Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

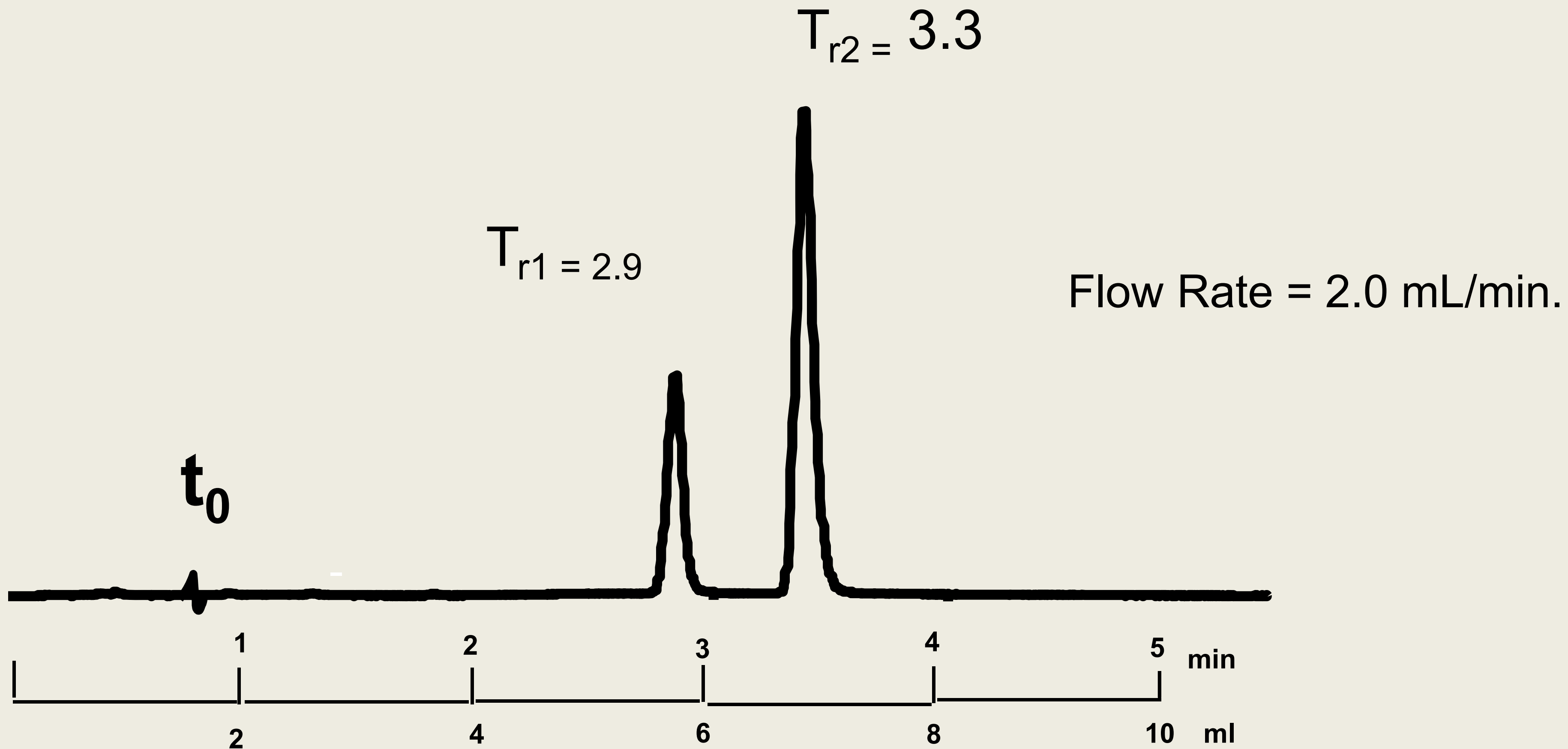
Basic Terms and Concepts

Chromatographic Parameters

- V_R = Retention volume
- V_M = Void volume
- t_M = Void time
- t_r = Retention time
- k = Retention factor or capacity factor
- α = Selectivity or separation factor
- N = Plate count
- H = Height Equivalent to a Theoretical Plate or Plate Height
- R = Resolution
- As = Asymmetry Factor (As) and Tailing Factor (Tf)

Retention Volume

The retention volume (VR) is the volume of mobile phase needed to elute the analyte at given flow rate (F).

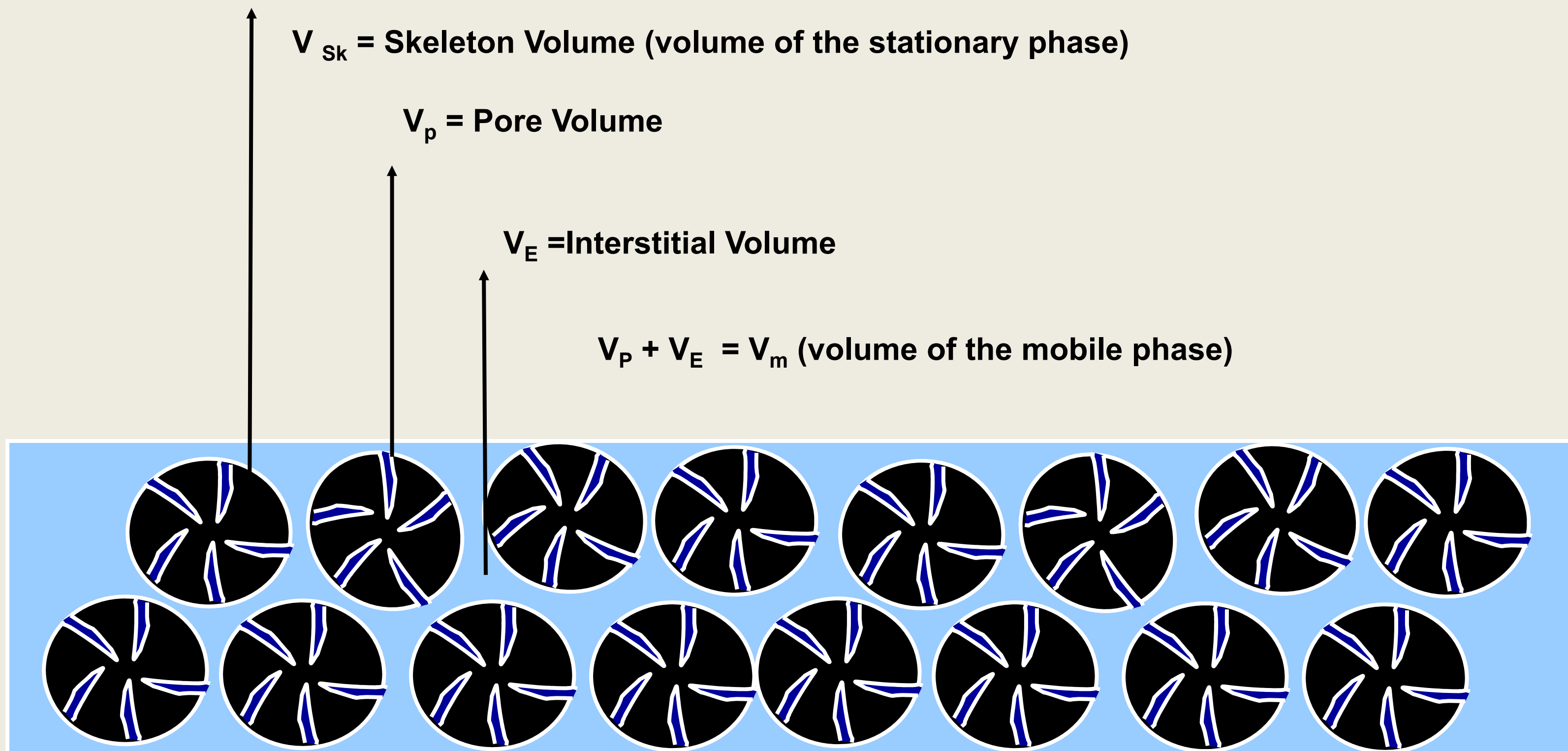


Void Volume

The void volume (VM) is the total volume of the liquid mobile phase contained in the column.

