

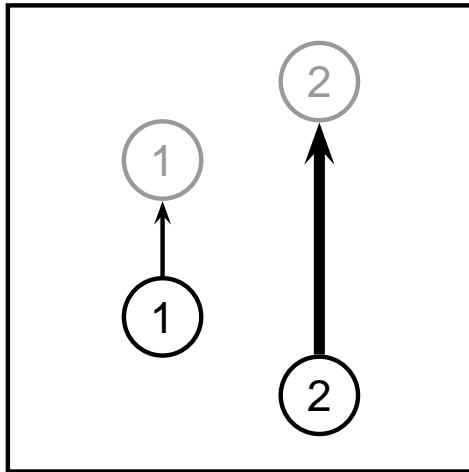
Separation by Chromatography

Lecture VAK 02-6007

May 17, 2005

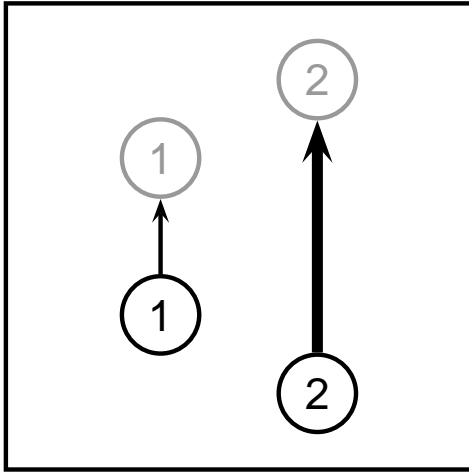
Johannes Ranke

1D Separation methods

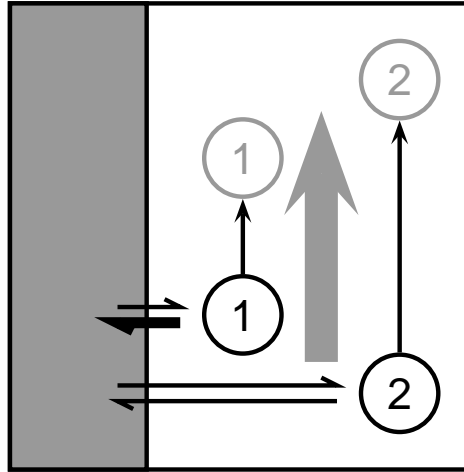


Electrophoresis

1D Separation methods

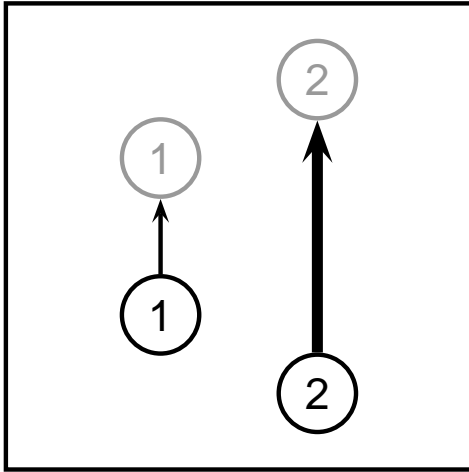


Electrophoresis

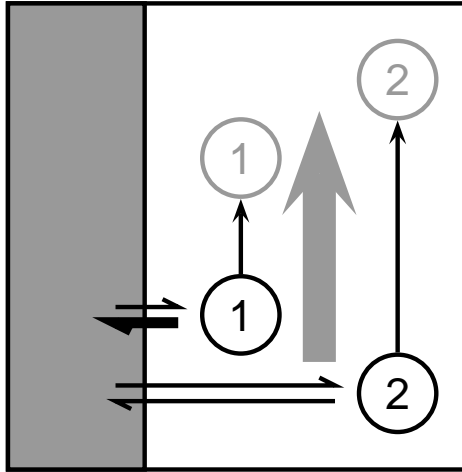


Chromatography

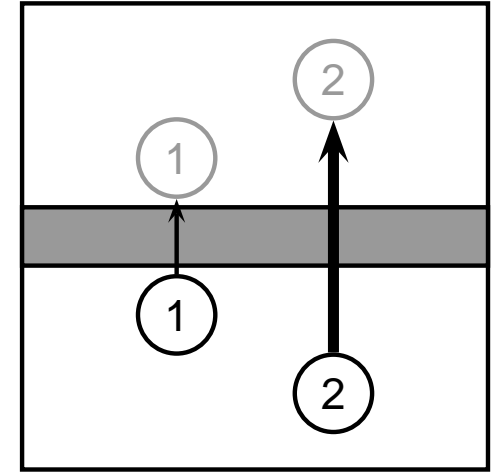
1D Separation methods



Electrophoresis

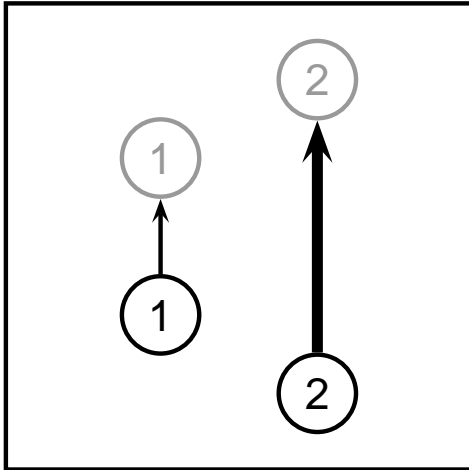


Chromatography

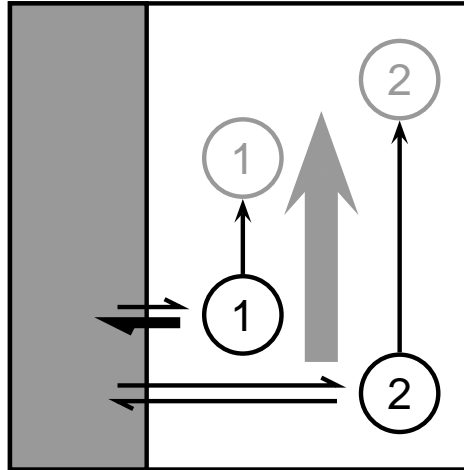


