

System Suitability : Numerical Criteria for the HPLC Analysis Performance

Efficiency- N

Asymmetry – Af; Tailing - T

Selectivity - a

Resolution - Rs

System Suitability

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Efficiency - N
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Selectivity - a
Resolution - Rs

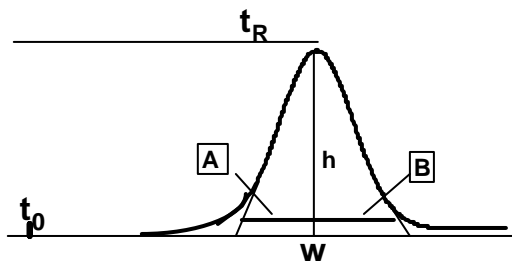
Quality Control

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	1.00000
2	System Suitability	20.0	1	Inject Samples	SST Method Set	10.00	1.00000	1.00000
				Clear Calibration	LC Demo Method Set			
3	Std1	20.0	5	Inject Standards	LC Demo Method Set	10.00	1.00000	1.00000
4	Std2	20.0	2	Inject Standards	LC Demo Method Set	10.00	1.00000	1.00000
				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	1.00000
2	Unk.1	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
3	Unk.2	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
4	Unk.3	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
5	Unk.4	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
6	Unk.5	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
7	Unk.6	20.0	2	Inject Samples	LC Demo Method Set	10.00	1.00000	1.00000
1	Std1	20.0	1	Inject Standards	LC Demo Method Set	10.00	1.00000	1.00000
				Clear Calibration	LC Demo Method Set			
				Calibrate	LC Demo Method Set			

ABSOLUTE RETENTION OF ONE PEAK

RETENTION FACTOR or CAPACITY RATIO

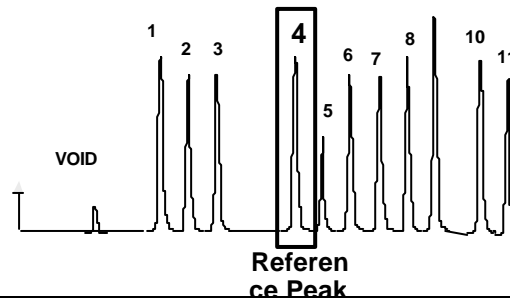
$$k' = \frac{t_R - t_0}{t_0} \quad k' = f \frac{C_s}{C_m}$$



Relative Retention Time - RRT

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

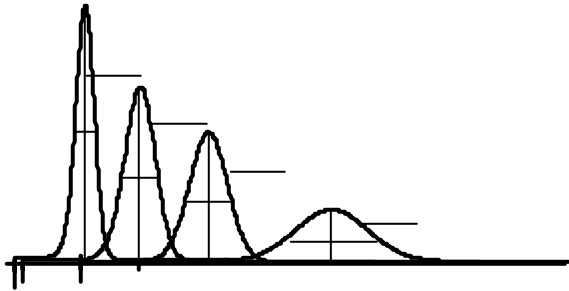
— RRT < 1 RRT > 1 —



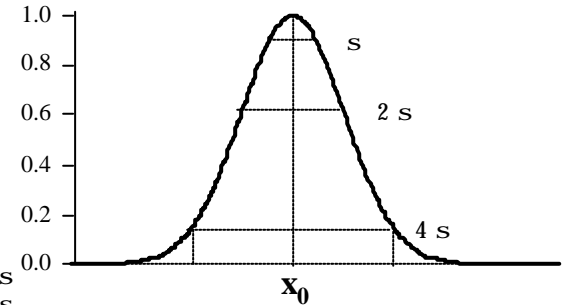
System Suitability

PEAK BROADENING

Diffusion while migrating in the column



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

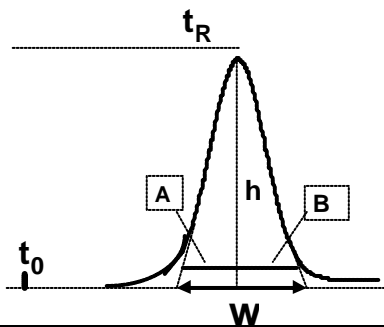


0.6065 h = 2 s
 0.1353 h = 4 s
 0.8825 h = 1 s

AREA 4s = 95.6%

PERFORMANCE BY ONE PEAK

NUMBER OF THEORETICAL PLATES



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

Or:

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

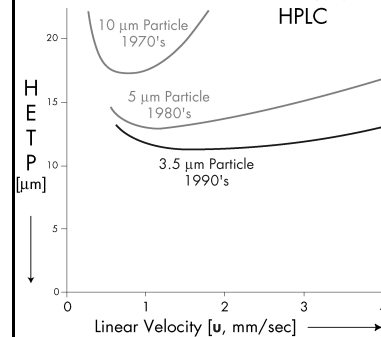
Smaller Particles

The theory

A term + B term + C term

$$H = a(dp) + \frac{b}{u} + c(dp)^2u$$

u = Linear Velocity (Proportional to Flow Rate, Column ID) dp = Particle Size

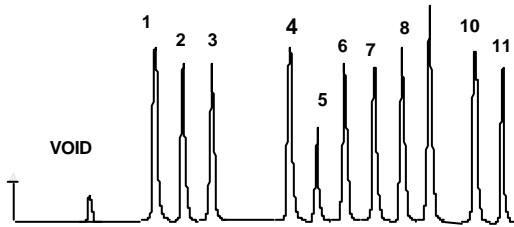


- **Smaller particles provide:**
 - increased efficiency
 - maintain efficiency over a wider linear velocity
 - ability for both added resolution and increased speed of separation
- **Particles are central to the quality of the separation**