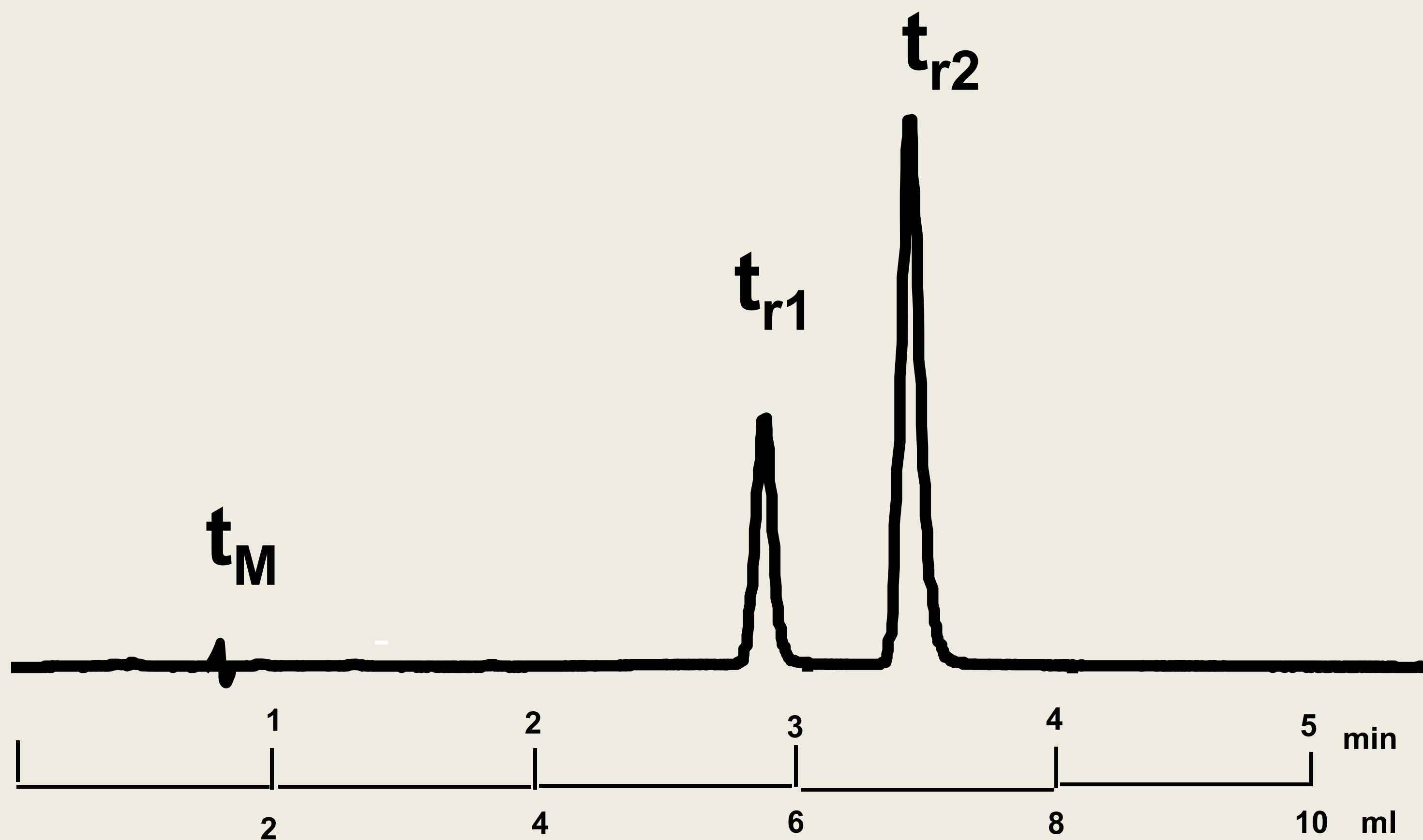
It is the volume of the empty column (Vc) minus the volume of the solid packing (V_{sk}). VM is the sum of the intraparticle volume (Vp) and the interstitial volumes (Ve). For most columns, the void volume can be estimated by the equation:

$$VM = 0.65\ \pi\ r2\ L$$



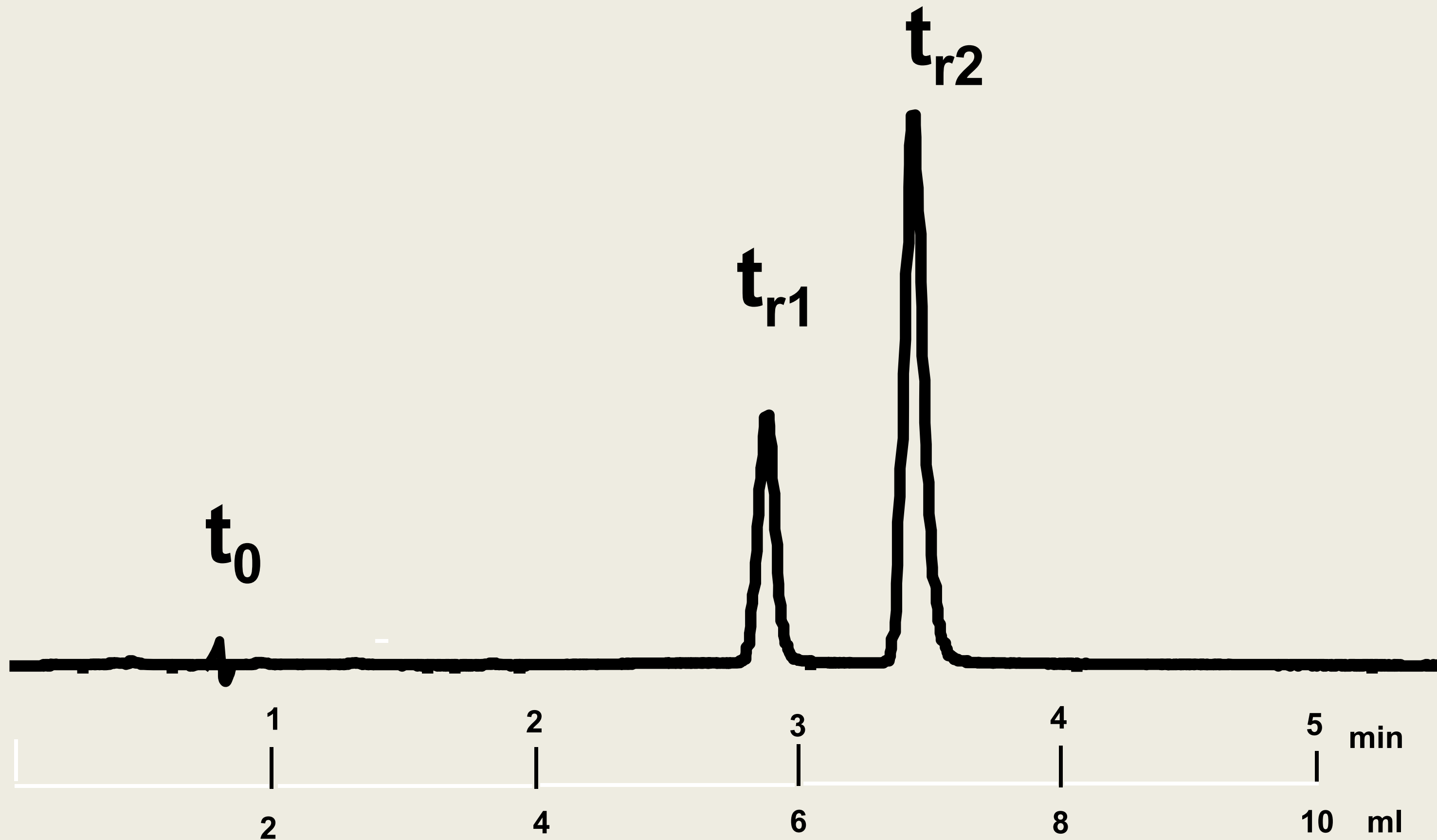
Void Time

The retention time of an unretained component or the first baseline disturbance by the sample solvent is called the void time (tM)



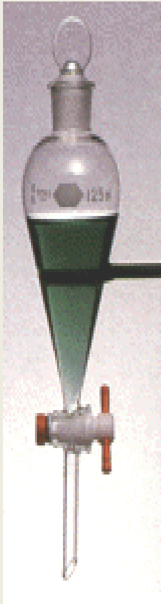
Retention Time

The time between the sample injection and the peak maximum is called the retention time (t_r)



Retention factor

The retention factor (k) is the degree of retention of the sample component in the column.
k′ is defined as the time the solute resides in the stationary phase (tR′) relative to the time it resides in the mobile phase (tM),



$$k' = K\frac{V_s}{V_m} = \frac{Amount\ \ stat.phase}{Amount\ \ mobile}$$

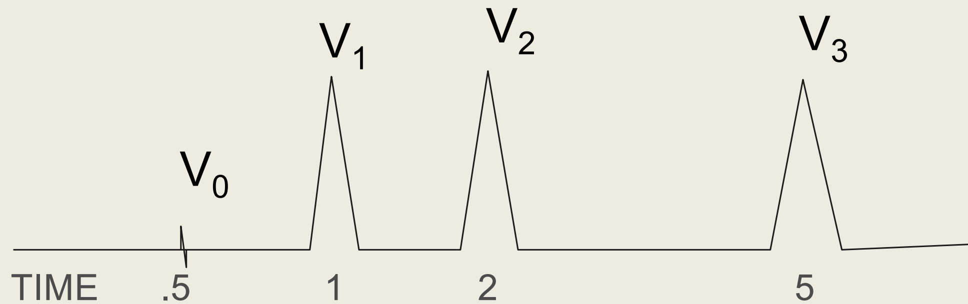
$$K = \frac{C_s}{C_m} = \frac{M_s\ V_m}{M_m\ V_s}$$

substitute

$$k' = \frac{v_r - v_0}{v_0} = \frac{t_r - t_0}{t_0}$$

k = Retention Factor

A Measure of Retention



$$k_1 = \frac{V_1 - V_0}{V_0}$$

$$k_1 = \frac{1-0.5}{0.5} = 1$$

$$k_2 = \frac{2-0.5}{0.5} = 3$$

$$k_3 = \frac{5-0.5}{0.5} = 9$$

- Describes how far the peak is from V_0
- No dimension, independent of flow, time and column size

