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Chapter 10
Introduction to Chromatography

Separation

Important areas in this chapter:

Basic concepts; partition coefficient; retention time; capacity factor; selectivity factor; band broadening; relationship between plate height and column variables; optimization of column performance; qualitative applications; quantitative applications: peak height; peak areas; internal calibration.

Chromatographic separations

Sample is dissolved in a mobile phase (a gas, a liquid or a supercritical fluid);

The mobile phase is forced through an immiscible stationary phase which is fixed in place in a column or on a solid surface.

The two phases are chosen so that the components of the sample distribute themselves between the mobile and stationary phase to a varying degree.

Classification of chromatographic methods:

1. physical means

column chromatography: **stationary phase is held in a narrow capillary through which the mobile phase is forced under pressure or by gravity;**

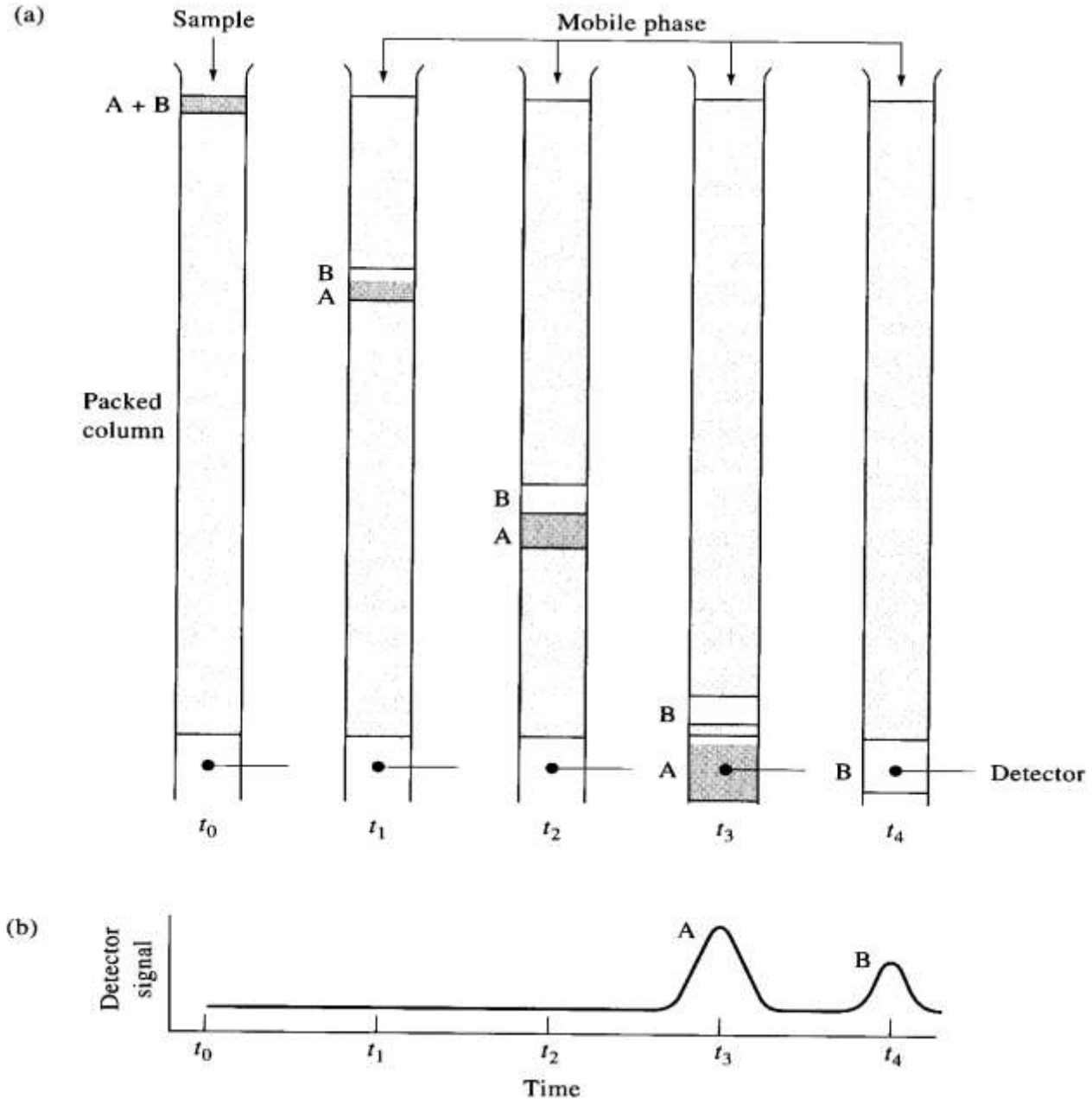
***planar chromatography*:** **stationary phase is supported on a flat plate or in the interstices of a paper. The mobile phase moves through the stationary phase by capillary action or under the influence of gravity.**

2. mobile and stationary phases

- Liquid chromatography
- Gas chromatography
- Supercritical-fluid chromatography

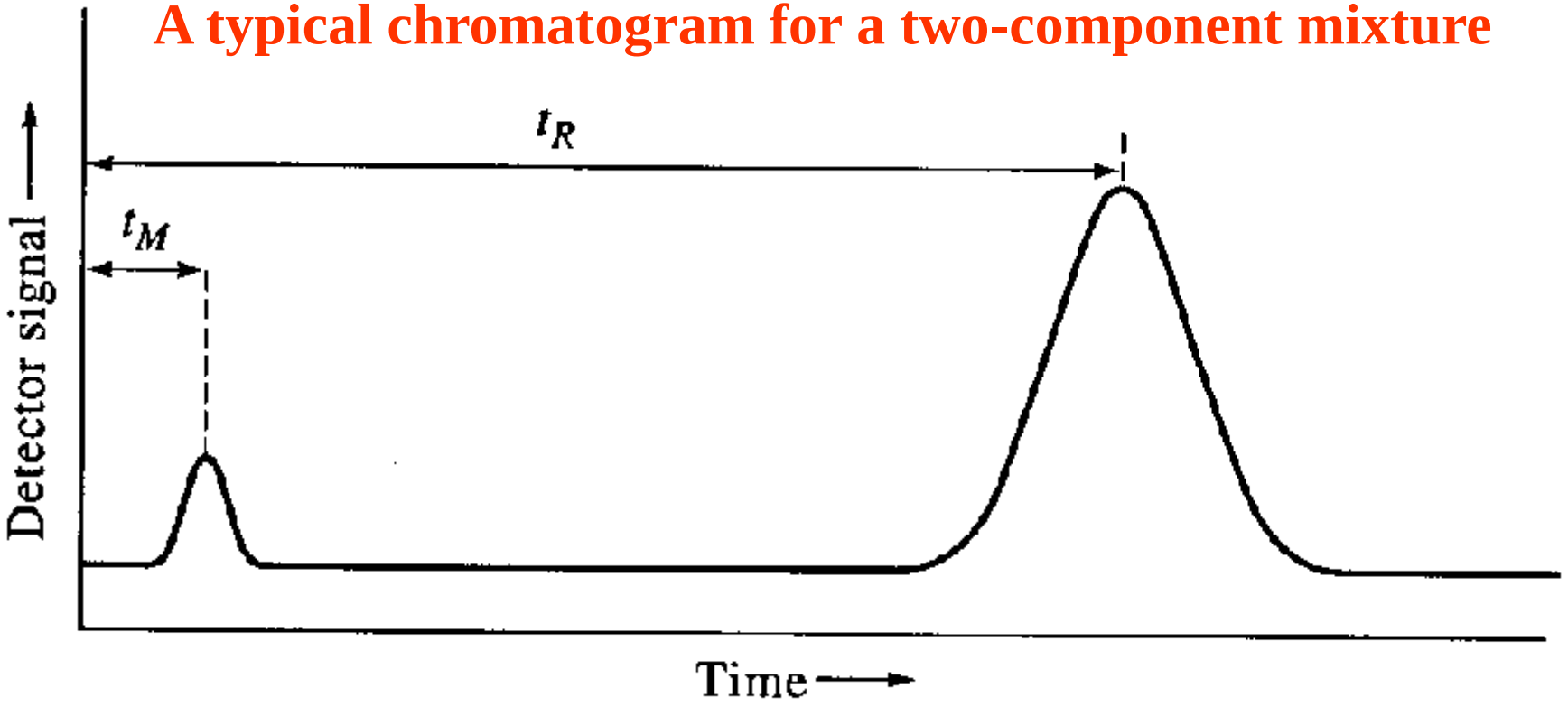
Classification of Column Chromatographic Methods

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



paration of a xture of nponents A and by column elution romatography. (b) e output of the nal detector at e various stages of tion shown in (a).

A typical chromatogram for a two-component mixture



The small peak on the left represents a species that is not retained on the column and so reaches the detector almost immediately after elution is started. Thus its retention time t_M is approximately equal to the time required for a molecule of the mobile phase to pass through the column.

Retention time, t_R:

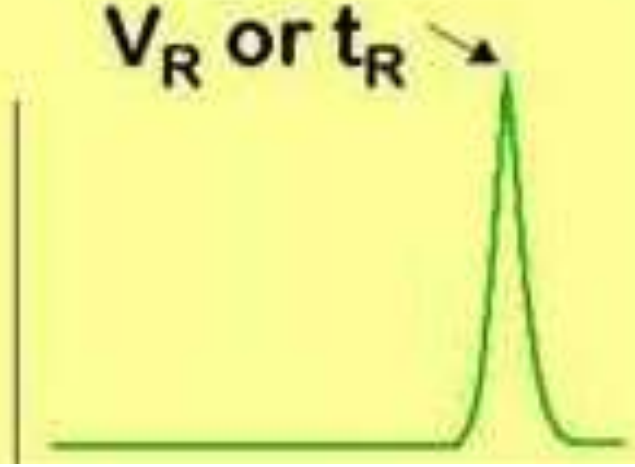
t_R is the time it takes after a sample injection for the analyte peak to reach the detector.

t_M is the time for the unretained species to reach the detector, dead time. The rate of migration of the unretained species is the same as the average rate of motion of the mobile phase molecules.

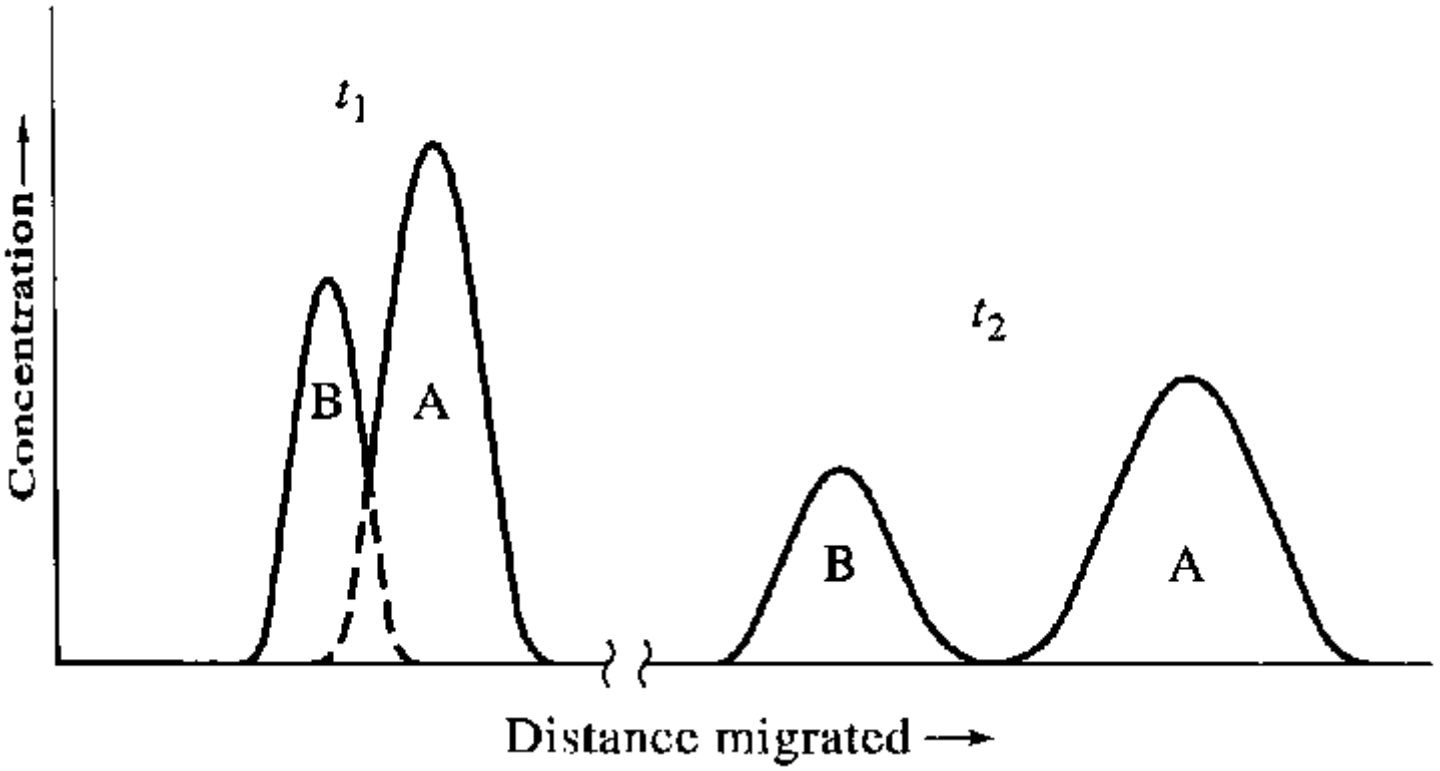
Retention time and retention volume

Retention volume, V_R - volume of mobile phase required to elute a solute to a maximum from a column.

