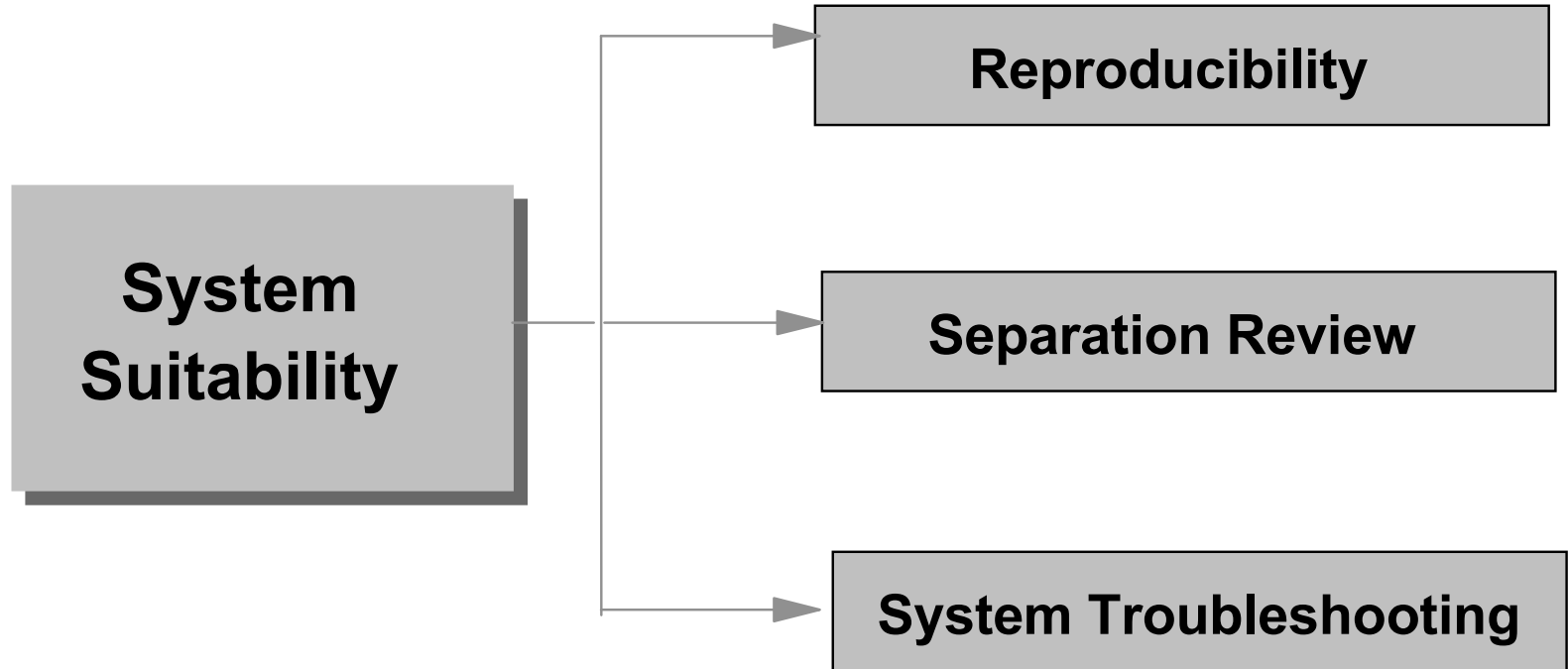


# Waters

## System Suitability

For Complete  Confidence

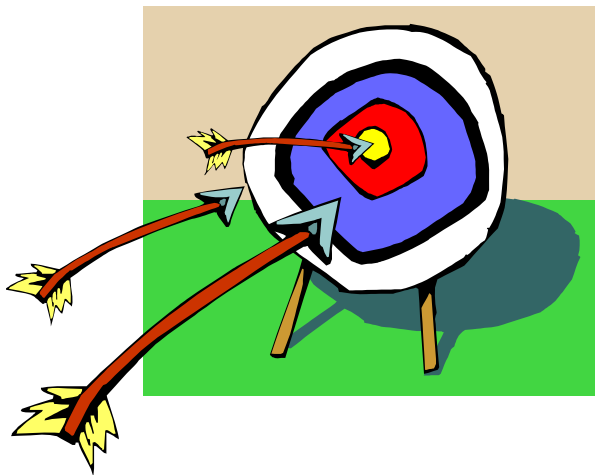
- System Suitability
  - ◆ The checking of a system, before or during analysis of unknowns, to insure system performance.
  - ◆ Plate Count, Tailing, Resolution, Capacity Factor
  - ◆ Determination of reproducibility (%RSD)
- System Suitability "Sample"
  - ◆ A mixture of main components and expected by-products utilized to determine system suitability



