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Liquid Chromatography: Separation Mechanisms

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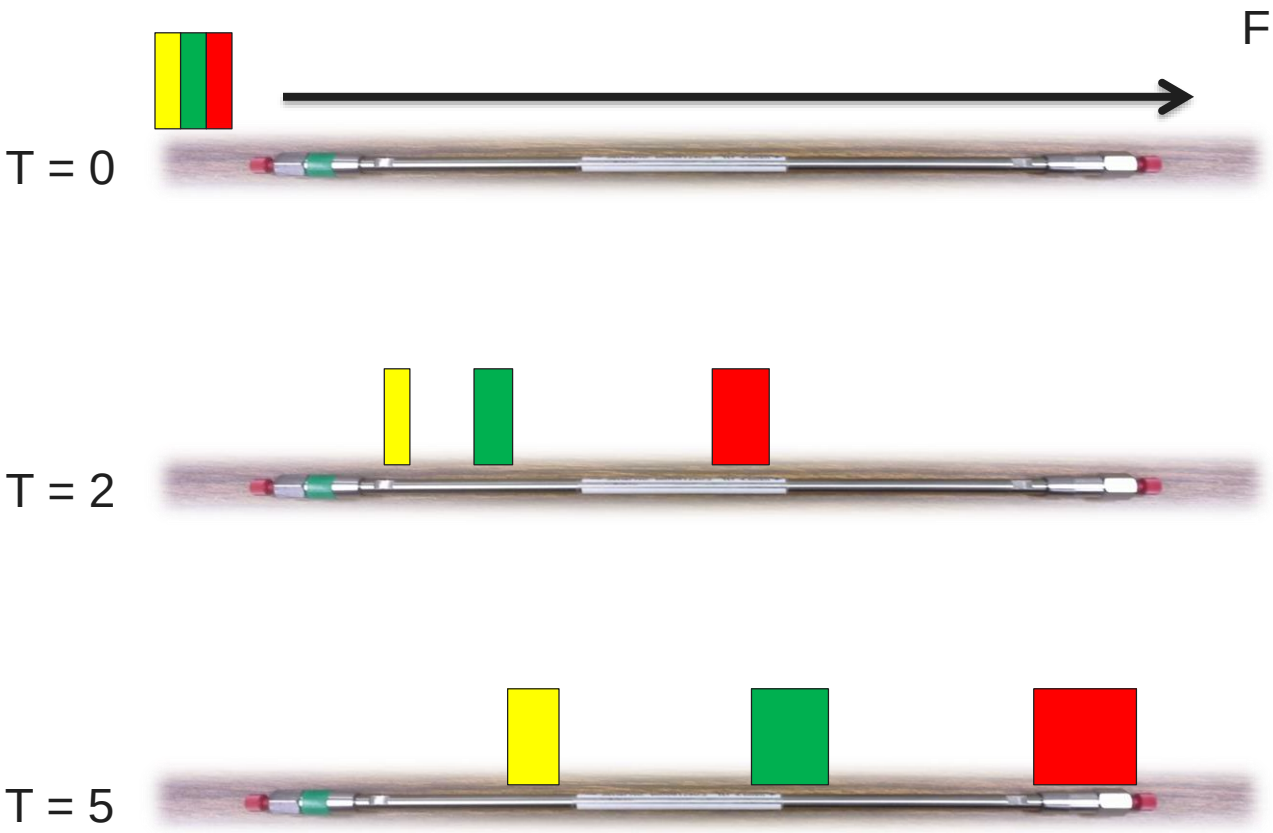
Outline

- LC Mechanisms
  - Overview
  - Mechanism: Reverse Phase
  - Isocratic vs. Gradient Separations

- LC Separation Parameters
  - Retention Time, t_R
  - Resolution, R_s
  - Efficiency, N
  - Retention Factor (Capacity), κ
  - Selectivity, α

- AND – learn to “think like a molecule!”

