

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

**Efficiency- N**

**Asymmetry – Af; Tailing - T**

**Selectivity - a**

**Resolution - Rs**

# Development and Validation of Chromatographic Methods

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

Efficiency- N

Asymmetry – Af; Tailing - T

Selectivity - a

Resolution - Rs

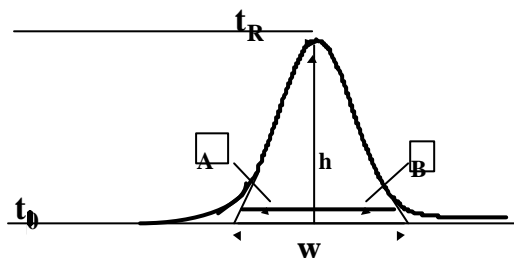
## Quality Control

VIAL	SAMPLE NAME	INJ VOL	No of Inj	Function	Method	Run Time	Sample Weight	Dilution
1	Blank	20.0	1	Inject Samples	LC Demo Method Set	10.00	1.00000	.00000
2	System Suitability	20.0	1	Inject Samples	SST Method Set	10.00	1.00000	.00000
				Clear Calibration	LC Demo Method Set			
3	Std1	20.0	5	Inject Standards	LC Demo Method Set	10.00	1.00000	.00000
4	Std2	20.0	2	Inject Standards	LC Demo Method Set	10.00	1.00000	.00000
				Report	LC Calibration Report			
				Report	Standard Comparison			
				Clear Calibration	LC Demo Method Set			
1	Std1	20.0	1	Inject Standards	LC Demo Method Set	10.00	1.00000	.00000
2	Unk.1	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	.00000
3	Unk.2	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	.00000
4	Unk.3	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	.00000
5	Unk.4	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	.00000
6	Unk.5	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	.00000
7	Unk.6	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	.00000
1	Std1	20.0	2	Inject Standards	LC Demo Method Set	10.00	1.00000	.00000
				Clear Calibration	LC Demo Method Set			
				Calibrate	LC Demo Method Set			

## ABSOLUTE RETENTION OF ONE PEAK

RETENTION FACTOR or CAPACITY RATIO

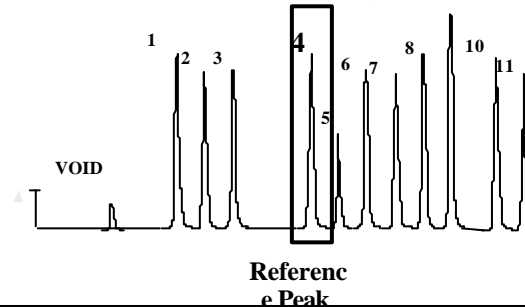
$$k' = \frac{t_R - t_0}{t_0} \quad k' = f \frac{C_s}{C_m}$$