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## ● Precision

- ◆ The measure of the degree of agreement among test results when the method is applied repeatedly to multiple samplings of a homogeneous sample
- ◆ Expressed as %RSD for a statistically significant number of samples



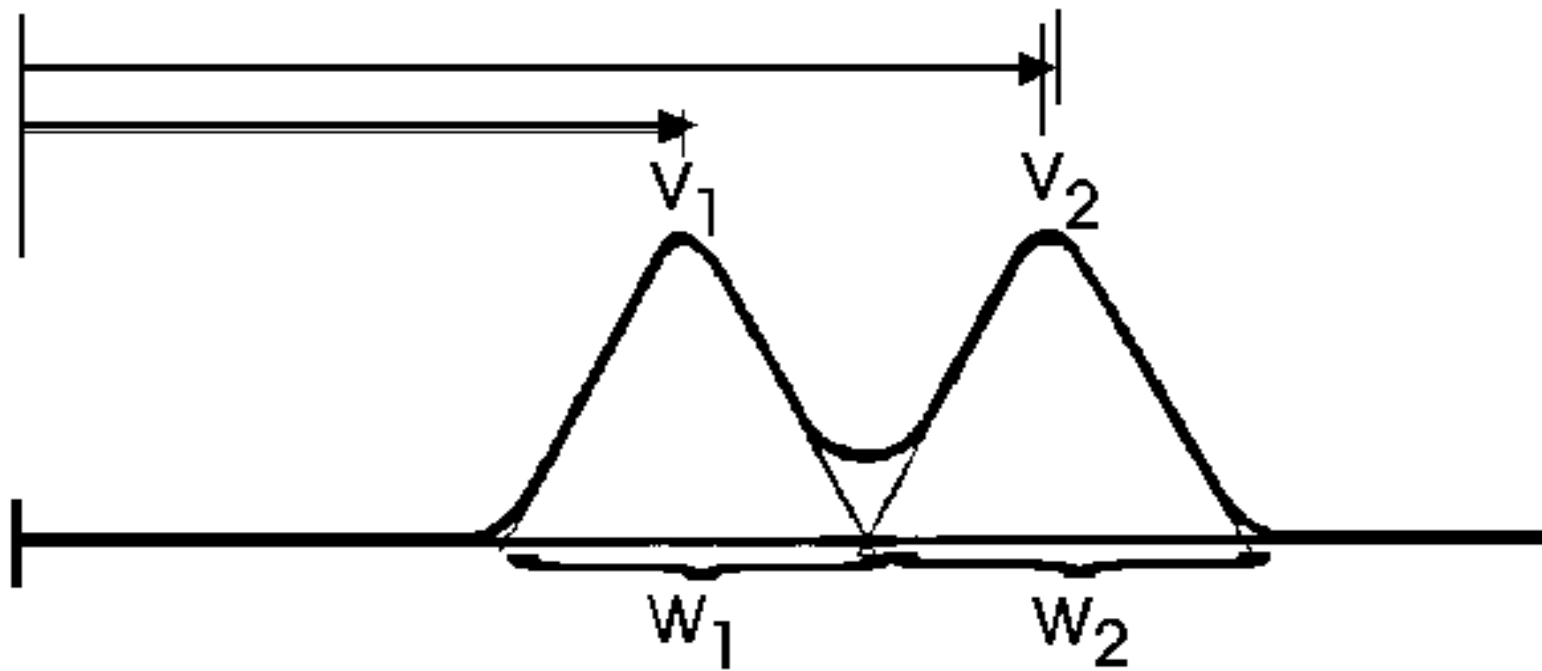
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- Specificity (Selectivity)
  - ◆ The ability to measure accurately and specifically the analyte in the presence of components that may be expected to be present in the matrix
  - ◆ The degree of interference
    - ◆ Active Ingredients
    - ◆ Excipients
    - ◆ Impurities
    - ◆ Degradation Products
    - ◆ Placebo Ingredients