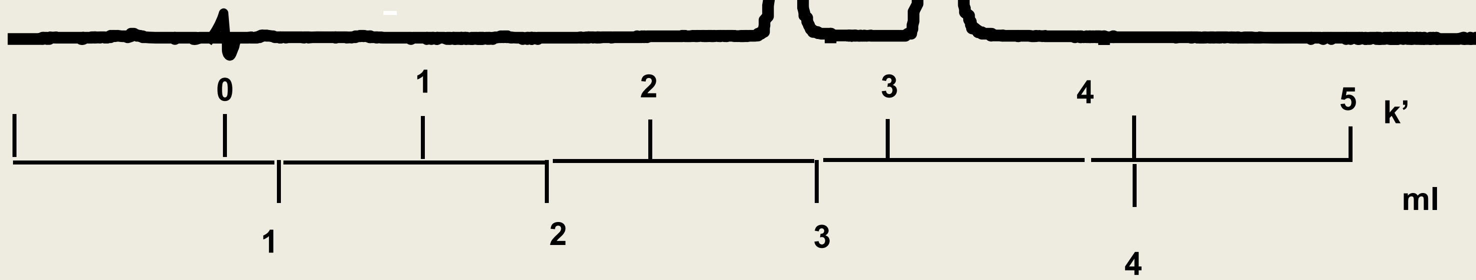
k´= Retention time measured in [t_0]
k´= Retention volume measured in [v_o]

$$k' = \frac{v_r - v_0}{v_0}$$

$k'_2 = (3.4 - 0.8) / 0.8 = 3.25$

$k'1 = (2.8 - 0.8) / 0.8 = 2.5$

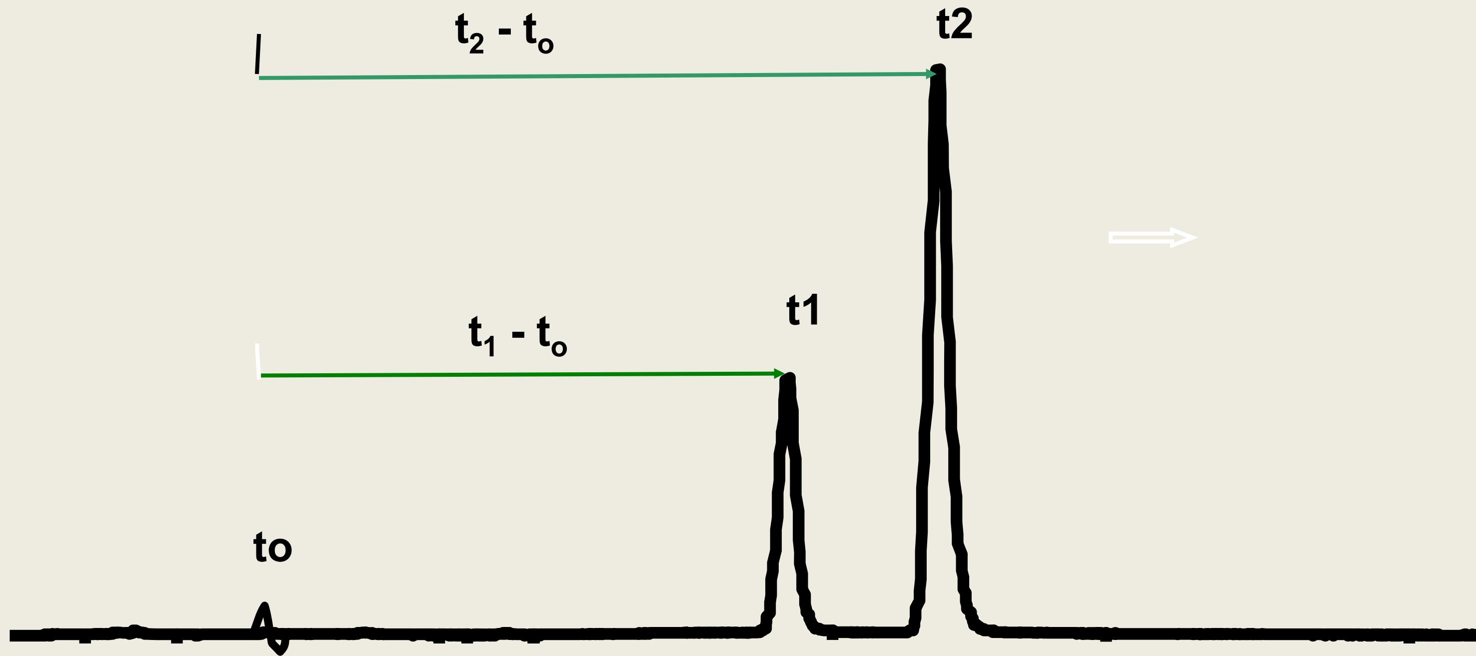
$V_0 = 0.8$



Separation Factor (Selectivity)

The separation factor (α) is a measure of relative retention k2/k1 of two sample components. Selectivity must be >1.0 for peak separation.

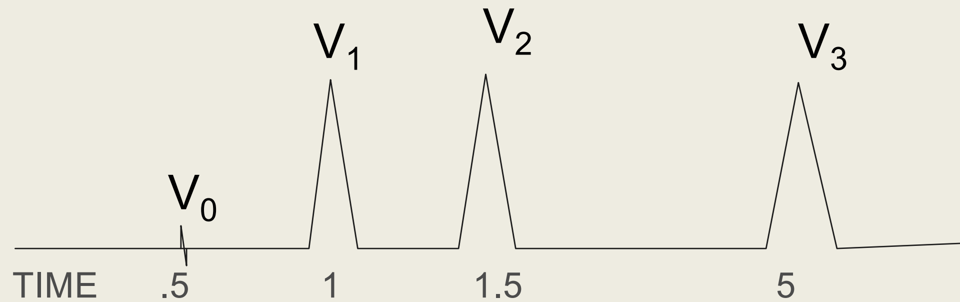
Selectivity is dependent on many factors that affect K such as the nature of the stationary phase, the mobile phase composition, and properties of the solutes.



$$\alpha = \frac{t_2 - t_o}{t_1 - t_o} = \frac{k'_2}{k'_1}$$

α = Selectivity Factor

A Measure of Peak Separation



$$\alpha_{1,2} = \frac{k_2}{k_1} \quad \text{or} \quad \alpha_{1,2} = \frac{V_2 - V_0}{V_1 - V_0}$$

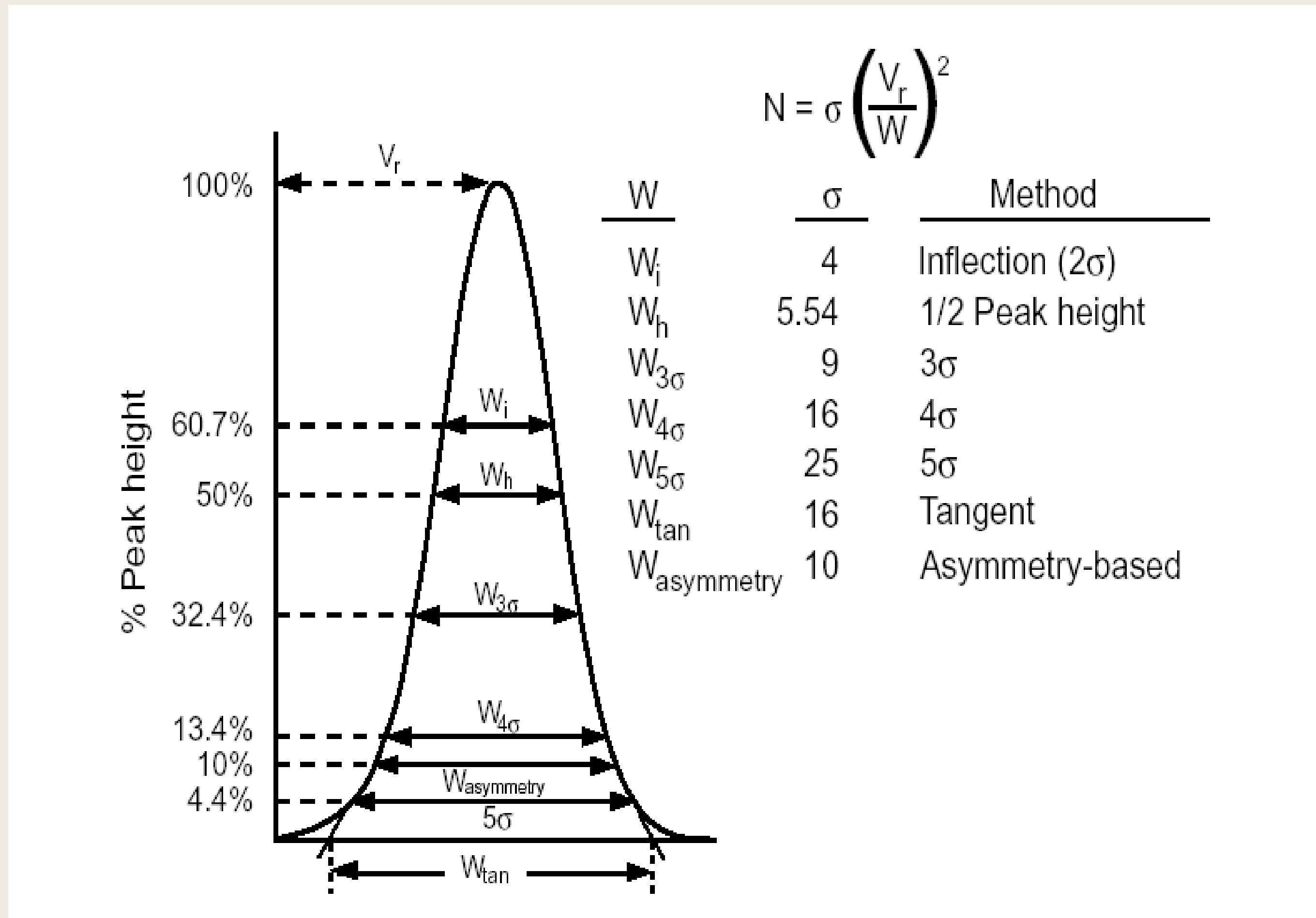
$$\alpha_{1,2} = \frac{1.5-0.5}{1-0.5} = 2$$

