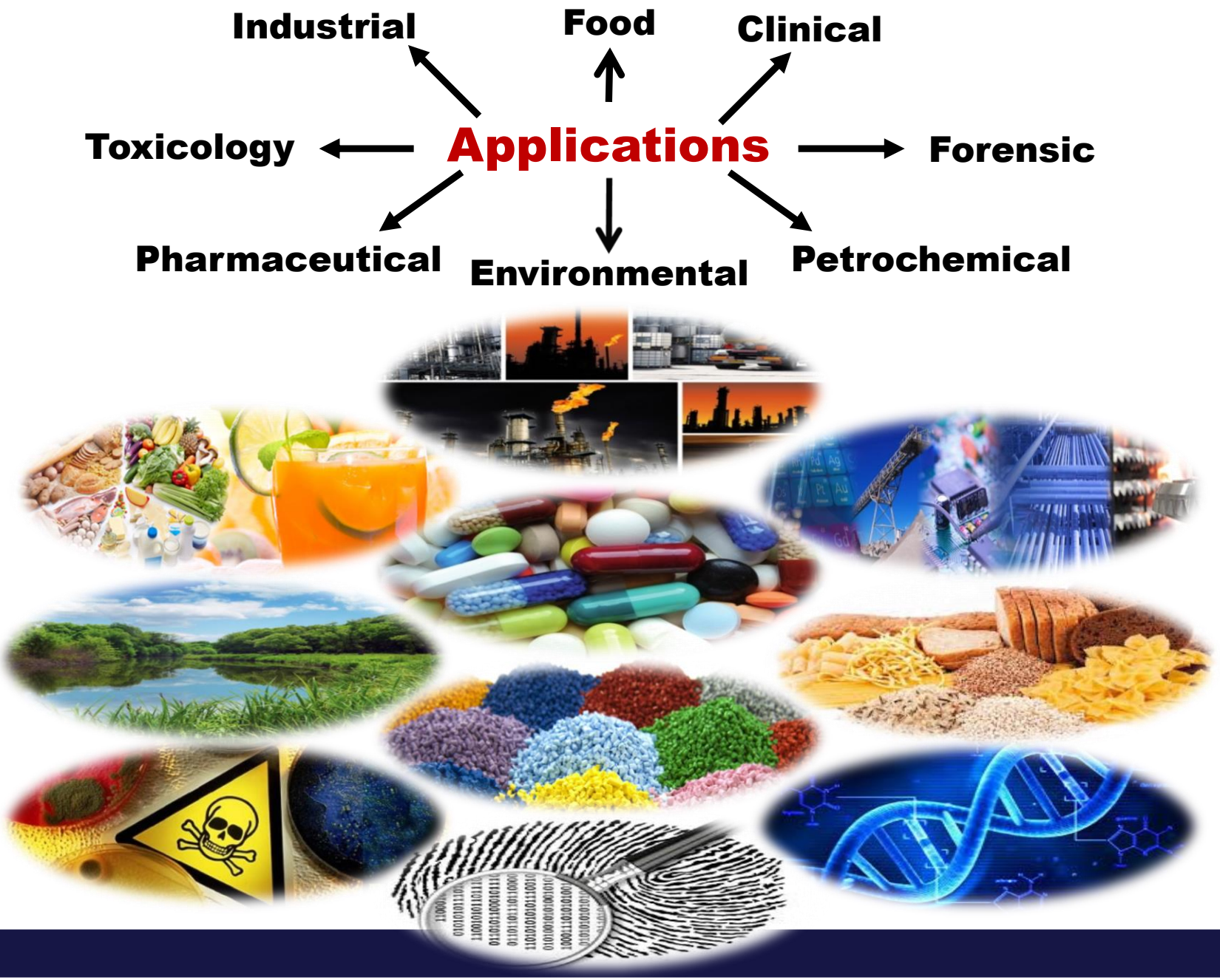




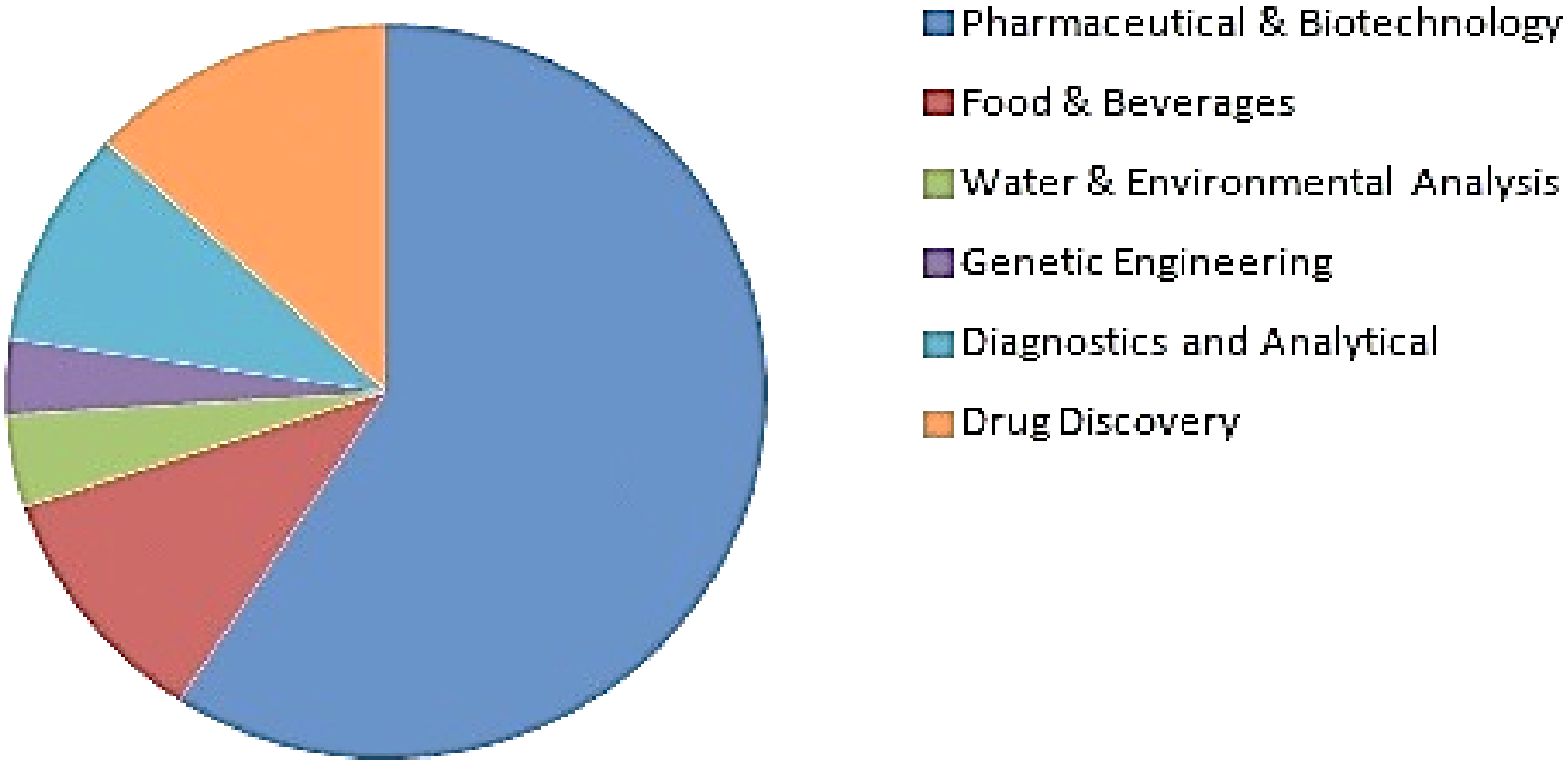
GC HPLC



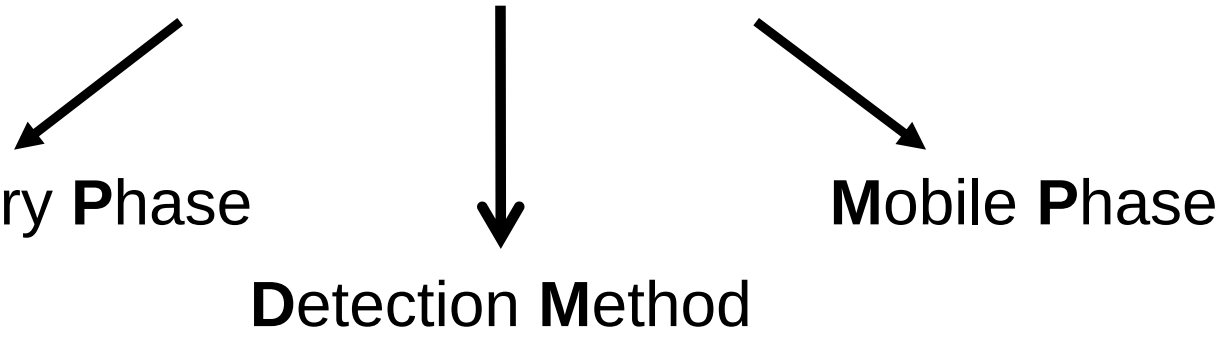
Application Areas



Application Segments



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 Techniques are Compromised Methods


Thank You!