Membrane separation

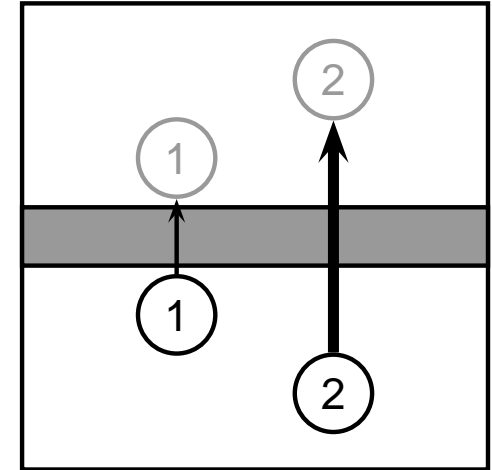
1D Separation methods



Electrophoresis



Chromatography

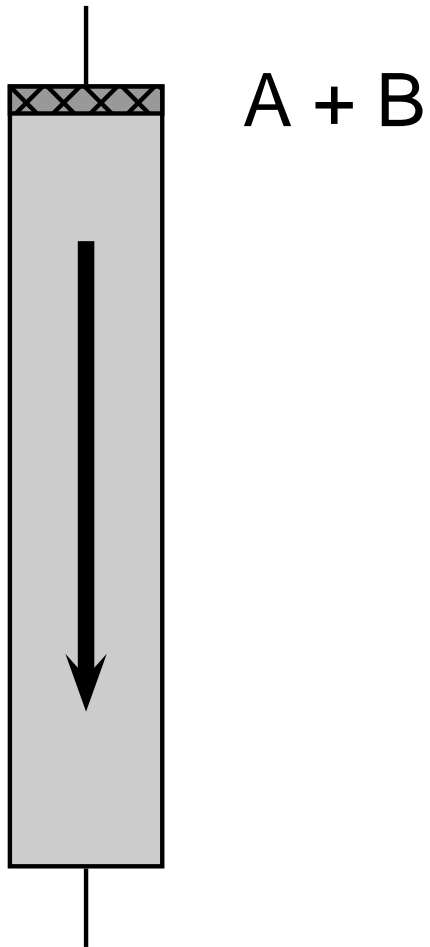


Membrane separation

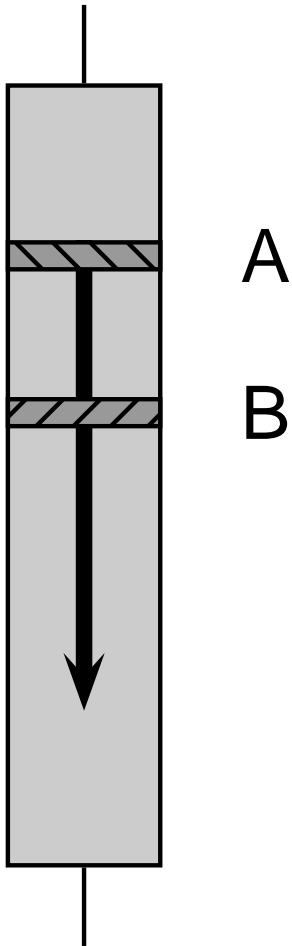
Separation according to

- Kinetic properties
- Equilibrium distribution
- Combinations thereof

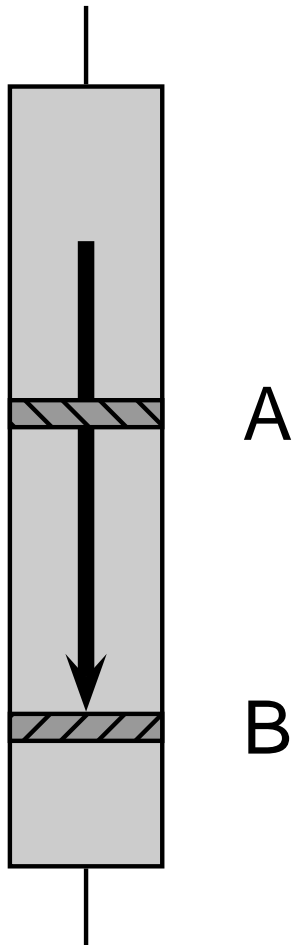
Elution techniques



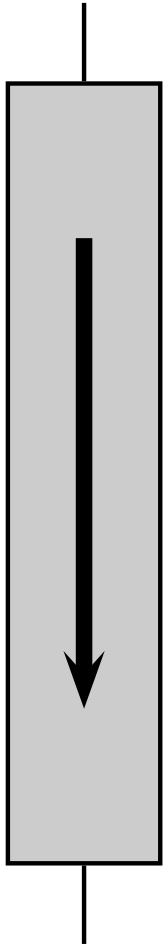
Elution techniques



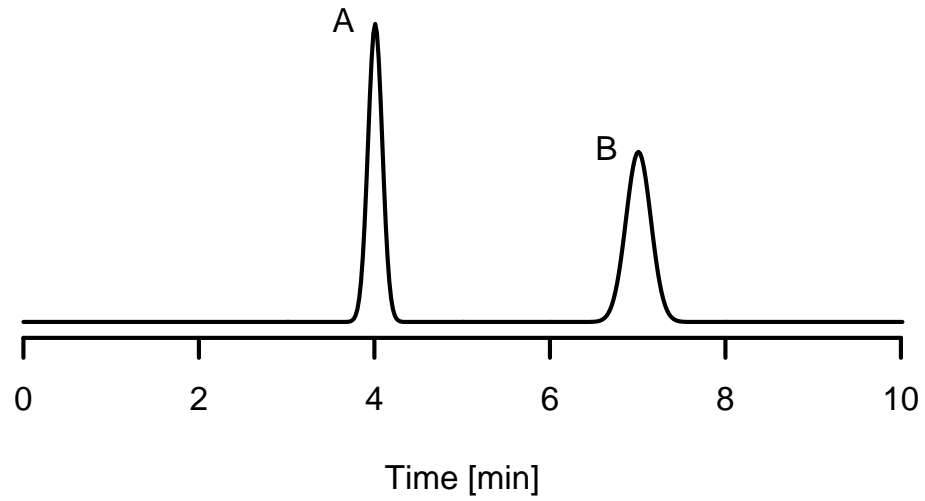
Elution techniques



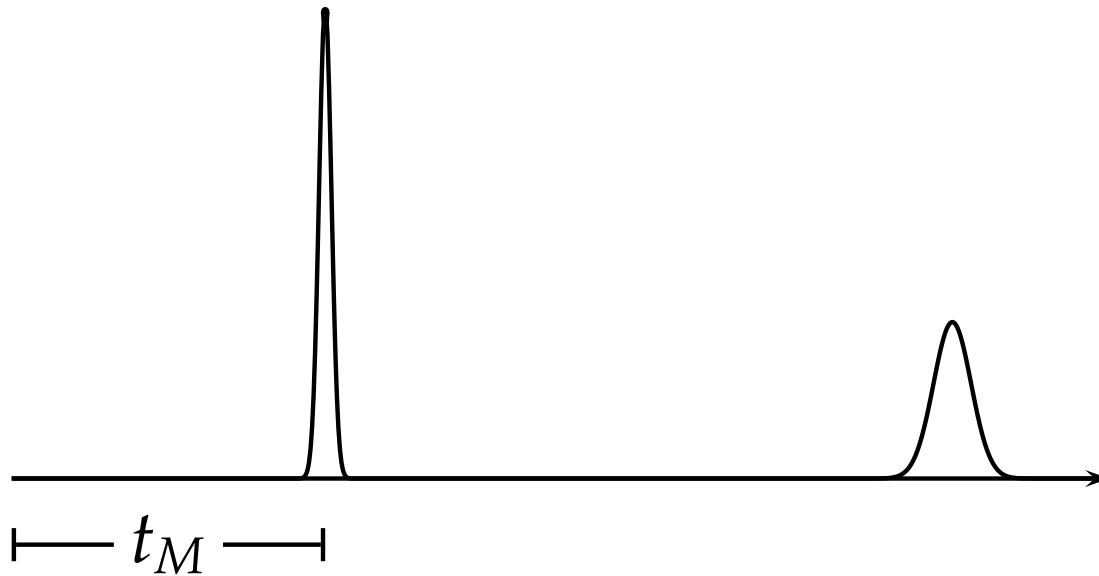
