

**System Suitability :
Numerical Criteria for the HPLC
Analysis Performance**

Efficiency- N

Asymmetry – Af; T

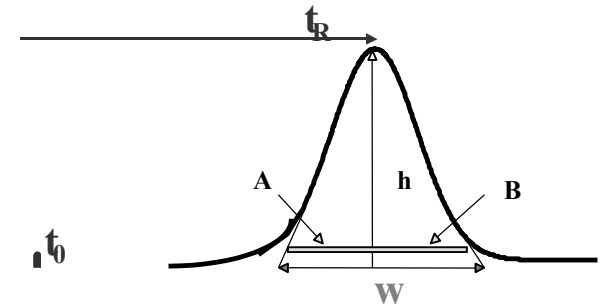
Selectivity - α

Resolution - Rs

RETENTION FACTOR or CAPACITY RATIO

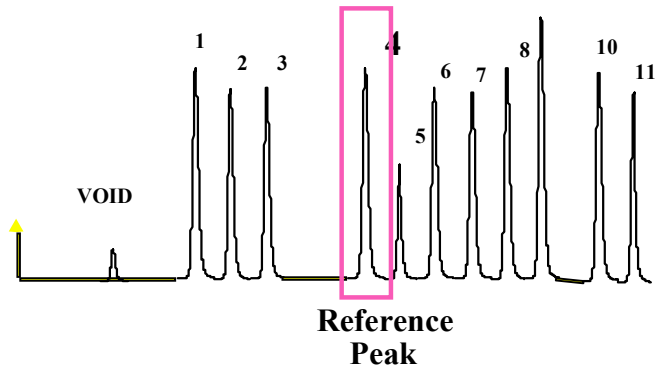
$$k' = \frac{t_R - t_0}{t_0}$$

$$k' = \phi \frac{C_s}{C_m}$$

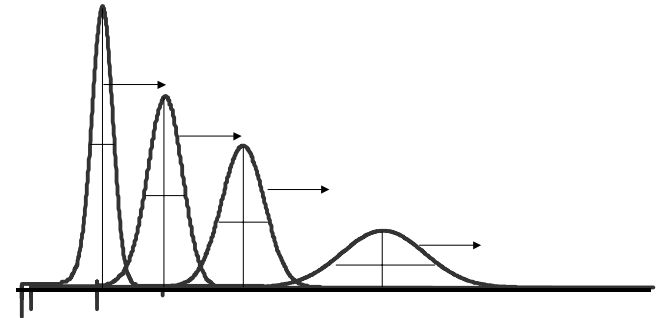


Relative Retention Time RRT

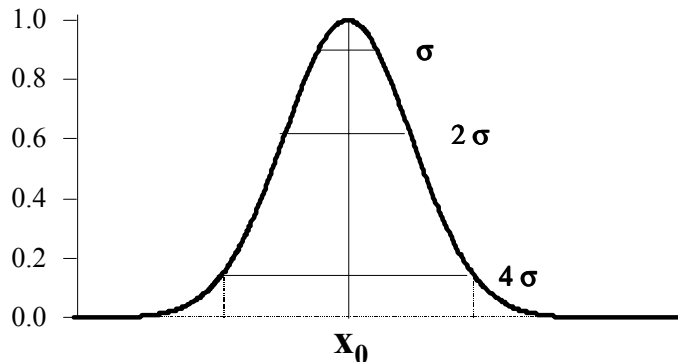
$$\frac{t_{R,i}}{t_{R,4}} = \text{RRT}$$



PEAK BROADENING



GAUSSIAN PEAK: $\frac{c}{c_0} = \exp\left(-\frac{ay^2}{2D\tau}\right)$
 ($y = x_0 - wt$)