$$\alpha_{2,3} = \frac{5-0.5}{1.5-0.5} = 4.5$$

- Ratio between k-values for two adjacent peaks
- Defines the position of two peaks relative to each other
- Depends on the “chemistry” of the system

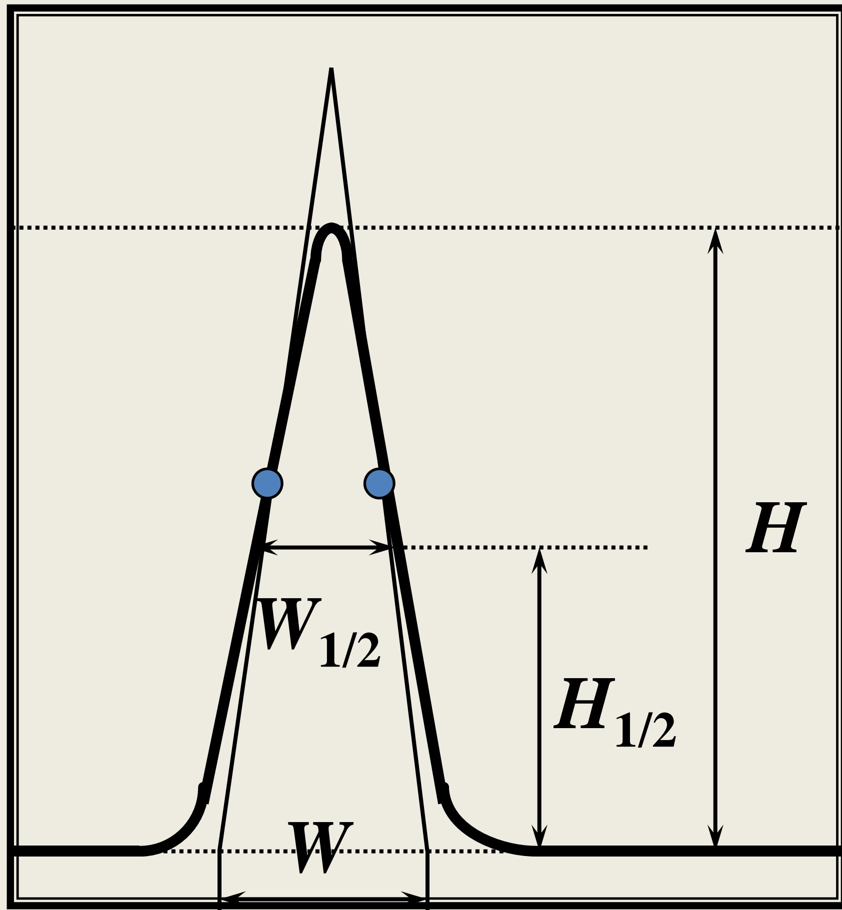
N = Theoretical Plates

The number of theoretical plates (N) is a measure of the efficiency of the column.



Theoretical plate

The number of theoretical plates (N) is a measure of the efficiency of the column.

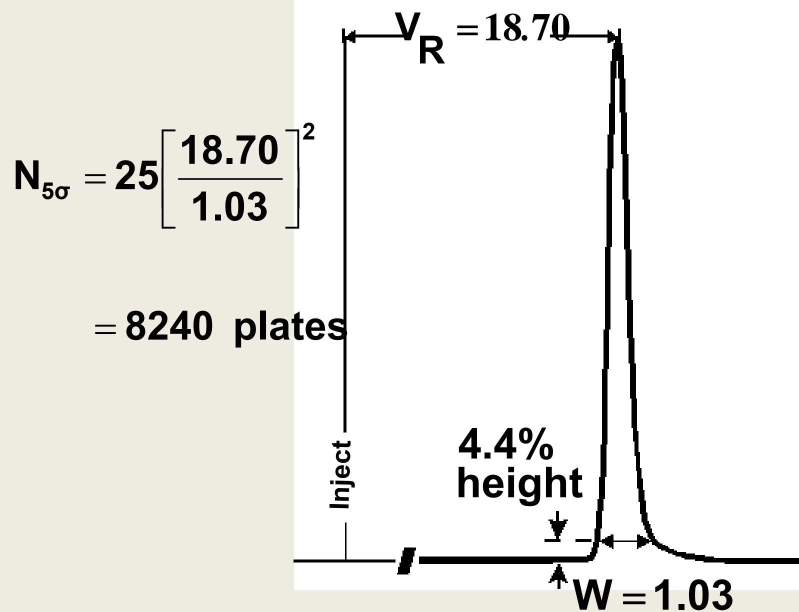


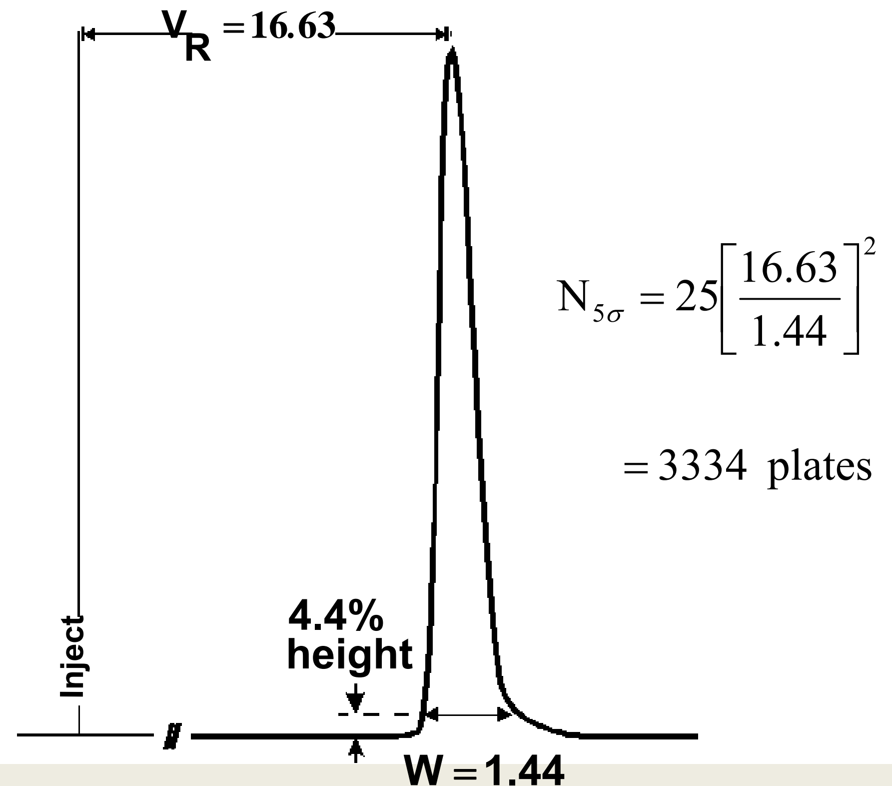
$$N = 16\left(\frac{t_R}{W}\right)^2$$

$$= 5.55\left(\frac{t_R}{W_{1/2}}\right)^2$$

$$= 2\pi\left(\frac{t_R \bullet H}{Area}\right)^2$$

Calculation of Column Efficiency





Good Column

Bad Column

Height Equivalent to a Theoretical Plate or Plate Height

The height equivalent to a theoretical plate (HETP or H) is equal to the length of the column (L) divided by the plate number (N).

$$H = L/N$$

In HPLC, the main factor controlling H is the particle diameter of the packing (dp).
For a well-packed column, H is roughly equal to 2.5 dp.

A 15-cm-long column packed with 5-µm materials should have
$N = L/H = 150{,}000\mu m/(2.5 \times 5\mu m)$, or about 12,000 plates.