Elution techniques



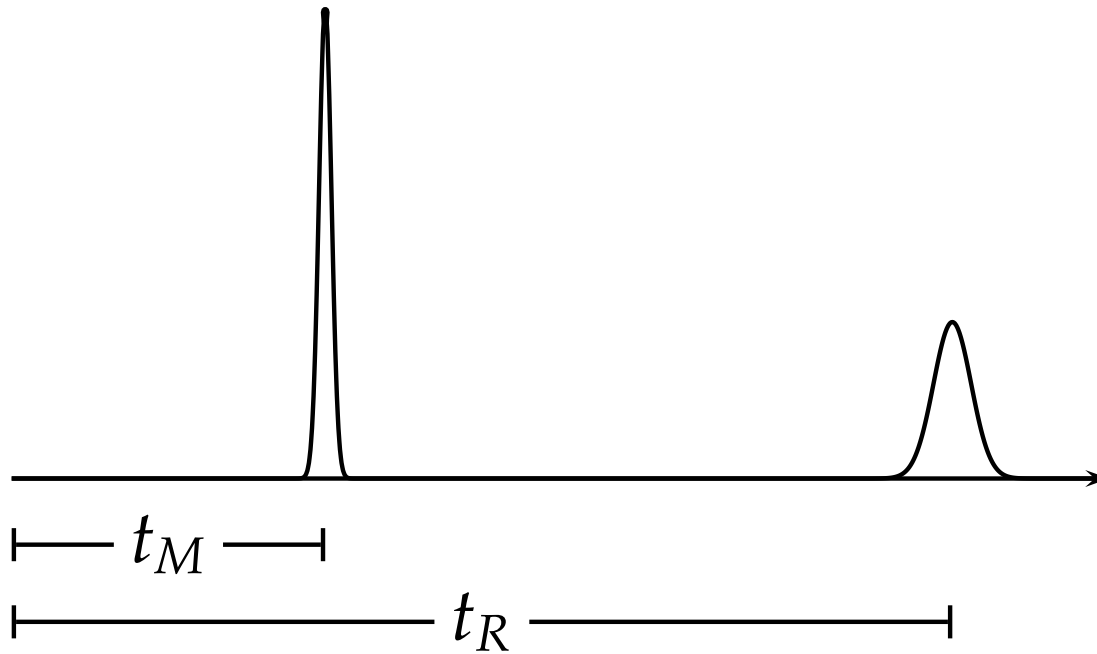
Detector signal



Equilibrium based separation

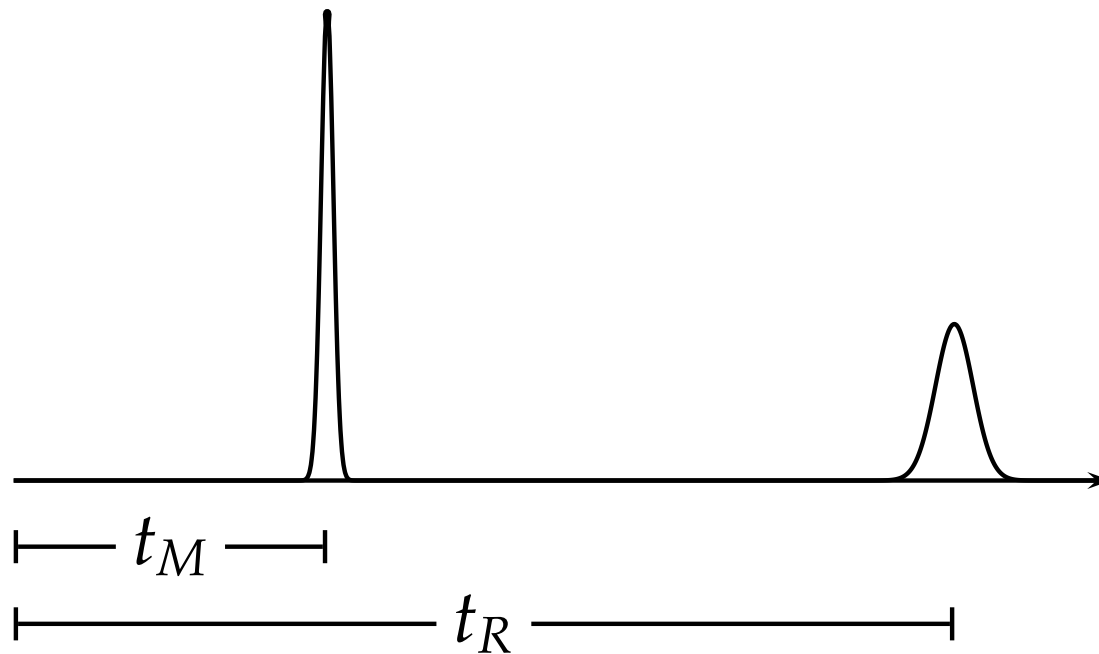


Equilibrium based separation



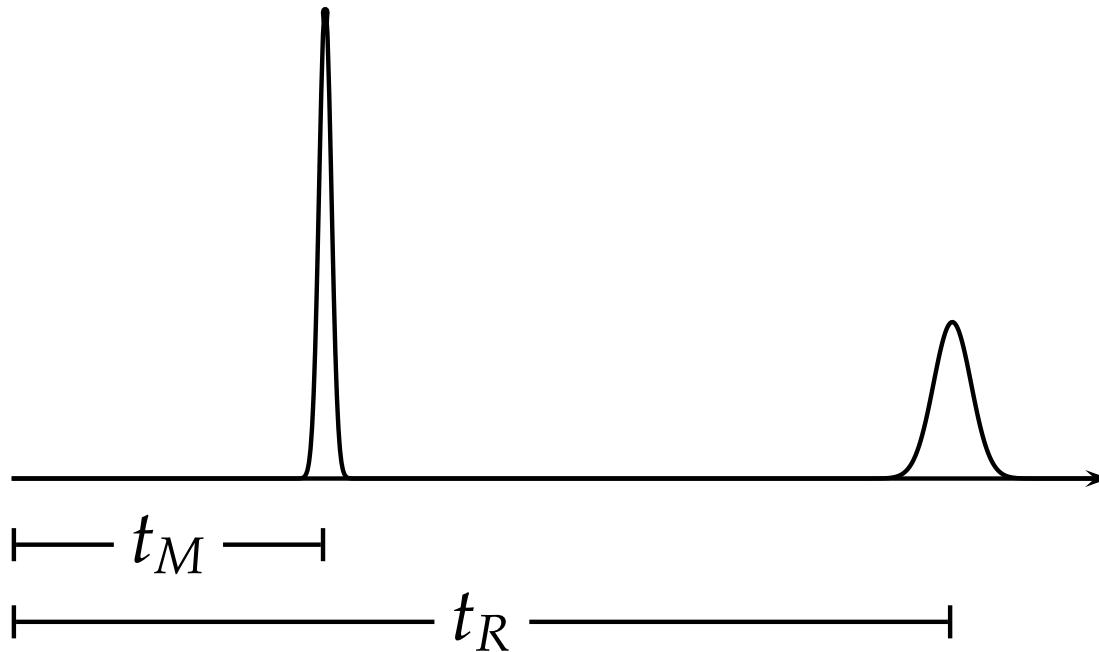
Equilibrium based separation

$$k' = \frac{n_S}{n_M}$$



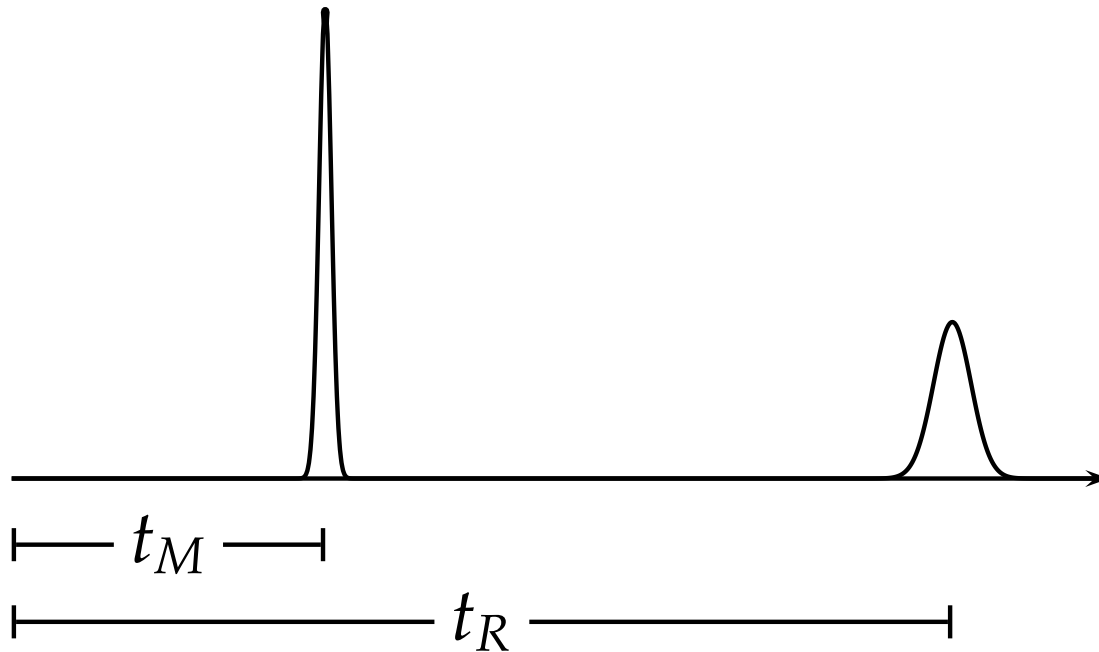
Equilibrium based separation

$$k' = \frac{n_S}{n_M} = \frac{\bar{t}_S}{\bar{t}_M}$$



Equilibrium based separation

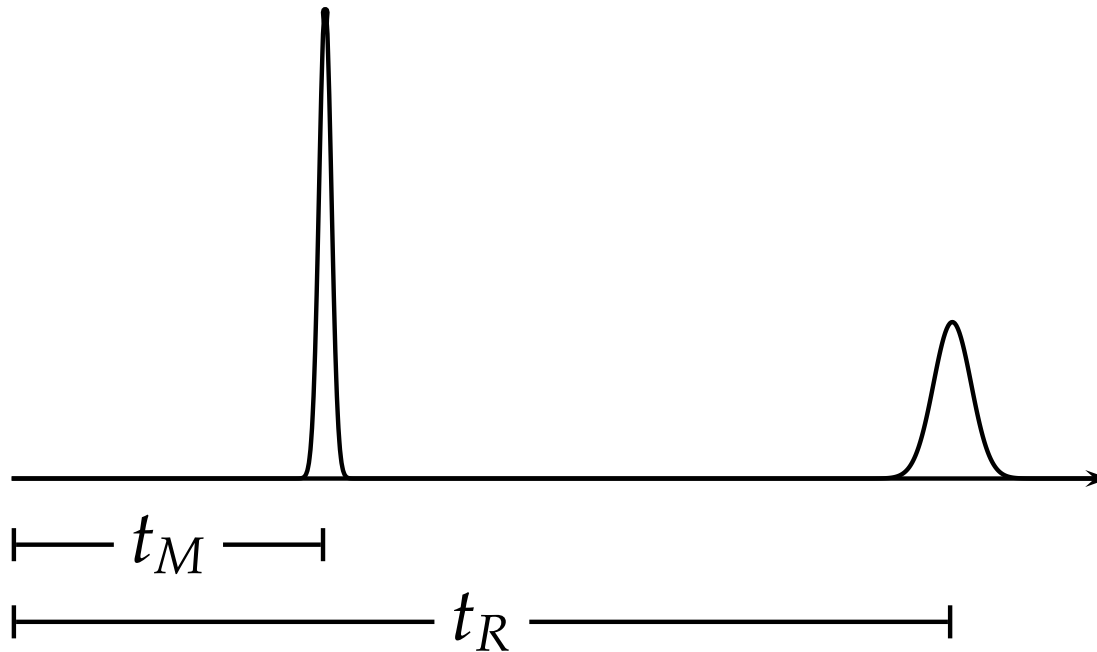
$$k' = \frac{n_S}{n_M} = \frac{\bar{t}_S}{\bar{t}_M} = \frac{t_R - t_M}{t_M}$$