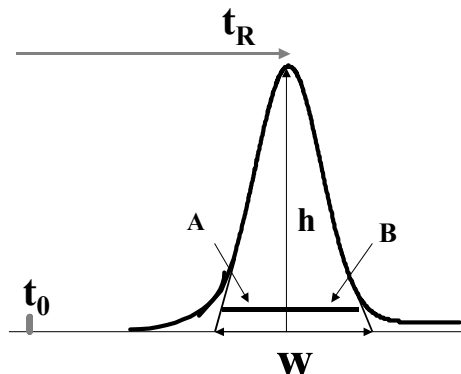


0.6065 h = 2 s
 0.1353 h = 4 s
 0.8825 h = 1 s

AREA 4s = 95.6%

PERFORMANCE BY ONE PEAK

NUMBER OF THEORETICAL PLATES



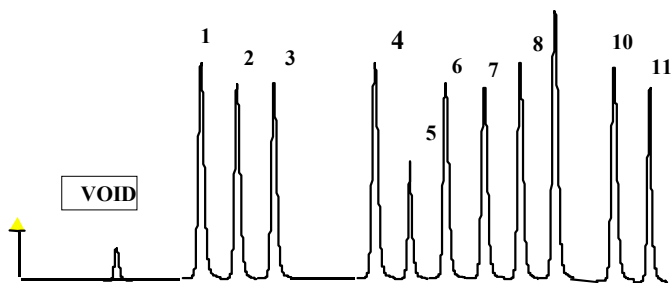
$$N = 16 \left(\frac{t_R}{W}\right)^2$$

Or:

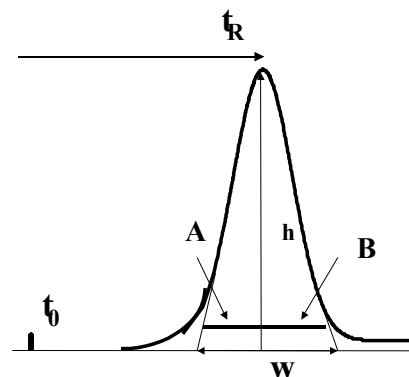
$$N = 5.54 \left(\frac{t_R}{W_{1/2}}\right)^2$$

ADVANTAGES OF HIGH EFFICIENCY

- * Better Resolution for peak identification and quantitation
- * Higher Peak capacity for analysis of many components at once
- * Higher Sensitivity for detection of low concentrations