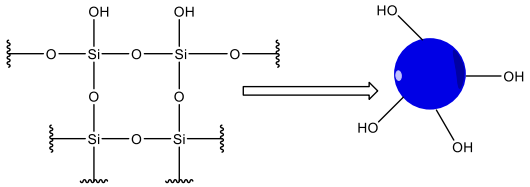
Mechanism: Overview



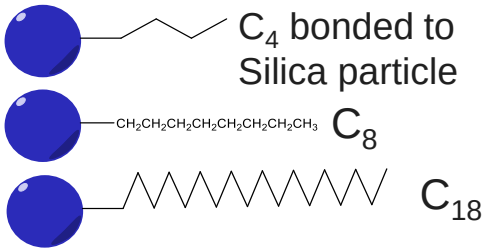
The analytes (RGY) partition between the mobile phase and the stationary phase

Reverse Phase Overview

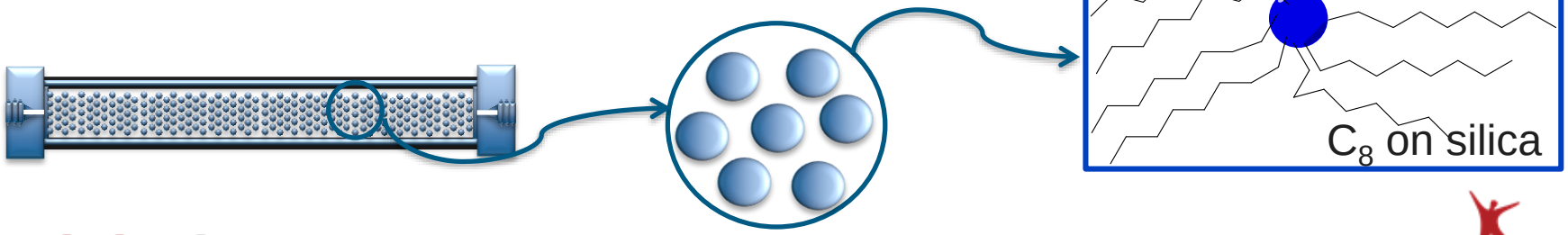
Packing materials are based on silica gel, a network polymer with –OH groups on the surface



Hydrocarbon chains of various lengths are bonded to the silica gel – different lengths give different retention properties

