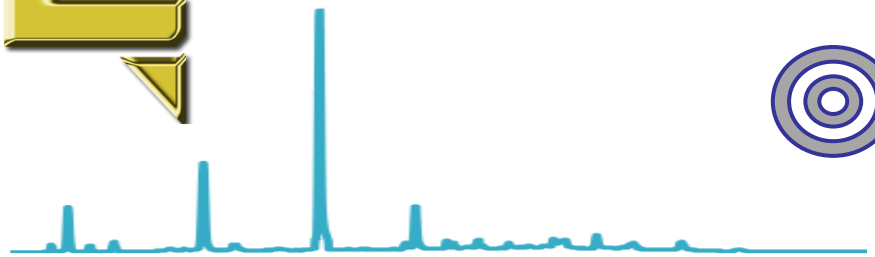
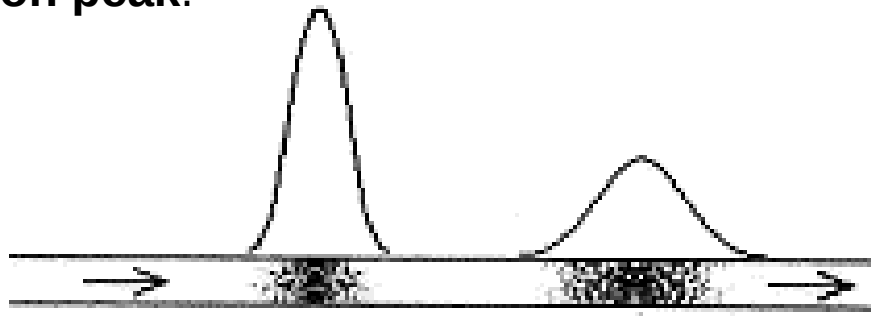
Chem 651
Advanced Studies in Instrumental Analysis

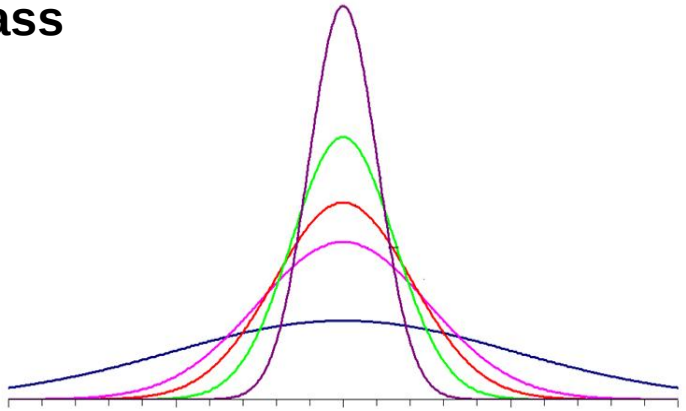


Ahmad Aqel Ifseisi
Professor of Analytical Chemistry
College of Science, Department of Chemistry
King Saud University
P.O. Box 2455 Riyadh 11451 Saudi Arabia
Building: 05, Office: 2A149, Lab: 2A127
Tel. 014674198, Fax: 014675992
Web site: http://fac.ksu.edu.sa/aifseisi
E-mail: ahmad3qel@gmail.com
aifseisi@ksu.edu.sa

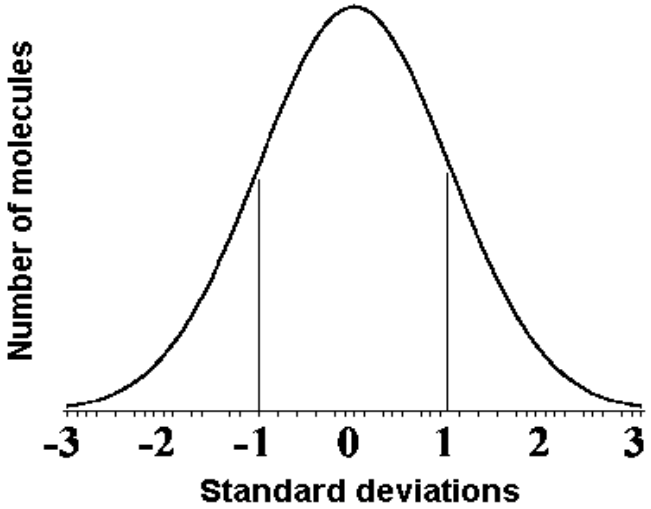
Peak shape …

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.

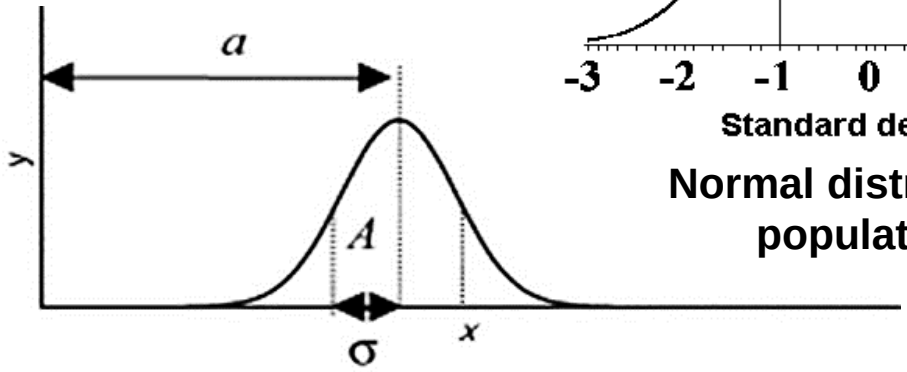


Normal distribution population

Basic Gaussian curve:

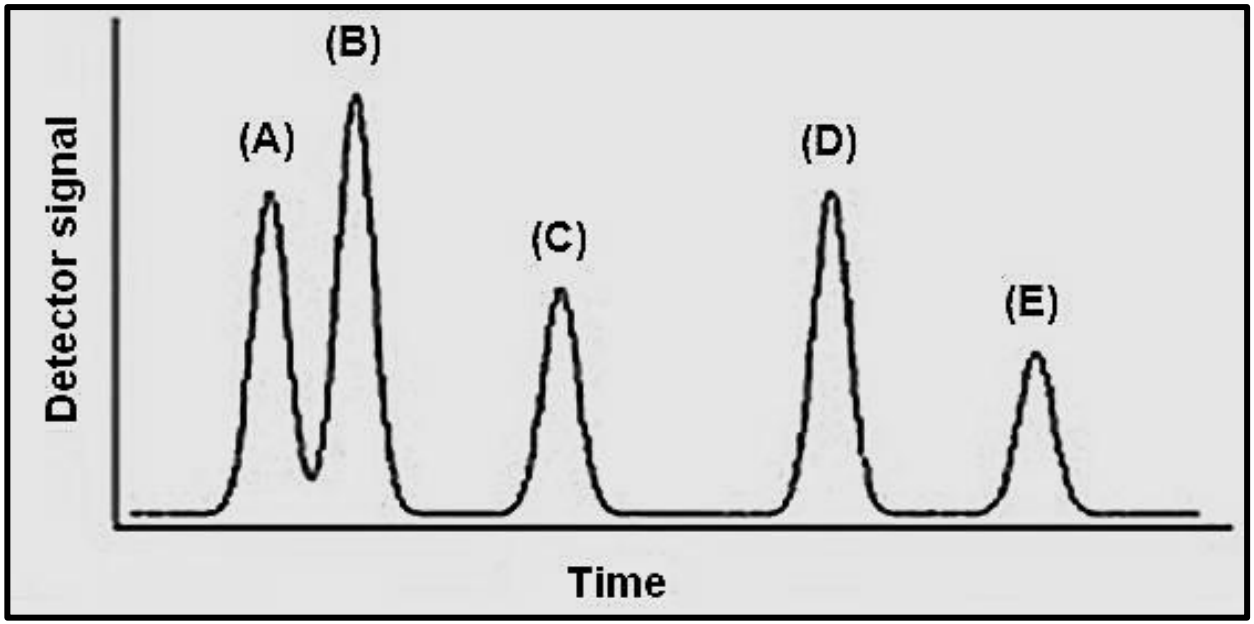
(A,a,σ are independent)

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



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.