A 15-cm column packed with 3-µm material should have
$N = L/H = 150{,}000\mu m/7.5\mu m$, or about 20,000 plates.

<u>Thus, columns packed with smaller particles are usually more efficient and have a higher plate number.</u>

Efficiency Expressed as HETP

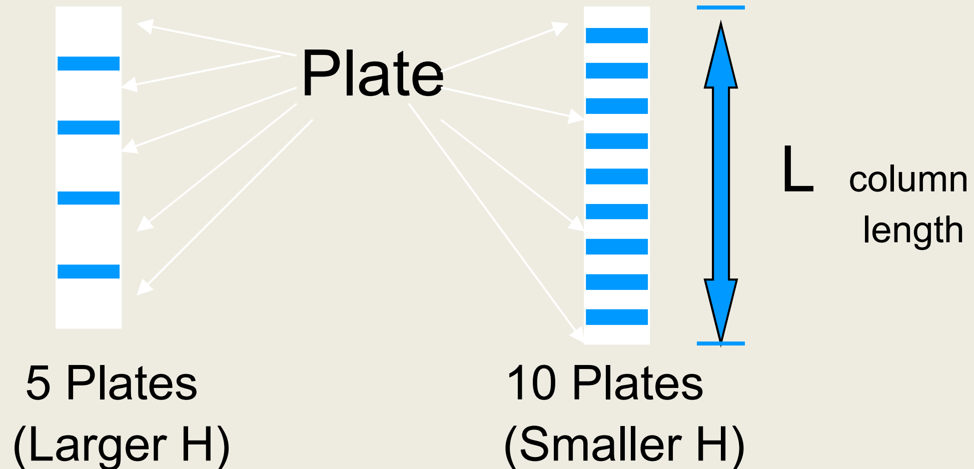
H = HETP (Height Equivalent to Theoretical Plate)

{Need H to be as Small as Possible so you can ‘fit” more “plates” into a column}

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

N= # Plates

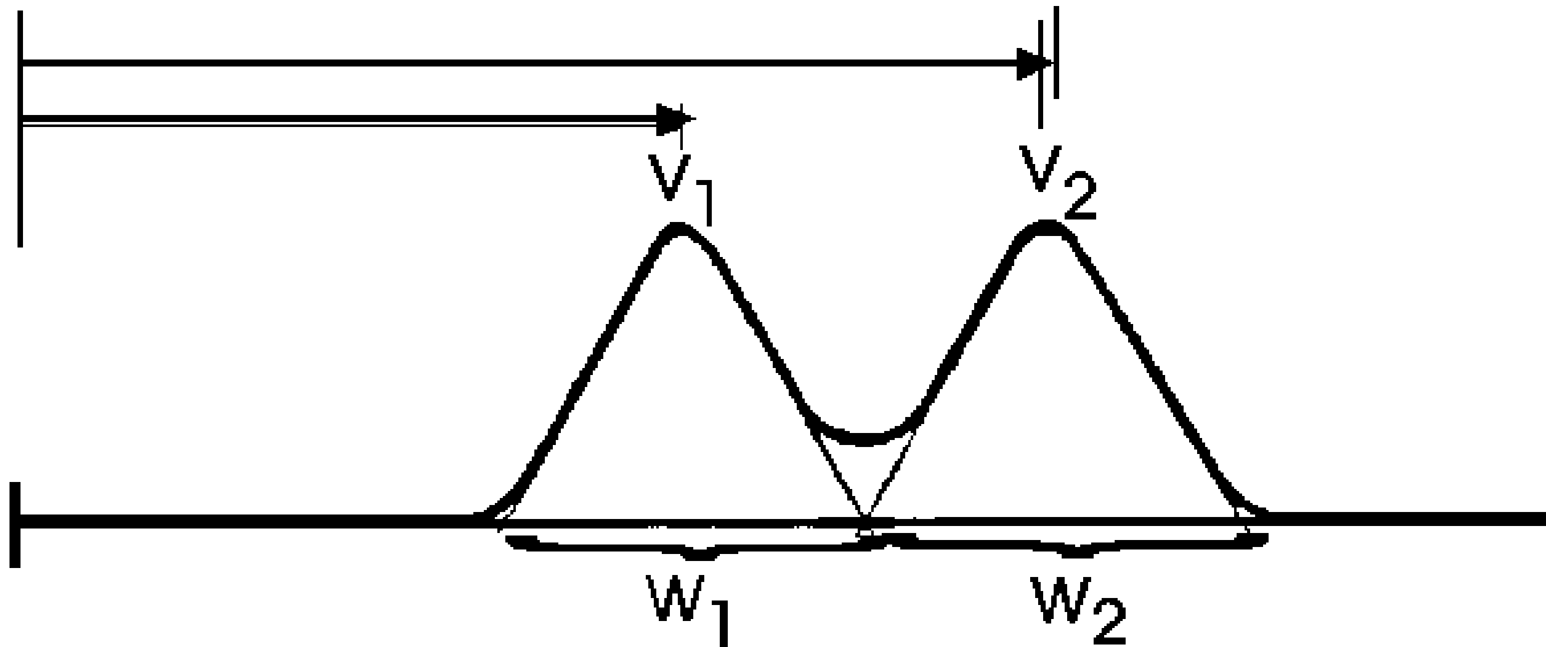
(From Distillation Theory)



Resolution R

The goal of most HPLC analyses is the separation of one or more analytes in the sample from all other components present.

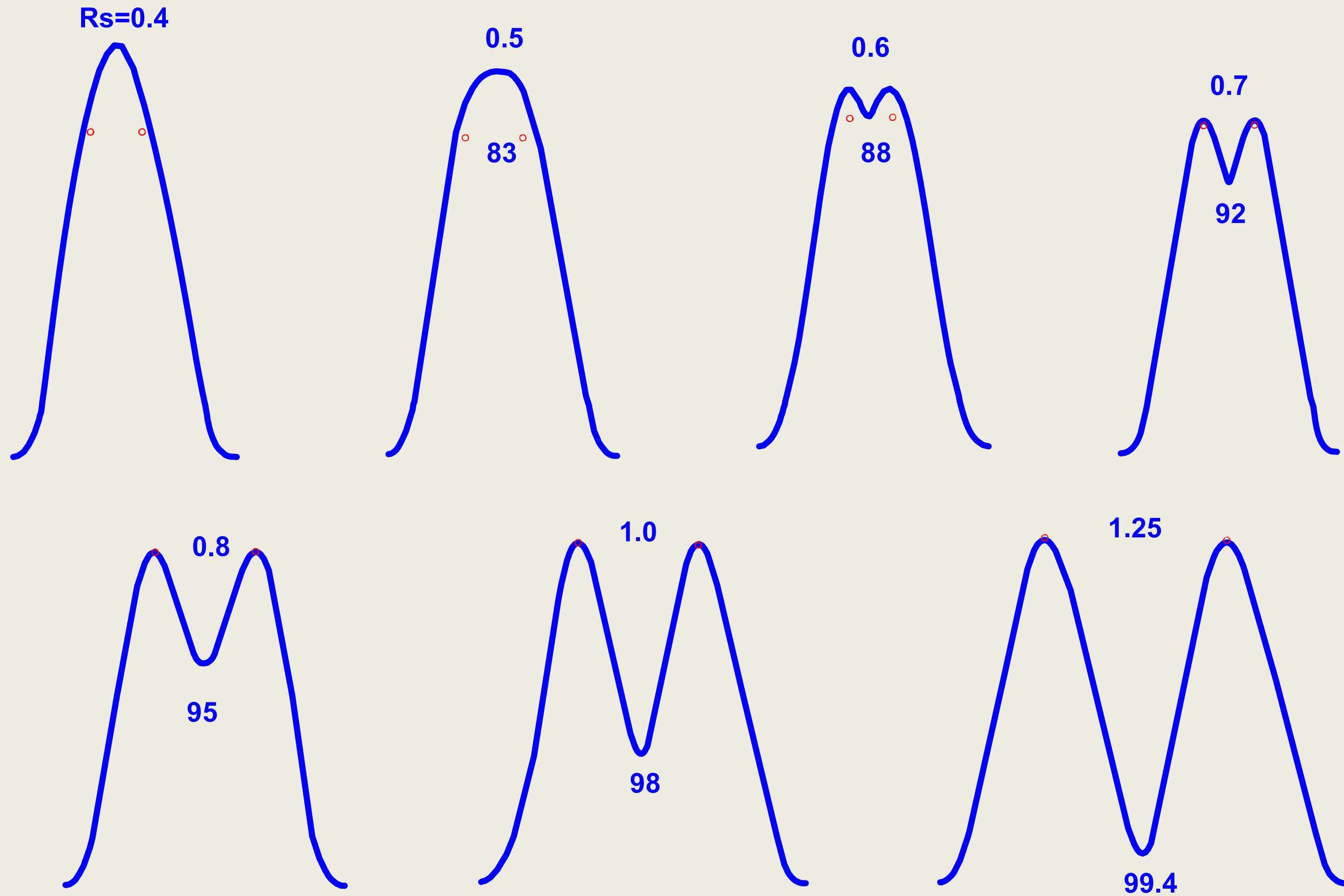
Resolution (Rs) is a measure of the degree of separation of two adjacent peaks.