- Separation
  - ◆ Resolution
    - ◆ Determination of separation between peaks
  - ◆ Plate Count
    - ◆ Determination of a systems efficiency
  - ◆ Tailing Factor
    - ◆ Calculation referencing peak shape
- PDA
  - ◆ Purity Angle
  - ◆ Purity Plots

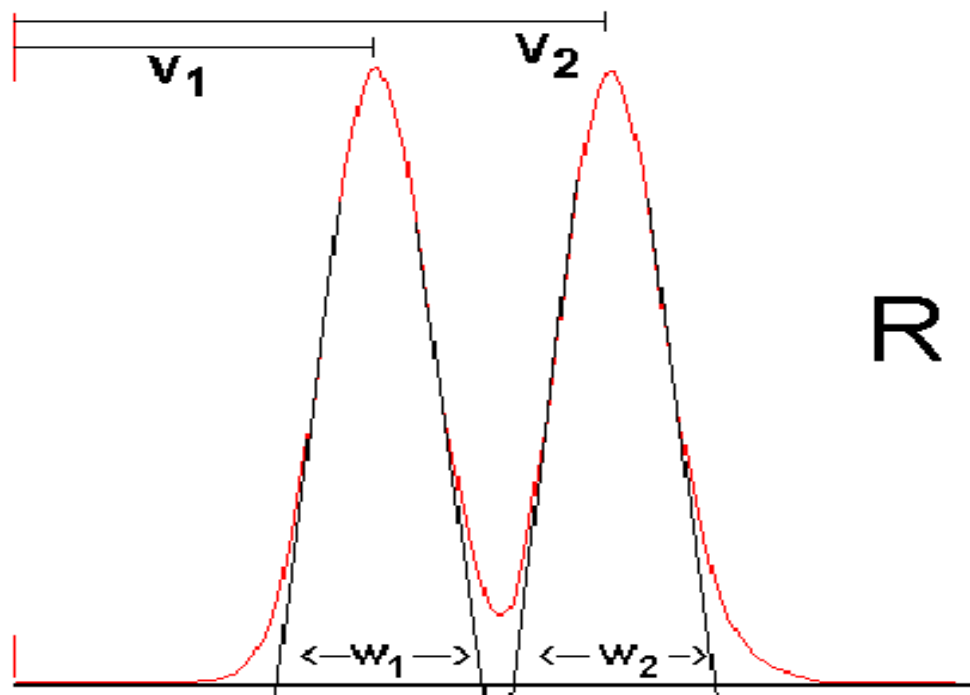
R = 1 2% Overlap 4σ  
R = 1.5 baseline separation 6σ