Retention time, t_R, time required to reach the same maximum at constant flow.



where $V_R = t_R \times$ flowrate

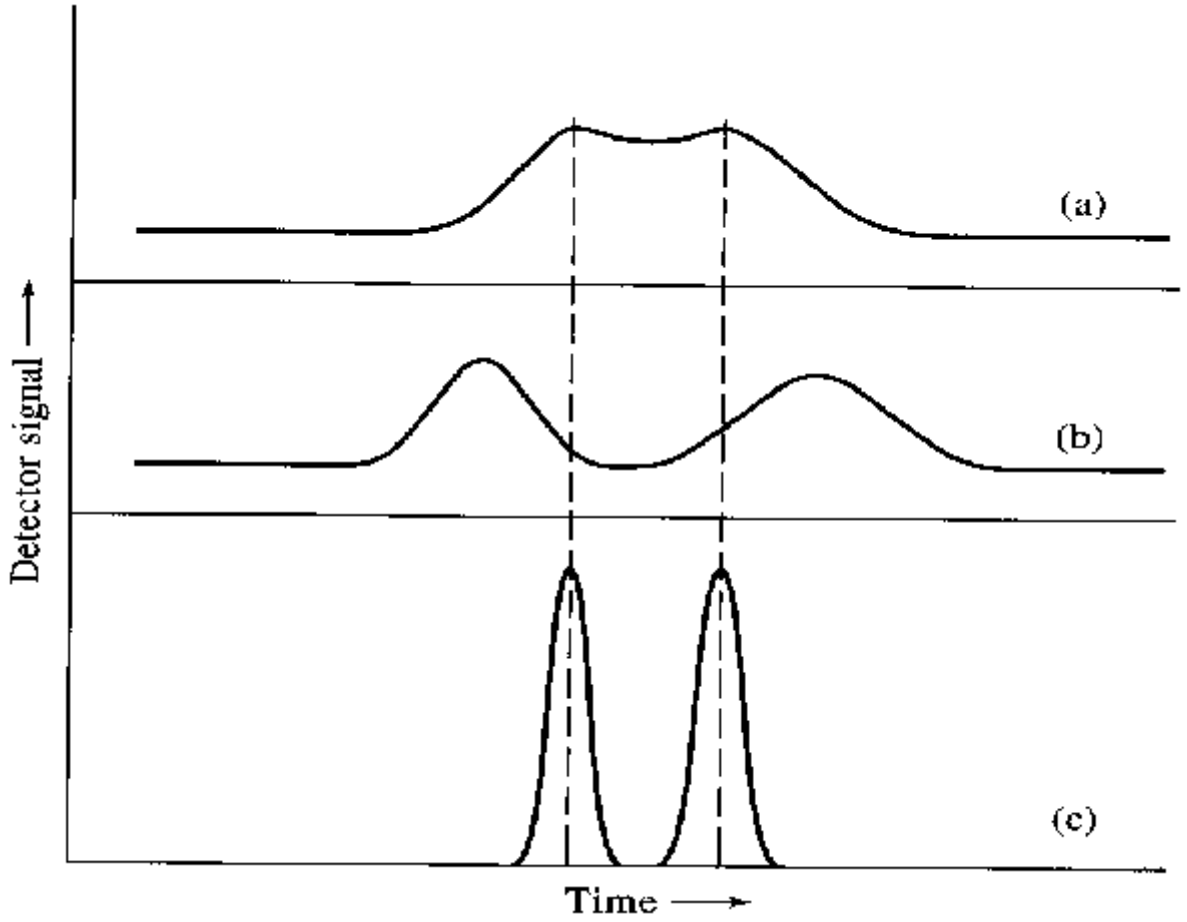


Concentration profiles of analyte bands A and B at two different times in their migration down the column. The times t_1 and t_2 are indicated in the figure above

Effects of migration rates and band broadening on resolution

longer column distance enable peaks separated better, but with more peak broadening, which lowers the efficiency of the column as a separating device.

Methods of improving separators



(a) original chromatogram with overlapping peaks; improvements brought about by (b) an increase in band separation, and (c) a decrease in band spread.

Partition (distribution) Coefficient

Distribution or Partition between phases

A(mobile) $\Leftrightarrow$ A(stationary)

$$K = \frac{c_S}{c_M}$$ Distribution **coefficient**

Partition Coefficient

Independent of conc., but Temperature dependent

Mobile **stationary**

As K increases the solute takes a longer time to elute

Partition Coefficient, K

- **K should be constant over a large range of concentration range.**
- **C_s is directly proportional to C_M**
- **Chromatography where this equation holds is linear chromatography**

The average linear rate of solute migration $\bar{v}$ is

$$\bar{v} = \frac{L}{t_R}$$

where L is the length of the column packing. Similarly, the average linear rate of movement u of the molecules of the mobile phase is

$$u = \frac{L}{t_M}$$

where t_M, the *dead time*, is the time required for an average molecule of the mobile phase to pass through the column.

The relationship between retention time and partition coefficients: relate t_R with K

$\bar{v}$ = u x fraction of time an analyte spends in mobile phase

= u x (moles of analyte in mobile phase/total mole of analyte)

= u x (C_M x V_M / (C_M x V_M + C_s x V_s))

= u x (1 / (1 + C_s x V_s /C_M x V_M))

$$\bar{v} = u \times \frac{1}{1 + KV_S/V_M} \qquad (1)$$

t_R = $\bar{v}$ X L

The rate of solute migration: Capacity (Retention factor)

The *retention factor,* or *capacity factor,* is an important parameter that is widely used to describe the migration rates of solutes on columns. For a solute A, the retention factor k'_A is defined as

$$k'_A = \frac{K_A V_S}{V_M} \qquad (2)$$

where K_A is the distribution constant for the species A. Substitution of Equation (2) into (1) yields

$$\bar{v} = u \times \frac{1}{1 + k'_A} \qquad (3)$$

Substituting $\bar{v}$ and u from the above equations into equation (3) yields

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k'_A}$$

This equation rearranges to

$$k'_A = \frac{t_R - t_M}{t_M}$$

- **When capacity factor is less than 1, elution occurs so rapidly that accurate determination of t_R is difficult as a result of peak broadening**
- **When capacity factor is >20 to 30, elution times become too long.**
- **Ideally, separation occurs under conditions of capacity factors in the range of 1 to 5**

The selective factor α

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

$$\alpha = \frac{K_B}{K_A}$$

K_B is the partition coefficient for the more strongly retained species. Thus, α, is always >1

$$\alpha = \frac{K_B}{K_A}$$

Since K'A can be expressed as:

$$k'_A = \frac{K_A V_S}{V_M}$$

Then,

$$\alpha = \frac{k'_B}{k'_A}$$

since

$$k'_A = \frac{t_R - t_M}{t_M}$$

Consequently,

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$

Retention Volume, V_r

- **V_r : Volume of mobile phase required to elute sample component (move completely from column)**
- **$V_r = t_r$ X F**
- **Since**

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1+k_A}$$

$$\frac{LXF}{V_r} = \frac{LXF}{V_M} X \frac{1}{1+k'_A}$$

$$\frac{1}{V_r} = \frac{1}{V_M} X \frac{1}{1+k'_A}$$

$$V_r = V_M (1 + K'_A) = Vm + K \; V_S$$

V_r can be obtained from the chromatogram since $V_r = t_r$ X F

- **Mobile phase volume is proportional to column length. So retention is also increased for longer columns**

- **As peaks travel through the column, they broaden.**

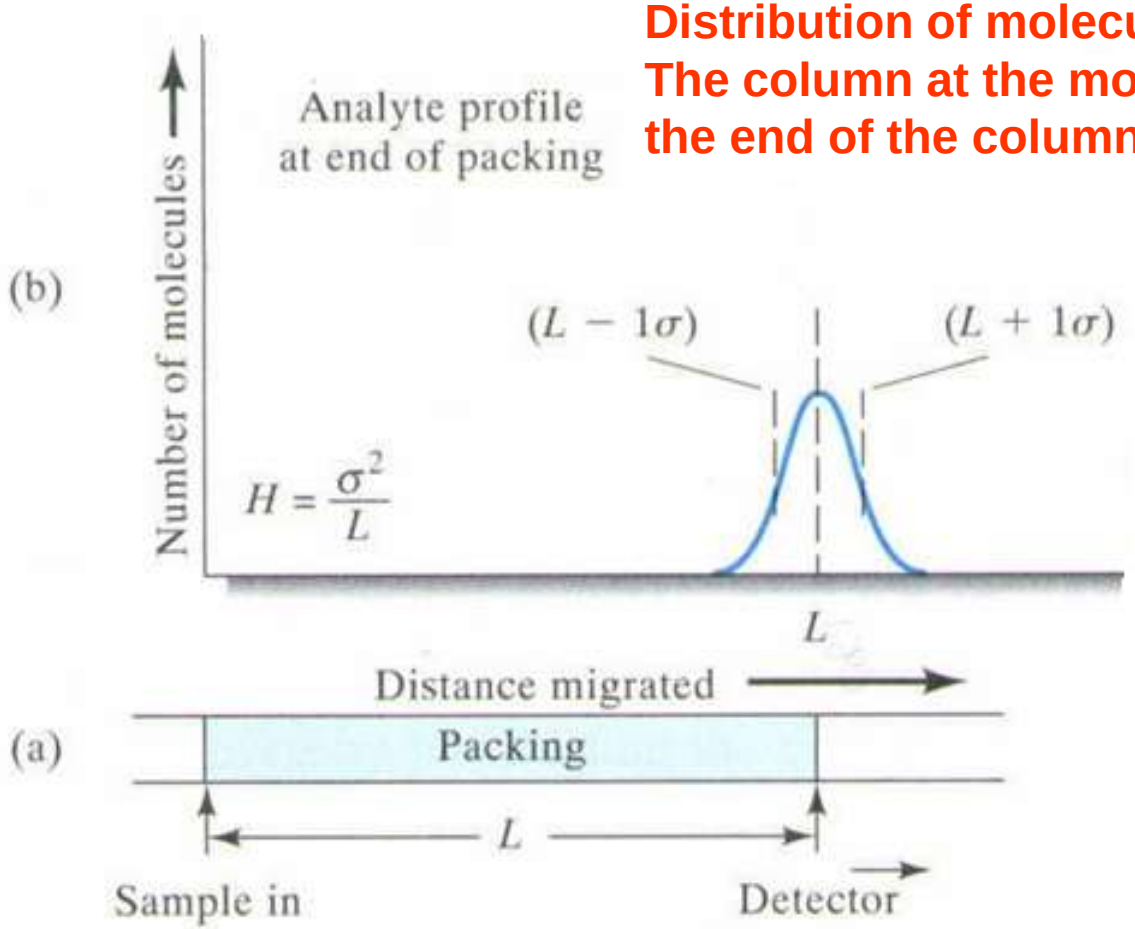
- **Peak width increases with the square root of column length**

- **Thus, longer columns wont give better resolution**

Methods for describing column efficiency Theoretical plates

- **In solvent extraction, a plate is represented by each equilibrium (extraction) is conducted**
- **In chromatographic column, the plates are theoretical**
- **The number of theoretical plates can be estimated based on peak retention times and widths**

Column Efficiency - Theoretical Plates
(Quantitative measures of separation efficiency)



Distribution of molecules along the length of The column at the moment the analyte peak reaches the end of the column

H = plate height

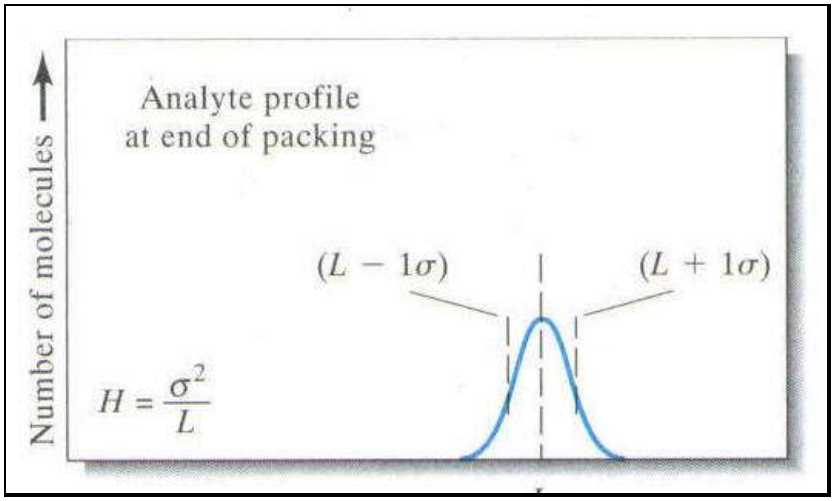
N = number of plates

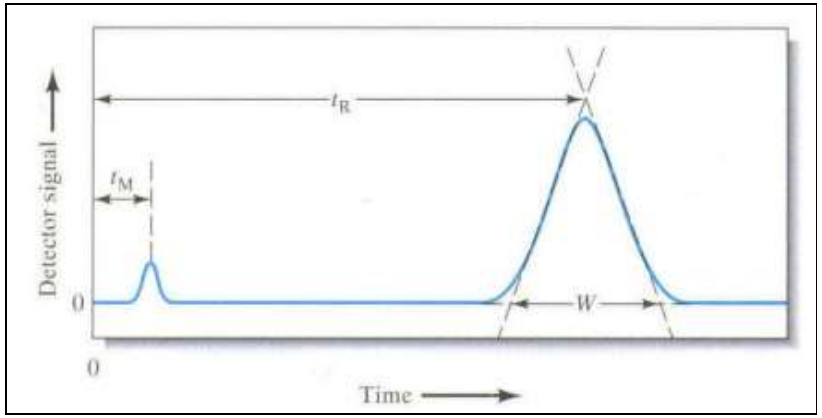
$$N = \frac{L}{H}$$

$$H = \frac{\sigma^2}{L}$$

σ is the standard deviation of measurements
Efficiency is defined in terms of variance per unit length

Relation between column length and retention times





$L =$ column length (distance)

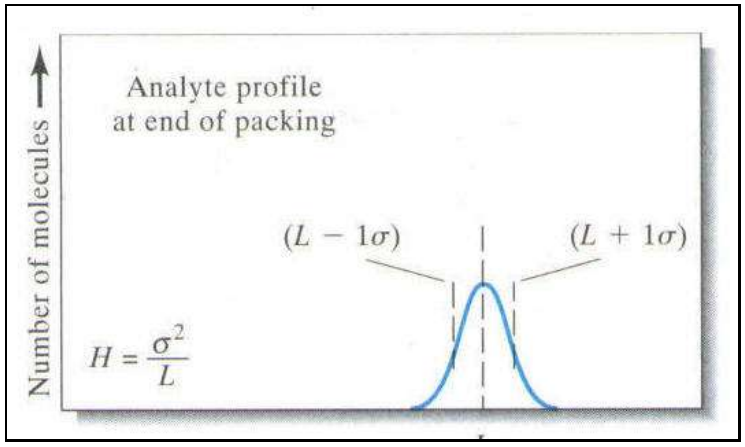
$\sigma =$ standard deviation in distance