Equilibrium based separation

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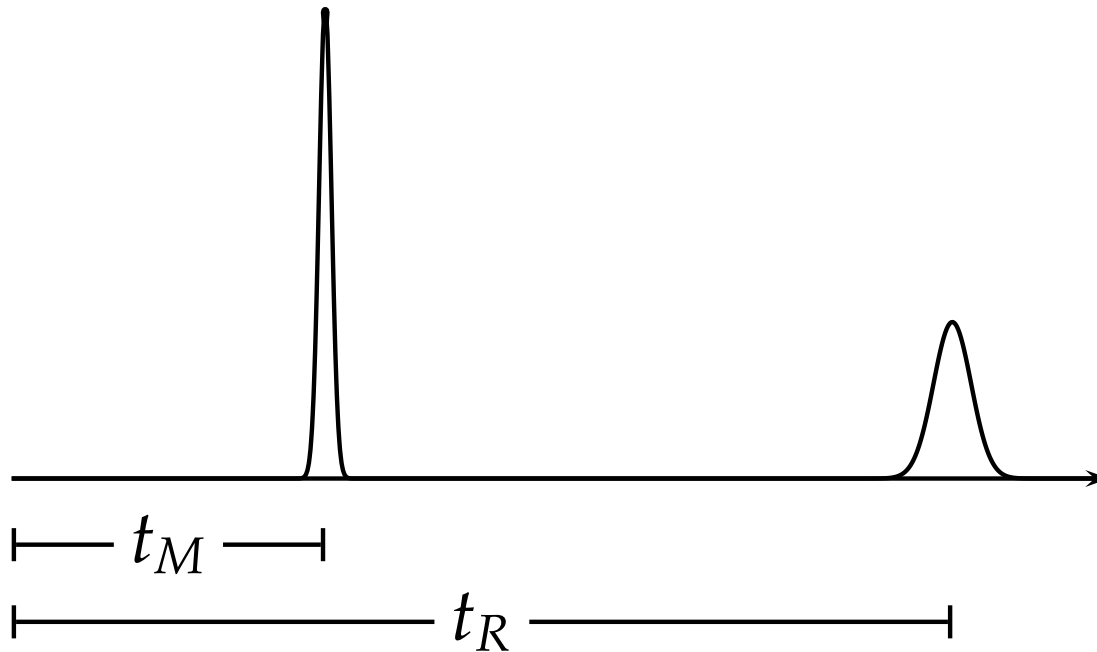
$$k' = \frac{c_S \cdot V_S}{c_M \cdot V_M}$$



Equilibrium based separation

$$k' = \frac{n_S}{n_M} = \frac{\bar{t}_S}{\bar{t}_M} = \frac{t_R - t_M}{t_M}$$

$$k' = \frac{c_S \cdot V_S}{c_M \cdot V_M} = K \cdot \frac{V_S}{V_M}$$



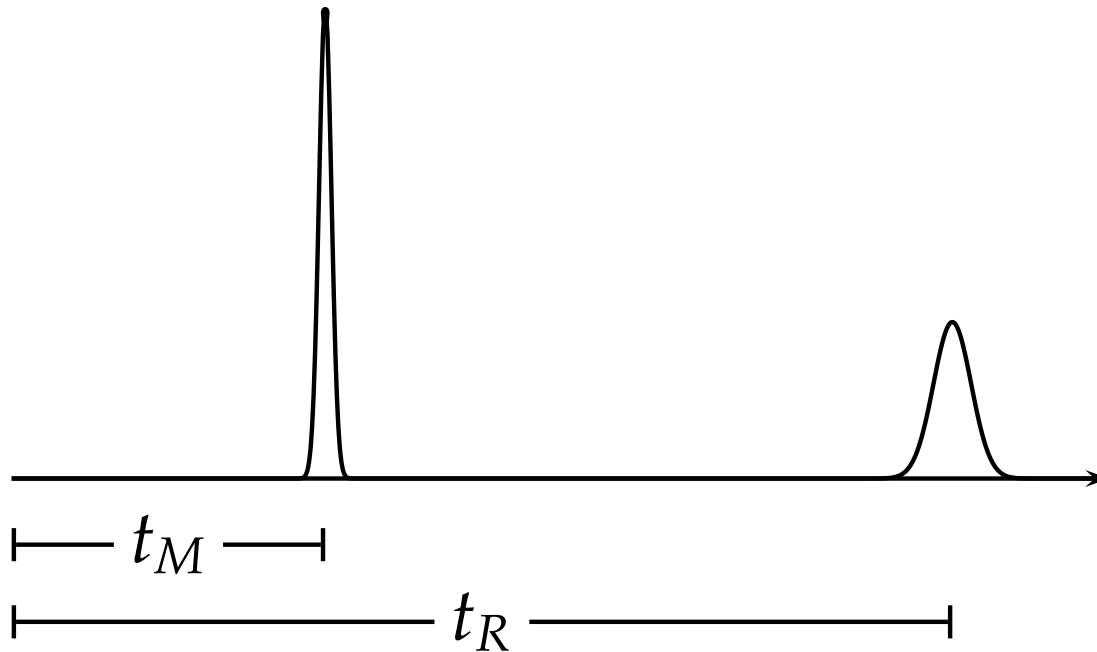
Equilibrium based separation

$$t_M = F \cdot V_M$$

$$t_R = F \cdot V_R$$

$$k' = \frac{n_S}{n_M} = \frac{\bar{t}_S}{\bar{t}_M} = \frac{t_R - t_M}{t_M} = \frac{V_R - V_M}{V_M}$$