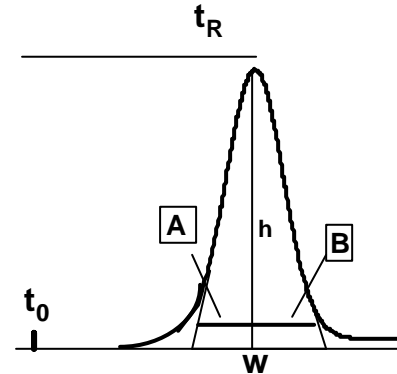
System Suitability

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



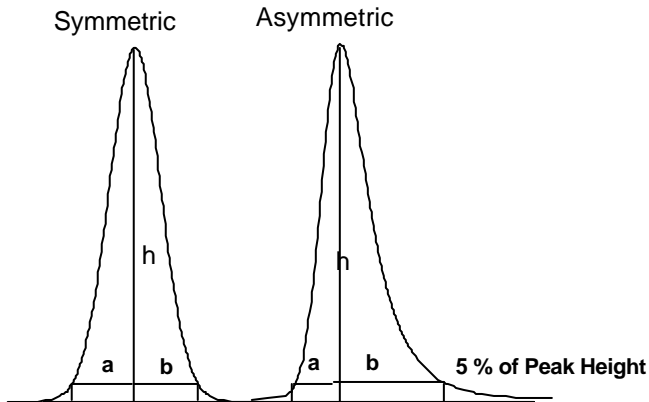
ASYMMETRY FACTOR

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

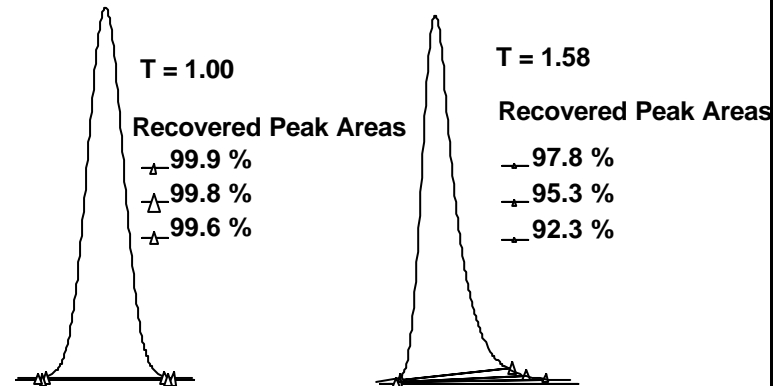
TAILING FACTOR

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

PERFORMANCE BY ONE PEAK: Asymmetric factor or Tailing factor



Integration Error Caused by Tailing of Peaks

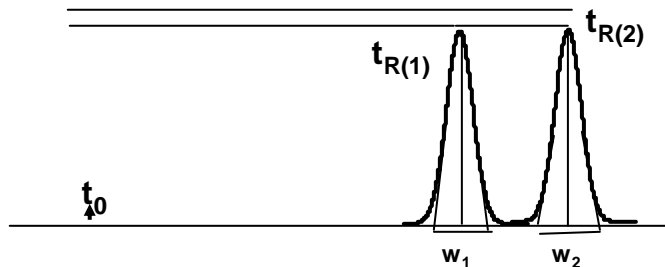


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PERFORMANCE BY TWO PEAKS

SELECTIVITY FACTOR

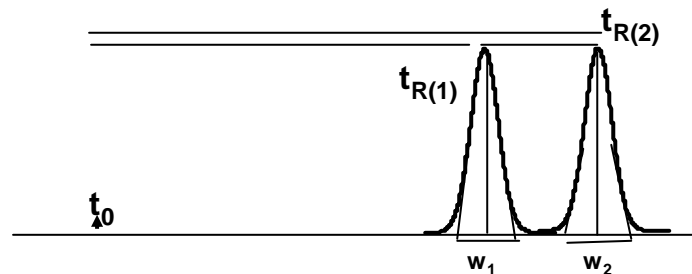
$$\alpha = \frac{k'_{(2)}}{k'_{(1)}}$$



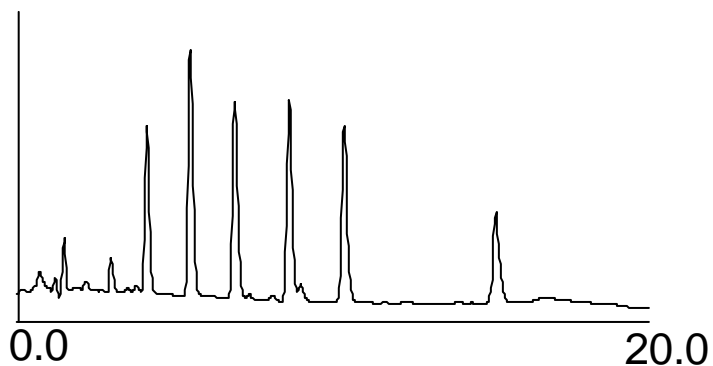
PERFORMANCE BY TWO PEAKS

EXPERIMENTAL RESOLUTION

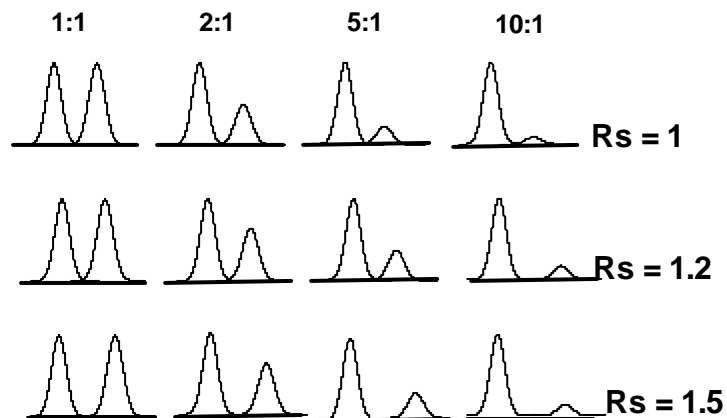
$$R_s = \frac{t_{R(2)} - t_{R(1)}}{1/2 (w_1 + w_2)}$$



ADVANTAGES OF HIGH RESOLUTION



Higher Components ratio - Higher Resolution Required



System Suitability

FACTORS CONTROLLING RESOLUTION

- k'** Strength of solvent - polarity
Strength of packing - surface area, carbon load
Temperature
- a** 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

