6. Introduction to chromatography

- Chromatography is the most frequently used analytical technique in pharmaceutical analysis
- Refers to any separation method in which the components are distributed between a stationary phase (SP) and mobile phase(MP)

  Mobile phase: a solvent that flows through the column

  Stationary phase: a coating on the supporting medium that interacts with the analytes (immobilized in the column)
- One of the prime analytical methods for identification and quantification of cpds

Principles of separation

- The samples are subjected to flow by mobile liquid phase through the stationary phase
- Separation is based on their relative affinity towards the two phases
- Separations occur because sample components have different affinities for the stationary phase and mobile phase, therefore moves at different rates along the column
  - i.e. those components of the sample having great affinity for the stationary phase will move very slowly and with less affinity travels faster
- The mobile phase is usually a liquid or a gas, and the stationary phase is a solid or a liquid film coated on a solid surface

Classification of chromatography

Chromatographic methods can be classified in various ways:

1. Based on geometry of the system
2. Based on separation mechanism
3. Based on Mobile phase

Based on **geometry** of the system : There are two basic geometries:

1. In a *column chromatography*

- A form of chromatography in which the stationary phase is retained in a column
- the stationary phase is placed in a narrow column through which the mobile phase moves under the influence pressure

2. planner chromatography

- A form of chromatography in which the stationary phase is immobilized on a flat surface
- the stationary phase coats a flat glass, metal, or plastic plate and is placed in a developing chamber
- A reservoir containing the mobile phase is placed in contact with the stationary phase, and the mobile phase moves by capillary action
    - Example: paper chromatography, thin-layer chromatography

Based on separation mechanism

Reading assignment

- chromatographic methods are classified as:

    1. Adsorption chromatography
    2. Partition chromatography
    3. Ion-exchange chromatography
    4. Size-exclusion chromatography

Based on Mobile phase

- Classification by mobile phases gives the physical state of the mobile phase followed by the stationary phase

1. Gas-chromatography

- Employed a gaseous fluid as the mobile phase, called carrier gas,
  - In gas–solid chromatography, the mobile phase is a gas whereas, the stationary phase is a solid and separation is based on adsorption
  - In gas-liquid chromatography, the mobile phase is gas whereas, the stationary phase is a liquid, and its separation is based on partition between gas and liquid

Based on Mobile phase Cont…

2. Liquid-chromatography

- This form of chromatography employs a liquid mobile phase
  - Liquid-solid chromatography utilizes a solid stationary phase, and the major mechanism of retention is adsorption
  - A liquid-liquid chromatography employs a liquid stationary phase, and its mechanism of separation is based on partition between two immiscible liquid phases

Some terminologies in chromatography

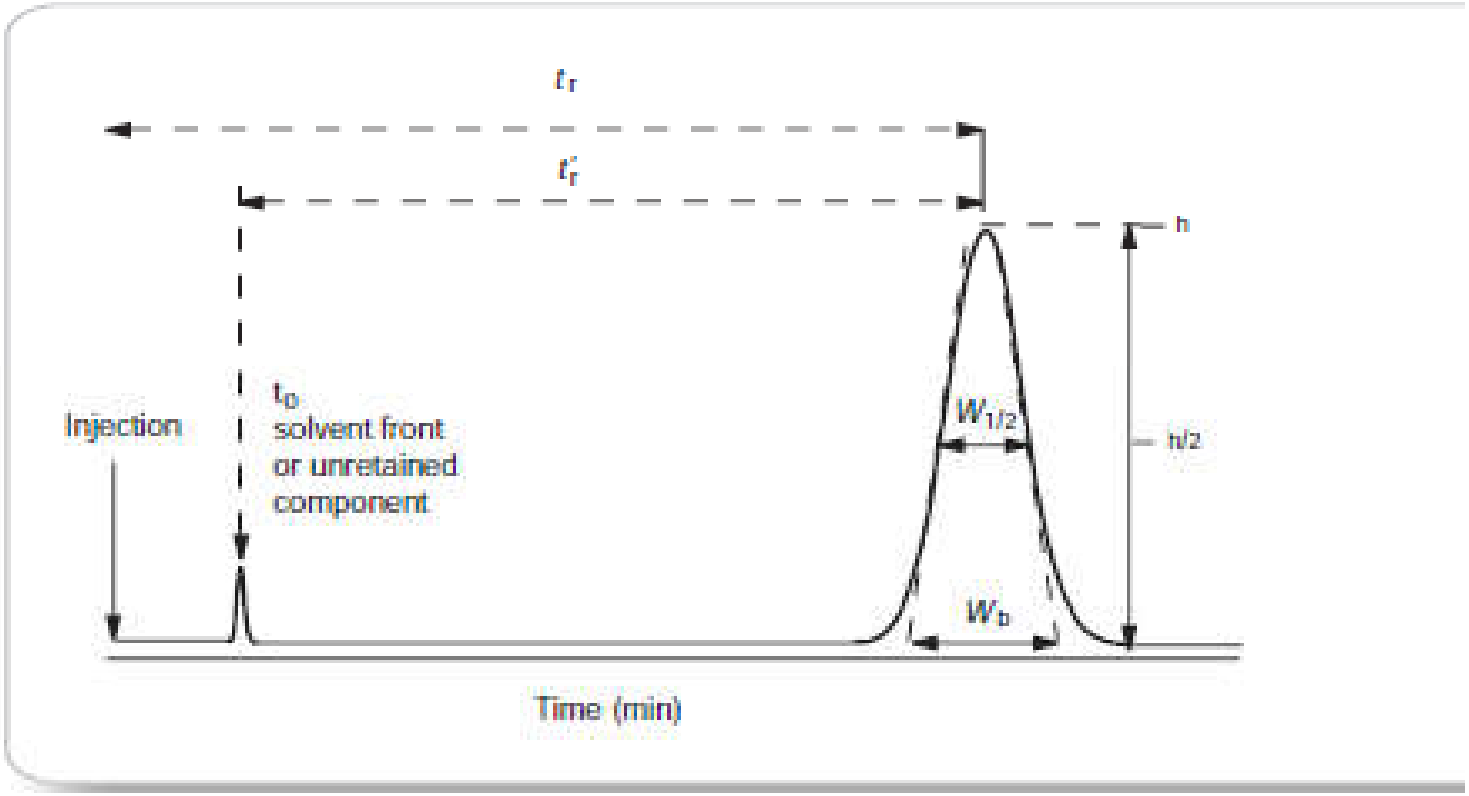
Retention time (t_R)