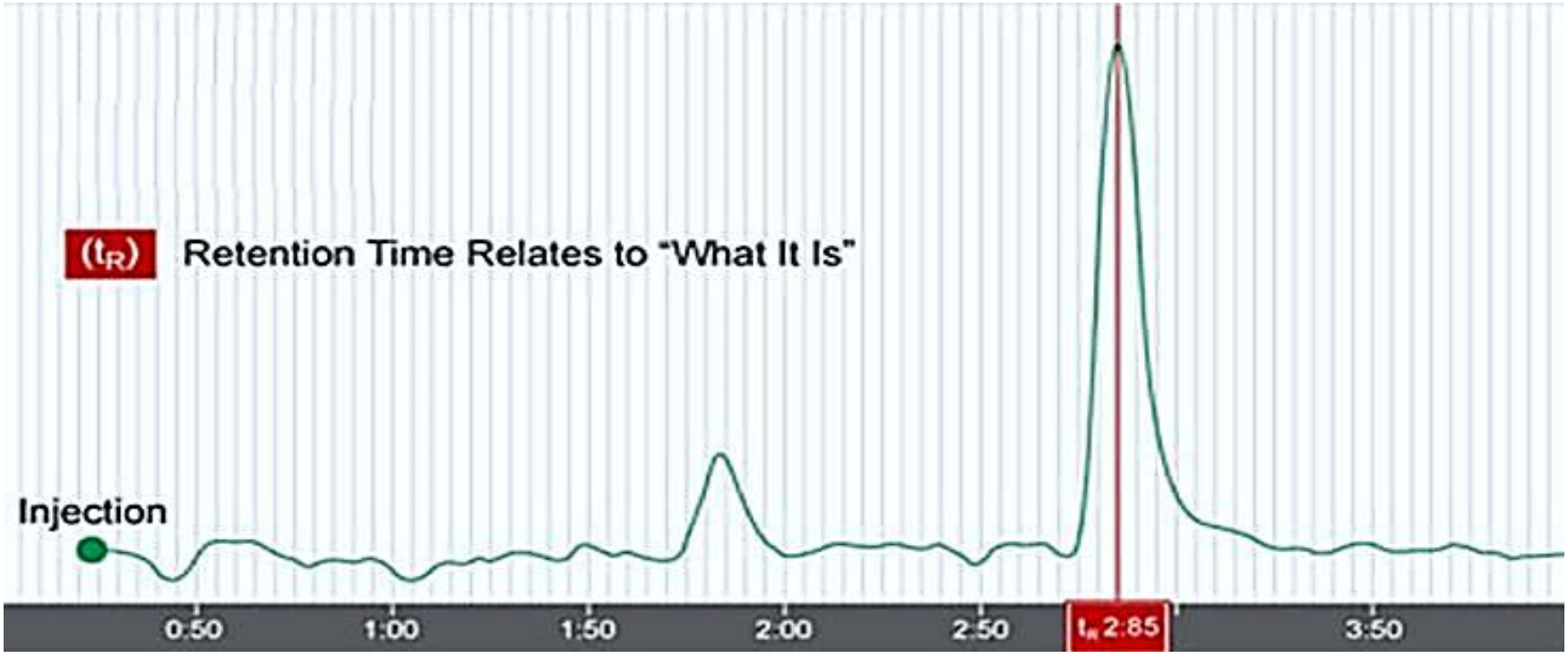
A chromatogram is useful for both **qualitative** and **quantitative** analysis;
-Every peak represents one component.
-The positions of peaks on the time axis may serve to identify the components of the sample.
-The peaks heights or areas provide a quantitative measure of the amount of each component.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

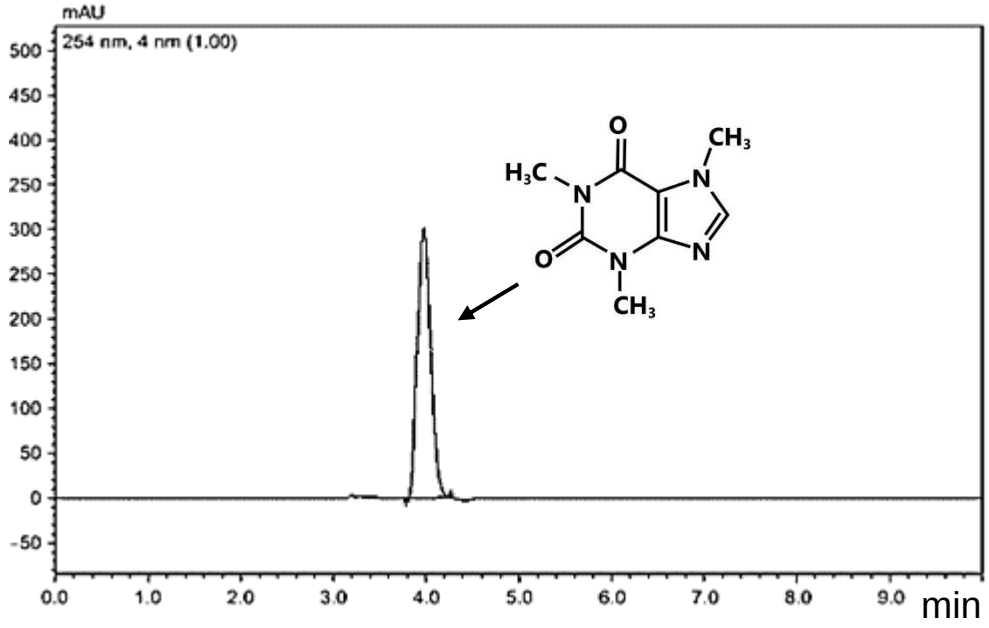
Qualitative Analysis

-Qualitative analysis is the identification of the mixture constituents separated by chromatography.

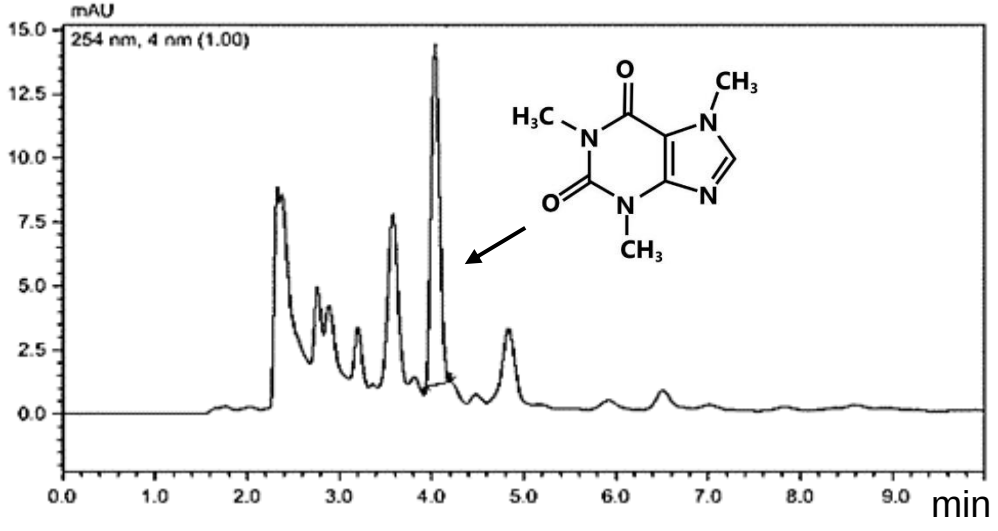
-It is generally based on comparison of reference standards to the unknown chromatogram peaks; components having the same **retention time** are assumed to be the same.

-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.