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

# Developing HPLC Separations

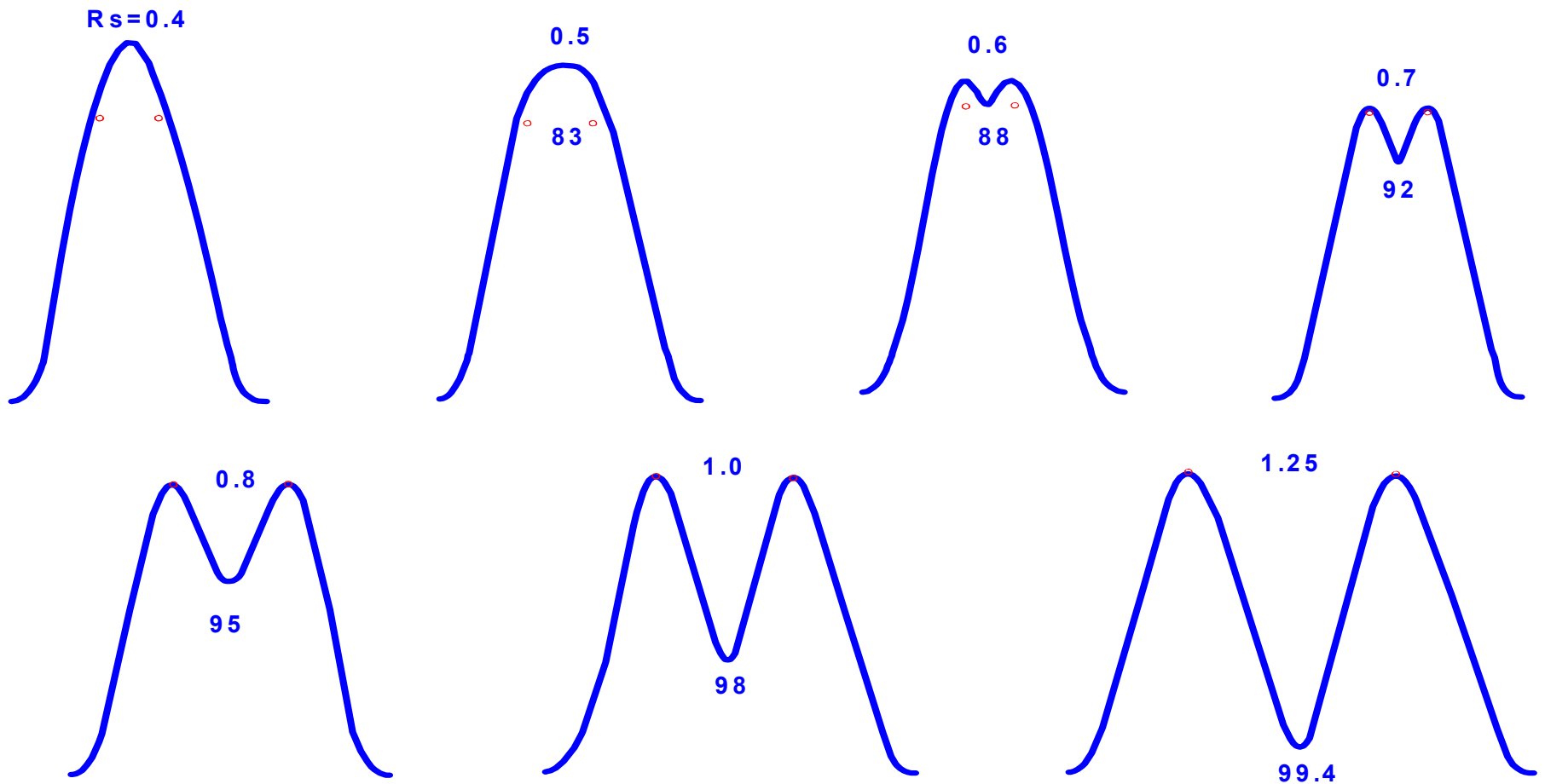
## Resolution Equation



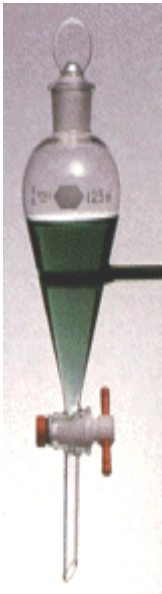
$$R = \frac{(V_2 - V_1)}{1/2 (W_1 + W_2)}$$



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



Capacity Factor describes the migration rate of an analyte through a column



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

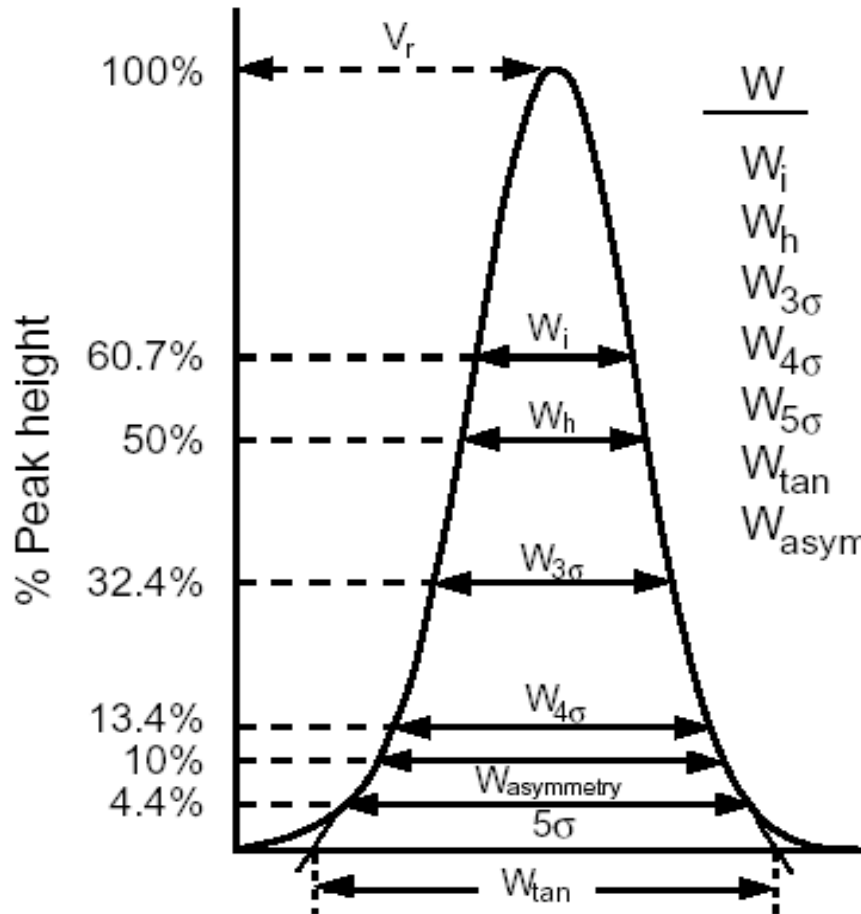
$$K = \frac{C_s}{C_m} = \frac{M_s V_m}{M_m V_s}$$