$$k' = \frac{c_S \cdot V_S}{c_M \cdot V_M} = K \cdot \frac{V_S}{V_M}$$



Equilibrium based separation

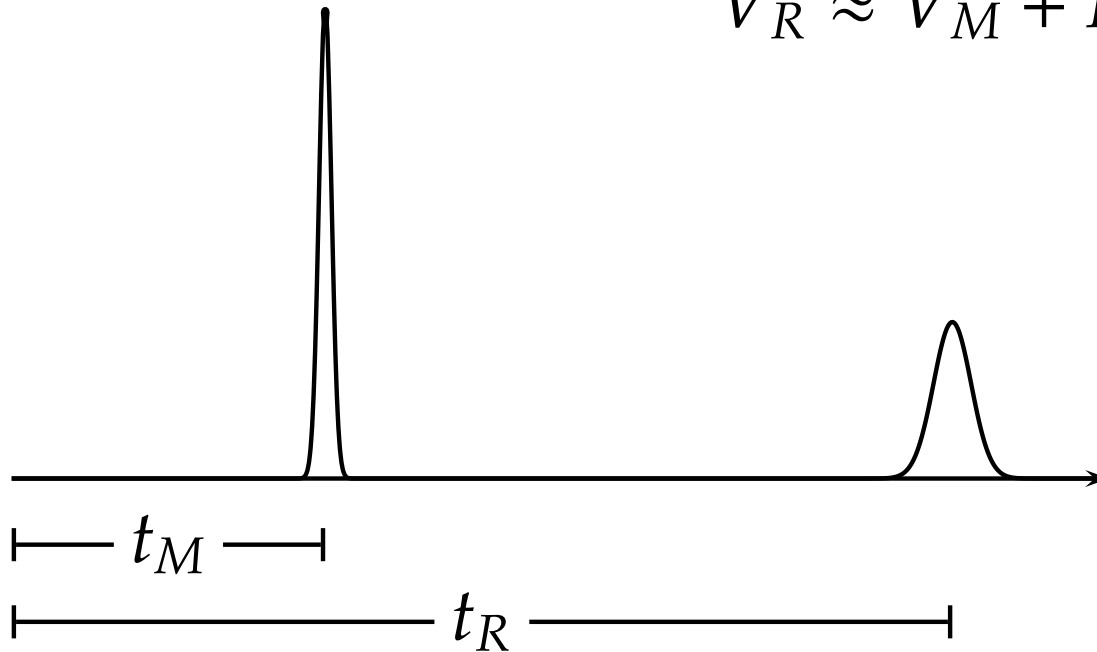
$$t_M = F \cdot V_M$$

$$t_R = F \cdot V_R$$

$$k' = \frac{n_S}{n_M} = \frac{\bar{t}_S}{\bar{t}_M} = \frac{t_R - t_M}{t_M} = \frac{V_R - V_M}{V_M}$$

$$k' = \frac{c_S \cdot V_S}{c_M \cdot V_M} = K \cdot \frac{V_S}{V_M}$$

$$V_R \approx V_M + K \cdot V_S$$



Equilibrium constant

$$K_i = \frac{c_S}{c_M}$$

$$K_i = \exp(-\Delta G_i^0 / RT) = \exp\left(-\frac{\Delta H_i^0 - T\Delta S_i^0}{RT}\right)$$

Equilibrium constant

$$K_i = \frac{c_S}{c_M}$$

$$K_i = \exp(-\Delta G_i^0 / RT) = \exp\left(-\frac{\Delta H_i^0 - T\Delta S_i^0}{RT}\right)$$

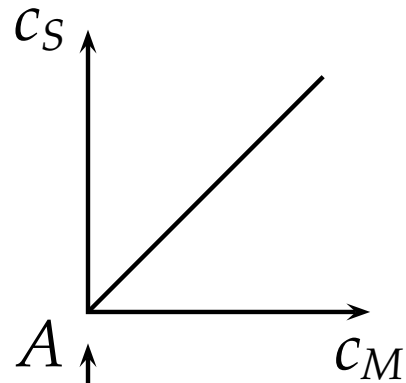
If $\Delta H_i^0 \approx 0$, then

$$K_i \approx \exp(-\Delta S_i^0 / R),$$

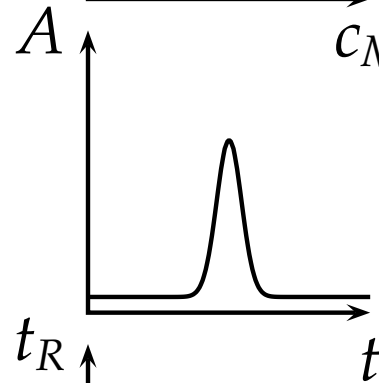
i.e. K_i is independent from temperature.

Peak shapes

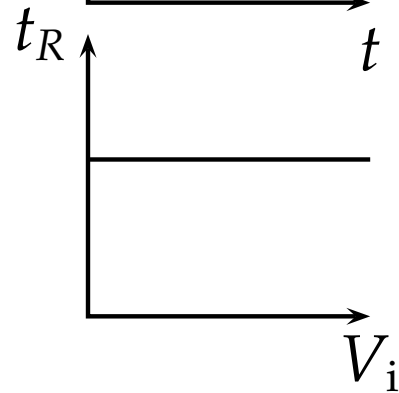
Isotherm



Signal shape

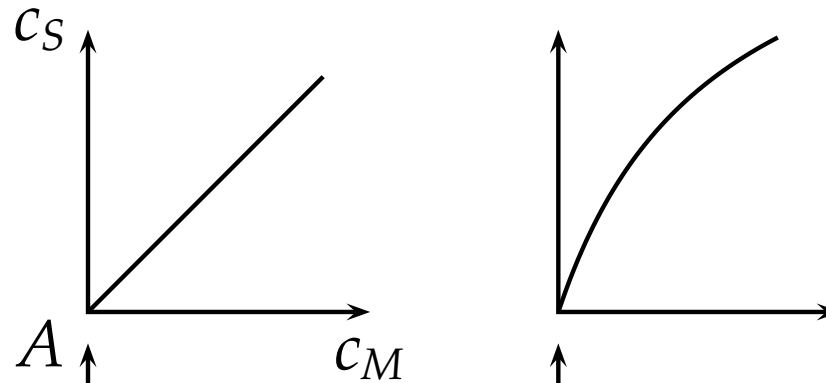


Retention time

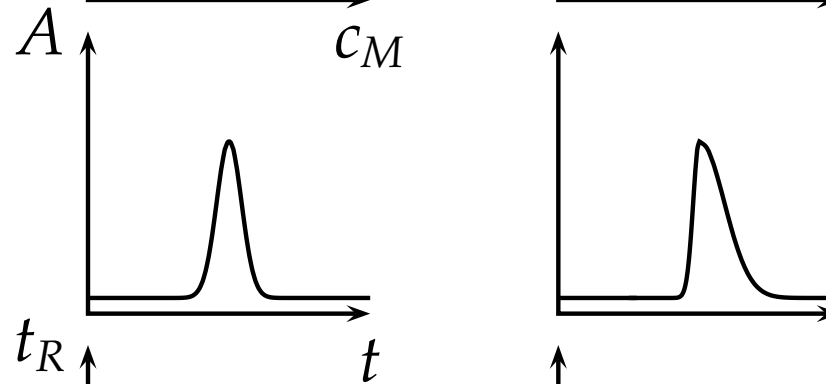


Peak shapes

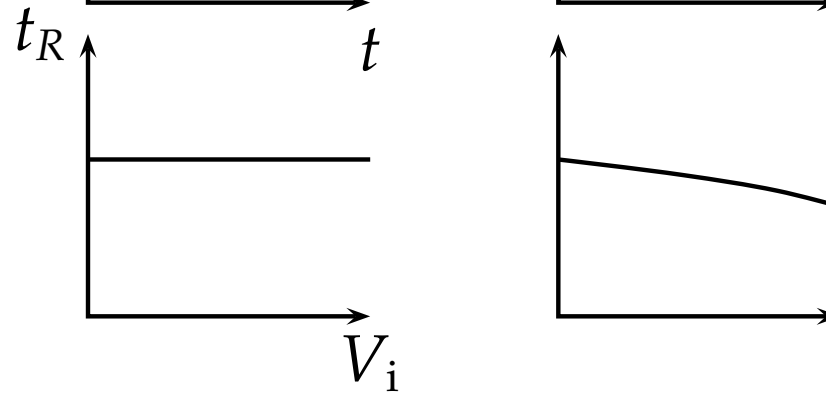
Isotherm



Signal shape

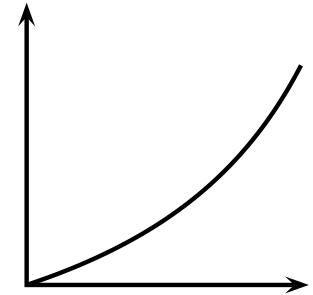
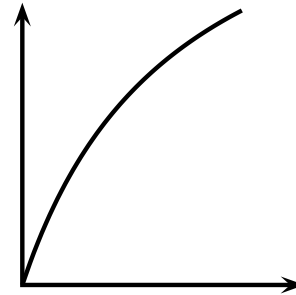
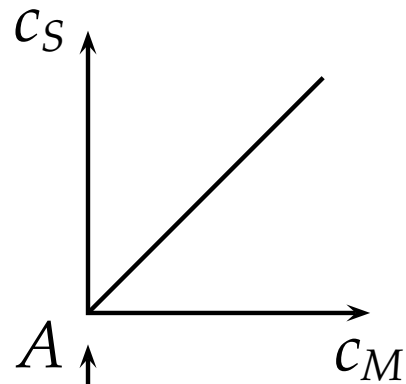