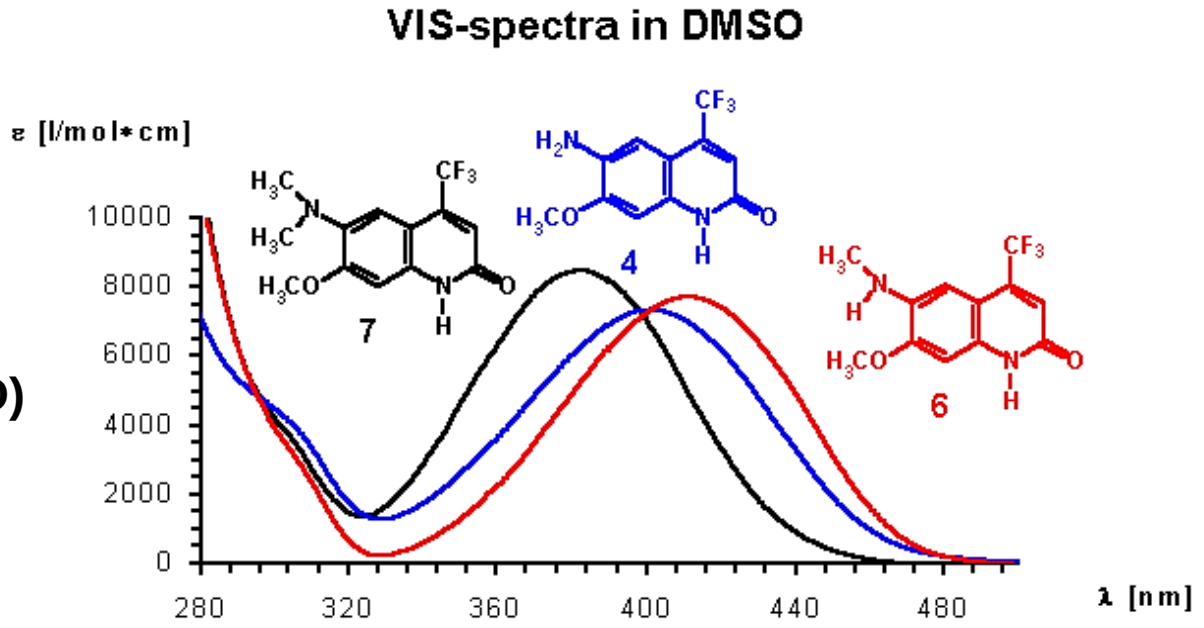


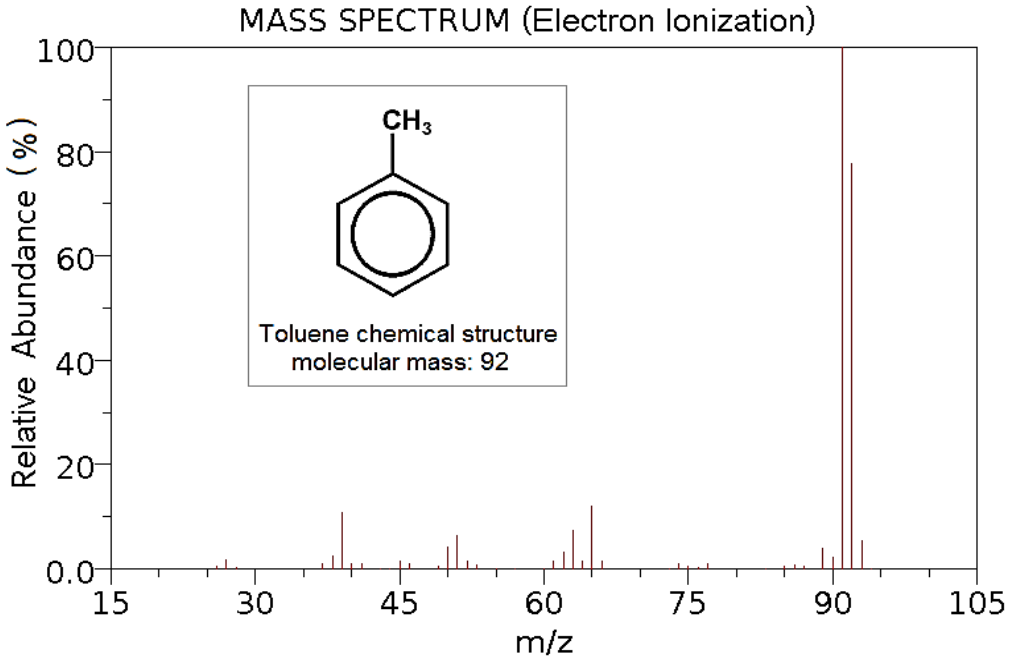
Chromatogram of standard caffeine



Chromatogram of real sample injected at the same conditions

UV-spectra
(diode array detector, DAD)

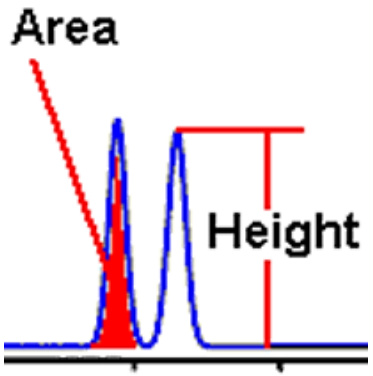


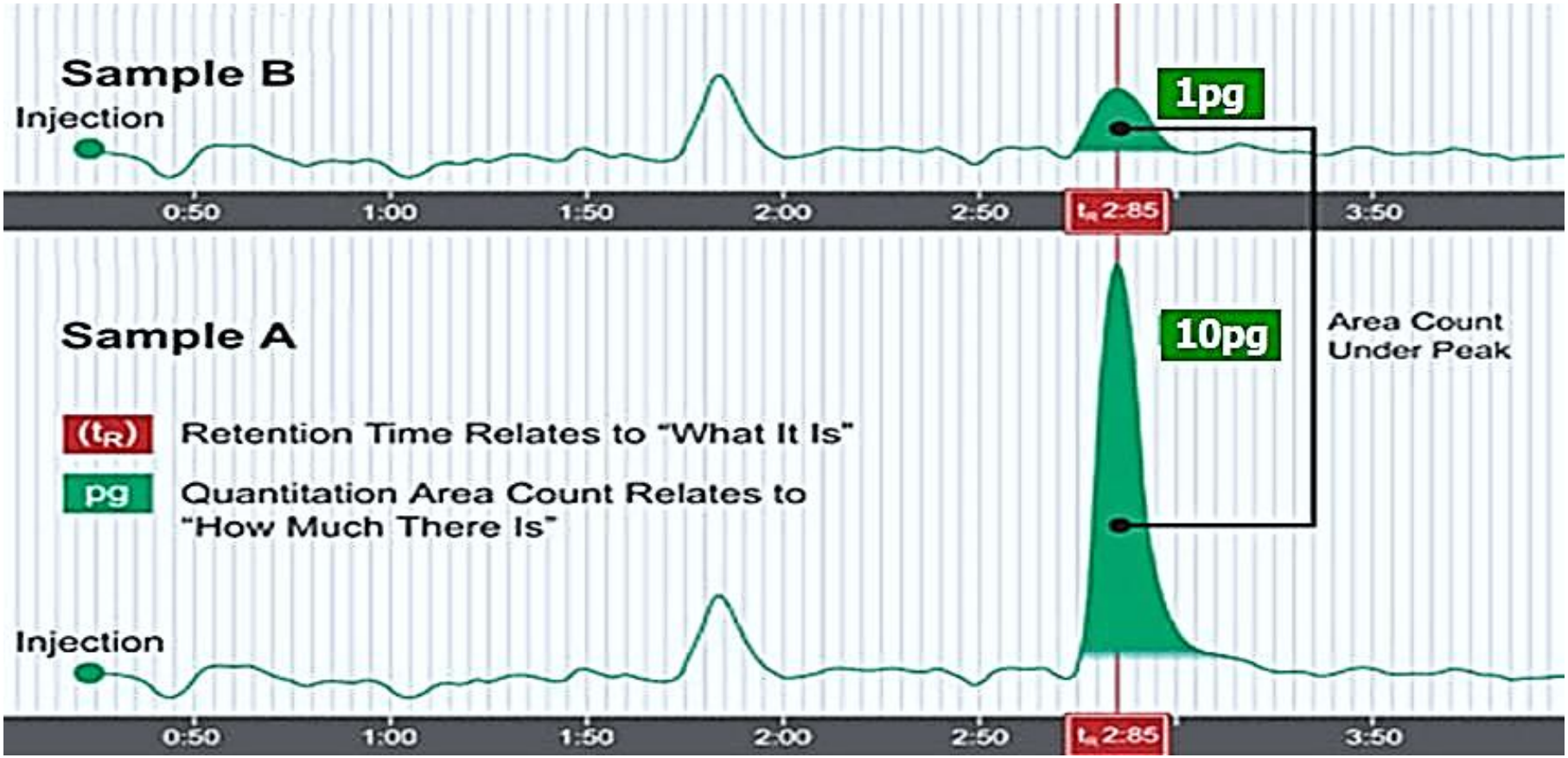


Mass spectra
(mass spectrometric detector, MS)

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.

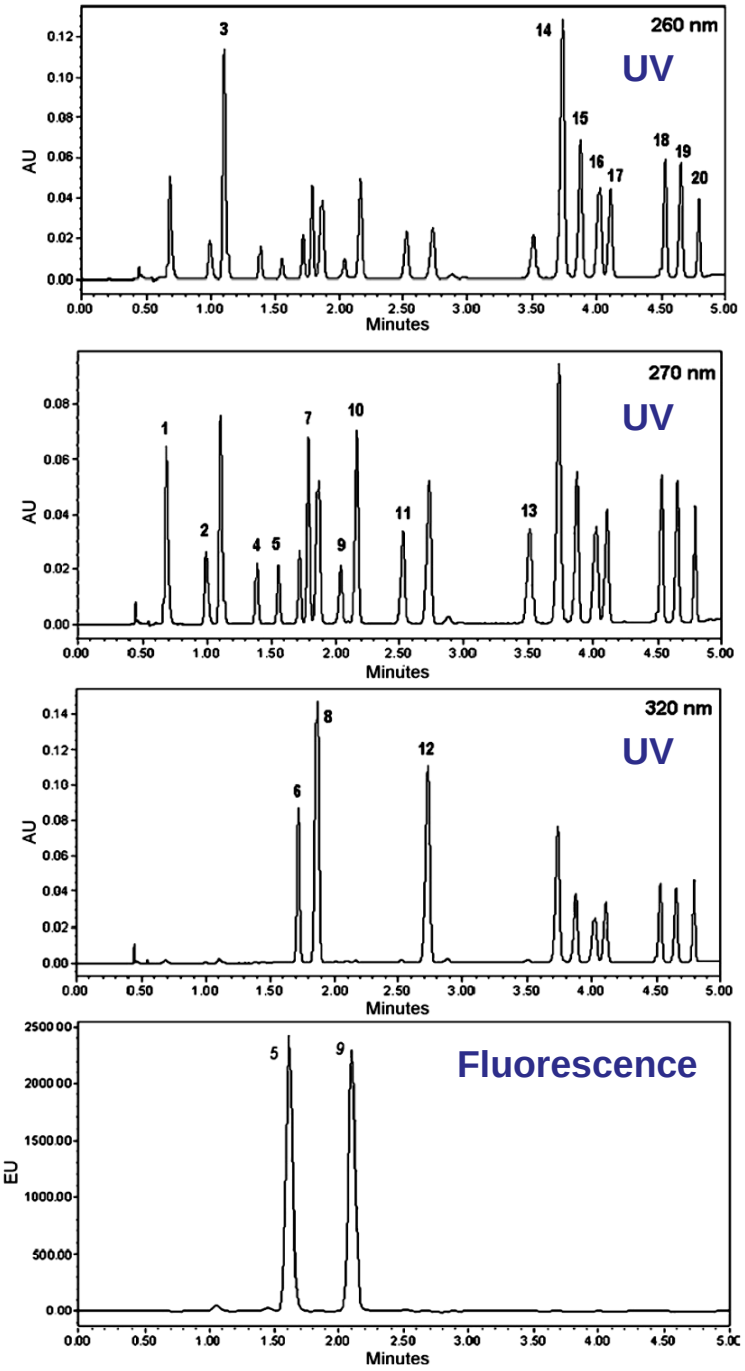




The correlation between various chromatographic detectors and detection parameters