PERFORMANCE BY ONE PEAK: Asymmetric factor or Tailing factor



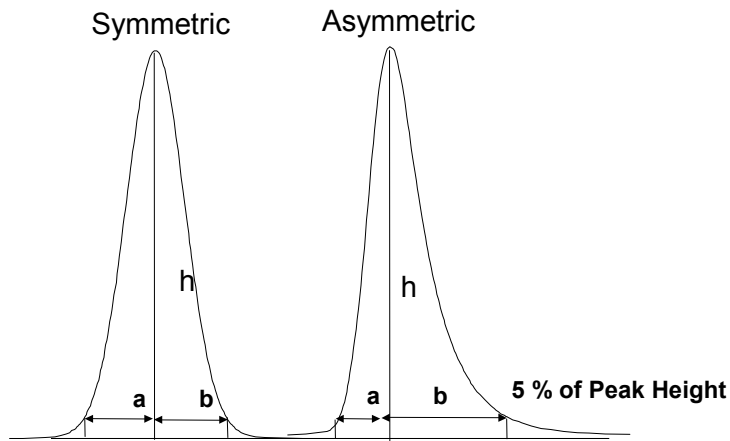
ASYMMETRY FACTOR

$$A_f = \frac{B_{(10\% h)}}{A_{(10\% h)}}$$

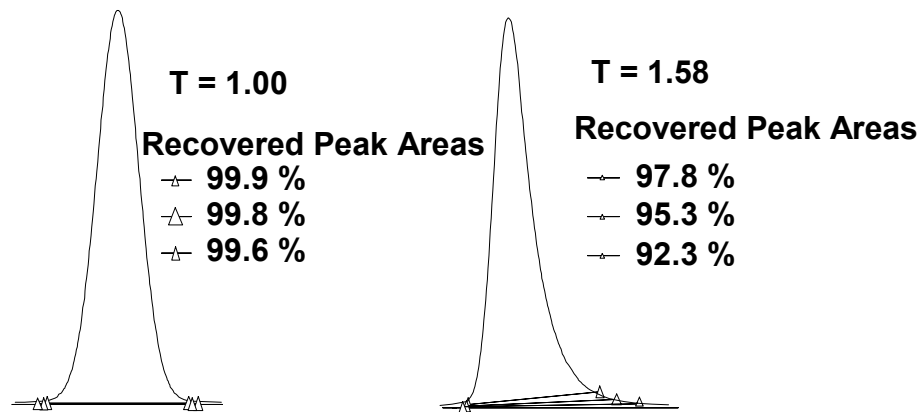
TAILING FACTOR

$$T = \frac{A_{5\% h} + B_{5\% h}}{2 \times A_{5\% h}}$$

PERFORMANCE BY ONE PEAK: Asymmetric factor or Tailing factor

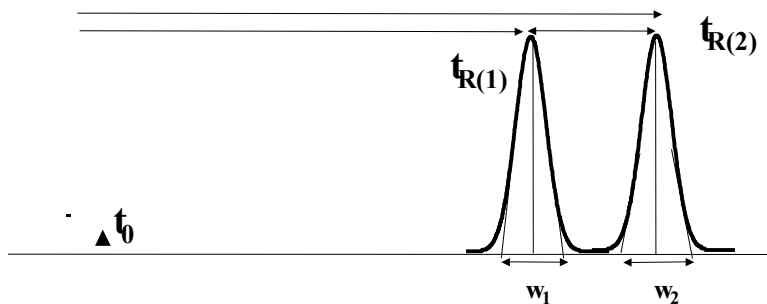


Integration Error Caused by Tailing of Peaks



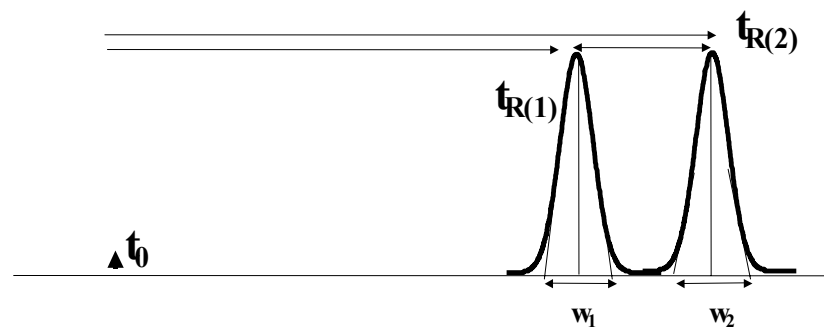
PERFORMANCE BY TWO PEAKS SELECTIVITY FACTOR

$$\alpha = \frac{k'(2)}{k'(1)}$$

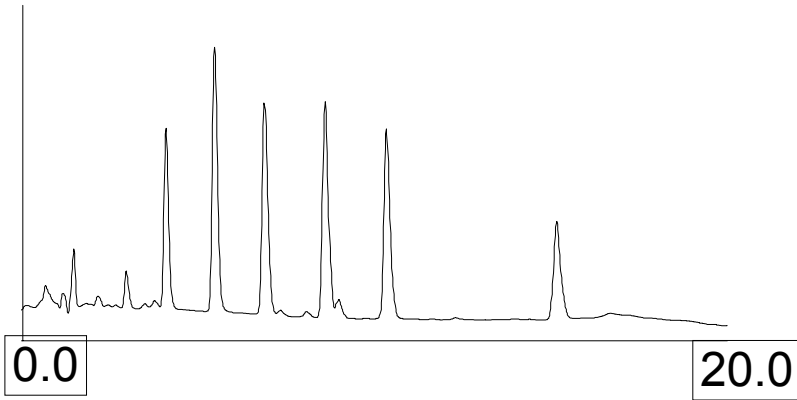


PERFORMANCE BY TWO PEAKS EXPERIMENTAL RESOLUTION

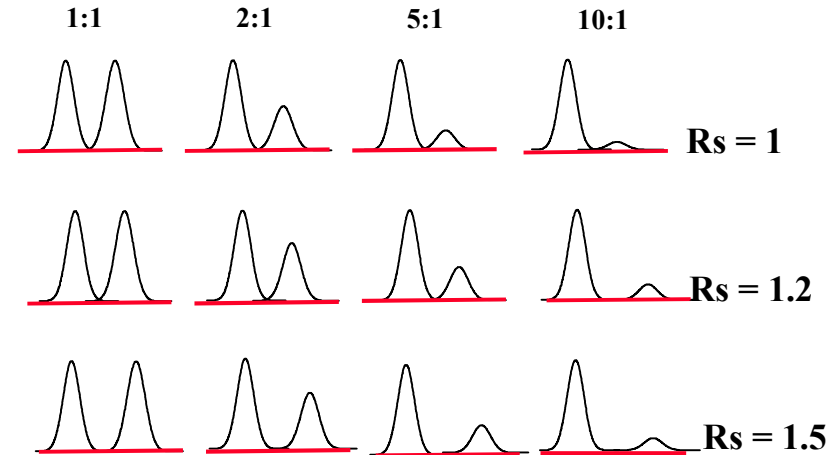
$$R_s = \frac{t_{R(2)} - t_{R(1)}}{1/2 (w_1 + w_2)}$$