Retention time

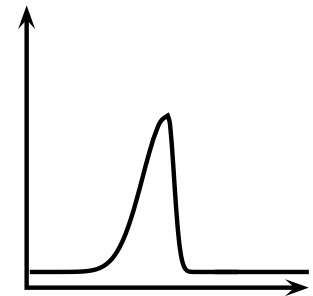
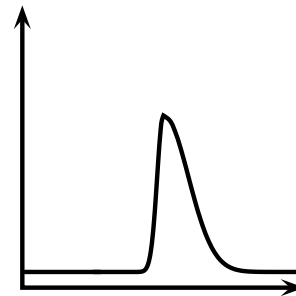
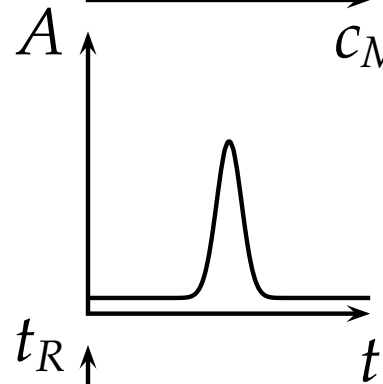


Peak shapes

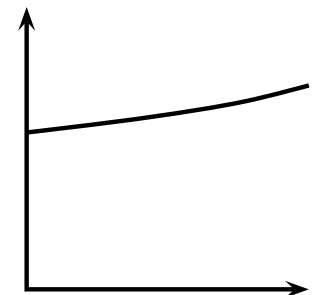
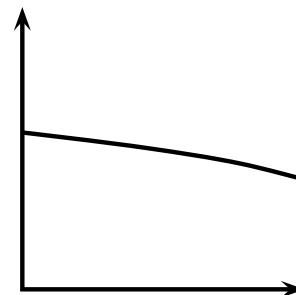
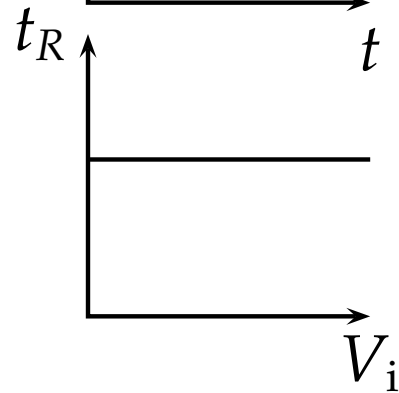
Isotherm



Signal shape



Retention time



Peak dispersion

- Injection

Peak dispersion

- Injection
- Dispersion in connecting tubes/capillaries

Peak dispersion

- Injection
- Dispersion in connecting tubes/capillaries
- **Dispersion in columns**

Peak dispersion

- Injection
- Dispersion in connecting tubes/capillaries
- **Dispersion in columns**
- Dispersion caused by signal detection

Dispersion in columns

van-Deemter equation:

$$H = A + B/v + C v$$

- Eddy diffusion

$$A = 2\lambda d_R$$

Dispersion in columns

van-Deemter equation:

$$H = A + B/v + C v$$

- Eddy diffusion
 $A = 2\lambda d_R$
- Longitudonal diffusion
 $B = 2\Psi D_M$

Dispersion in columns

van-Deemter equation:

$$H = A + B/v + C v$$

- Eddy diffusion

$$A = 2\lambda d_R$$

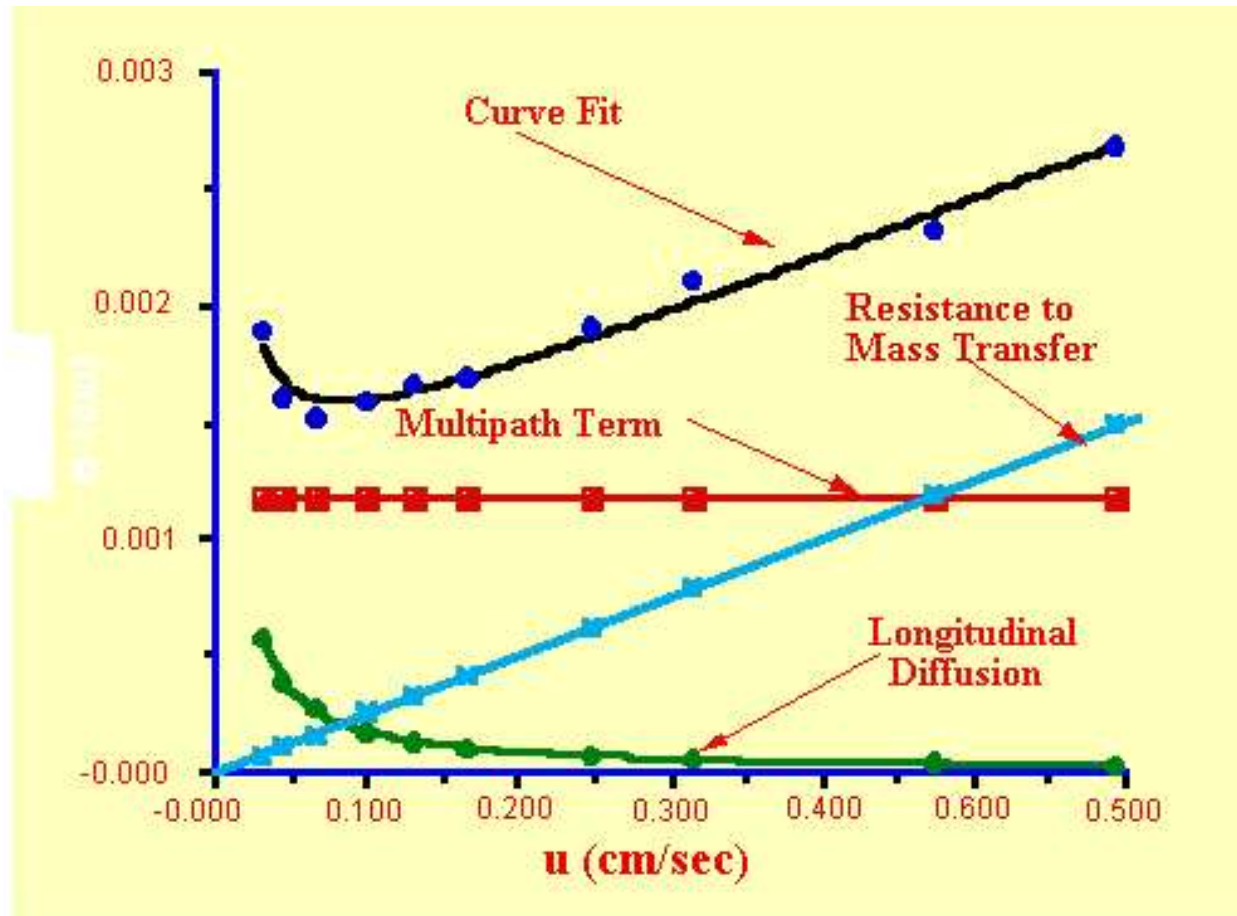
- Longitudinal diffusion

$$B = 2\Psi D_M$$

- Lateral diffusion, disequilibrium

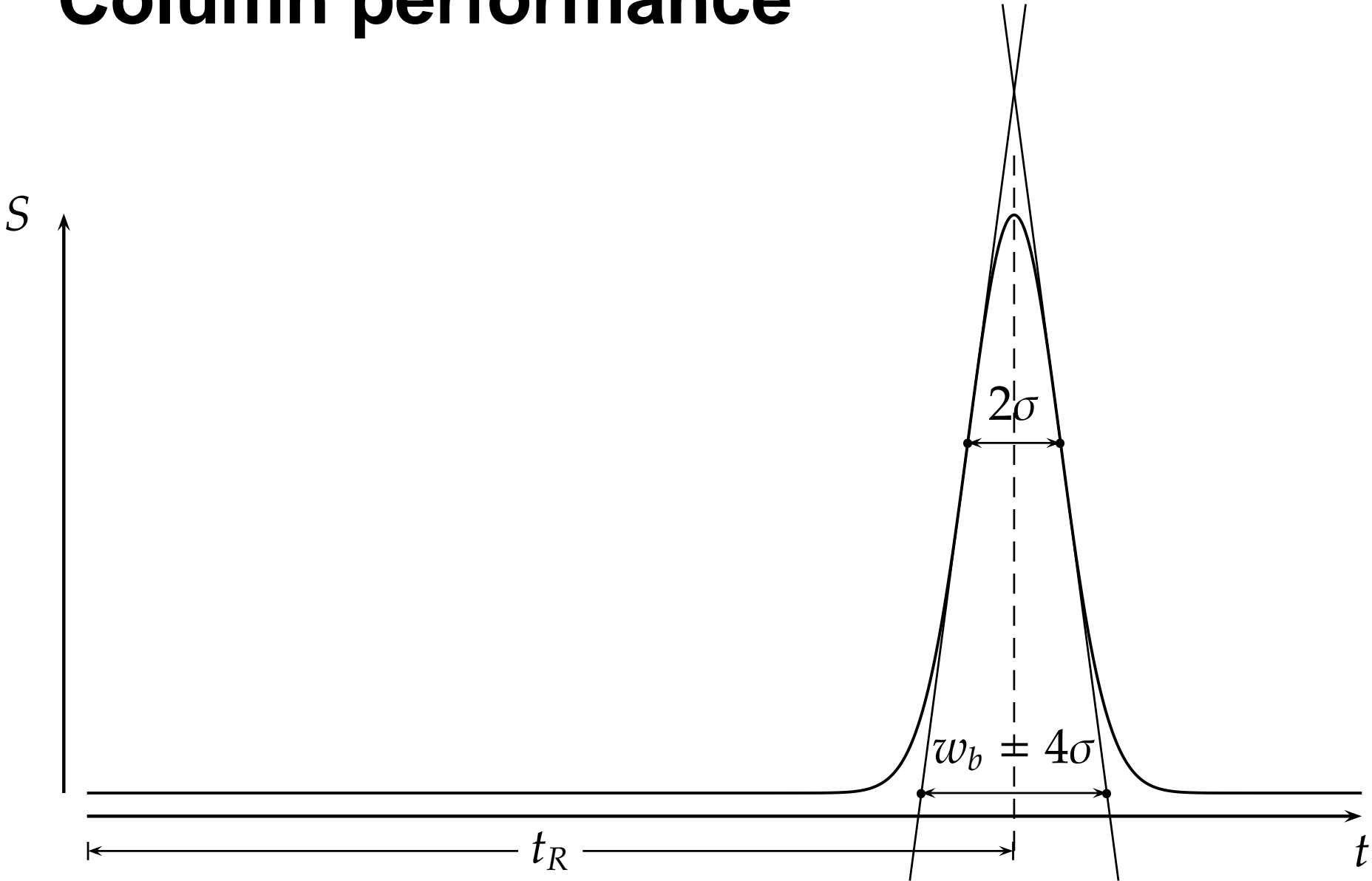
$$C = K_1 \cdot R \cdot (1 - R) \cdot d_f^2 / D_S + K_2 / D_M$$