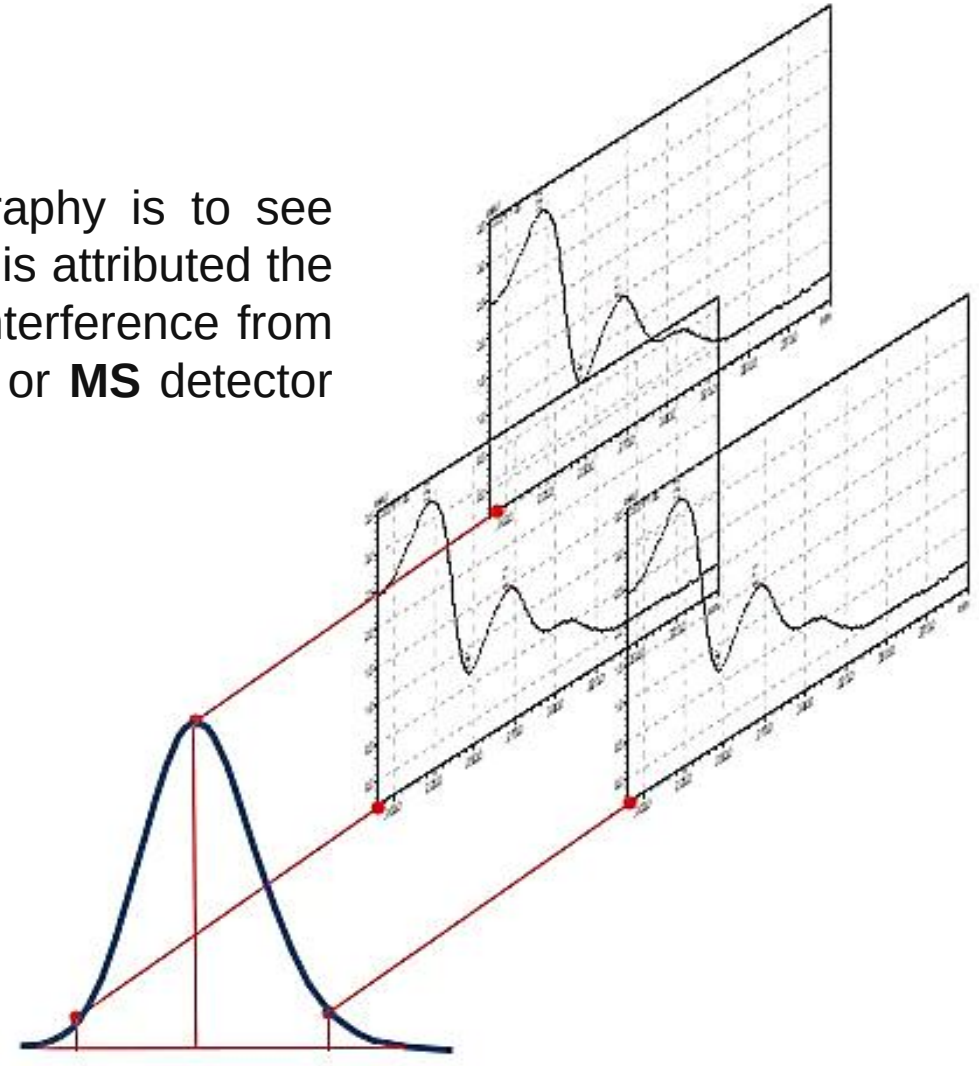
Peak #	Compound	Detection (nm)	RT (min)	Asymm.	Rs
1	Gallic acid	270	0.68	1.53	–
2	(–) Gallocatechin	270	0.99	1.24	6.7
3	Protocatechuic acid	260	1.11	1.12	2.1
4	(–) Epigallocatechin	270	1.39	1.00	7.3
5	(–) Catechin	270	1.56	1.01	4.6
6	Chlorogenic acid	320	1.72	0.94	4.0
7	Caffeine	270	1.79	0.95	1.8
8	Caffeic acid	320	1.86	0.91	1.8
9	(–) Epicatechin	270	2.04	1.01	4.0
10	(–) Epigallocatechin gallate	270	2.16	0.93	2.6
11	(–) Gallocatechin gallate	270	2.52	0.90	7.3
12	p-Coumaric acid	320	2.73	0.85	3.4
13	(–) Epicatechin gallate	270	3.51	0.86	13.1
14	Myricetin-3-O-rhamnoside	260	3.74	0.89	2.9
15	Quercetin-3-O-rutinoside	260	3.87	0.86	2.3
16	Quercetin-3-O-glucopyranoside	260	4.02	0.84	3.3
17	Luteolin-7-O-glucoside	260	4.11	0.89	1.5
18	Kaempferol-3-O-rutinoside	260	4.53	0.91	8.5
19	Kaempferol-3-O-glucoside	260	4.66	0.87	2.8
20	Apigenin-7-O-glucoside	260	4.80	0.90	3.2
5	(–) Catechin	Fluoresc.	1.62	1.05	–
9	(–) Epicatechin	Fluoresc.	2.12	1.00	2.25

Analysis of tea sample using HPLC-UV-Flu



Peak-purity

Peak purity analysis in chromatography is to see that is chromatographic peak eluted is attributed the analyte peak only and there is no interference from other peaks. You require e.g., **PDA** or **MS** detector to determine the peak purity.



For example, **UV** or **MS** spectra are taken at various points across a chromatographic peak and compared. If the spectra are sufficiently alike, the peak is considered pure; if the differences in spectra are large enough, the peak is not pure.

Chromatographic properties and evaluation

The effectiveness of a chromatographic column in separating two solutes depends in part upon the relative rates at which the two species are eluted.

These rates are determined by the magnitude of the equilibrium constants for the reactions by which the solutes distribute themselves between the mobile and stationary phases.

- **Partition coefficients**
- **Retention time**
- **Capacity factor**
- **Selectivity factor**
- **Resolution**
- **Efficiency**
- **Peak symmetry**

Partition coefficients …

The distribution equilibria in chromatography involve the transfer of an analyte between the mobile and stationary phases.

For the analyte species, ***A***

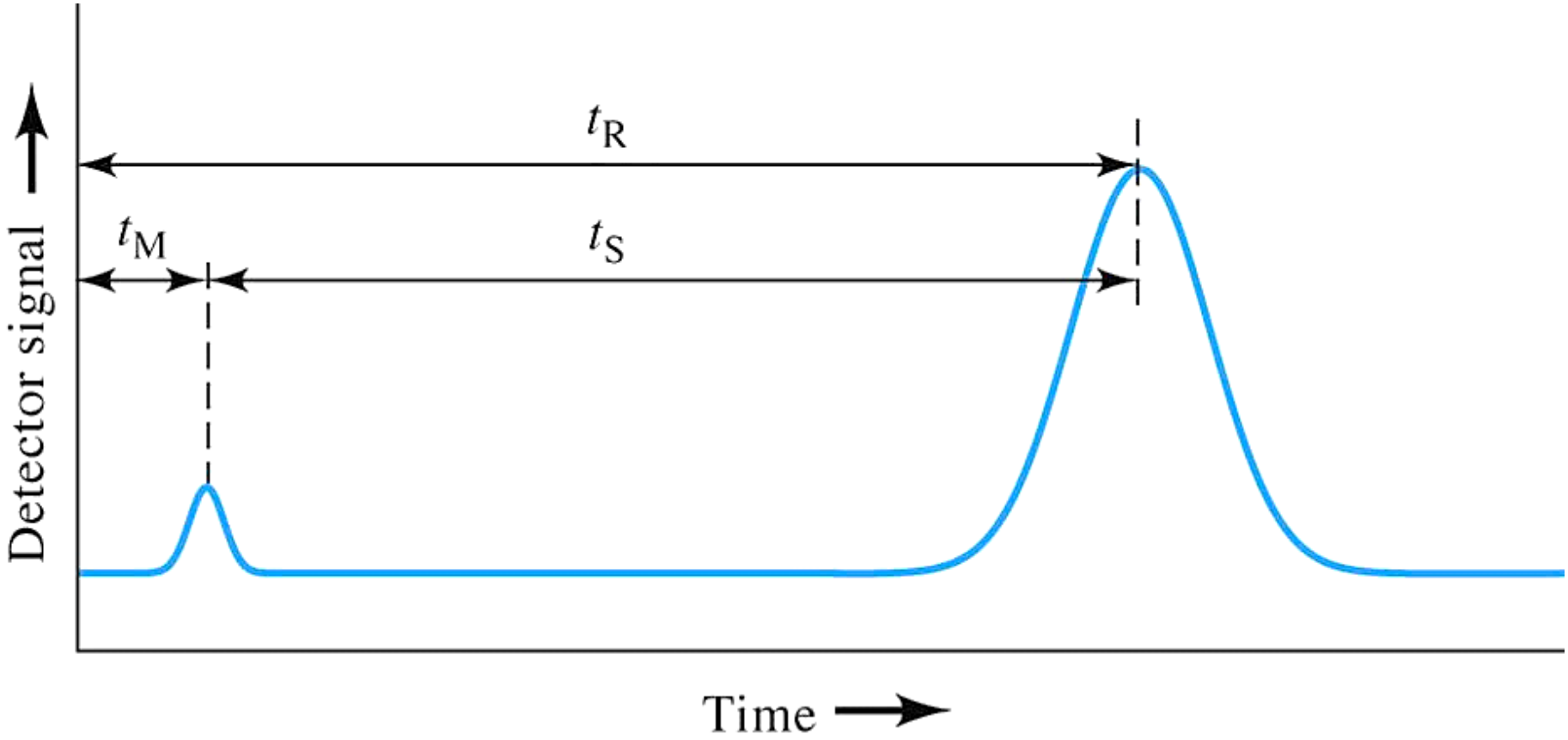
The equilibrium constant, ***K*** for this equilibrium is called the **distribution constant**, the **partition ratio**, or the **partition coefficient**,

$$K = \frac{C_S}{C_M}$$

Where, c_s is the molar concentration of the solute in the stationary phase and c_M is its molar concentration in the mobile phase.