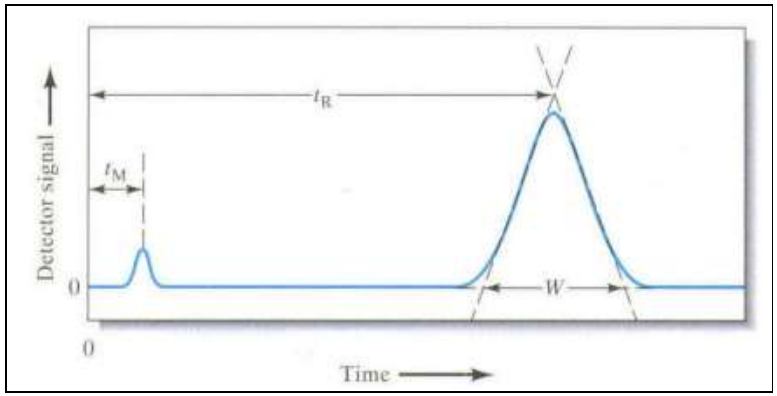
$t_R =$ retention time

$\tau =$ standard deviation in time

$$\frac{\sigma}{L} = \frac{\tau}{t_R}$$

$$\tau = \frac{\sigma}{L / t_R}$$





$$\frac{\sigma}{L} = \frac{\tau}{t_R}$$

$$\sigma = \frac{\tau L}{t_R}$$

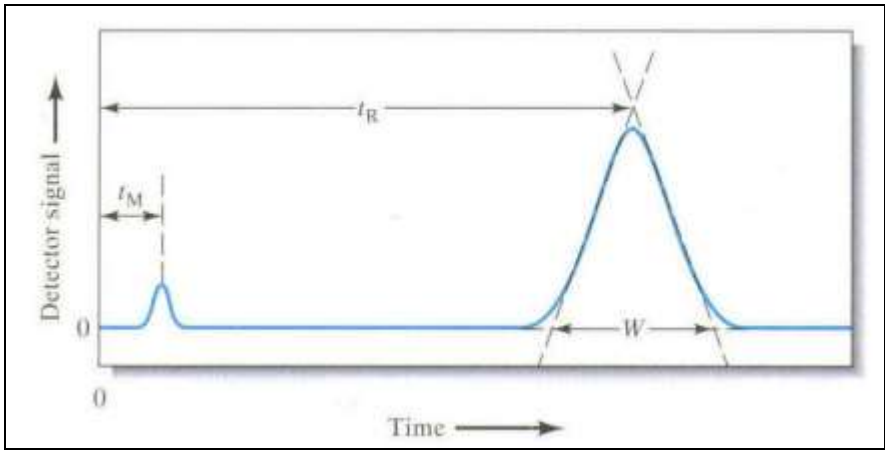
$$W = 4\tau$$

$$\sigma = \frac{W L}{4 t_R}$$

$$H = \frac{\sigma^2}{L} = \frac{W^2 L}{16 t_R^2}$$

- **Low H values achieve better separation**
- **In GC H < 1 to 3 mm**
- **In HPLC, H is one to two orders of magnitude lower**

Determining the Number of Theoretical Plates



N = number of pates

$$N = 16\left(\frac{t_R}{W}\right)^2 \qquad = \text{L/H}$$

where

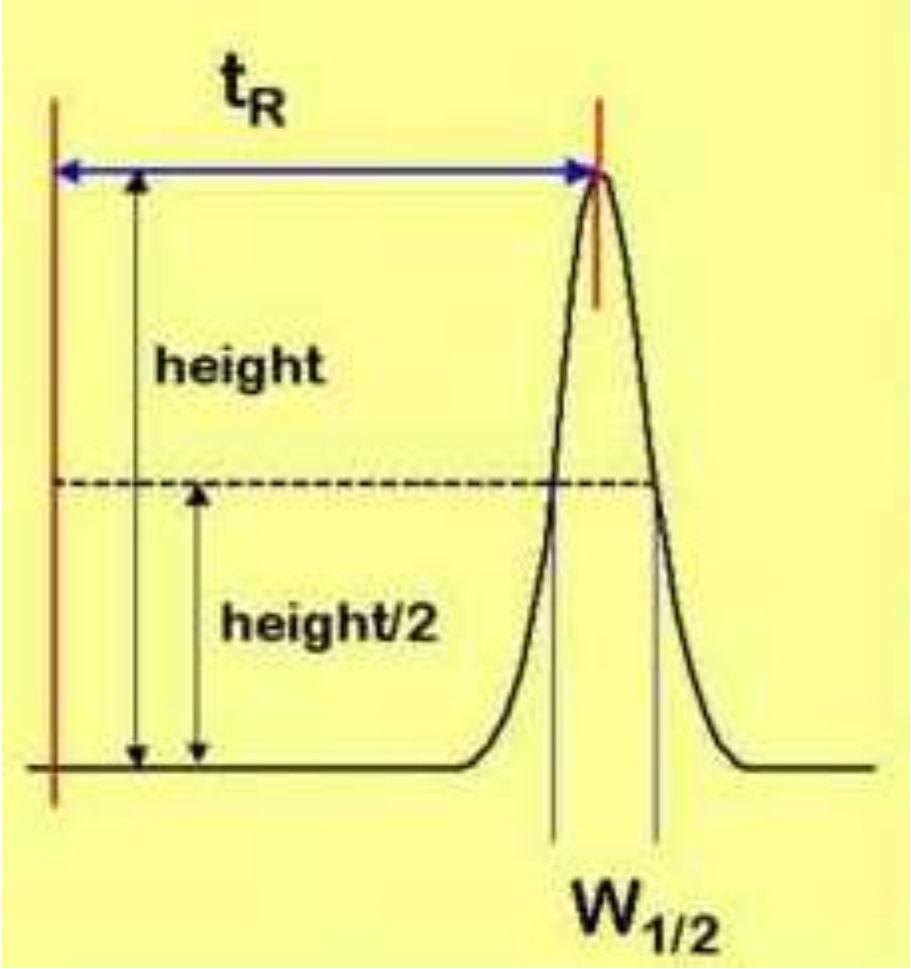
t_R = retention time

W = approximate base width of peak

W is derived from the intersection of the baseline with tangents drawn through inflection points on the sides of each peak

Experimental determination of N

- **Measuring the peak width is not always simple**
- **The peak may co-elute with another**
- **The low detector sensitivity may result in a difficulty in finding the start and the end of the peak**
- **Is there an alternative way?**



- The width at half height of the peak is measured
- Thus, the measurements are made well above the background and away from any detector sensitivity limit problem

- **Since the peak is Gaussian, the number of plates, N, can be determined by the following modified formula**

$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)$$

For a fixed length column the height equivalent to a theoretical plate , H, can be determined from the equation:

H = column height/N

Column Resolution

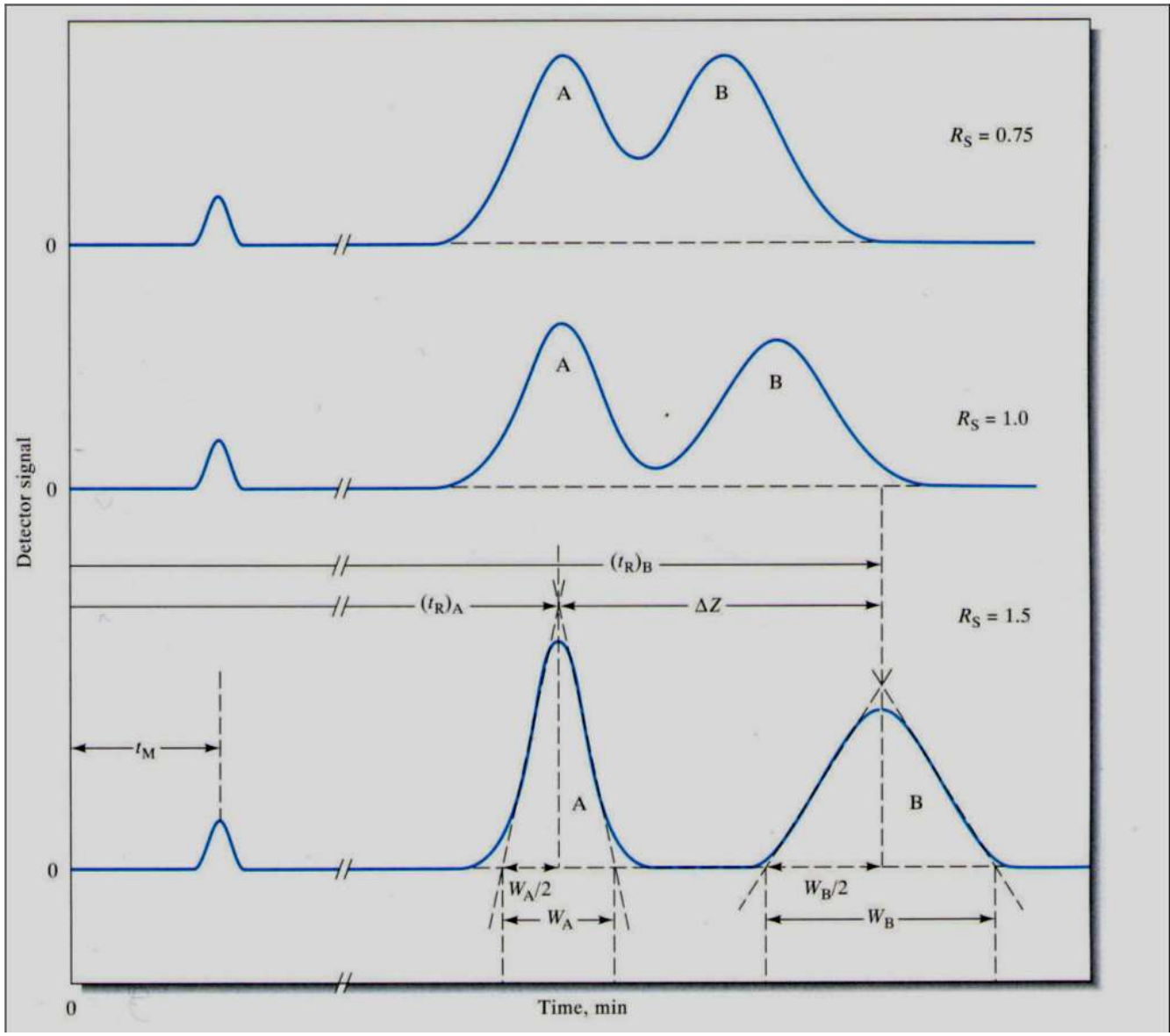
- **Column resolution, R_s, provides a quantitative measure of the ability of the column to separate two analytes**
- **It is s measure of how completely two neighboring peaks are separated from one another**

Column resolution

$$R_s = \frac{\Delta Z}{W_A/2 + W_B/2}$$

$$R_s = \frac{2\Delta Z}{W_A + W_B}$$

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



Column resolution

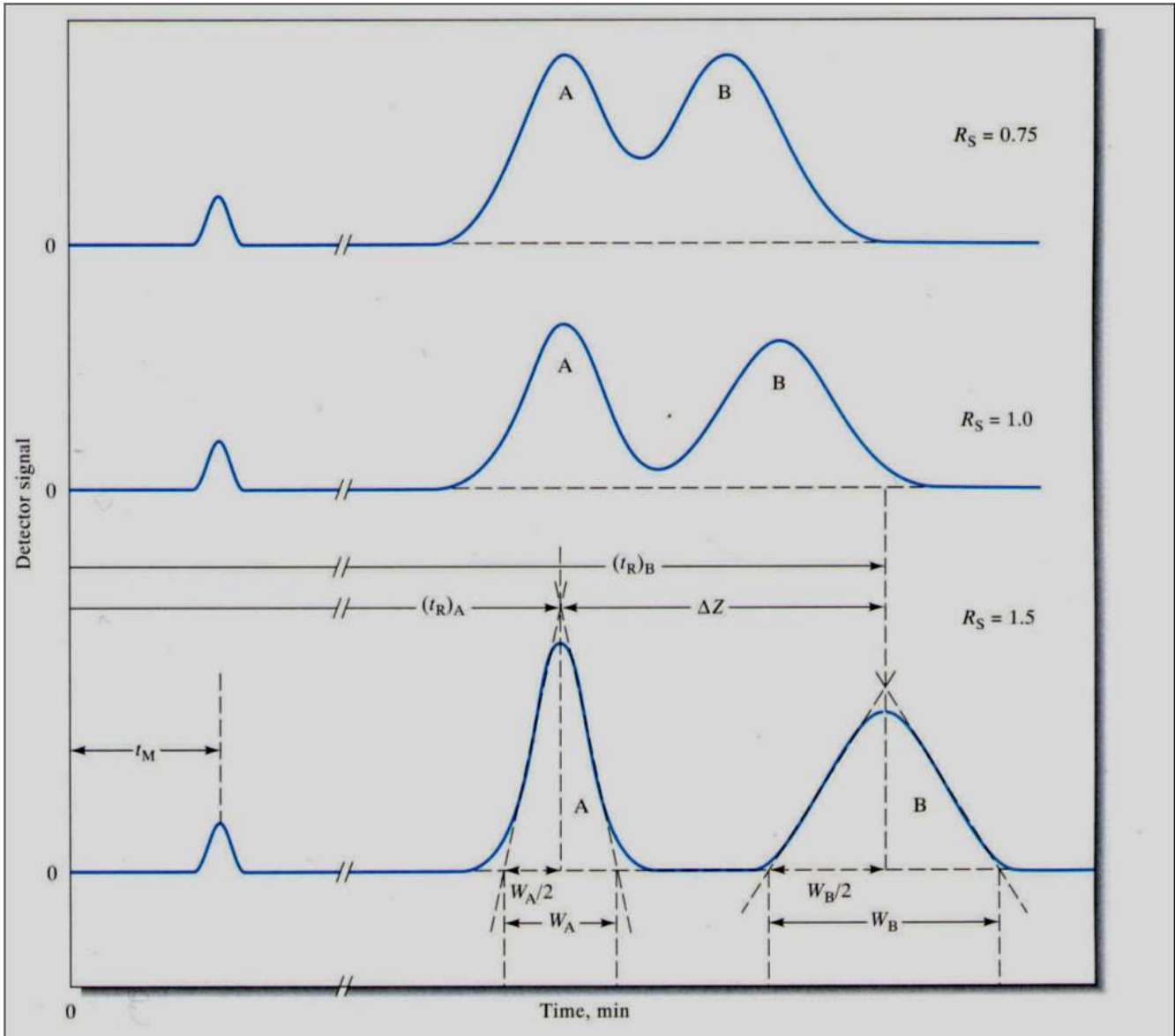
$$W_A = W_B = W$$

$$R_s = \frac{(t_R)_B - (t_R)_A}{W}$$

$$N = 16\left(\frac{t_R}{W}\right)^2$$

$$W = \frac{4t_R}{\sqrt{N}}$$

$$R_s = \frac{(t_R)_B - (t_R)_A}{(t_R)_B} \times \frac{\sqrt{N}}{4}$$



Sample Capacity

- **It is the amount of sample that can be sorbed onto a particular stationary phase before overloading occurs**
- **Exceeding the capacity results in : asymmetric peaks, change in retention time, loss of resolution.**
- **Sample capacity is proportional to V_s**