$$R = \frac{V_2 - V_1}{\frac{1}{2}(W_1 + W_2)}$$

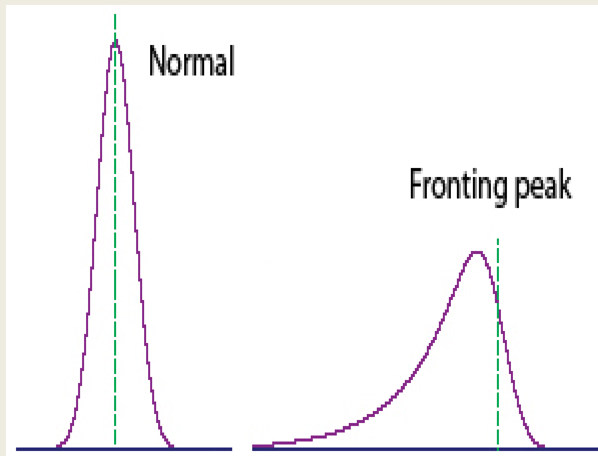
Mathematical Effect of Increasing Resolution

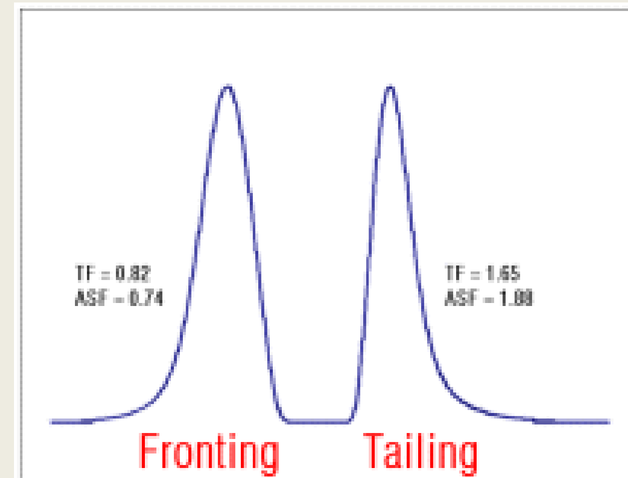
The value of 1.5 or more, indicates that peaks are separated through base line.



Peak Symmetry: Asymmetry Factor and Tailing Factor

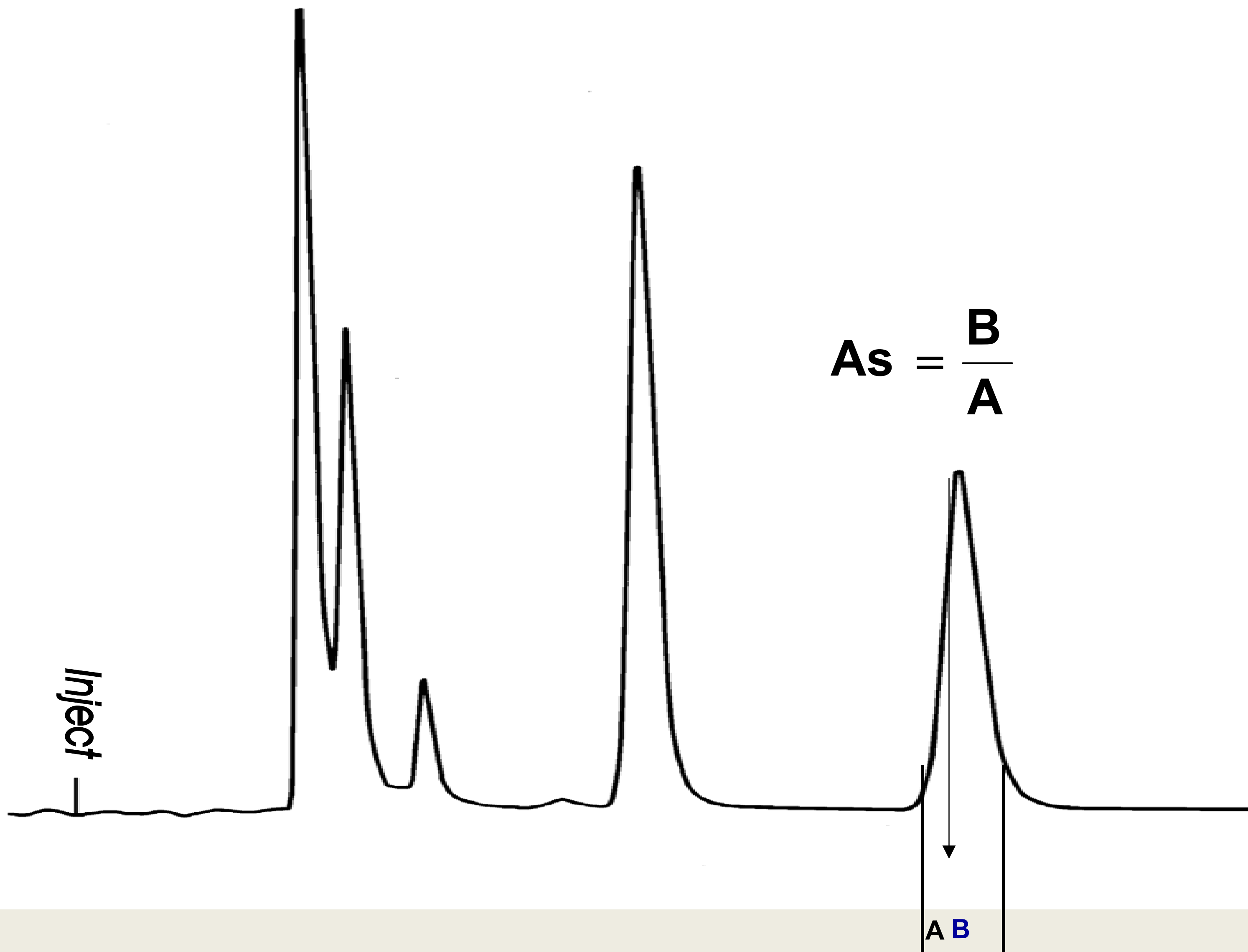
Under ideal conditions, chromatographic peaks should have Gaussian peak shapes with perfect symmetry.
In reality, most peaks are not perfectly symmetrical and can be either fronting or tailing .





The asymmetry factor (As) is used to measure the degree of peak symmetry.

Asymmetry Factor



THE RESOLUTION EQUATION

The degree of separation or resolution (Rs) between two solutes is dependent on both thermodynamic factors (retention, k, and selectivity, α) and kinetic factors (peak width and column efficiency, N).

Resolution is controlled by three somewhat independent factors (retention, selectivity, and efficiency) as expressed quantitatively in the resolution equation:

$$R = 1/4 \left(\frac{\alpha - 1}{\alpha}\right) \left(\sqrt{N}\right) \left(\frac{\kappa'}{1 + \kappa'}\right)$$

Chemistry
pH
Nature of Solvent
Modifier

Physics
Mechanics of packing
Particle size

Physical Chemistry
Polarity
Solvent strength

THE VAN DEEMTER EQUATION

The van Deemter equation was developed in the 1950s to explain band broadening in chromatography by correlating HETP or plate height with linear flow velocity (u).