ADVANTAGES OF HIGH RESOLUTION



Higher Components ratio - Higher Resolution Required



FACTORS CONTROLLING RESOLUTION

- k'** Strength of solvent - polarity
Strength of packing - surface area, carbon load
Temperature
- α** Chemistry of solvent - functionality
Chemistry of packing - functionality
Chemistry of sample - hydrogen bonding or derivatization
- N** Flow rate - linear velocity
Average particle size
Column bed configuration
Viscosity of solvent
Temperature (viscosity)
Particle shape
Viscosity of sample
Mixed mechanisms
- Column length
Particle size distribution
Volume of injection
Mass of injection
Column diameter
System dead volume
Residence on column
Pellicular vs. porous

RESOLUTION

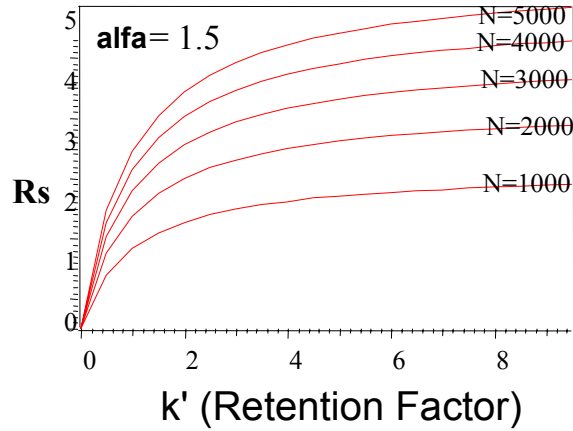
$$R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k'_2}{k'_2 + 1} \right)$$

Resolution between two peaks is affected by the efficiency (N), selectivity (α) and the retention parameter (k')

TYPICAL VALUES

alfa	k'	N
1	0	500
1.1	0.5	1000
1.2	1	1500
1.3	1.5	2000
1.4	2	2500
1.5	2.5	3000
1.6	3	3500
1.7	3.5	4000
1.8	4	4500
1.9	4.5	5000
2	5	5500
2.1	5.5	6000
2.2	6	6500
2.3	6.5	7000
2.4	7	7500
2.5	7.5	8000
2.6	8	8500
2.7	8.5	9000
2.8	9	9500
2.9	9.5	10000

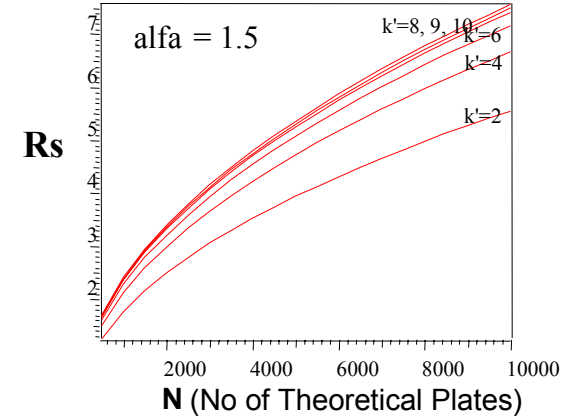
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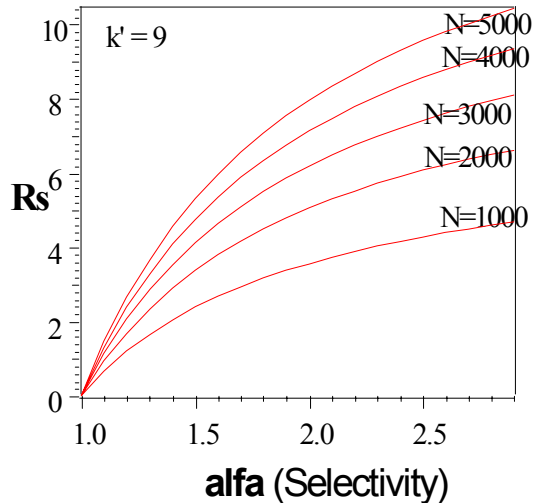