- Is the time taken between the point of injection and the peak maximum recorded on the chromatogram
- The total time that a compound spends in both the mobile phase and stationary phase
- is the time taken for the analyte to pass through the column
- It is generally reported in minutes

Dead time (t_m)

- is the time taken for an unretained molecule to pass through the column
- the time a non retained compound spend in the mobile phase
- Reported in minutes

Some terminologies in chromatography……



Some terminologies in chromatography……

Adjusted retention time (t'_R):

- It is the time the compound spends in the stationary phase
- Is the difference b/n the dead time and the retention time for a cpd

Capacity factor (K):

- It is the ratio of the time the component spends in the stationary phase to the time in the mobile phase
- A measure of how strongly a solute is retained by the stationary phase

$$k = (t_R - t_M)/t_M$$

- It is a measure of the column's retention of a cpd
- It is unit less
- Small k = the compound is poorly retained
- Large k values imply a good separation but longer analysis times
- High retention factors (greater than 20) mean that elution takes a very long time
- Ideally, the retention factor for an analyte is between 1 and 5.

Example:

In a chromatographic analysis of a drug sample , the drug elutes with a retention time of 7.63 min. The column's dead time is 0.31 min. Calculate the capacity factor for the drug.

Selectivity (separation) factor, α

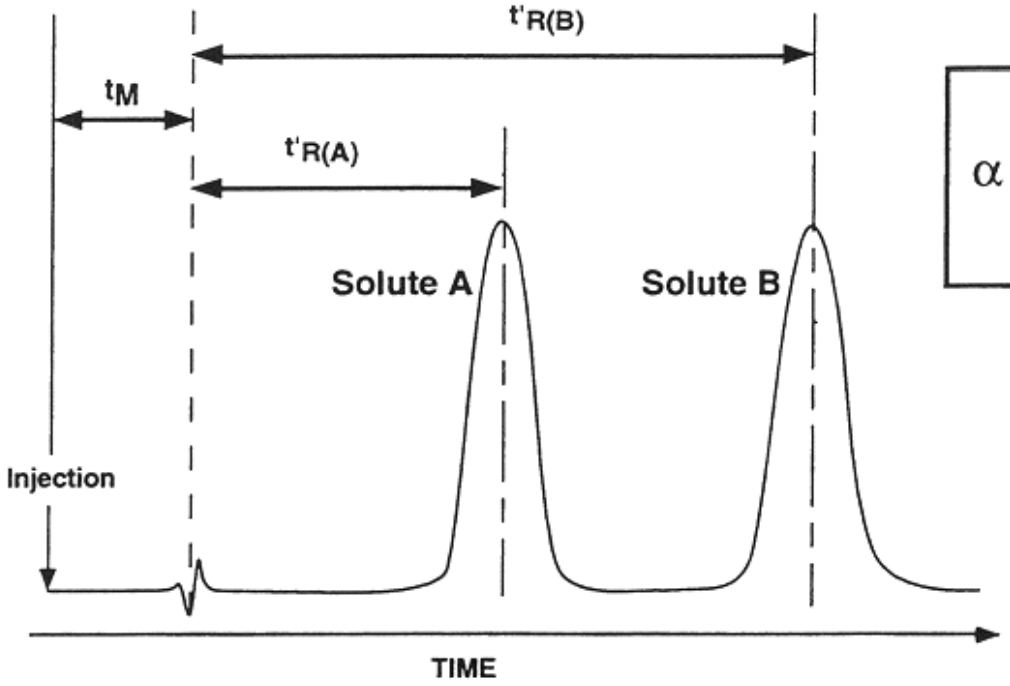
- Is the ratio of capacity factor of the more strongly retained compound to that of less strongly retained(or more rapidly eluted) cpd.
  - Let cpd B- be the more strongly retained compound and
  - Let cpd A- be the less retained cpd