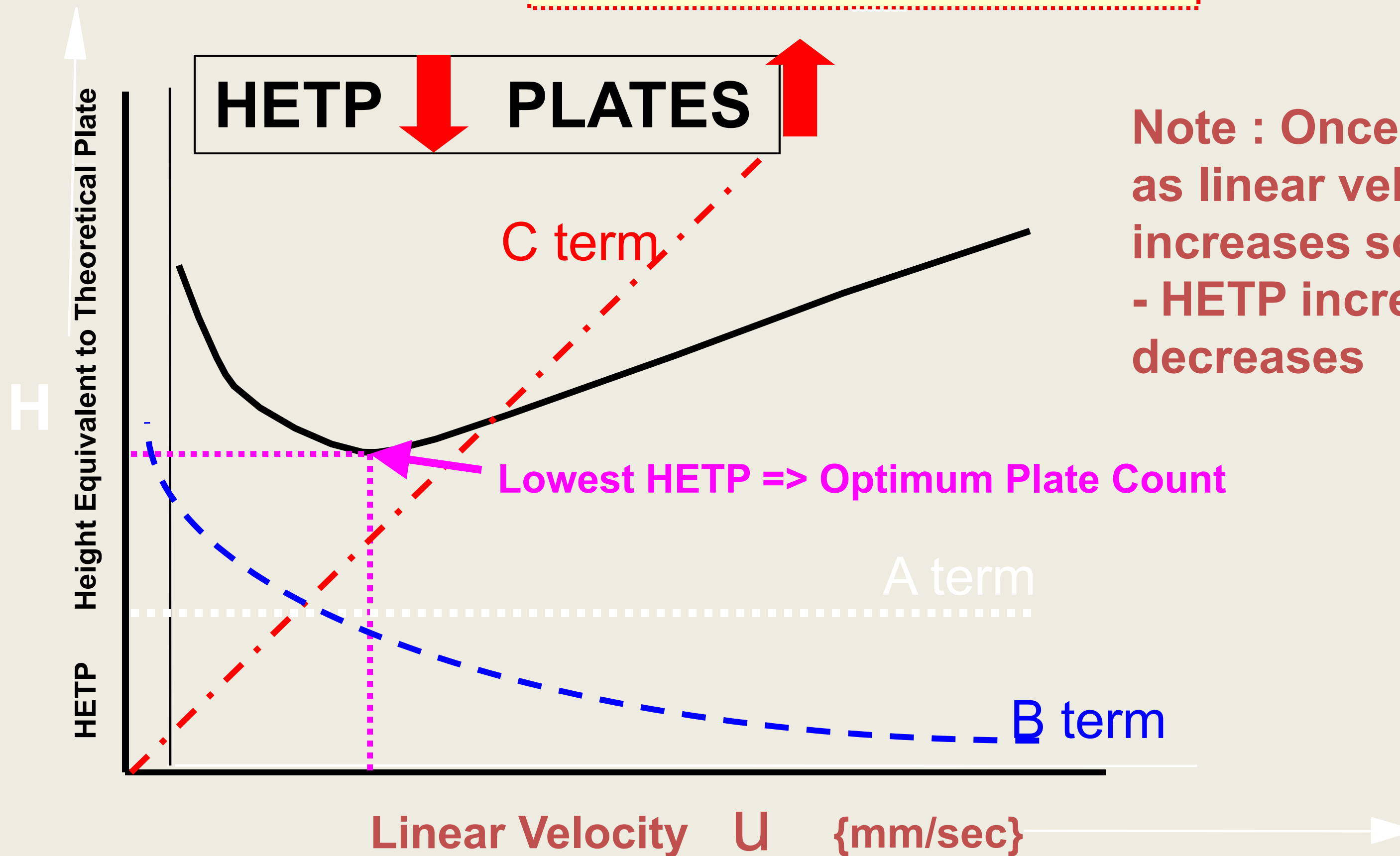
The Van Deemter plot describes about the Optimum mobile phase flow rate.

$$H = A + \left(\frac{B}{\mu}\right) + C\mu$$

μ = ***linear velocity***

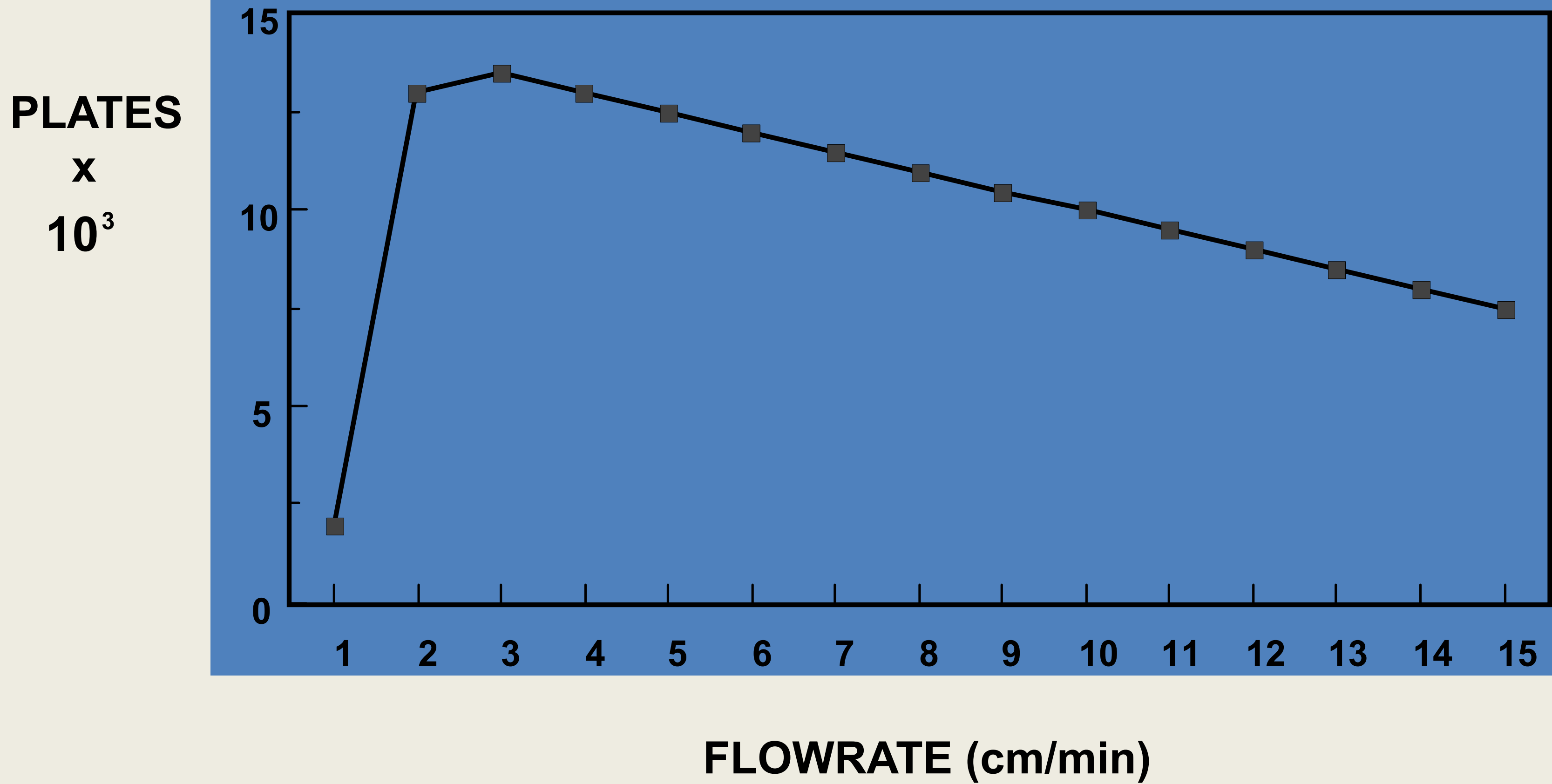
The van Deemter Equation

$$H = A + \frac{B}{u} + uC$$

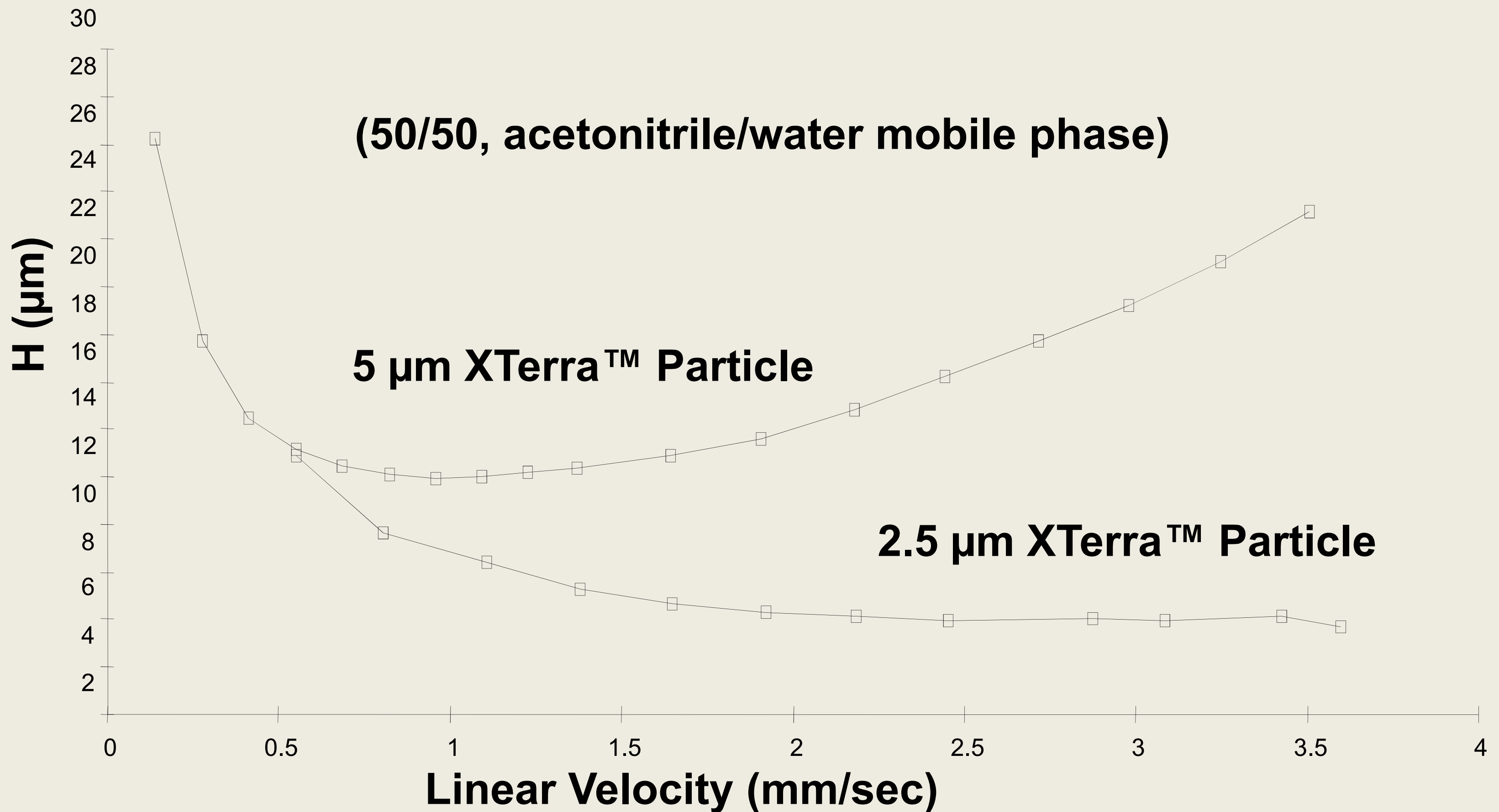


Note : Once past optimum as linear velocity (flow rate) increases so does
- HETP increases - plate count decreases