Chromatographic Compromise

- **Sample capacity, speed and resolution are dependent. Any one of the 3 can be improved at the expense of the other 2.**
- **Always there is a compromise**
- **In LC, speed and resolution are desired. Sample capacity is not important provided that detectable amount of sample is separated**
- **In preparative LC, speed is usually sacrificed**

Kinetic variables affecting band broadening

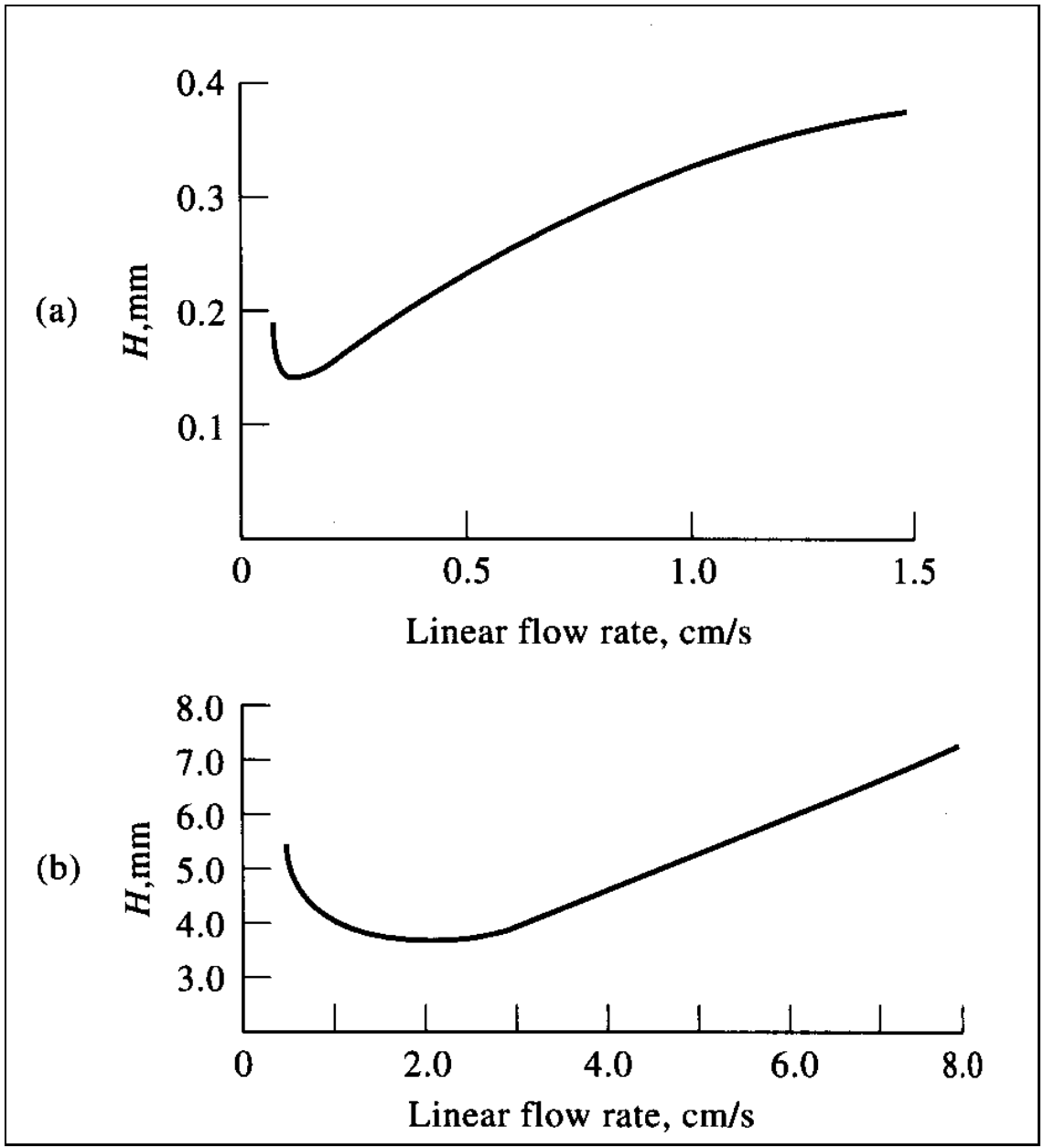
- **Band broadening is a consequence of the finite rate at which several mass-transfer processes occur during migration of a species down a column.**
- **Some of theses rates are controllable by adjustment of experimental variables, thus permitting improvement of separation**

Variables That Affect Column Efficiency

Variable	Symbol	Usual Units
Linear velocity of mobile phase	u	$cm \cdot s^{-1}$
Diffusion coefficient in mobile phase*	D_M	$cm^2 \cdot s^{-1}$
Diffusion coefficient in stationary phase*	D_S	$cm^2 \cdot s^{-1}$
Retention factor (Equation 26-8)	k'	unitless
Diameter of packing particle	d_p	cm
Thickness of liquid coating on stationary phase	d_f	cm

*Increases as temperature increases and viscosity decreases.

Effect of flow rate of mobile phase



Liquid chromatography

Gas chromatography

Relationships between plate heights and column variables

$$H = A + B/u + Cu$$

Van Deemter Equation

$$= A + B/u + (C_S + C_M)u$$

Modified Van Deemter Equation

Van deemter equation

We commonly group the various constants into single terms and reduce the equation to:

$$H = A + B/u + Cu$$

A - multipath or eddy diffusion

B - molecular diffusion **(Longitudinal diffusion)**

C - resistance to mass transfer **to and from liquid Stationary phase and in mobile phase**

Note that A, B and C are constants but the effect of B and C is dependent of the velocity of the mobile phase.

relationship between plate height and column variables

$$H = A + B/u + Cu$$
$$= A + B/u + (C_S + C_M)u$$

Van Deemter Equation

Modified Van Deemter Equation

Multiple flow path

(Eddy diffusion)

Longitudinal diffusion

Mass transfer between phases

$$H = A + B/u + Cu$$
$$= A + B/u + (C_S + C_M)u$$

Process	Term in Van Deemter eq.	Relationship to Column* and Analyte Properties
Multiple flow paths	A	$A = 2\lambda d_P$
Longitudinal diffusion	B/u	$\frac{B}{u} = \frac{2\gamma D_M}{u}$
Mass transfer to and from liquid stationary phase	$C_S u$	$C_S u = \frac{f_S(k')d_f^2}{D_S} u$
Mass transfer in mobile phase	$C_M u$	$C_M u = \frac{f_M(k')d_p^2}{D_M} u$

*u, D_S, D_M, d_f, d_p, k' are as defined in the Table above

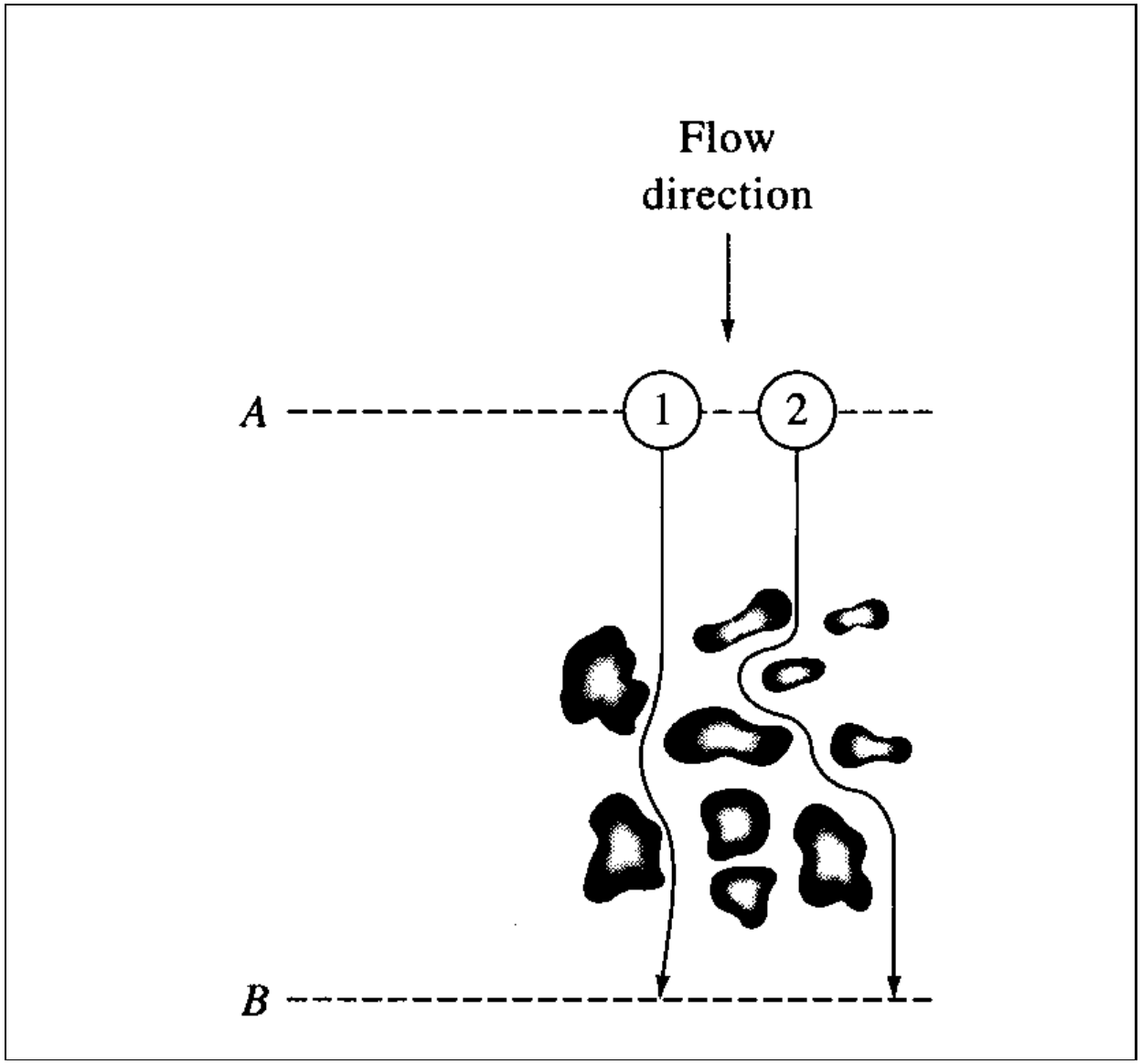
$f(x)$ = function of x.

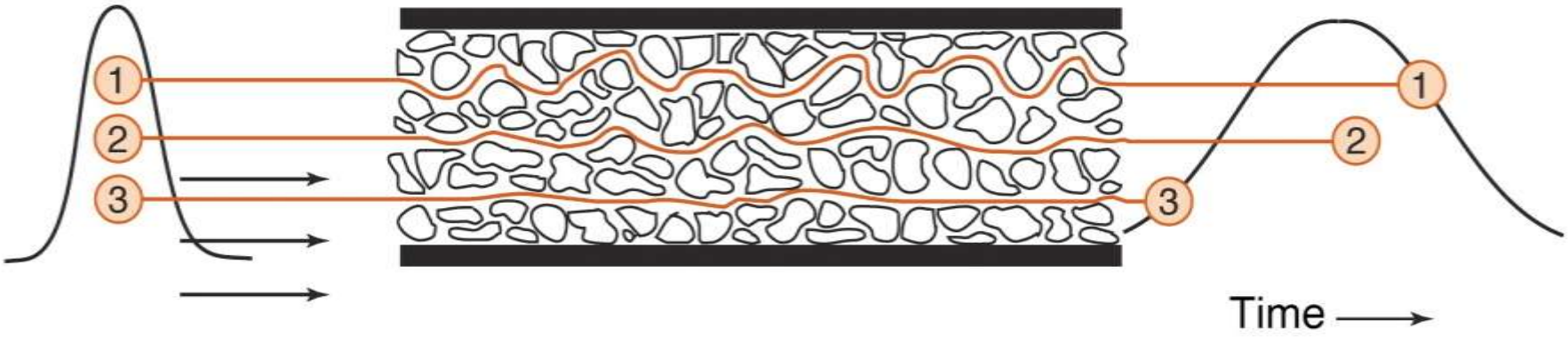
λ, γ: constants that depend on the quality of the packing.

B: coefficient of longitudinal diffusion.

C_S, C_M: coefficients of mass transfer in stationary and mobile phases, respectiv

Multiple Pathways (Eddy diffusion)

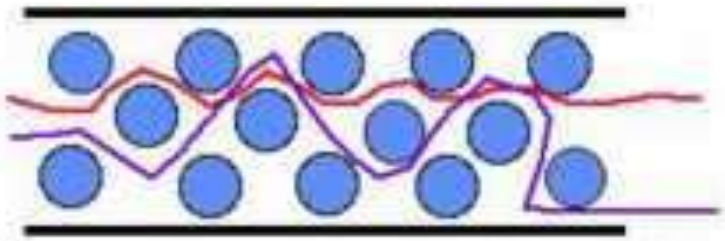


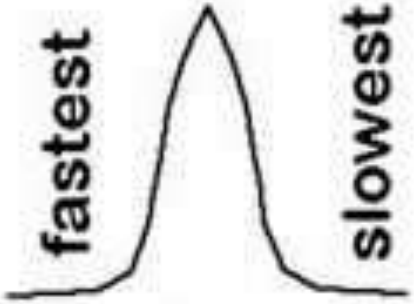

1
2
3
1
2
3
Time

Eddy Diffusion A term

Multipath or eddy diffusion

This term accounts for the effects of packing size and geometry.





The range of possible solute paths results in a minimum peak width.