  $\alpha = k'_B / k'_A = t_{R2}-t_M / t_{R1}-t_M$

- Selectivity factor describes the separation of two species (A and B) on the column

Selectivity (separation) factor, α

- The selectivity factor is always equal to or greater than one;
- If α =1, the compounds can not be separated
- The higher α, the more separation between two cpds
- α value of 1.1 is usually indicative of a good separation



$$\alpha = \frac{t'_{R(B)}}{t'_{R(A)}} = \frac{k_{R(A)}}{k_{R(B)}}$$

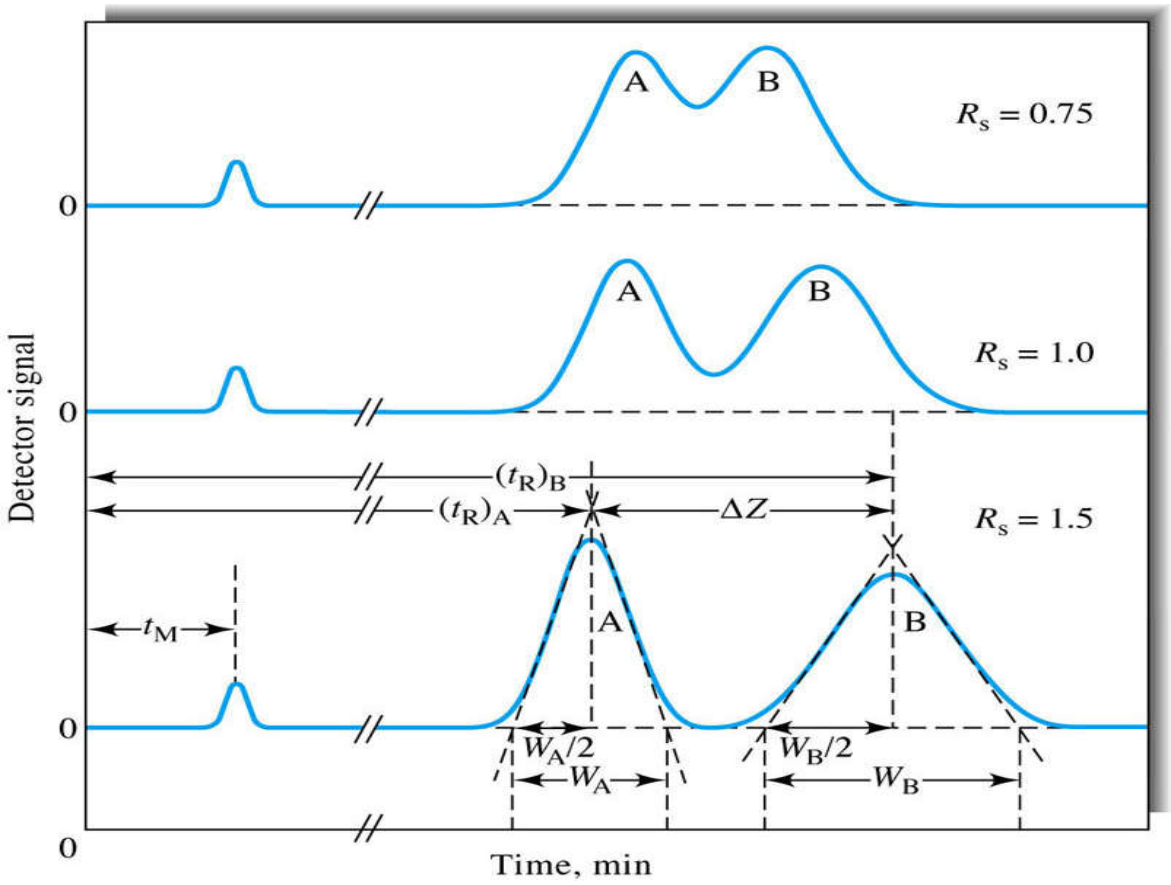
Resolution

- **Resolution** is a quantitative measure of the degree of separation between two chromatographic peaks, A and B, and is defined as

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

- resolution between two peaks is a second measure of how well two peaks are separated

- R_s = 1.5 represents *baseline resolution*, or complete separation of two neighboring solutes → ideal case.
- R_s =1.0 considered adequate for most separations.



Self-test 10.4

The BP assay of betamethasone 17-valerate states that it must be resolved from betamethasone 21-valerate so that the resolution factor is > 1.0. Which of the following ODS columns meet the specification?

Retention time of betamethasone 21-valerate (min)	Retention time of betamethasone 17-valerate (min)	Width at base of bet 21-valerate (min)	Width at base of bet 17-valerate (min)
1. 9.5	8.5	0.4	0.5
2. 9.3	8.6	0.4	0.4

Answer: 1 and 2

chromatogram

- A plot of the detector's signal as function of elution time