Retention time …

The time it takes after sample injection for the analyte peak to reach the detector (t_R).
The time t_M for the unretained species to reach the detector is called the **dead time**.



t_R: retention time; time elapsed from point of injection to maximum of peak = $t_M + t_S$
t_M: the time the component resides in the mobile phase
t_S: the time the analyte is retained on the stationary phase
also called **adjusted retention time** = $t_R - t_M$

The capacity factor …

The **retention factor**, or **capacity factor**, is an important parameter that used to describe the migration rates of solutes on columns.

For a solute **A**, the retention factor k_A is defined as the ratio between the reduced and the unretained time:

$$k_A = \frac{V_R - V_M}{V_M} = \frac{t_R - t_M}{t_M} = \frac{t_S}{t_M}$$

$\bar{u} = V_M / t_M$

$F = V_R / t_R$

Where, V_R is the retention volume, V_M is the mobile phase volume (also called void volume).

When the retention factor for a solute is much less than unity, elution occurs so rapidly that accurate determination of the retention times is difficult. When the retention factor is larger than perhaps 20, elution times become inordinately long. Typically, a k value between 2 and 5 represents a good balance between analysis time and resolution.

The selectivity factor …

The **selectivity factor**, or **separation factor** is the ability of the chromatographic system to discriminate different analytes.

The selectivity factor α of a column for the two species A and B is defined as:

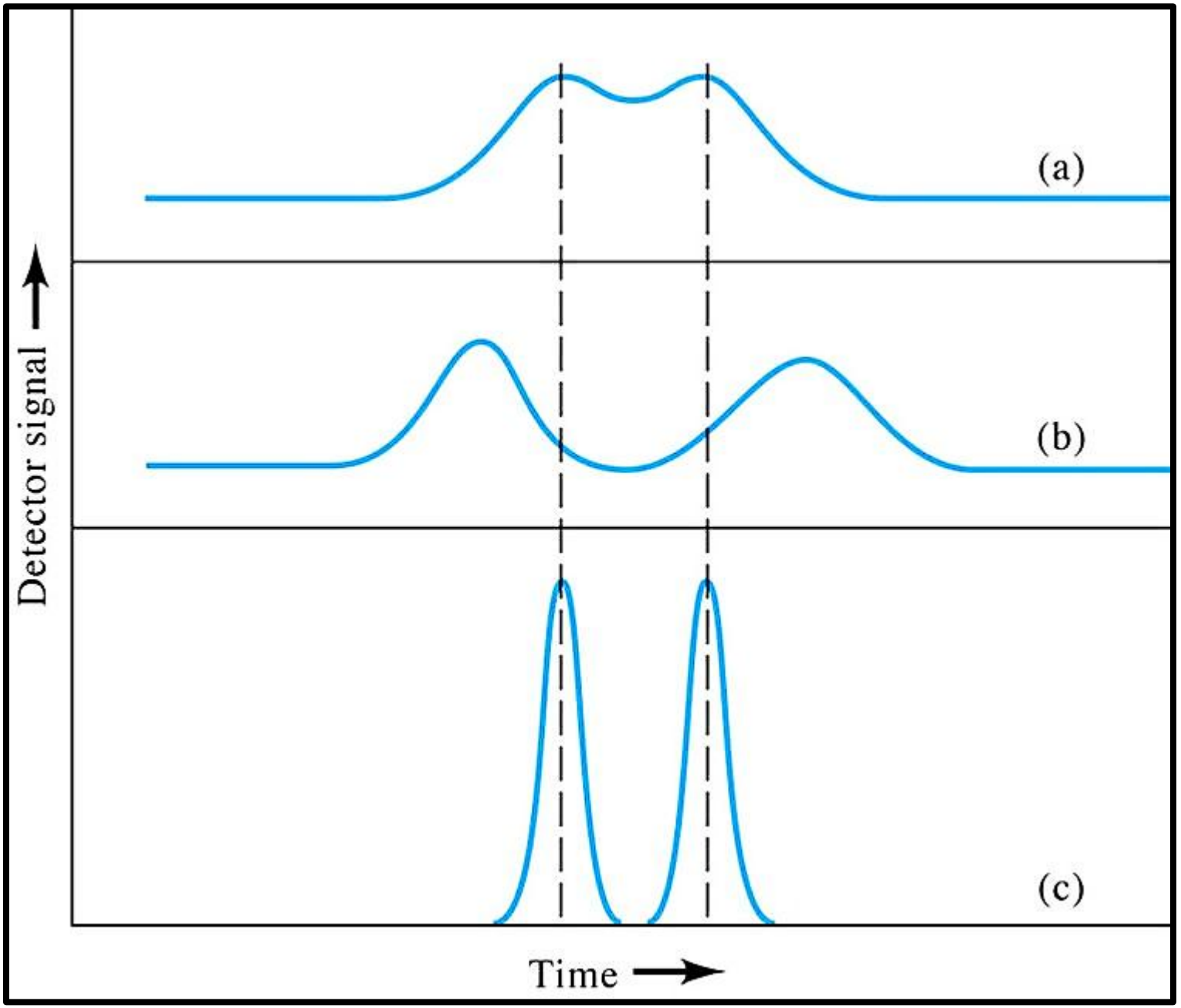
$$\alpha = \frac{k_B}{k_A} = \frac{t_{RB} - t_M}{t_{RA} - t_M}$$

The distance between the peak maxima reflects the selectivity of the system.

The greater the distance, the higher the selectivity. α is always greater than unity.

Selectivity factor not take into account the **peak width**.

Selectivity vs. Resolution



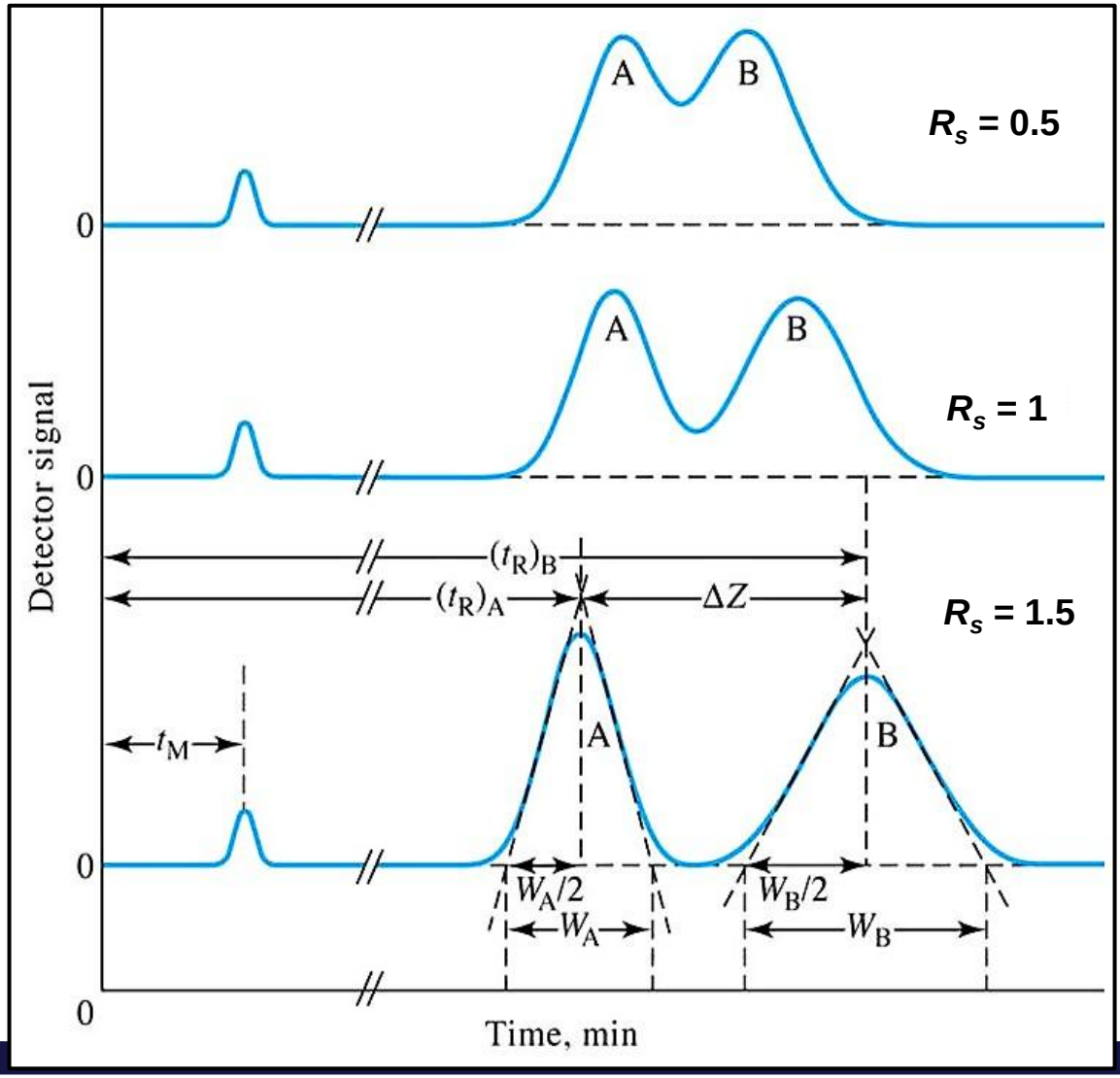
Resolution …

Resolution (R_s) is used to describe the degree of separation between neighboring solute bands or peaks (ability of the column to resolve two analytes into separate peaks; distance between the peaks).