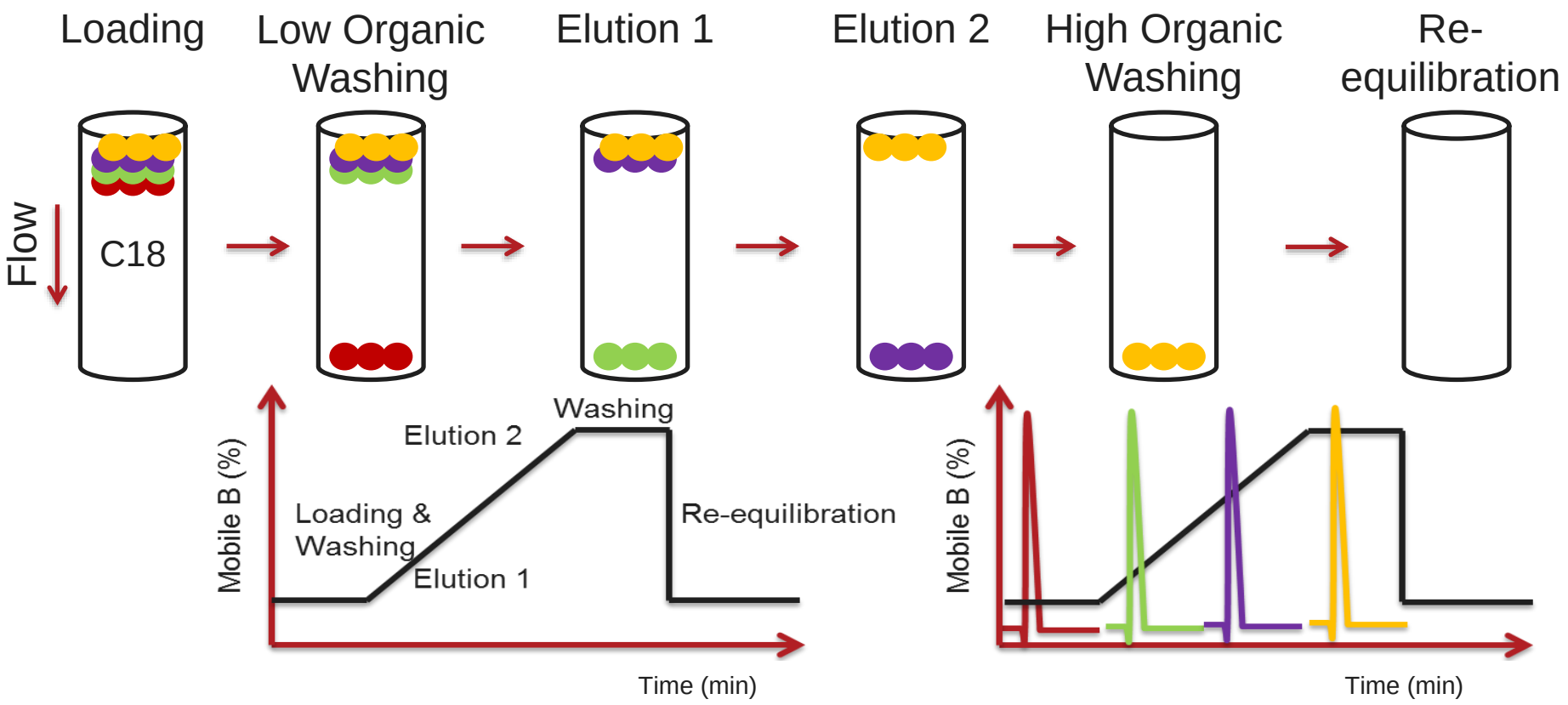


Any one silica gel particle has many, many hydrocarbon chains bonded to it

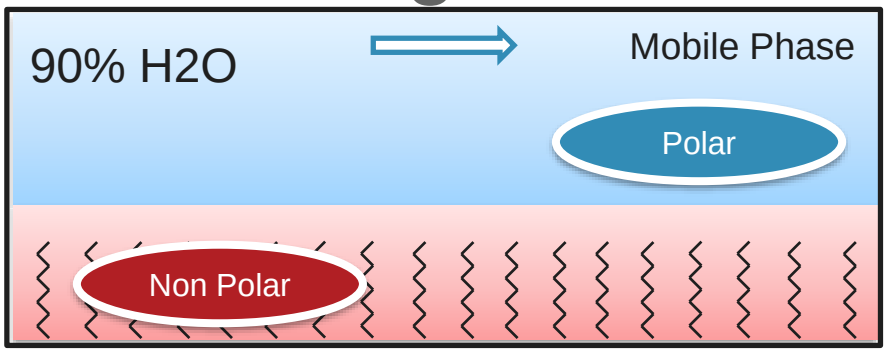


Mechanism of Reverse Phase

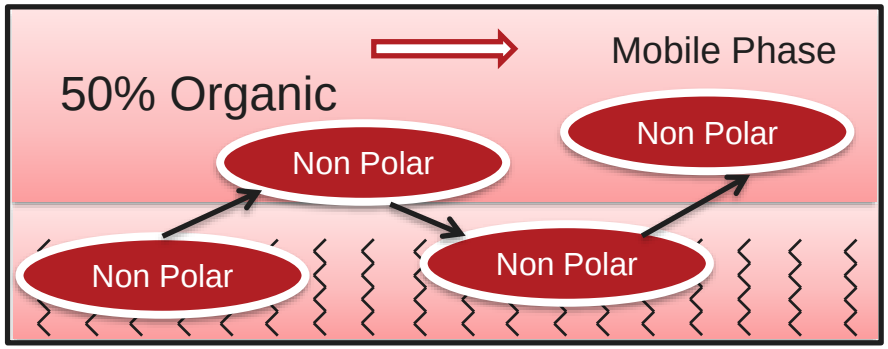
Stationary Phase: C18 bounded silica | Mobile Phase: Organic Hydrophobic Compound