Eddy Diffusion

$A = 2\lambda d_R$

where λ => packing factor

d_R => average diameter of particle

- caused by many pathways
- minimized by careful packing

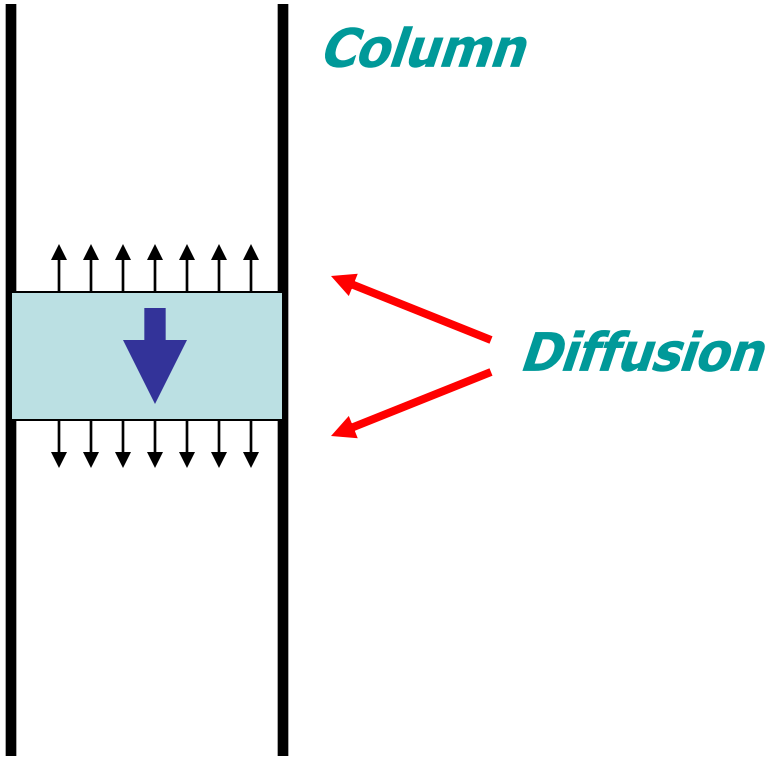
A term

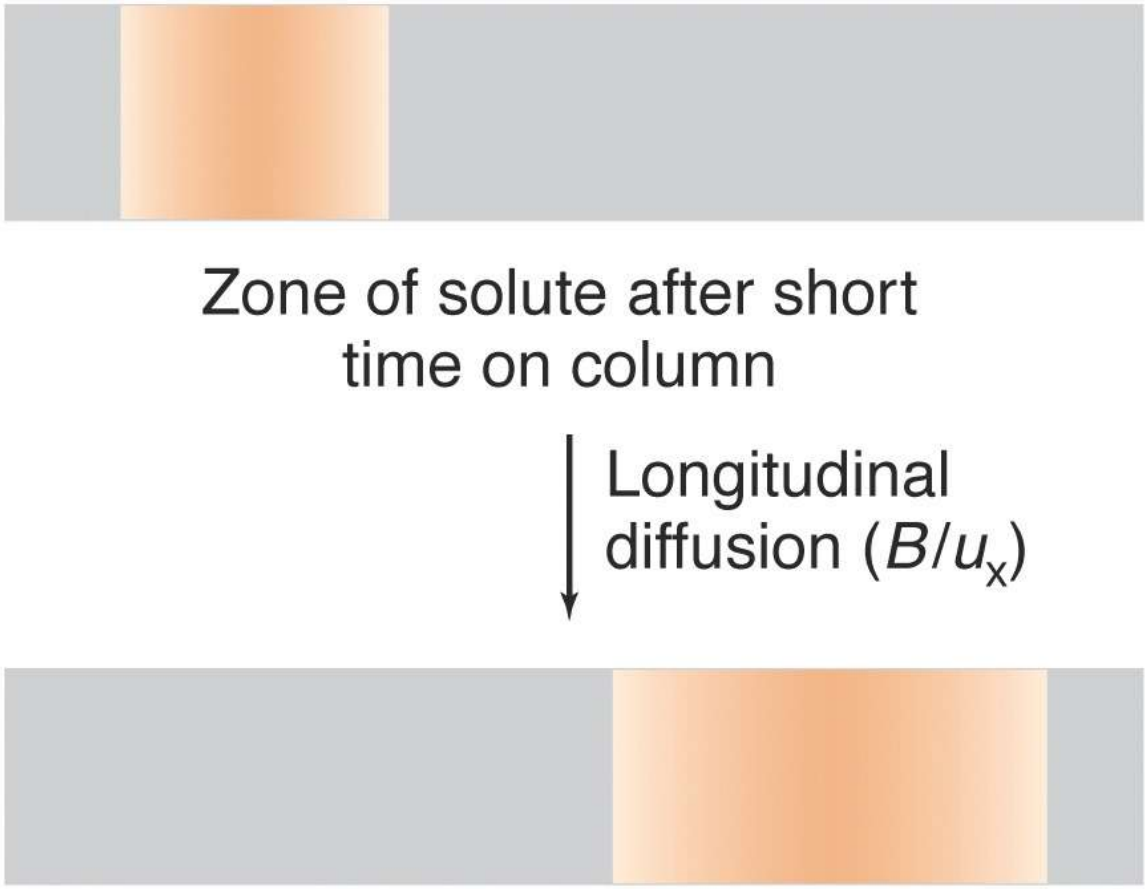
Once the column is packed, nothing can be done to reduce the A term.

Its effect can be reduced by using

regular sized packing
small diameter packing
not allowing any loose packing or dead space in the column

Longitudinal Diffusion: B/u




Zone of solute after short time on column
Longitudinal diffusion (B/u_x)
Zone of solute after longer time on column
Direction of travel

Longitudinal Diffusion

Represents broadening due to diffusion in the mobile phase.

Reverse diffusion is more significant than forward to to mobile phase movement.

Longitudinal Diffusion B//u

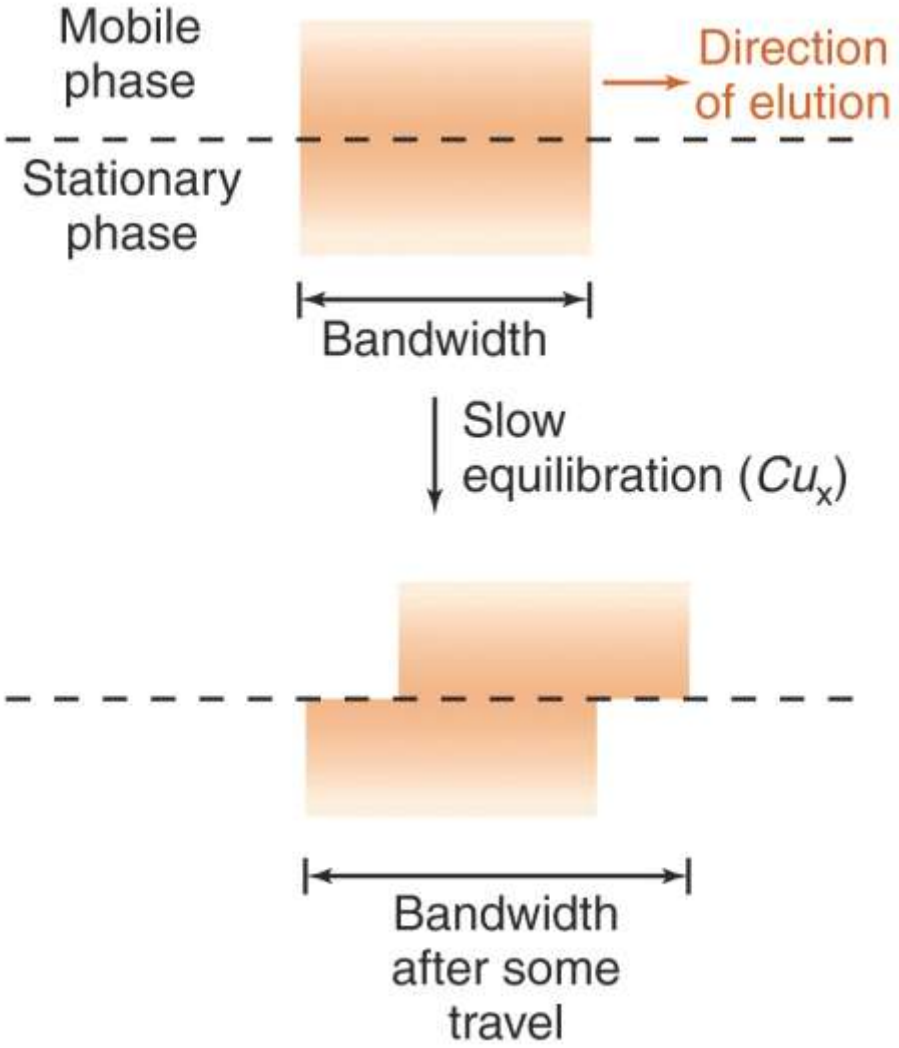
$B = 2\psi D_M/u$

where ψ =>obstruction factor

D_M => diffusion coefficient of solute in the mobile phase

- minimized by lowering temperature of column oven and decreasing flow rate

$(C_s + C_M)u$ Term: Resistance to mass transfer



It takes time for a solute to reach equilibrium between the two phases

Thick or viscous stationary phases have larger terms

Thus, there is a lower rate of mass transfer and an increase in plate height

$C_s u$ Term

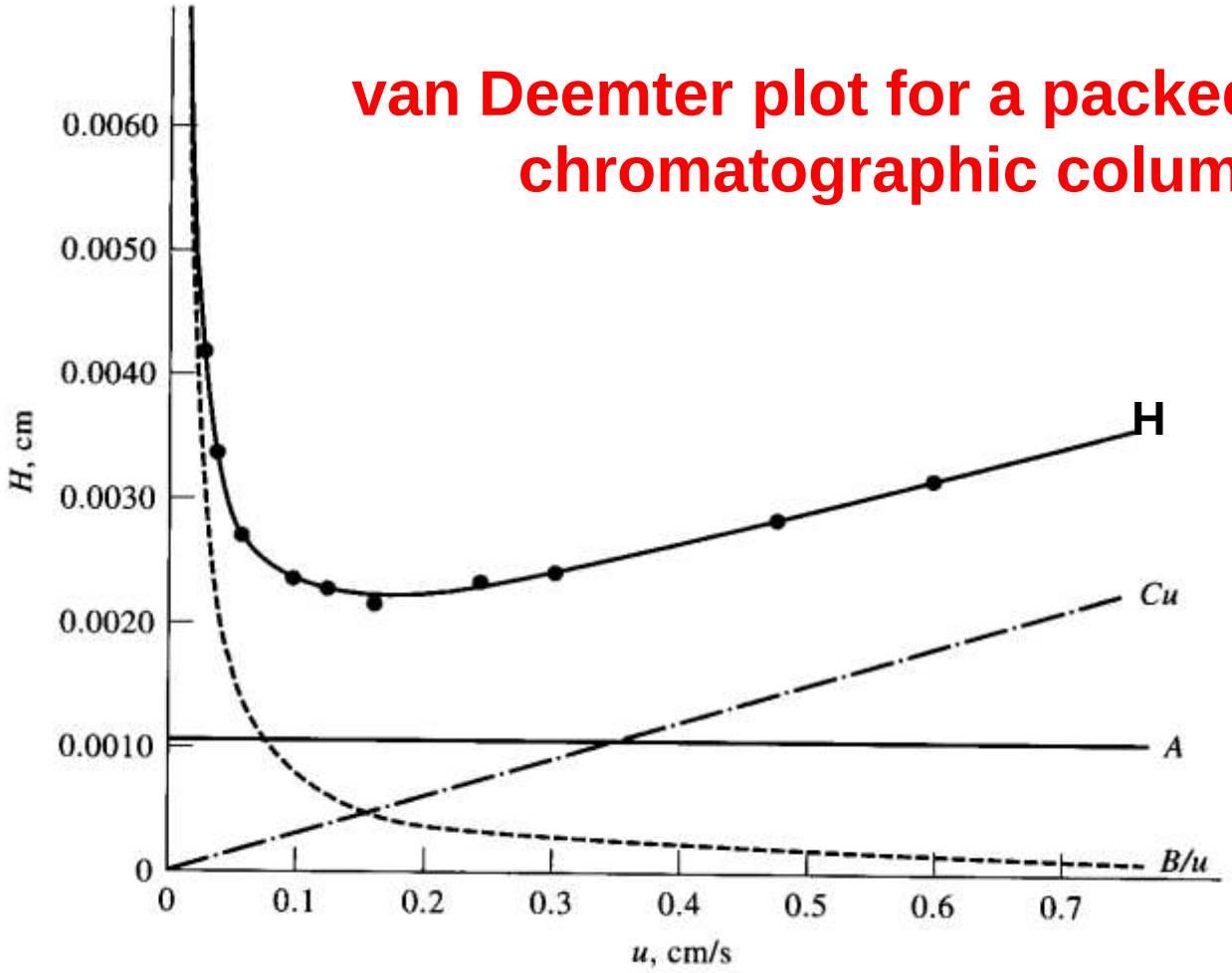
- **$C_s u$ differs depending upon the stationary phase (l or s)**
  - **When stationary phase is liquid, $C_s u$ is directly proportional to d^2_f and inversely proportional to diffusion coefficient of species in the film**
  - **When stationary phase is solid, $C_s u$ is directly**