## Relative Retention Time - RRT

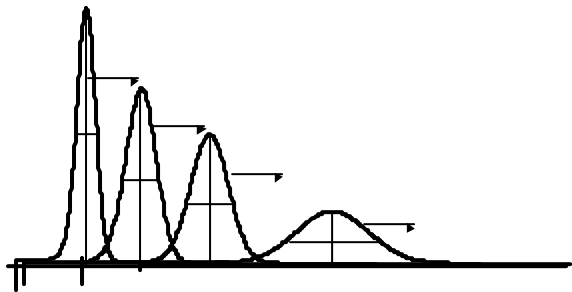
$$\frac{t_{R,i}}{t_{R,4}} = RRT$$

← RRT < 1      RRT > 1 →

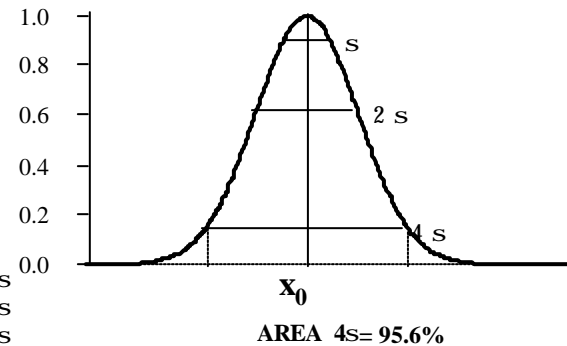


# Development and Validation of Chromatographic Methods

## PEAK BROADENING Diffusion while migrating in the column

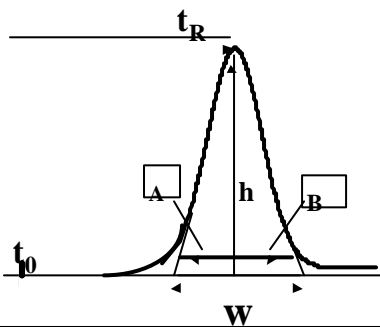


**GAUSSIAN PEAK:**  $\frac{c}{c_0} = \exp\left(-\frac{ay^2}{2DT}\right)$   
( $y = x_0 - wt$ )



## PERFORMANCE BY ONE PEAK

### NUMBER OF THEORETICAL PLATES

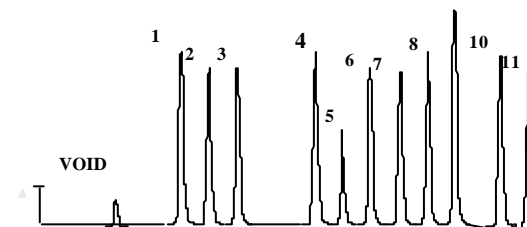


$$N = 16 \left( \frac{t_R}{W} \right)^2$$

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

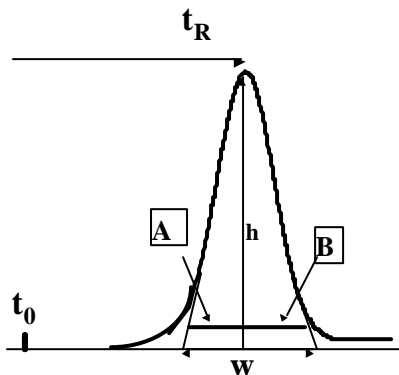
## ADVANTAGES OF HIGH EFFICIENCY

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



# Development and Validation of Chromatographic Methods

## PERFORMANCE BY ONE PEAK: Asymmetric factor or Tailing factor



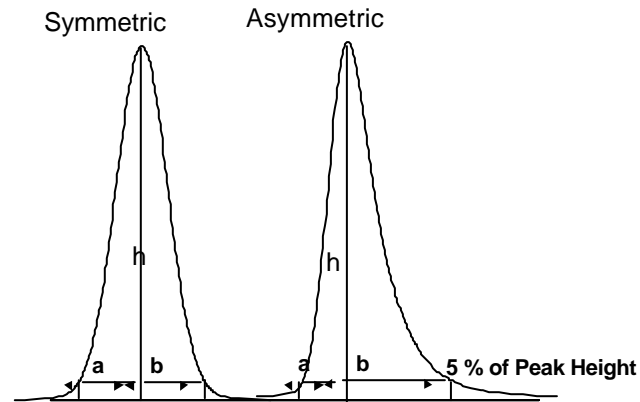
### ASYMMETRY FACTOR

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

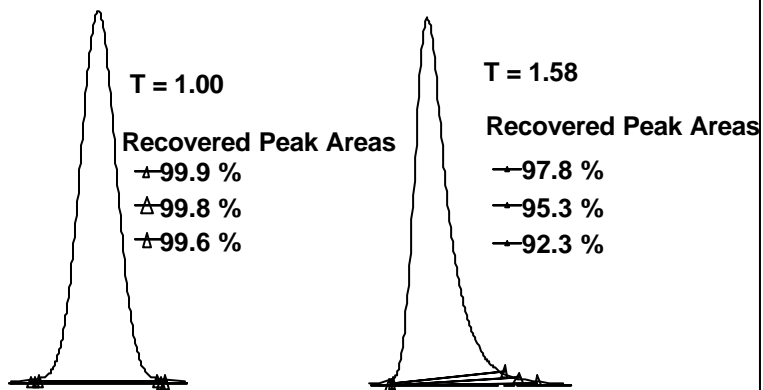
### TAILING FACTOR

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

## PERFORMANCE BY ONE PEAK: Asymmetric factor or Tailing factor



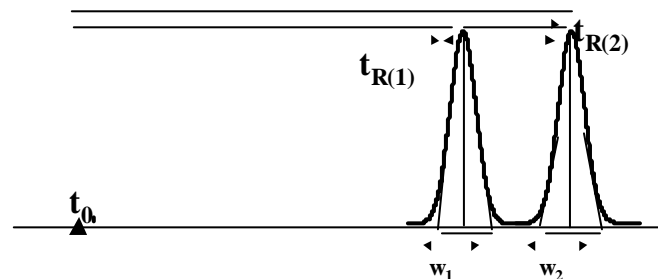
## Integration Error Caused by Tailing of Peaks