$$R_s = 2\,\frac{t_{RB} - t_{RA}}{w_A + w_B}$$

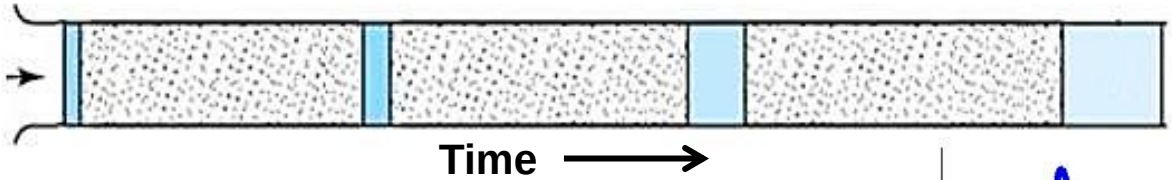
$$= 1.18\,\frac{t_{RB} - t_{RA}}{w_{\left(\frac{1}{2}\right)A} + w_{\left(\frac{1}{2}\right)B}}$$

Typically, an R_s value greater than 1.0 is required for accurate quantification of two peaks. A complete separation requires at least R_s > 1.5 units.

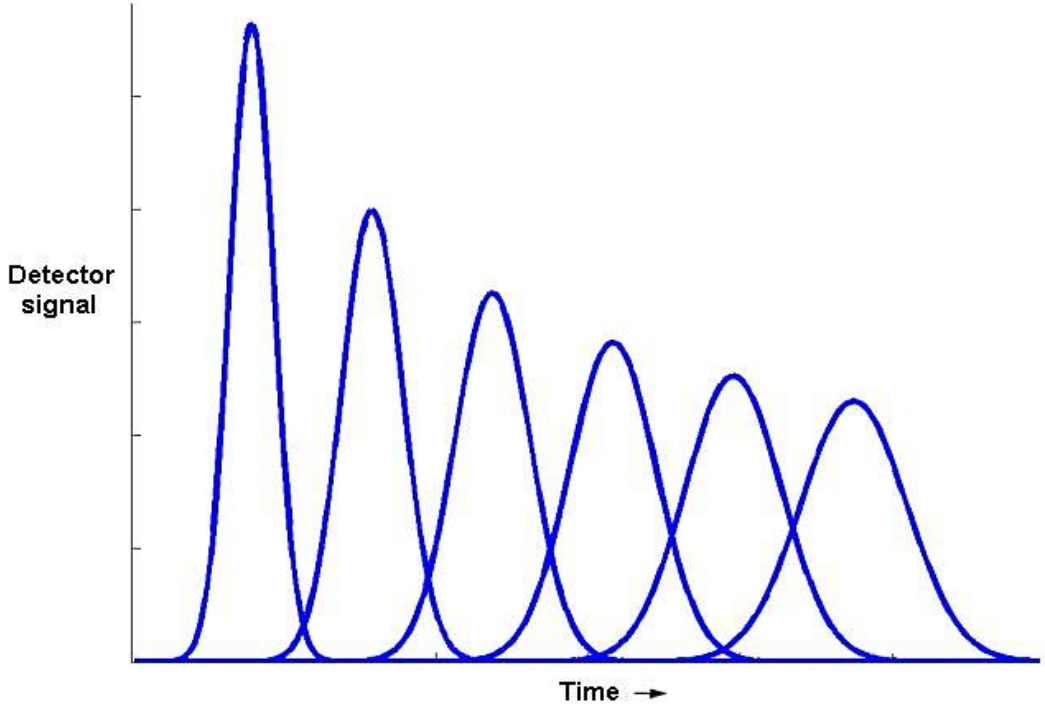


Column efficiency …

The efficiency of a column is a number that describes **peak broadening** as a function of **retention**.



Band or peak broadening



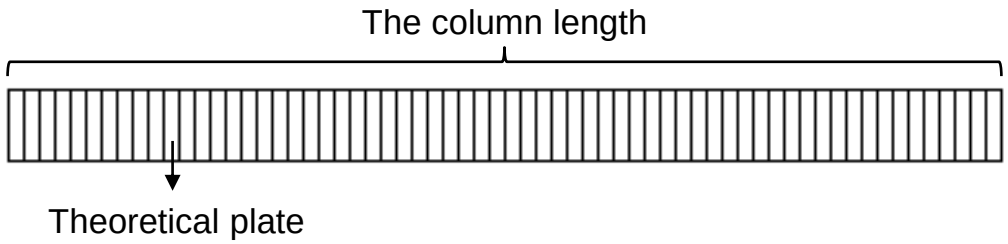
Two major theories have been developed to describe column efficiency:

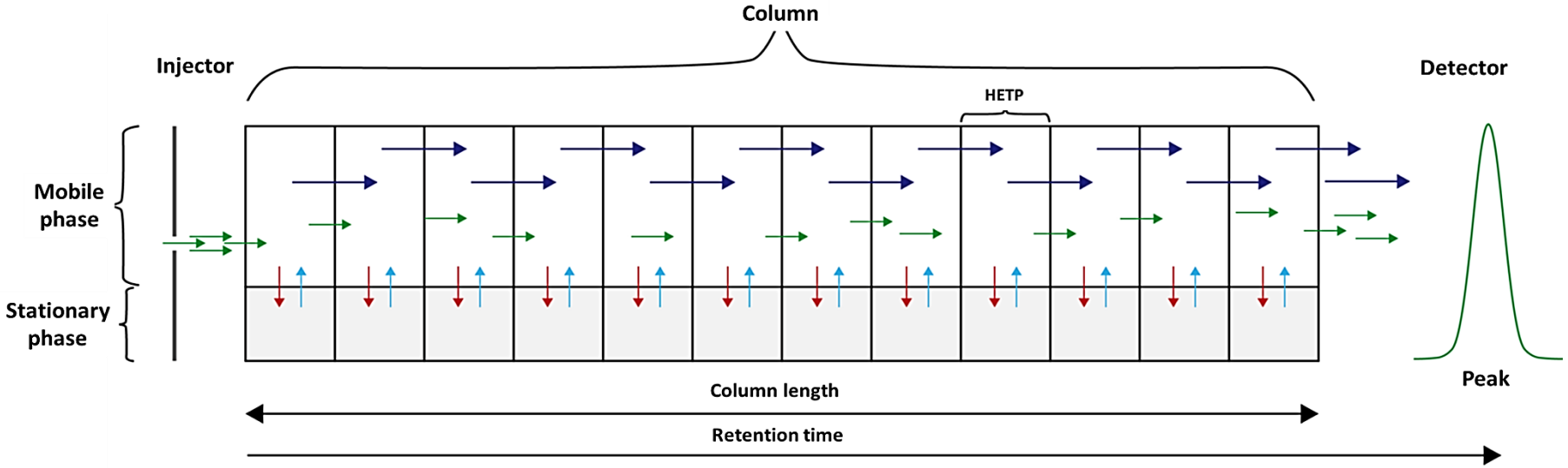
(1) The plate theory (Proposed by Martin and Synge, 1941).
Provides a simple and convenient way to measure column performance and efficiency.

(2) The rate theory (Developed by van Deemter, Zuiderweg, and Klinkenberg, 1956).
Provides a means to measure the contributions to band broadening and optimize the efficiency.

Plate theory ...

The major assumption in the plate theory is that there is an instantaneous equilibrium set up for the solute between the stationary and mobile phases.





The column is considered to consist of a number of hypothetical thin sections or "**plates**", and the solute spends a finite time in this plate. This time is considered to allow the solute to equilibrate between the stationary and mobile phases.

$$H = L / N$$

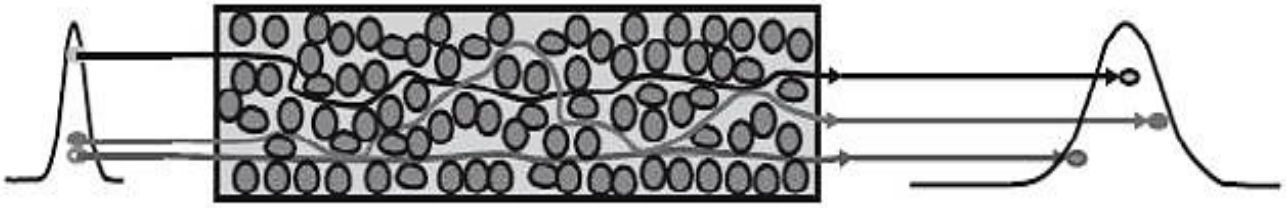
$$N = \left(\frac{t_R}{\sigma}\right)^2 = 16\left(\frac{t_R}{w}\right)^2 = 5.54\left(\frac{t_R}{w_{1/2}}\right)^2$$

The smaller the plate height (***H***) or the greater the number of theoretical plates (***N***), the more efficient the analyte exchange is between two phases and the greater is the efficiency of the column, which mean the better the separation.