    proportional to the time required for the species to be adsoebed or desorbed

$C_m u$ Term

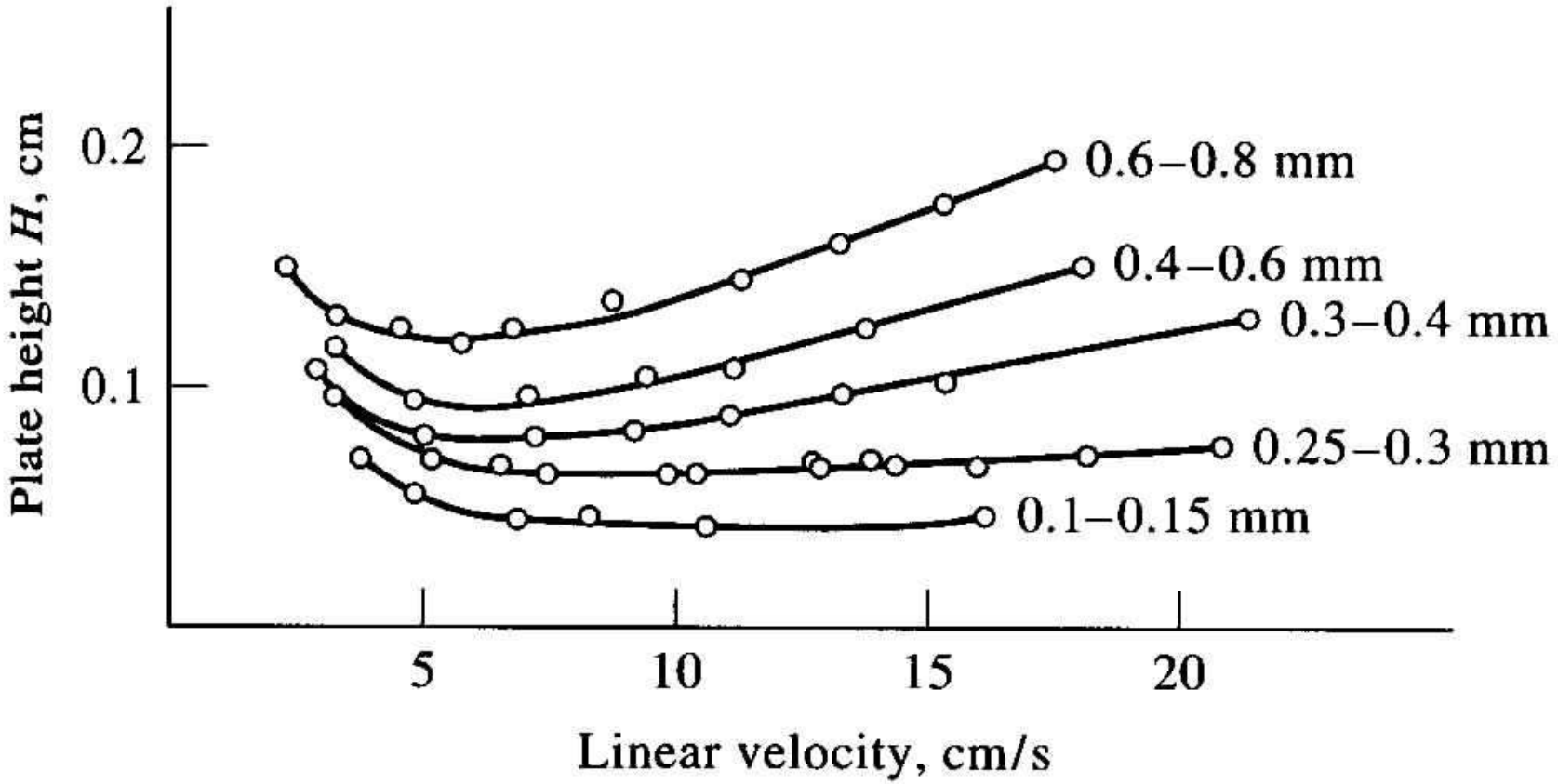
- **$C_m u$ is inversely proportional to diffusion coefficient of the analyte in the mobile phase and some function of the square of the particle diameter of the packing d^2_p**

van Deemter plot for a packed liquid chromatographic column



- **The points on the upper curve are experimental.**
- **The contributions of the various rate terms are shown by the lower curves: *A*, multiple path effect; *B/u*, longitudinal diffusion; *Cu*, mass transfer for both phases.**

Two important variables: diameter of the column and the diameter of the particles packed in the column



Effect of particle size on plate height. The numbers to the right are particle diameters.

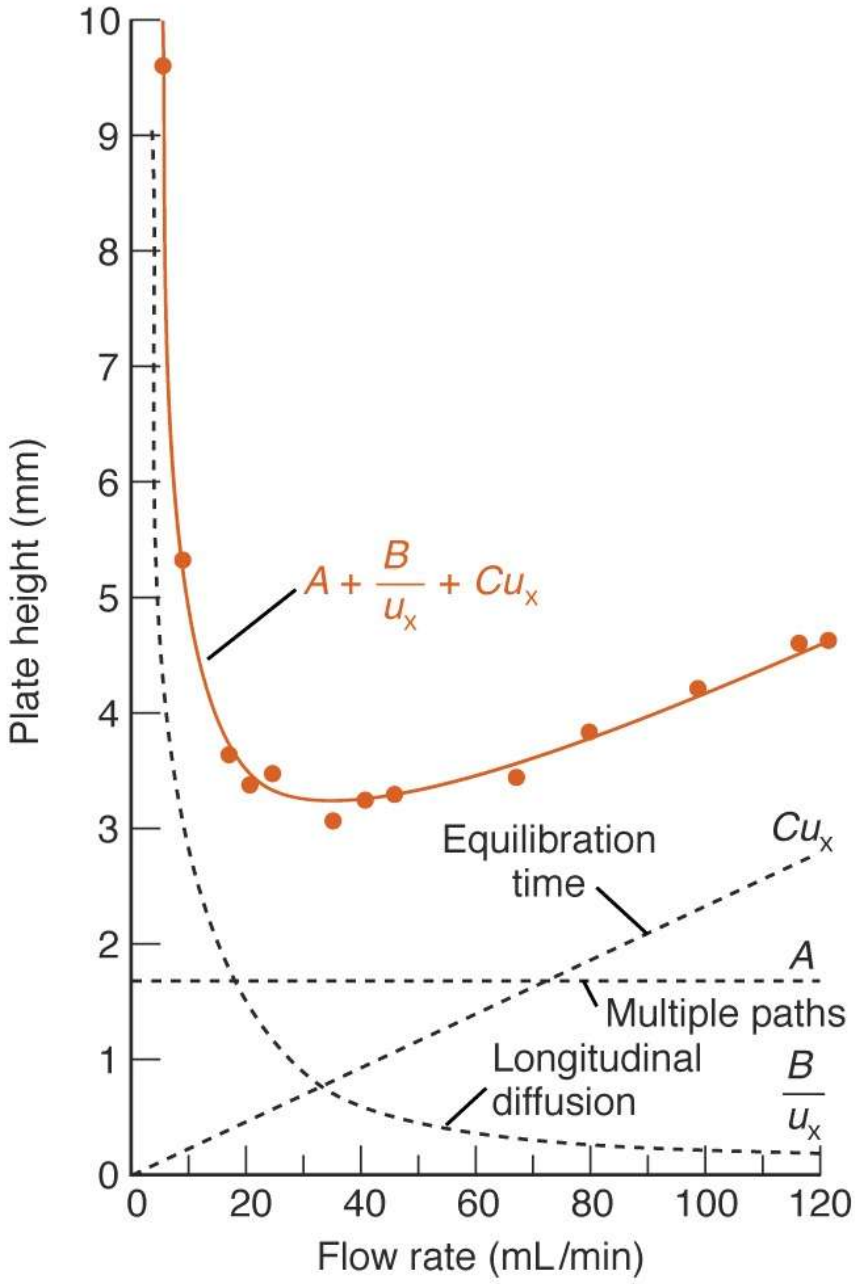
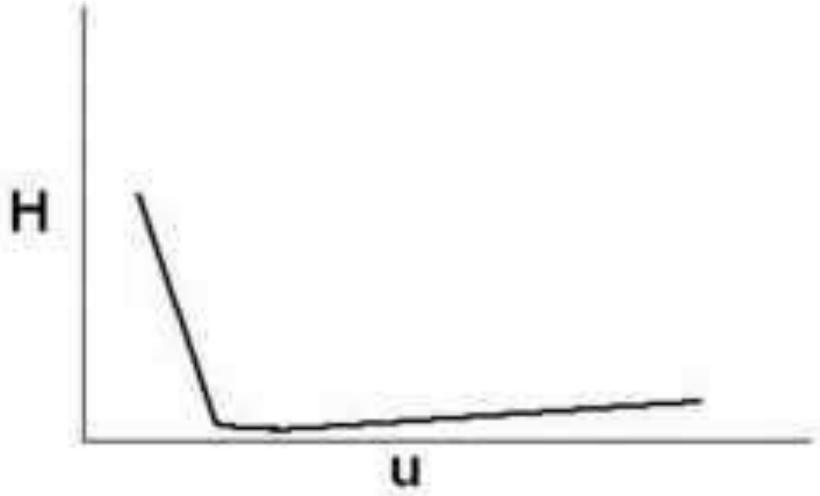


Plate height (mm)
10
9
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
120
Flow rate (mL/min)
$A + \frac{B}{u_x} + Cu_x$
Equilibration time
Cu_x
A
Multiple paths
Longitudinal diffusion
$\frac{B}{u_x}$

Capillary columns

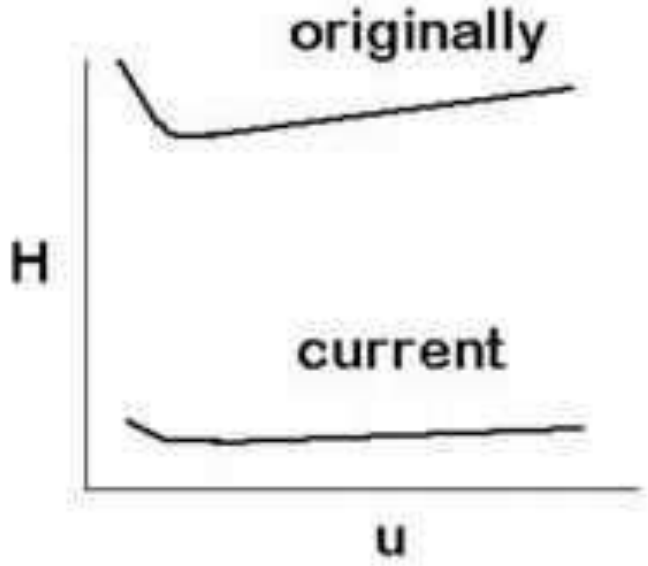
Not much effect from A or C. There is no packing and the phase is very thin



Liquid chromatography

At first, LC relied on irregular packing. Now the packings are pretty good so the A term is very low.

The B and C terms are low because liquids diffuse much more slowly than gases.



Applications of Chromatography

- **Qualitative Analysis**
- **Quantitative Analysis**
  - **Analyses Based on Peak Height**
  - **Analyses Based on Peak Areas**
  - **Calibration and Standards**
  - **The Internal Standard Method**
  - **The Area Normalization Method**

GC & LC Qualitative and Quantitative Analysis

Qualitative analysis: Main approach

- **GC is a blind method that indicates the presence of a substance but not what it is.**
- **Qualitative analysis is based upon comparison of retention data that are characteristics but not unique**
- **Retention data used :**
  - **Retention time,**
  - **retention distance,**
  - **retention volume**
- **These are dependent upon: Column dimensions, liquid phase (type and amount), column temperature, flow rate, type of carrier gas, packing density, pressure drop**

Factors affect t_R from two different columns of the same type

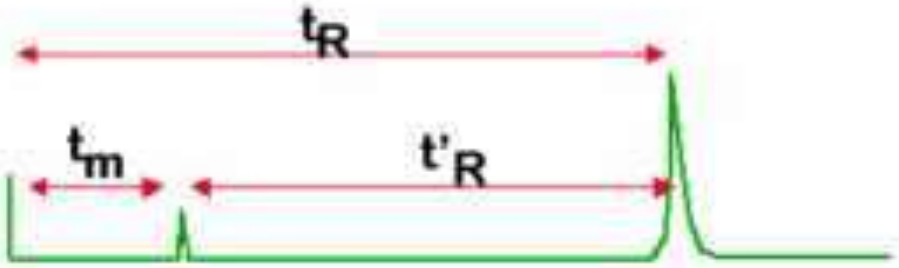
- Packing density
- Liquid loading
- Activity of the support
- Age & previous use of the packing
- Variation in composition of the column wall

- Thus, when two separate columns must be used, Relative Retention date is preferred.
- Since t_R values are characteristic of a particular sample in the column conditions but are not unique!!
  - run the unknown sample immediately before or immediately after the standard; and all conditions are the same.

Retention time

Retention time - t_R - time elapsed from point of injection to maximum of peak.

Adjusted t_R - t'_R - time from maximum of unretained peak to maximum of eluent.



Hold up time - t_M - time required for mobile phase to traverse the column.

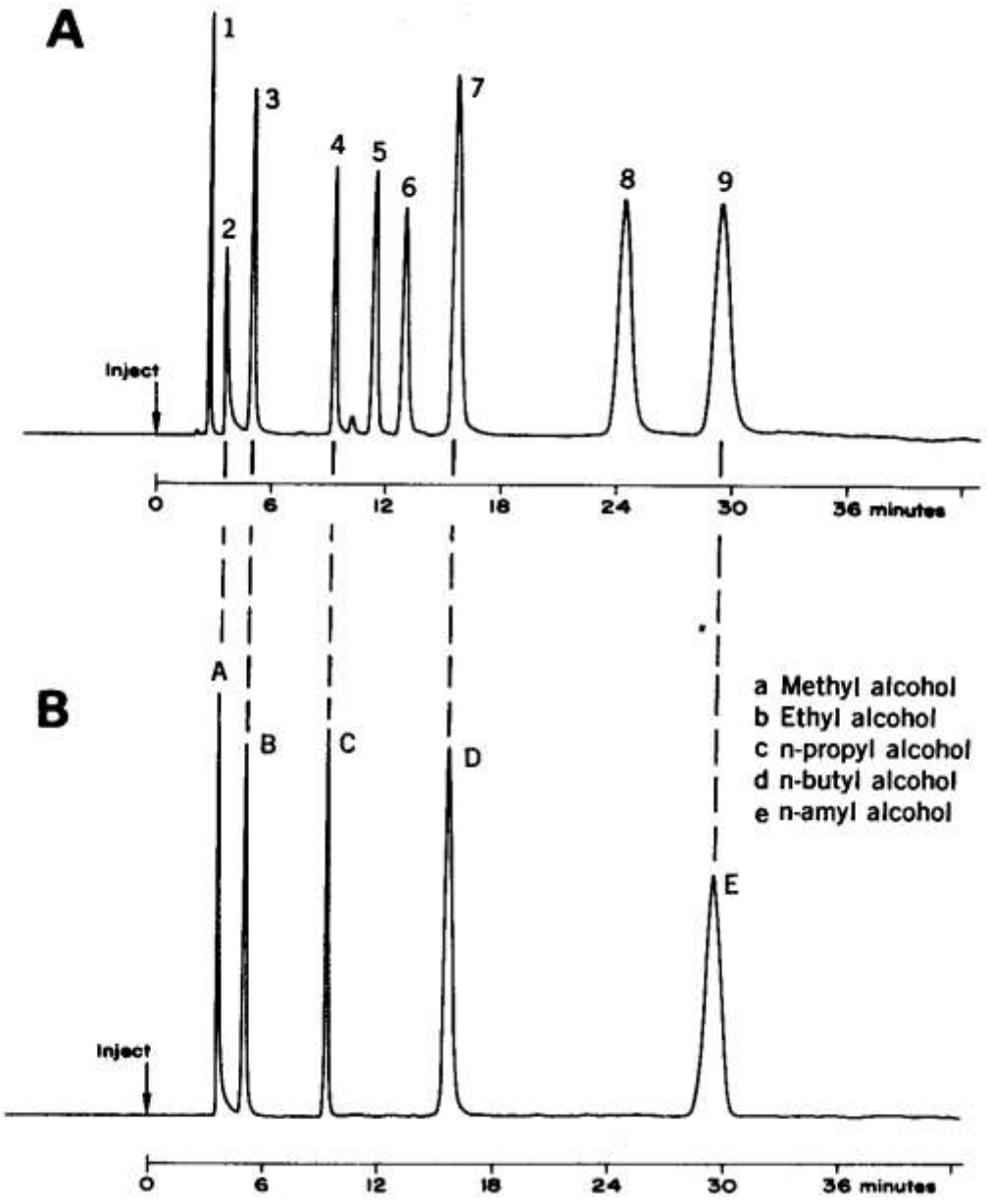
Retention time, t_R

Simple retention time data is adequate for simple assays like process quality control.