## PERFORMANCE BY TWO PEAKS

### SELECTIVITY FACTOR

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

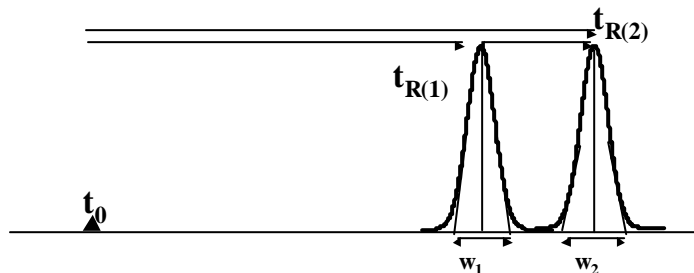


# Development and Validation of Chromatographic Methods

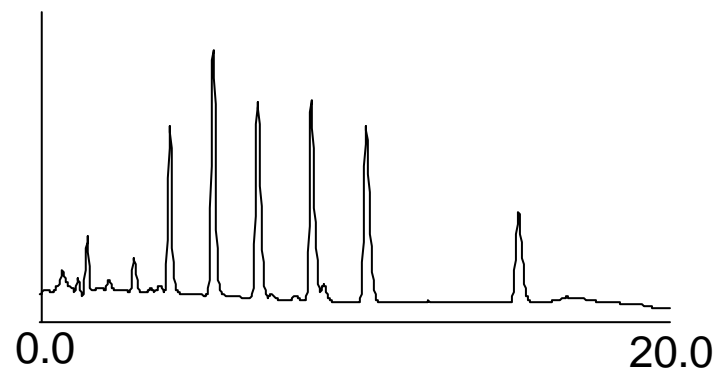
## PERFORMANCE BY TWO PEAKS

### EXPERIMENTAL RESOLUTION

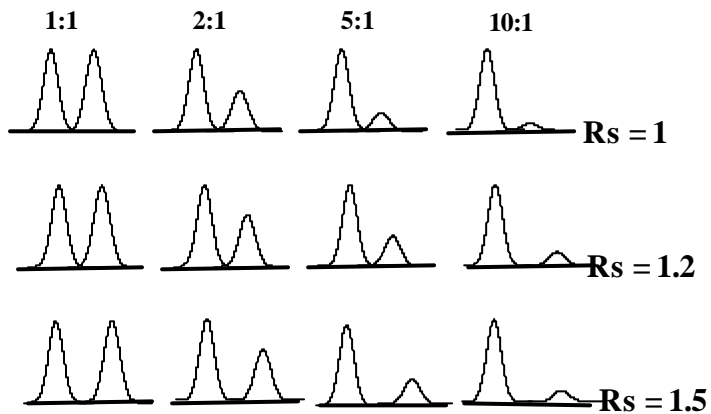
$$R_s = \frac{t_{R(2)} - t_{R(1)}}{1/2 (w_1 + w_2)}$$



## ADVANTAGES OF HIGH RESOLUTION



## Higher Components ratio - Higher Resolution Required



## FACTORS CONTROLLING RESOLUTION

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

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## RESOLUTION

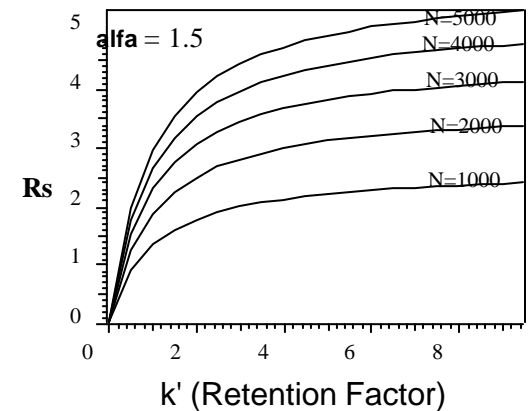
$$R_s = \frac{\sqrt{N}}{4} \left( \frac{a-1}{a} \right) \left( \frac{k'_2}{k'_2+1} \right)$$

Resolution between two peaks is affected by the efficiency (N), selectivity (a) 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

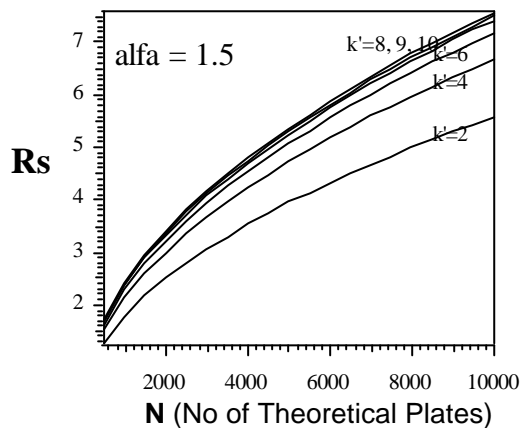
$$R_s = \frac{\sqrt{N}}{4} \left( \frac{a-1}{a} \right) \left( \frac{k'_2}{k'_2+1} \right)$$