substitute

$$k' = \frac{V_r - V_0}{V_0} = \frac{t_r - t_0}{t_0}$$

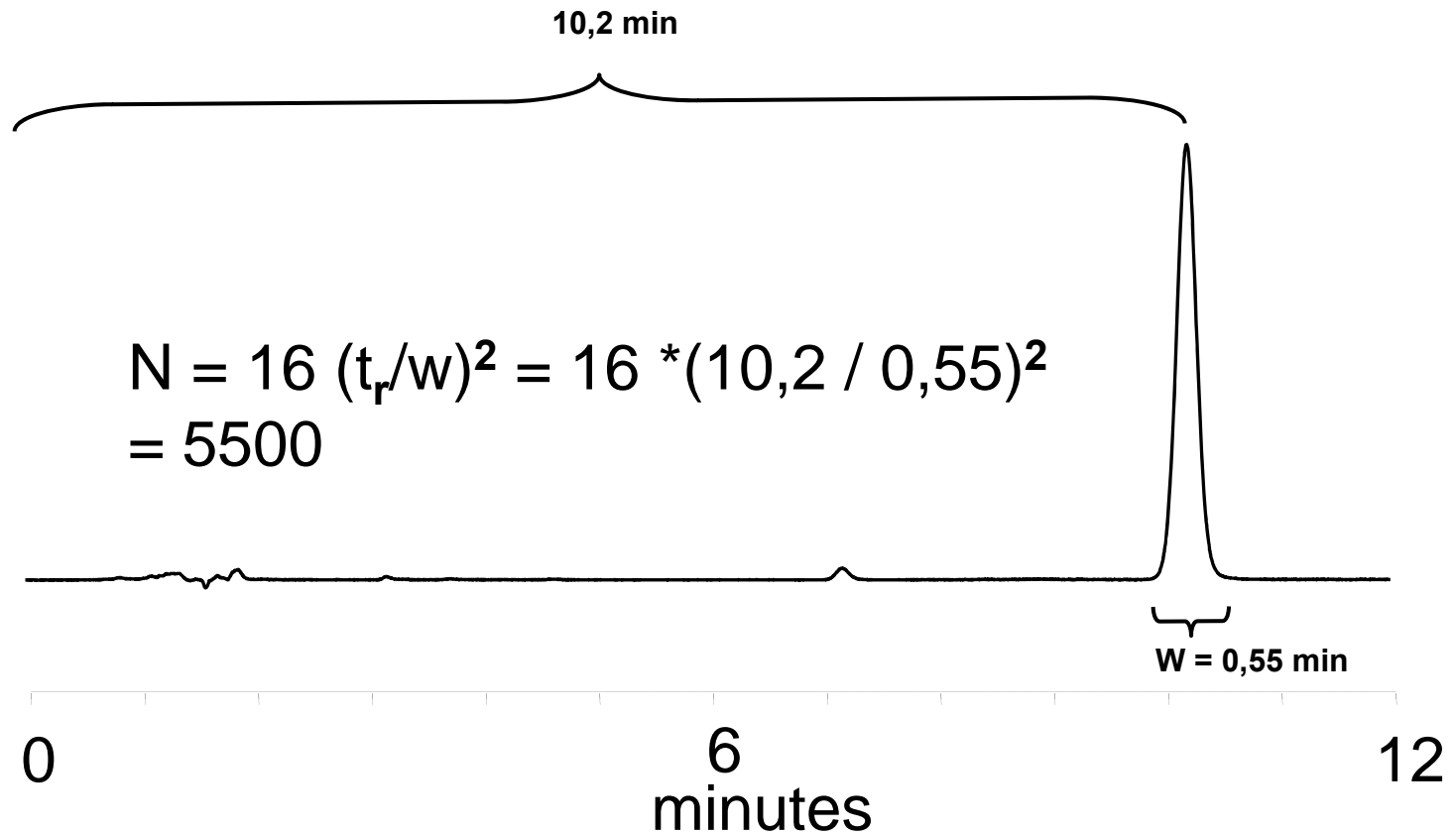
## N = Theoretical Plates A Measure of Efficiency