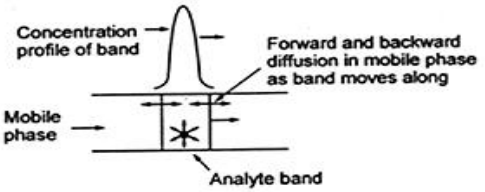
Rate theory ...

The rate theory considers the diffusional factors that contribute to band broadening in the column and avoids the assumption of an instantaneous equilibrium.

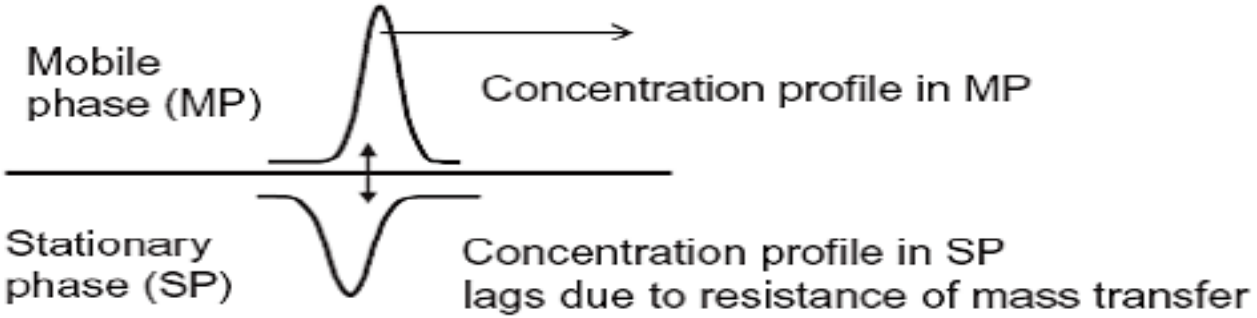
(1) Eddy diffusion or multipath effect (***A*** term)



(2) Longitudinal diffusion (***B*** term)



(3) Resistance to mass transfer (***C*** term)



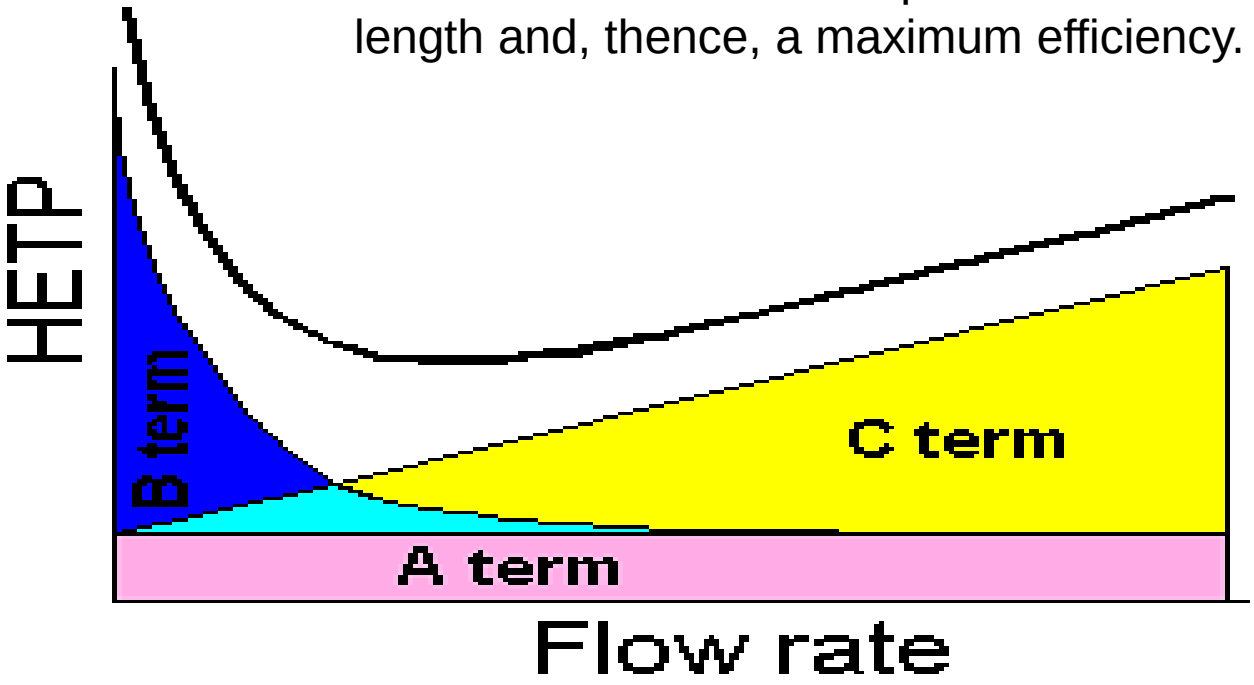
Van Deemter equation expresses the extent to which a component band spreads as it passes through the column in terms of physical constants and the velocity of the mobile phase.

$$H = 2\lambda d_p + \frac{2\gamma D_m}{u} + f(k)\frac{d_p^2}{D_m}u$$

$$H = A + \frac{B}{u} + C \cdot u$$

van Deemter equation

The van Deemter equation is a **hyperbolic function** that predicts that there is an **optimum velocity** at which there will be the minimum variance per unit column length and, thence, a maximum efficiency.



van Deemter curve
H vs. u

Effect of capacity and selectivity factors on resolution

The efficiency and resolution could also expressed in terms of capacity factor and selectivity by the **Purnell equation**:

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)$$

Effect of resolution on retention time

$$t_{R2} = \frac{16R_s^2 H}{u}\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{1 + k_2}{k_2}\right)^2$$

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_2}{1 + k_2}\right)$$

Resolution

Efficiency

Selectivity

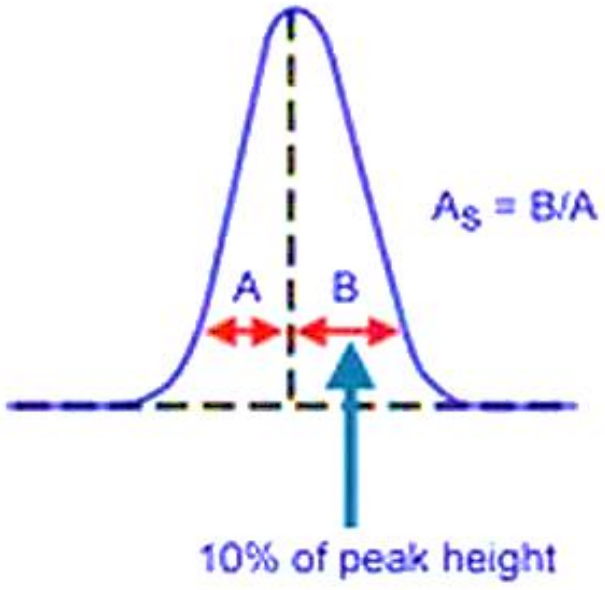
Retentivity

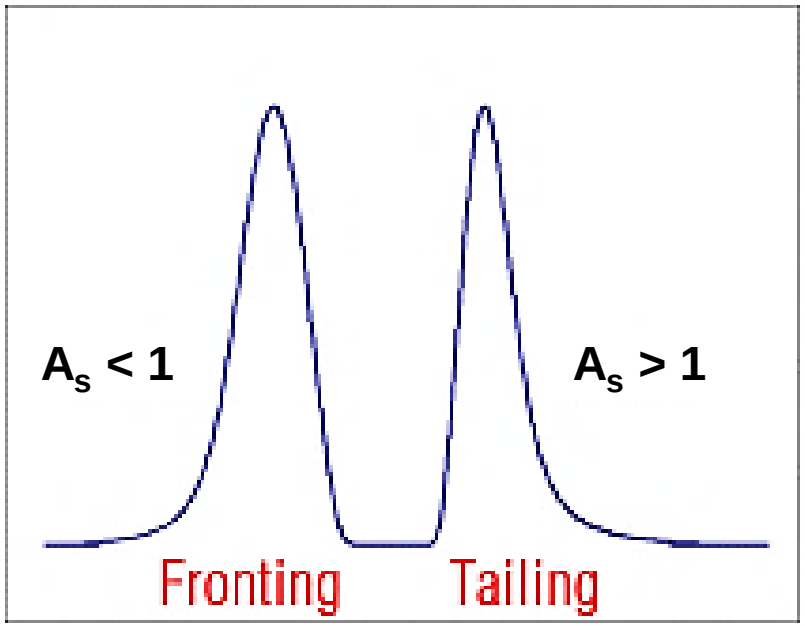
Peak symmetry ...