Van Deemter plot

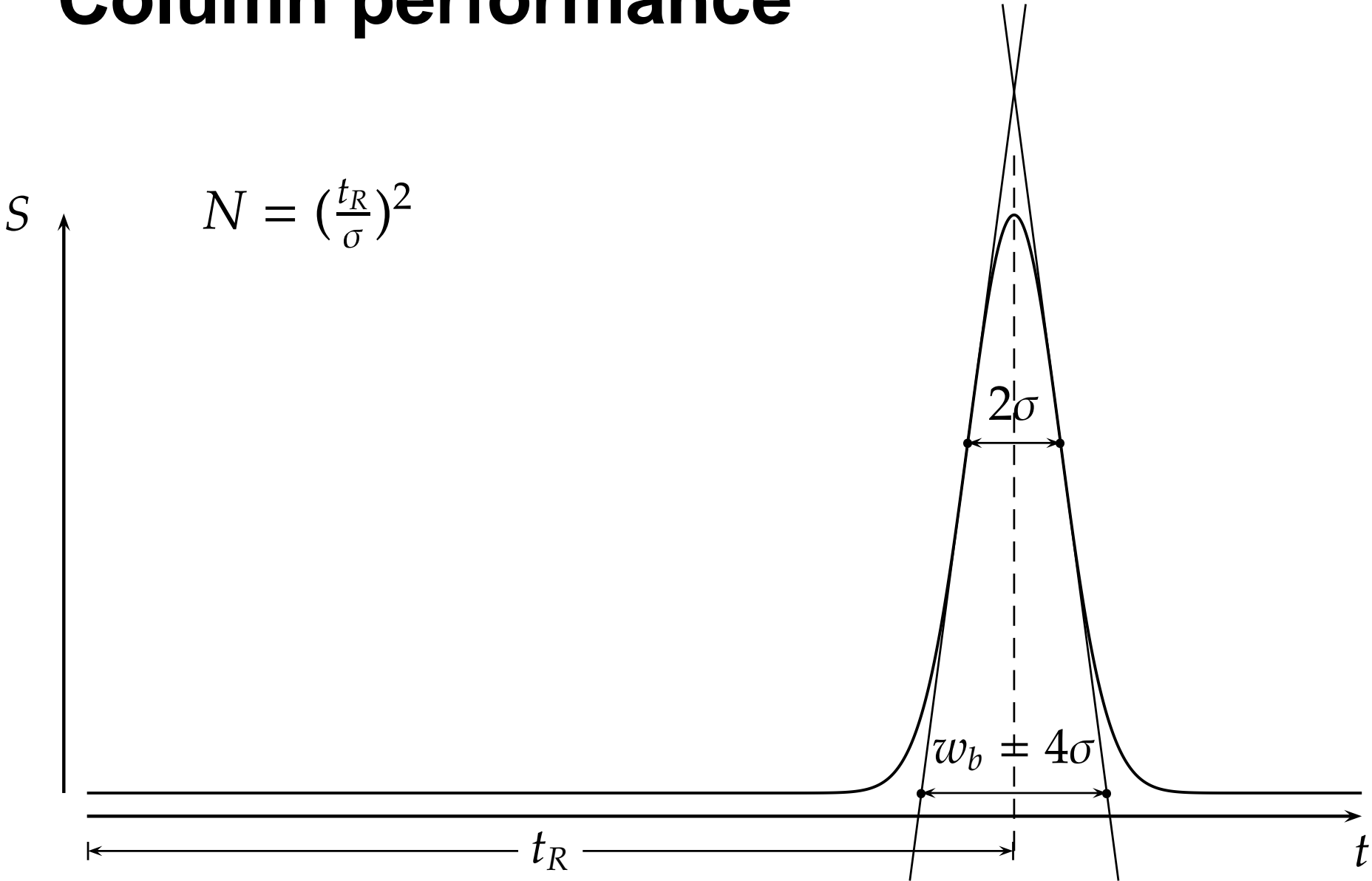


<http://www.chromatography-online.org/Dispersion/Van-Deemter-Equation/rs49.html>

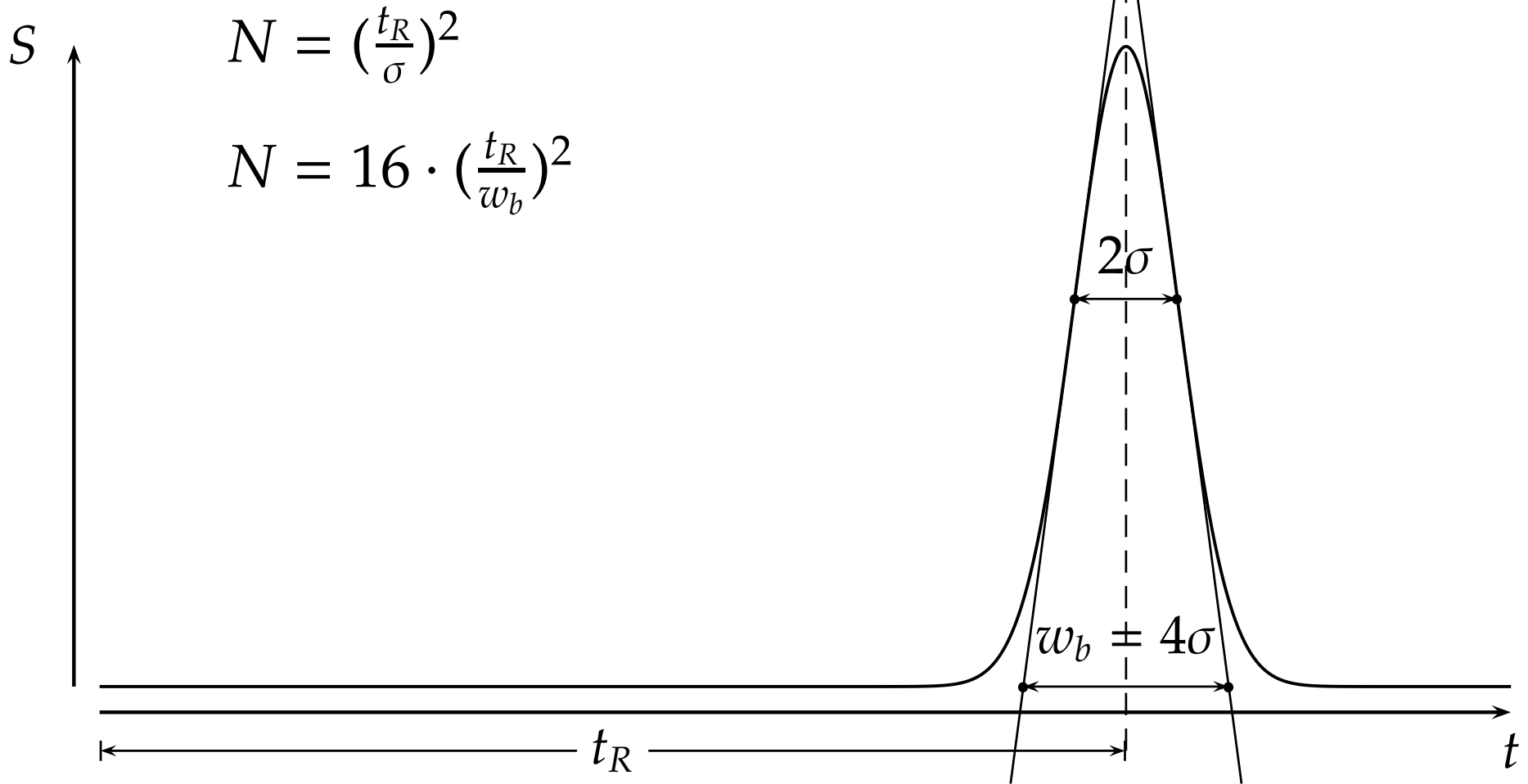
Column performance



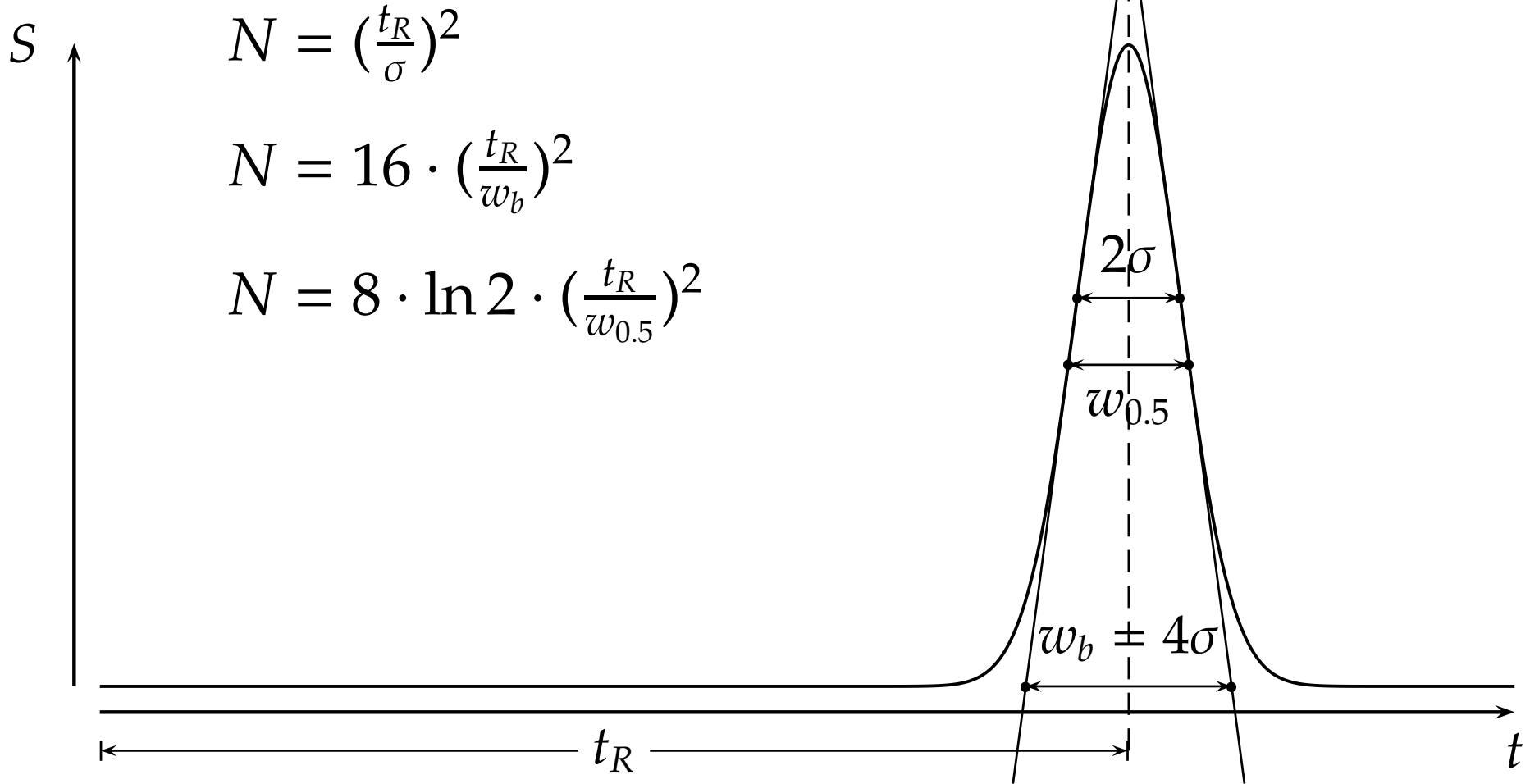
Column performance



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

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$$N_{\text{eff}} = 16 \cdot \left(\frac{t_R - t_0}{w_b}\right)^2$$

Column performance

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$$N_{\text{eff}} = 16 \cdot \left(\frac{t_R - t_0}{w_b}\right)^2$$

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

H is the Height Equivalent of a Theoretical Plate (HETP).

Column performance

	H [mm]	\dot{N} [s ⁻¹]	n_{sample}
TLC	0.7	1	$\mu\text{g} - \text{mg}$
HPTLC	0.02	50	20 ng - mg
Column	20	0.0005	mg - kg
HPLC	0.01	400	ng - mg
packed GC	0.5	100	$\mu\text{g} - \text{g}$
capillary GC	0.25	400	pg - μg

Column performance

$$\text{Resolution } R_S = \frac{t_{R,1} - t_{R,2}}{0.5 \cdot (w_{b,1} + w_{b,2})}$$

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$$\text{Separation factor } \alpha = \frac{k'_2}{k'_1} = \frac{t_{R,2} - t_0}{t_{R,1} - t_0} = \frac{K_2}{K_1}$$

Column performance

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$$\text{Separation factor } \alpha = \frac{k'_2}{k'_1} = \frac{t_{R,2} - t_0}{t_{R,1} - t_0} = \frac{K_2}{K_1}$$

$$R_S = \frac{\sqrt{N_{\text{eff}}}}{4} \frac{\alpha - 1}{\alpha}$$