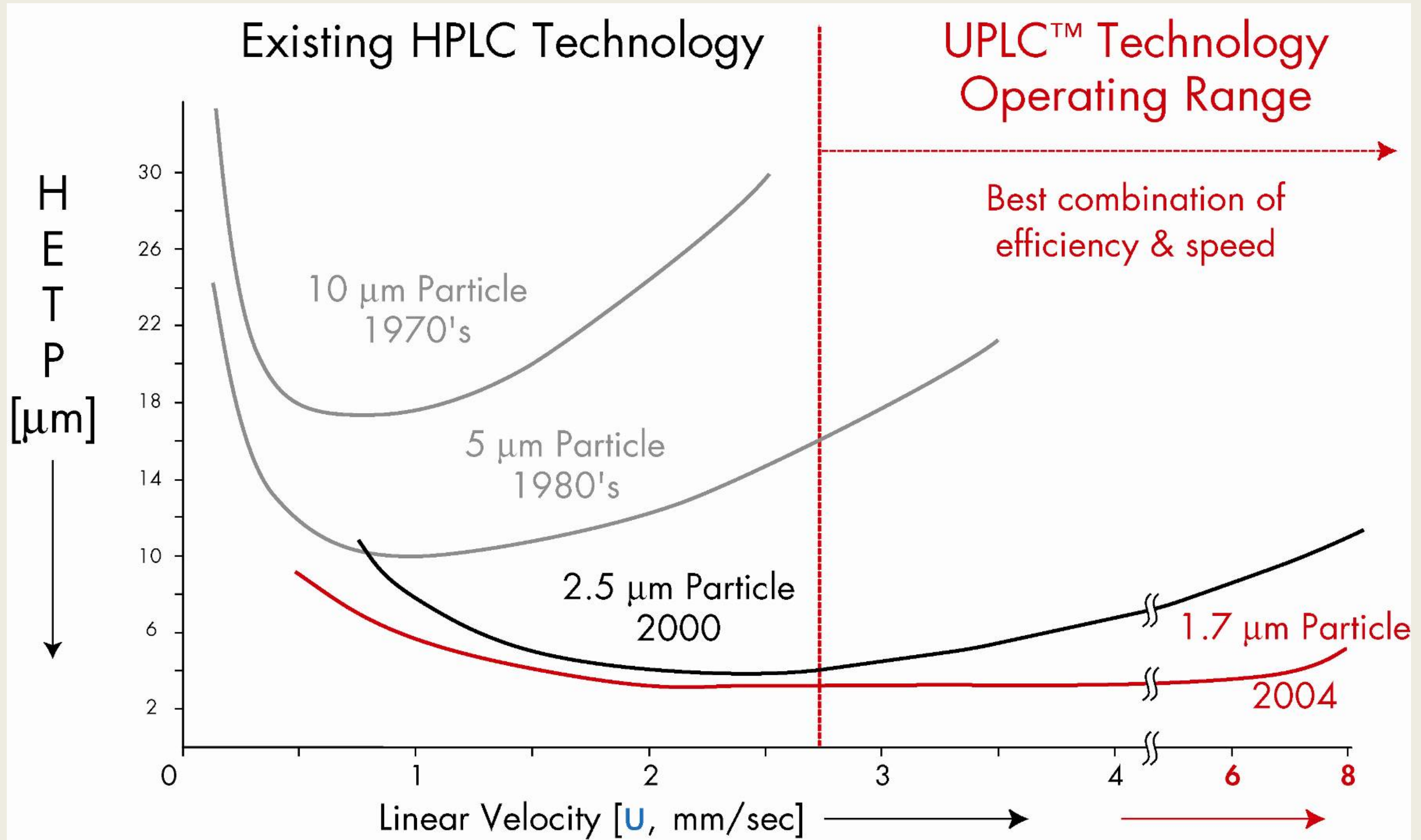
Plates vs. Flow rate



Comparison of the van Deemter Plots for 5 µm and 2.5 µm Particles

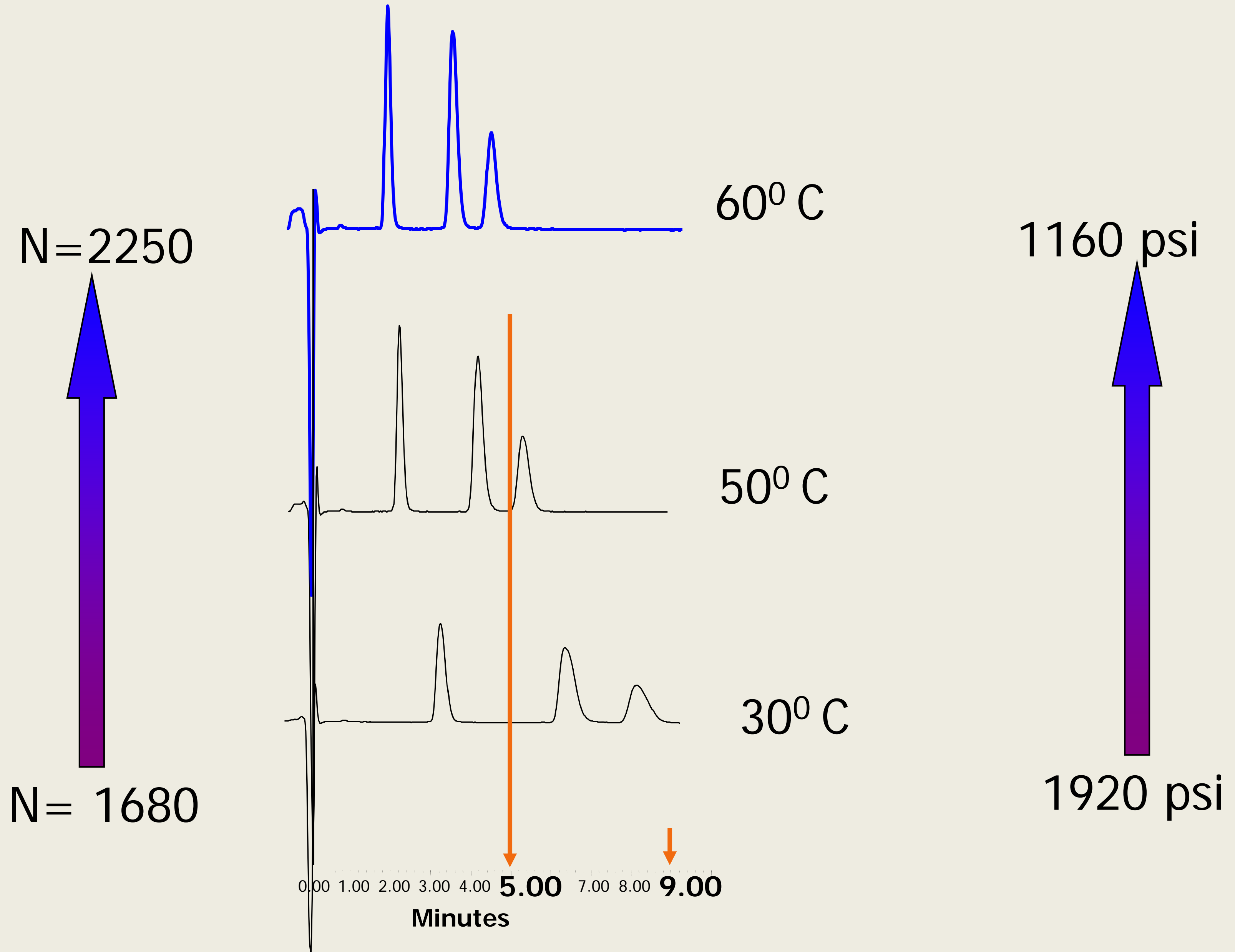


Particle Size Evolution



Properly designed small particles allow us to achieve higher speeds, AND great resolution

Effect of Temperature



FDA Guidelines for System Suitability test

Parameters	Guidelines
Repeatability of peak response	1.0% for 5 replicates
Resolution	Minimum 1.5
Tailing factor	Less then or equal to 1.0
Column efficiency	Greater then 2000 (plate count)
Capacity factor	Greater then 2