$$N = \sigma \left( \frac{V_r}{W} \right)^2$$

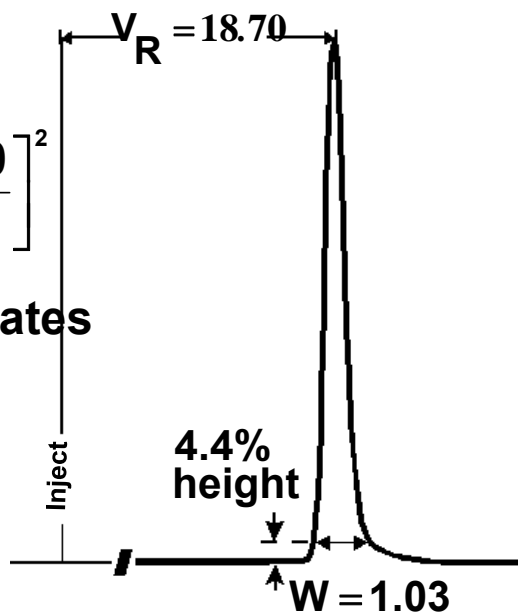


<u>W</u>	<u>σ</u>	<u>Method</u>
$W_i$	4	Inflection ( $2\sigma$ )
$W_h$	5.54	1/2 Peak height
$W_{3\sigma}$	9	$3\sigma$
$W_{4\sigma}$	16	$4\sigma$
$W_{5\sigma}$	25	$5\sigma$
$W_{tan}$	16	Tangent
$W_{asymmetry}$	10	Asymmetry-based