- You already know what is there.
- There are only a few components in the sample (or only a few of interest).

If a true unknown is observed, you can't do much more than note its presence!

IDENTIFICATION BY RETENTION TIMES



Relative retention data

To be useful

Standard should be a part of the sample or added to it - internal standard

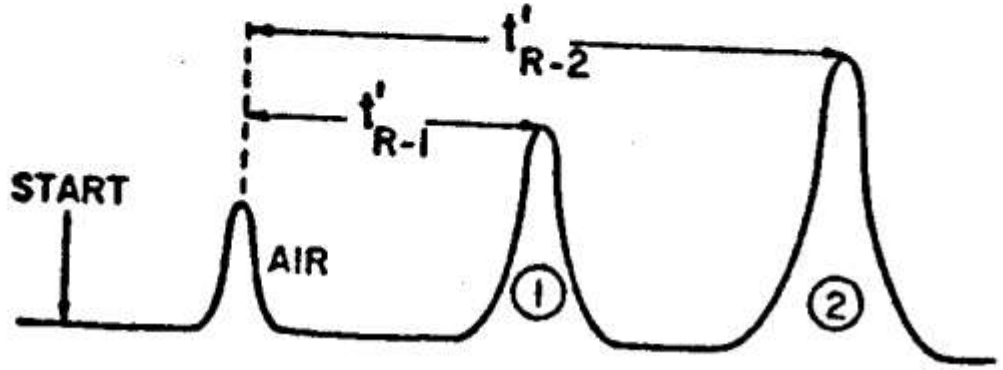
It should be something that elutes near center of an analysis - although you can use more than one.

Sample size should be small.

Values will remain pretty constant between runs - may vary with a new column.

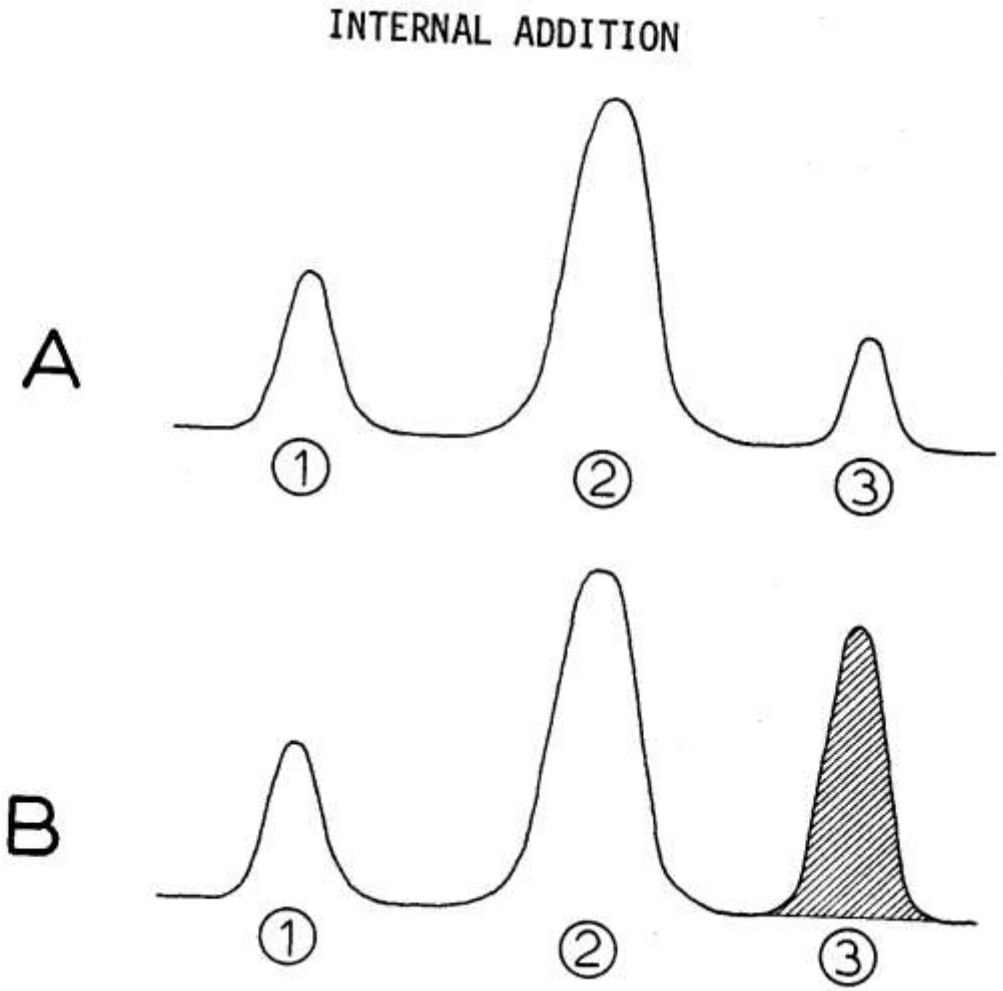
RELATIVE RETENTION

$$\alpha = t'_{R-2} / t'_{R-1} = K_2 / K_1$$



- **Component 1 is used as the reference; it should be present or added to the sample and compatible with the sample**
- **Peak of component 1 must be close (but resolved) to the sample peak**

- **Relative retention eliminates variations in**
  - **Column diameter and length**
  - **liquid phase loading**
  - **Carrier flow rate**
  - **Others**
  - **Referring the sample to 2 references gives different results**



When component 3 is suspected add more of this component to the sample and watch any change in its peak

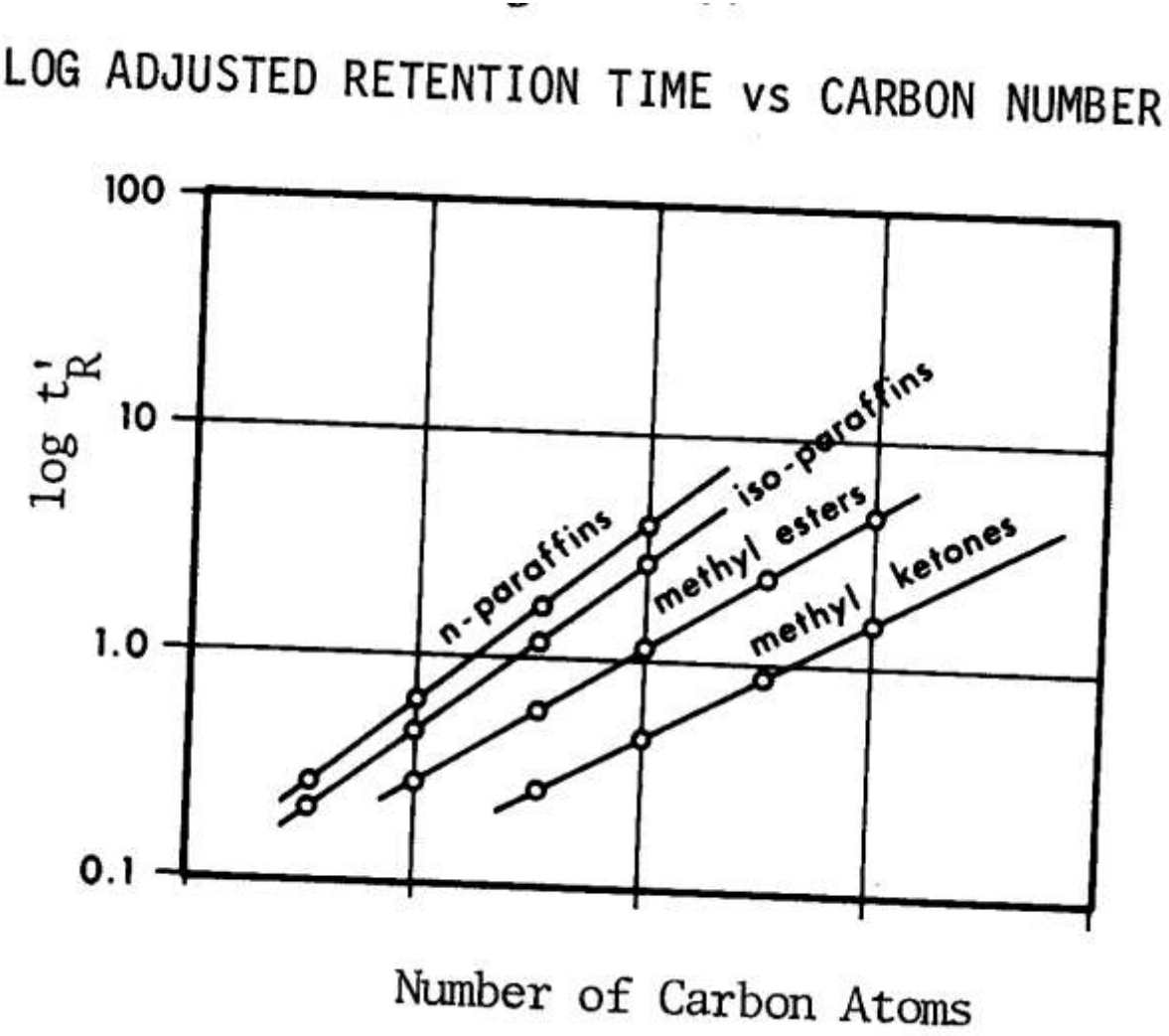
Other methods of qualitative analysis

Retention plots

Retention values of materials belonging to a homologous series can usually be related to physical characteristics.

A many cases, a semi log plot of t_R vs carbon number will give a linear relationship for earlier members of a series.

This can be used to pick out potential series members.



- **Only 3 compounds are needed to establish the line**
- **The line can be used to identify other members of the Same homologous series**

Homologous and Pseudo-homologous Series:

Alkanes,
Olefins
Aldehydes,
Ketones,
Alcohols,
Acetates
Acetals,
Esters,
Sulfoxides
Nitroderivatives
Aliphatic amines
Pyridine homologs.
Aromatic hydrocarbons, dialkyl ethers, thiols, alkylnitrates, substitute tetrahydrofuran, tetrahydrofuran

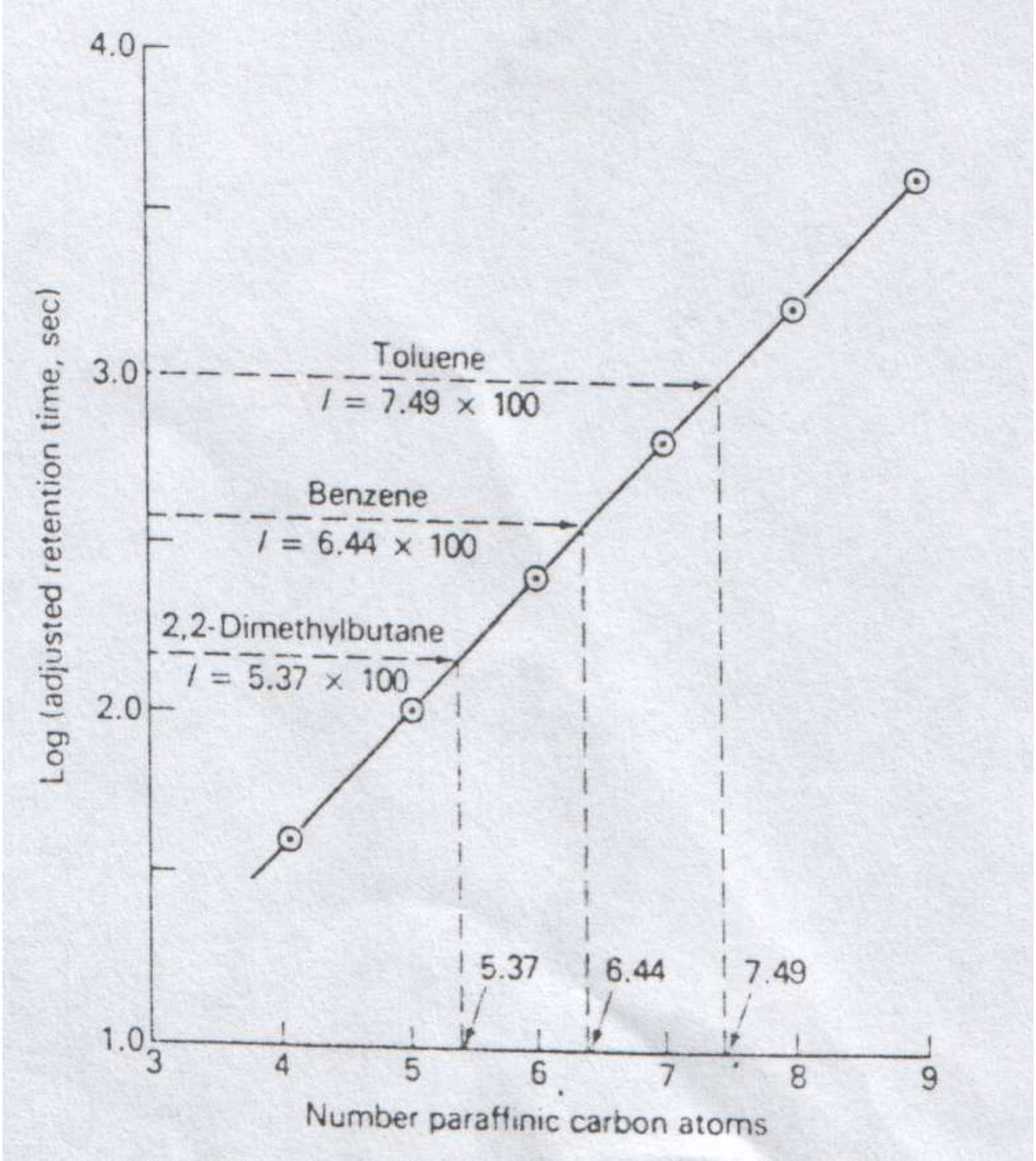
Kovats Retention Index, R.I.