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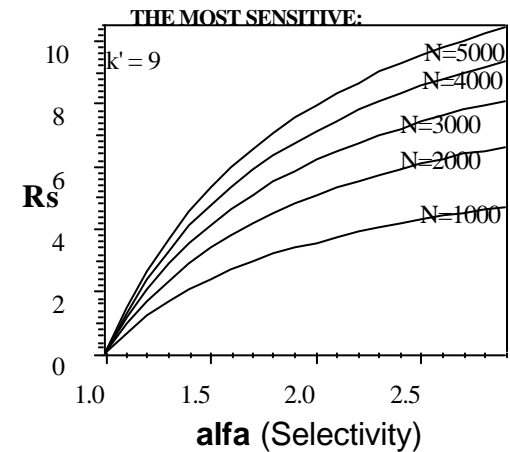
$$R_s = \frac{\sqrt{N}}{4} \left( \frac{a-1}{a} \right) \left( \frac{k'_2}{k'_2+1} \right)$$



### TYPICAL VALUES

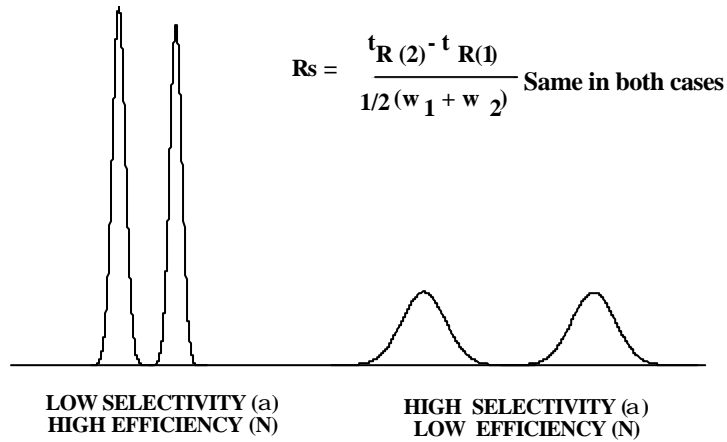
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$$R_s = \frac{\sqrt{N}}{4} \left( \frac{a-1}{a} \right) \left( \frac{k'_2}{k'_2+1} \right)$$

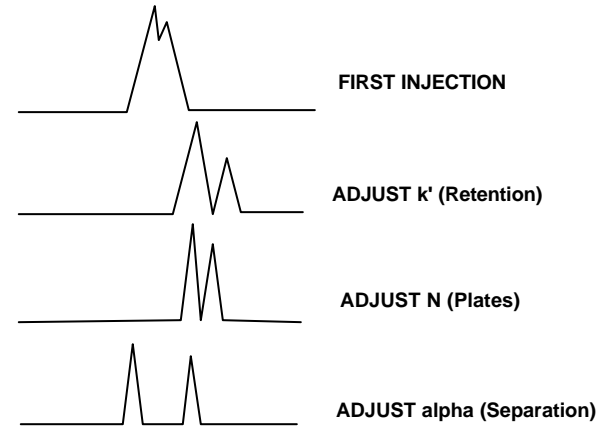


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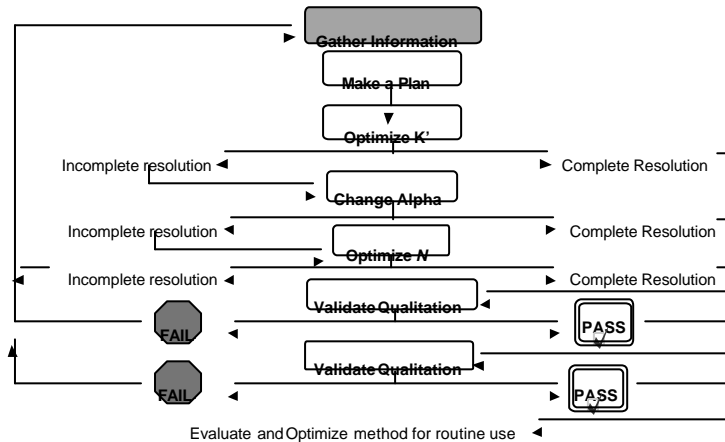
## SELECTIVITY vs EFFICIENCY



## Separation Development Sequence



## Methods Development Strategy



- Step by step method development strategy -