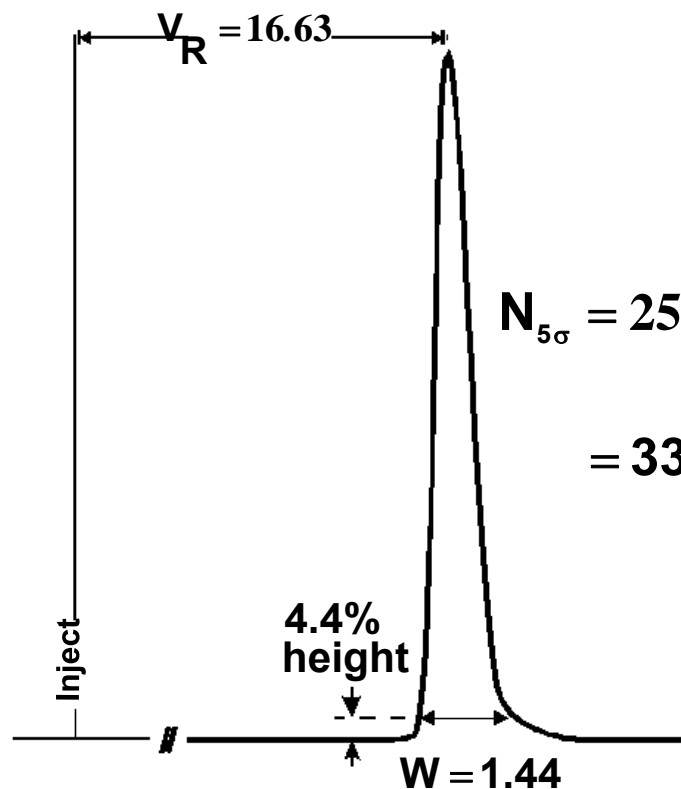
# Peak broadening during separation



$$N_5 = 25 \left[ \frac{18.70}{1.03} \right]^2 = 8240 \text{ plates}$$



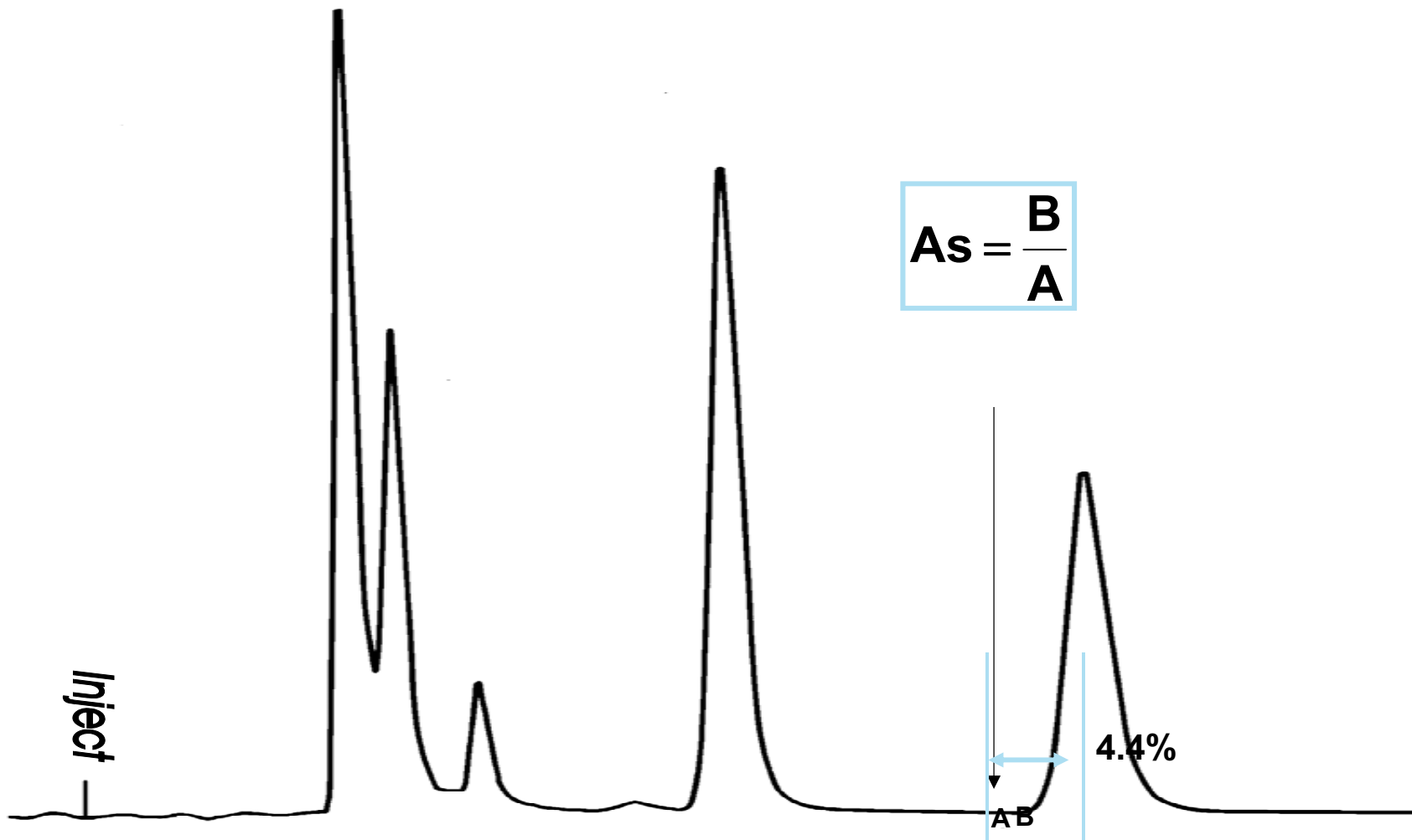
**Good Column**



$$N_{5\sigma} = 25 \left[ \frac{16.63}{1.44} \right]^2 = 3334 \text{ plates}$$

**Bad Column**

- Factors that will influence the efficiency, N :
  - Flow rate (linear velocity)
  - Column length
  - Particle size
  - Particle size distribution
  - Porosity
  - Injection volume
  - Solvent viscosity
  - Column temperature
  - Particle shape (spherical versus irregular)
  - Retention
  - Extra column band spreading (in tubing and instruments)



### Parameters

### Guidelines

Repeatability of peak response

1.0% for 5 replicates

Resolution

Minimum 2.0

Tailing factor

Less than or equal to 1.0

Column efficiency

Greater than 2000 (plate count)

Capacity factor

Greater than 2