- **R.I. indicates where a compound will appear with respect to normal paraffins**
- **R. I (n-paraffins) (by definition) =**

  # C atoms x 100

  (regardless of column or conditions)

- **R.I of any solute is derived from a chromatogram of a mixture of the solute + at least two normal paraffins having t'_R close to that of the solute.**
- **R.I. values change with column**
- **They are used for qualitative analysis**
- **R.I values are meaningless without the conditions being reported**


4.0
3.0
2.0
1.0
Log (adjusted retention time, sec)
Toluene
I = 7.49 × 100
Benzene
I = 6.44 × 100
2,2-Dimethylbutane
I = 5.37 × 100
5.37
6.44
7.49
3
4
5
6
7
8
9
Number paraffinic carbon atoms

Quantitative Analysis

Steps :

- **Sampling**
- **Sample preparation**
- **Chromatography**
- **Integration**
- **Calculations**

- **Peak height or peak area are the basis for quantitative analysis**

Basis for Quantitative Analysis

- **The peaks in the chromatogram are the basis for quantitative analysis**

Peaks of interest should fulfill the following requirements:

- **must be undistorted**
- **must be well separated**
- **Must have a large S/N ratio**
- **must have a flat aseline**

Peak shape: The ideal chromatographic peak is symmetric and narrow

To get symmetrical peaks following should emphasized:

*** Clean entry through the septum**

*** Rapid depression of the syring plunger**

*** Quick withdrawal of the syringe**

*** Choosing proper column conditions**

*** Temperature programming helps avoiding the broadening of the later peaks in an isothermal techniques.**

Peak separation

- **The resolution of at least 1 must be achieved for all peaks of interest**
- **If the two peaks are fused together, dropline technique should be considered first, where the fused peaks are separated by dropping a perpendicular from the vally between them to the baseline.**

Peak size

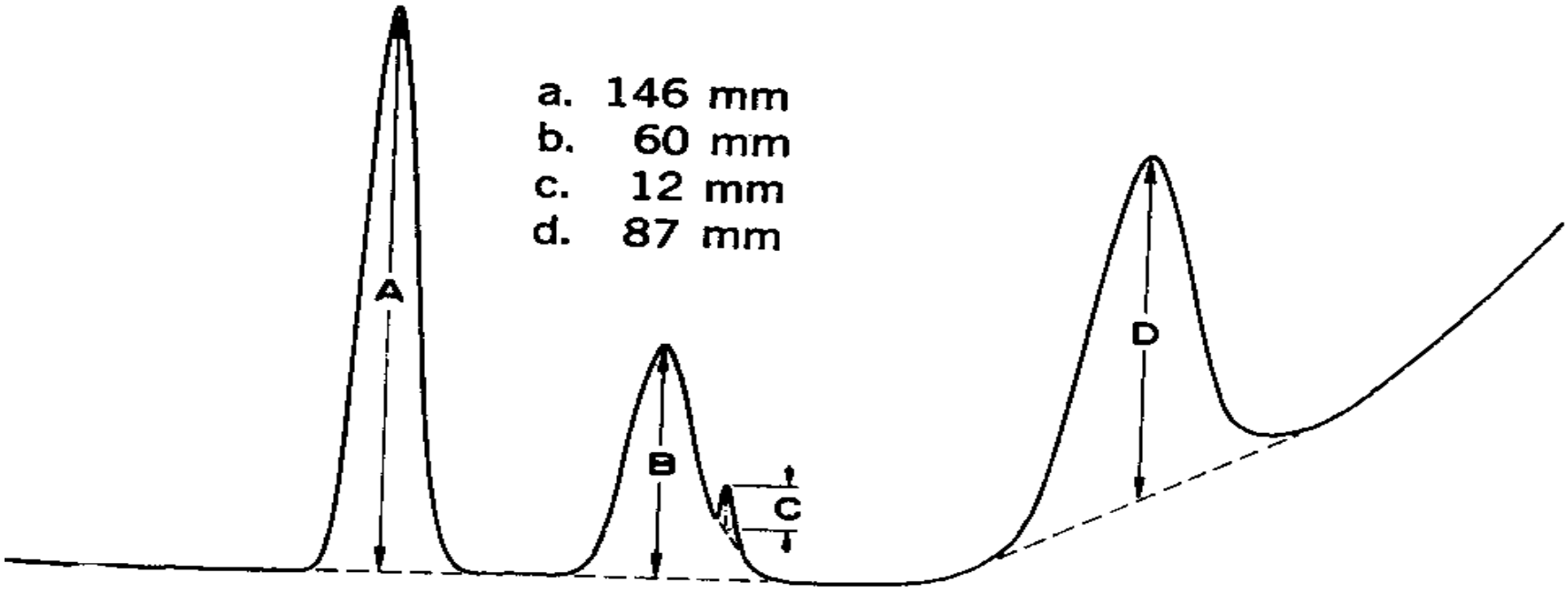
- **Most appropriate peak is a big one on a quite flat baseline.**

Linear range

- **It is desirable to operate in the linear range of the detector system.**
- **When the linear range of the detector is narrow it is often necessary to make several dilutions until a linear range is found.**

Response Measurements:

The computer will take care of the chromatographic peaks and give information like: retention times; peak heights; peak areas; calculations and comparison with memory values



Peak height

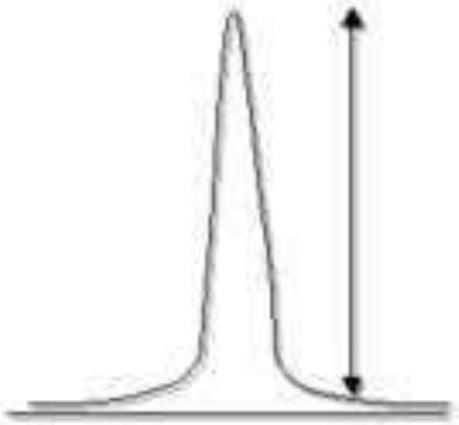
In some cases, you can assume that peak height is proportional to concentration.

Advantages

- Simplicity
- Rapid calculations

Disadvantages

- Height is more variable than area



Typically used only with capillary columns

Peak area

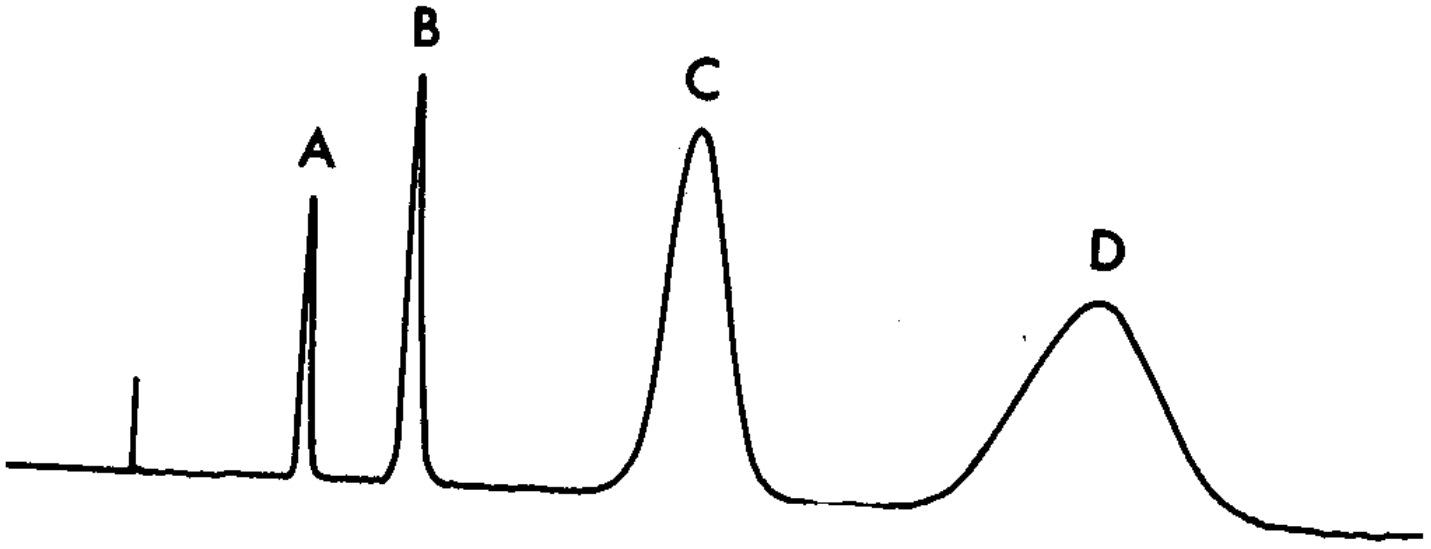
This is the major approach for establishing a relationship between peaks and concentration.

$$\text{area} \propto \text{concentration}$$

Area is determined from a large number of measurements and detectors usually have very large dynamic ranges. This results in a very reliable measurement.

Calculation Methods

1. Area normalization
2. Normalization with response factors
3. External standard method
4. Internal standard method
5. Standard addition method



1. Area normalization (Internal normalization)

$$\%A = \frac{\text{Area A}}{\text{Total Area}} \times 100$$

2. Normalization with response factors

$$\%A = \frac{\text{Area A} \cdot \text{Factor A}}{\Sigma \text{ Areas x Factors}} \times 100$$

Detector response factors

Determines the actual response per unit concentration for a set of sample components.

detector response, f = concentration/area

Let's work with a set of three species.

We'll call them 1, 2 and 3

Detector response factors

Now, when assaying a sample containing these three components, the corresponding concentrations (%C) are calculated using these factors.

$$A'_1 f_1 + A'_2 f_2 + A'_3 f_3 = \sum_{i=1}^{n} A'_i f_i$$

and

$$\%C_x = 100 \frac{A'_x f_x}{\sum A'_i f_i}$$

Detector response factors

OK, how about we use some numbers!

Example

A sample consists of three component:
X, Y and Z.

The standard contains 200 mg of each component in 100 ml of an appropriate solvent.

Detector response factors

Injection of 5 μl of the standard produces the following peak areas:

Component	Area
X	238
Y	660
Z	1190

We'll make Z the NORM for this example.

Detector response factors

Determine the relative response factors for the other components.

X	f_X	=	1190 / 238	= 5.0
Y	f_Y	=	1190 / 660	= 1.8

Now run your actual sample.

Component	Area
X	90
Y	265
Z	460

Detector response factors

Multiply each peak area by the appropriate response factor and calculate the total corrected area.

X	90 x 5.0	=	450
Y	265 x 1.8	=	477
Z	460 x 1.0	=	460
	Total area	=	1387

Detector response factors

Finally, calculate the corrected % by weight for each of the species:

$$\%\,X = 100\,(\,450 / 1387\,) = 32.4$$

$$\%\,Y = 100\,(\,477 / 1387\,) = 34.4$$

$$\%\,Z = 100\,(\,460 / 1387\,) = 33.2$$

Detector response factors

So why bother?

- First, Z must be present in each sample assayed. It is actually serving as a type of internal standard.
- This method corrects for variations in the amount of sample injected.
- Is there a better way? Sure - the internal standard method.

External standard method

Requirements for proper use:

Standard solution containing all eluents to be quantified.

Standard eluents should be of similar concentration as unknowns.

The standard and sample matrix should be as similar as possible

Analysis conditions must be identical - stable instrument, same sample size ...

External standard method

You either assume that response is linear over the entire concentration range or measure it. Then:

$$conc_{unknown} = \frac{Area_{unknown}}{Area_{known}} \; conc_{known}$$

This is assuming that the same injection volume was used for both the unknown and standard.

External standard method

Example - determination of X in $MeCl_2$

Prepare a standard of X

(20.0 mg in 100 ml $MeCl_2$) - 0.200 μg/μl

Use an injection volume of 5 μl for both the standard and the unknown.

Measure the areas produced by both the sample and the unknown.

Area X_{std} = 2000 units

Area X_{unk} = 3830 units

External standard method

Now, determine the concentration of X in you unknown.

$$conc_{unknown} = \frac{Area_{unknown}}{Area_{known}} \; conc_{known}$$

$$conc_{unknown} = \frac{3830}{2000} \; 0.200 \; \mu g / \mu l$$

$$= 0.384 \; \mu g / \mu l$$

You can now convert to a more appropriate concentration if required.

Internal standard method

Overall, the most reliable approach.

Basis

A known substance is added at a constant concentration to all standards and samples - internal standard.

Since the internal standard is always present at a constant amount, it can be used to account for variations such as injection volume during an analysis.

Internal standard method

Requirements for an internal standard.

- Must be present at a constant concentration in all samples and standards.
- Must be stable and measurable under the analysis conditions.
- Must not interfere with the analysis or co-elute with sample components.

Internal standard method

Three common approaches are used

Classical method - weighed portions of the standard and sample are combined

Stock solution - a known volume of the sample is 'spiked' with a known volume of the standard

Calibration plot - a series of standards are run and a curve plotted based on corrected peak areas.

Internal standard method

Regardless of the method for introducing the standard or calibrating, the calculations are the same.

They are the same as with the detector response factor method.

Our NORM substance is now predetermined and a fix value.

Internal standard method

$$C_{ISTD} = f_{ISTD} A_{ISTD}$$

$$C_{unk} = f_{unk} A_{unk}$$

Since the internal standard is assigned a value of 1.00 and is held constant, we can correct for run to run variations by:

$$C_{unk} = \frac{A_{ISTD1}}{A_{ISTD2}} \frac{A_{unk}}{A_{known}} C_{known}$$

known & ISTD1 are obtain from the standard, unk & ISTD2 from the unknown

Internal standard method

It is assumed that variations in the internal standard area are representative of the whole analysis.

Accounts for factors such as:

Sample injection errors or changes
Slow detector variations
Slow column changes

Internal standard method

Example

Prepare a standard that contains 11.3 mg of X and 12.00 mg of ISTD.

Make several 2 μl injections and calculate an average response for each component.

Component	Average area
X	635
ISTD	1009

Internal standard method

Now, inject your unknown.

$Area_X = 990$

$Area_{ISTD} = 1031$

$C_X =$ (1009/1031) (990/635) x 11.3 mg

= 17.24 mg X in the unknown.

Internal standard plot method

- Hold the ISTD constant but vary the amount of the target species in a series of standards.
- Create a calibration curve using the corrected areas.
- Useful when the linearity of the detector is in question.