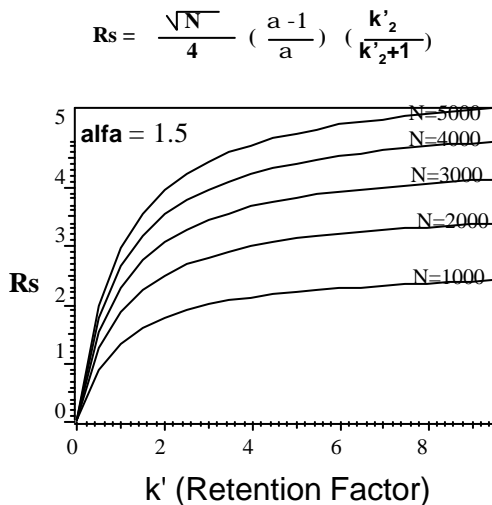
RESOLUTION

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

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

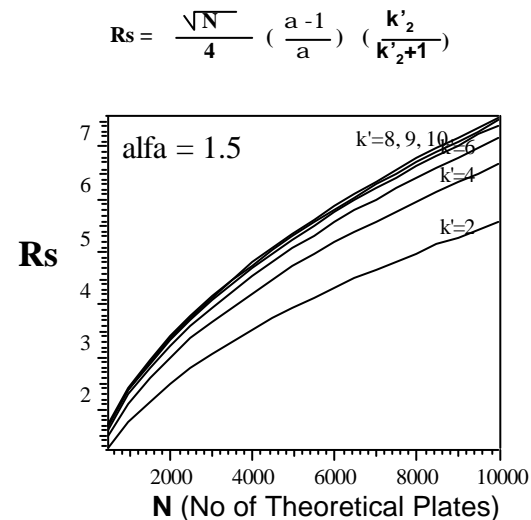
TYPICAL VALUES

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



TYPICAL VALUES

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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



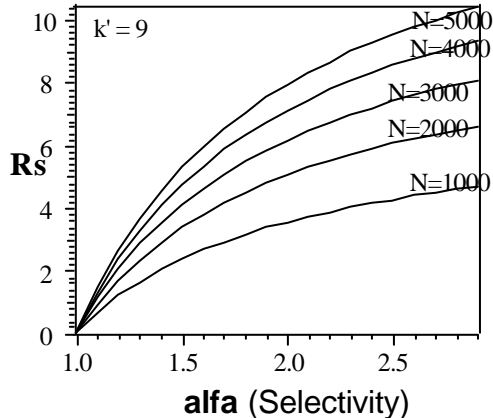
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$$R_s = \frac{\sqrt{N}}{4} \left(\frac{a-1}{a} \right) \left(\frac{k'_2}{k'_2+1} \right)$$

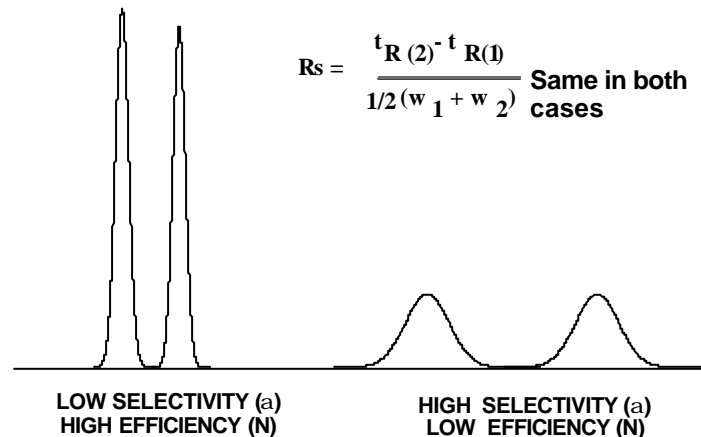
THE MOST SENSITIVE:



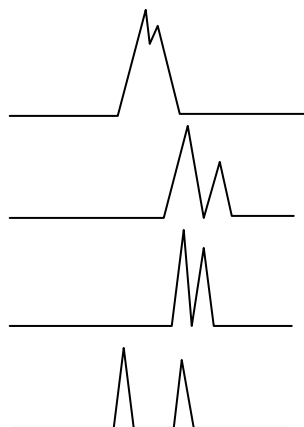
SELECTIVITY vs EFFICIENCY

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

Same in both cases



Separation Development Sequence



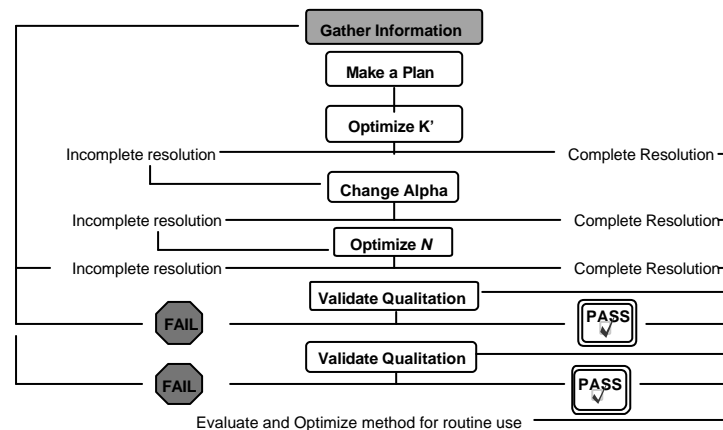
FIRST INJECTION

ADJUST k' (Retention)

ADJUST N (Plates)

ADJUST alfa (Separation)

Methods Development Strategy



- Step by step method development strategy -