TYPICAL VALUES

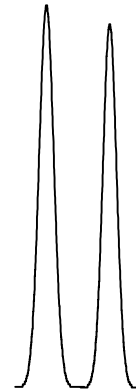
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THE MOST SENSITIVE:



SELECTIVITY vs EFFICIENCY



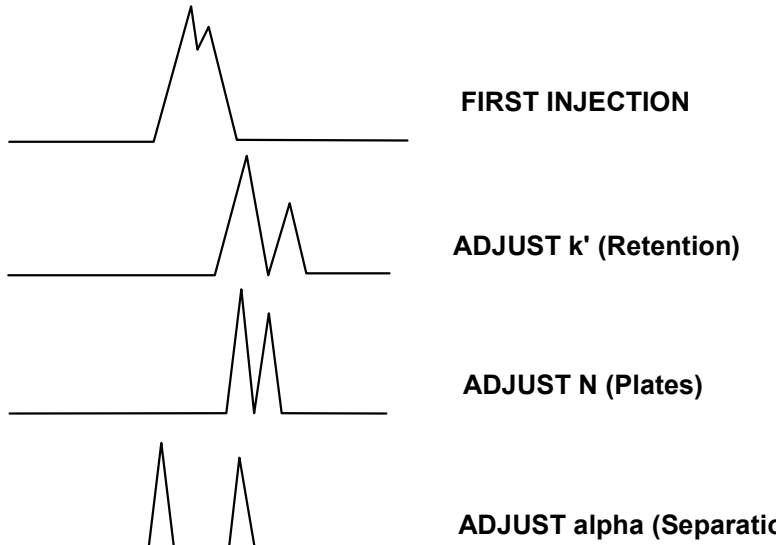
LOW SELECTIVITY (α)
HIGH EFFICIENCY (N)

$$R_s = \frac{t_{R(2)} - t_{R(1)}}{1/2(w_1 + w_2)} \text{ Same in both cases}$$

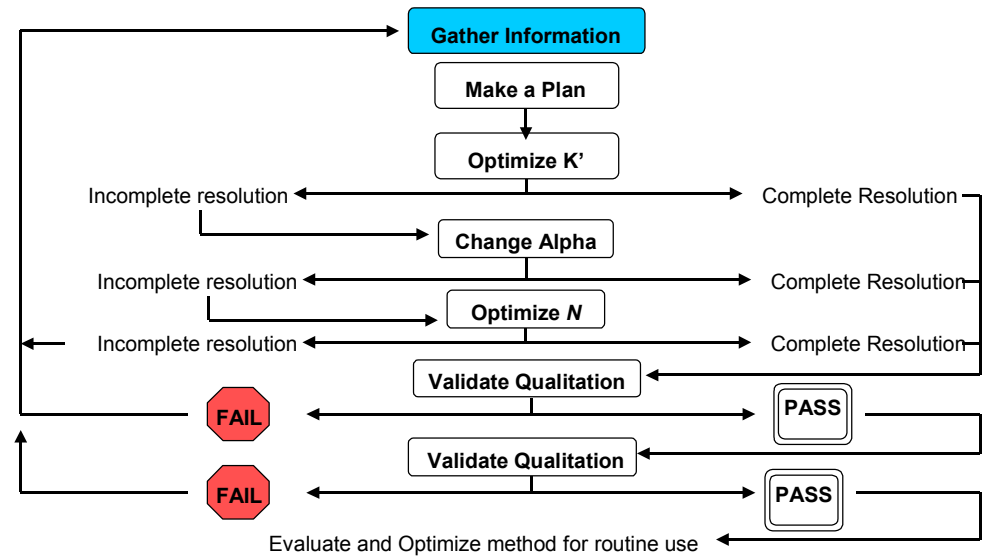


HIGH SELECTIVITY (α)
LOW EFFICIENCY (N)

Separation Development Sequence



Methods Development Strategy



- Step by step method development strategy -