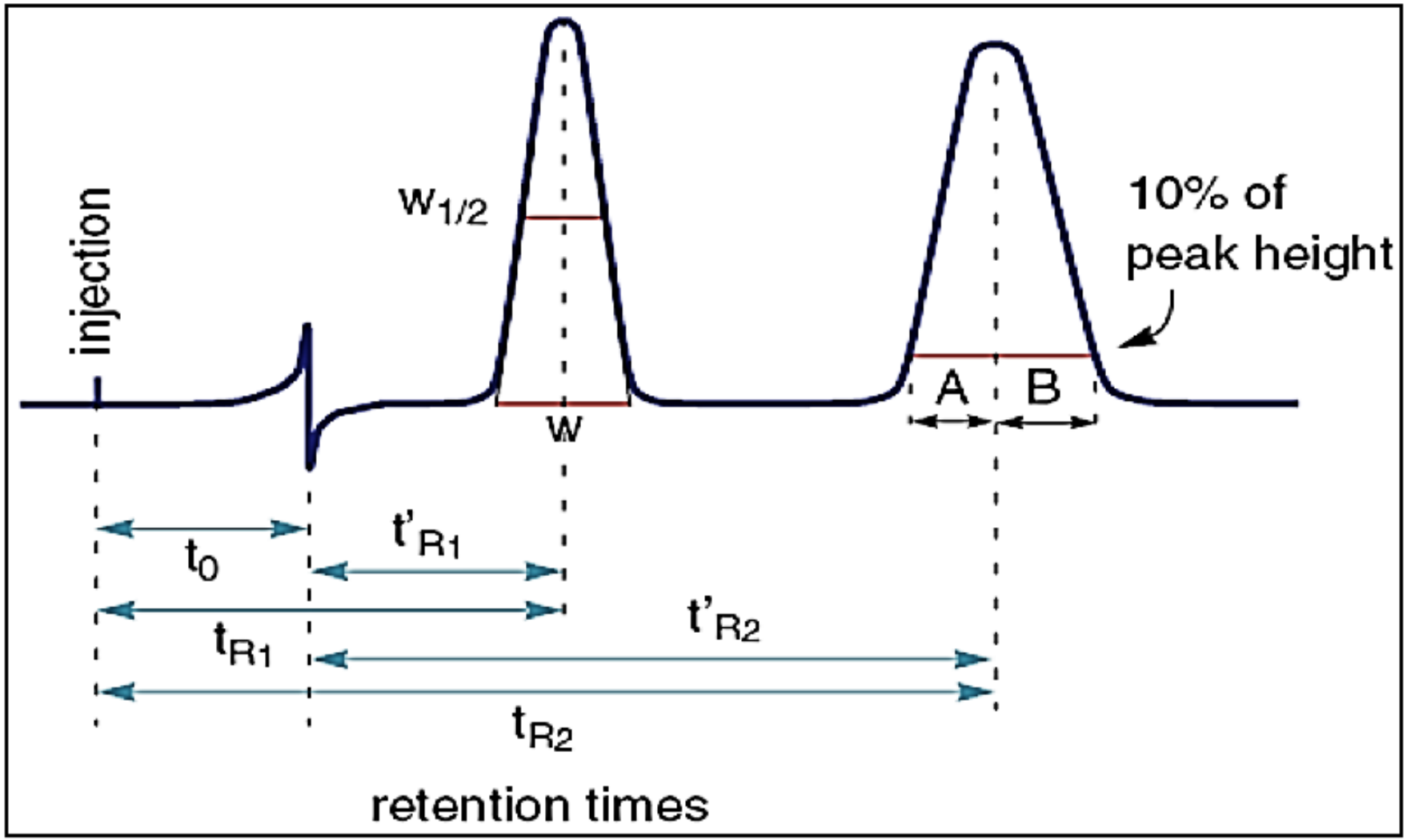
For practical reasons, the peak symmetry is usually measured at 10% of peak height, where ***A*** is the distance from peak front to peak maximum, and ***B*** is the distance from peak maximum to peak end.

$$symmetry = B / A$$

Ideally symmetry should be 1, i.e., ***A*** = ***B***.
Values > 1 indicate peak tailing,
whilst values < 1 characterize peak fronting.







Typical chromatogram and its characteristic parameters.

Example:

Substances **A** and **B** have retention times of 16.40 and 17.63 min, respectively, on a 30.0 cm column. An unretained species passes through the column in 1.30 min. The peak widths (at base) for A and B are 1.11 and 1.21 min, respectively.
Calculate: (a) column resolution, (b) average number of plates in the column, (c) plate height, (d) length of column required to achieve a resolution of 1.5, and (e) time required to elute substance B on the longer column.

(a) $R_s = 2(17.63 - 16.40) / (1.11 + 1.21) = 1.06$
(b) $N = 16\,(16.40 / 1.11)^2 = 3493$ and $N = 16\,(17.63 / 1.21)^2 = 3397$
$N_{avg} = (3493 + 3397) / 2 = 3445$
(c) $H = L / N = 30.0 / 3445 = 8.7 \times 10^{-3}$ cm
(d) k' and α do not change greatly with increasing N and L. Thus,

$$\frac{(R_s)_1}{(R_s)_2} = \frac{\sqrt{N_1}}{\sqrt{N_2}} \quad == \quad \frac{1.06}{1.5} = \frac{\sqrt{3445}}{\sqrt{N_2}} \quad == \quad N_2 = 3445\left(\frac{1.5}{1.06}\right)^2 = 6.9 \times 10^3$$

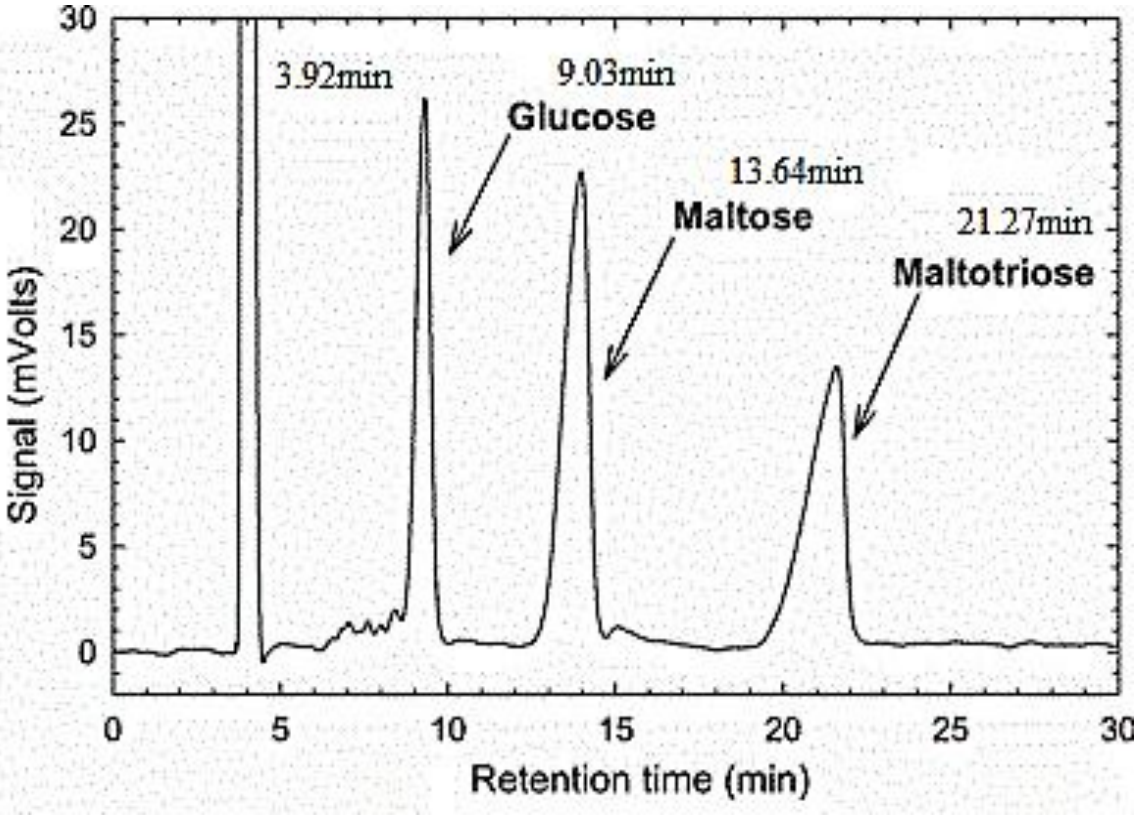
$L = N H = 6.9 \times 10^3 \times 8.7 \times 10^{-3} = 60$ cm

(e) $$\frac{(t_R)_1}{(t_R)_2} = \frac{(R_s)_1^2}{(R_s)_2^2} = \frac{17.63}{(t_R)_2} = \frac{(1.06)^2}{(1.5)^2}$$

$(t_R)_2 = 35$ min
Thus, to obtain the improved resolution, the separation time must be doubled.

Example:

A chromatograph of three sugars separated using LC column with 250 mm long. The flow rate was 0.50 mL/min. Knowing that width of the peaks at half ($w_{1/2}$) for Glucose, Maltose and Maltotriose are 0.33, 0.67 and 1.32min, respectively.



Calculate:

(a) The time of Glucose spends on the stationary phase
(b) The retention factor for Maltotriose
(c) The resolution between Maltose and Maltotriose
(d) The selectivity factor for Maltose and Maltotriose
(e) An average number of plates for the three components
(f) An average plate height for the column


Thank You!