Partitioning

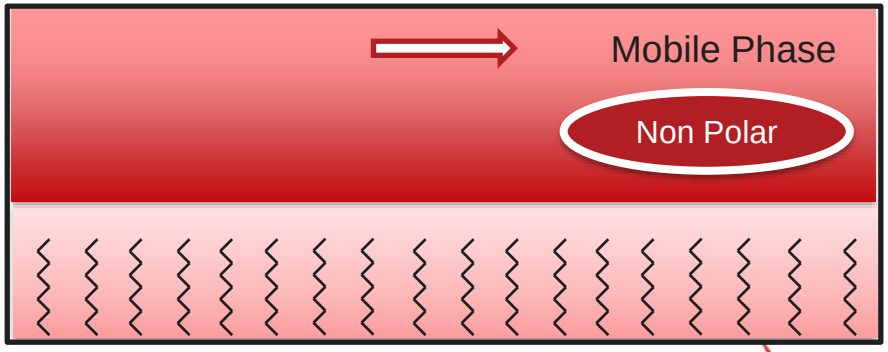


Retention

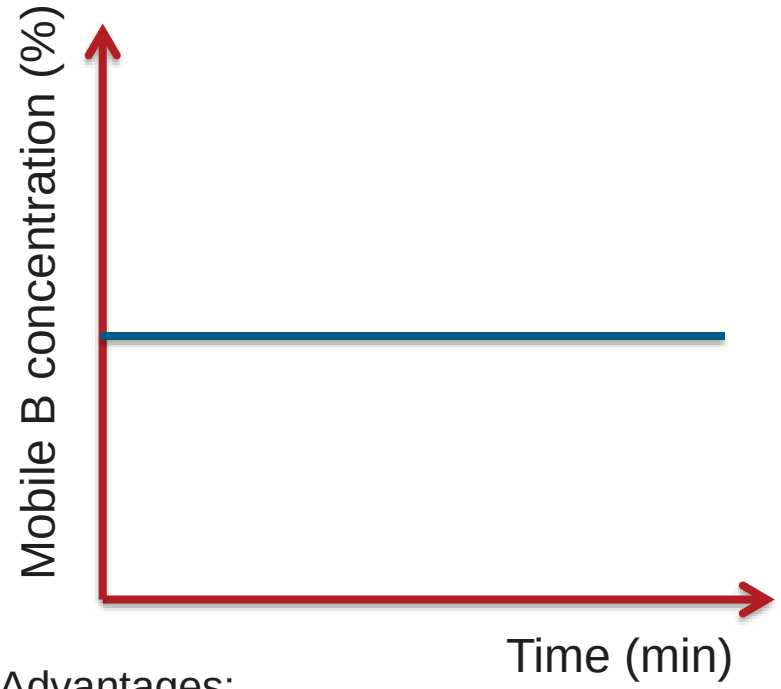


Partitioning

Elution

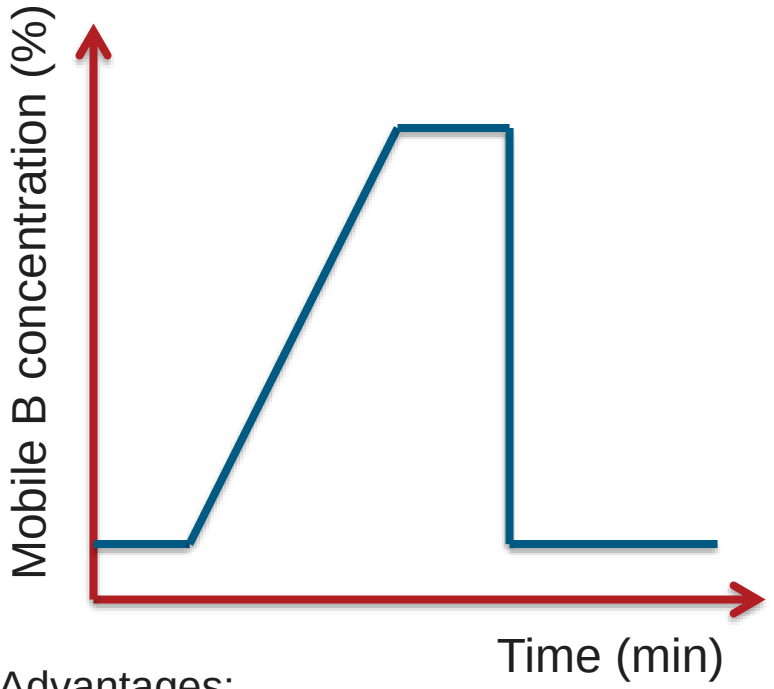


Isocratic vs. Gradient Separation



Advantages:
- Simple – one bottle

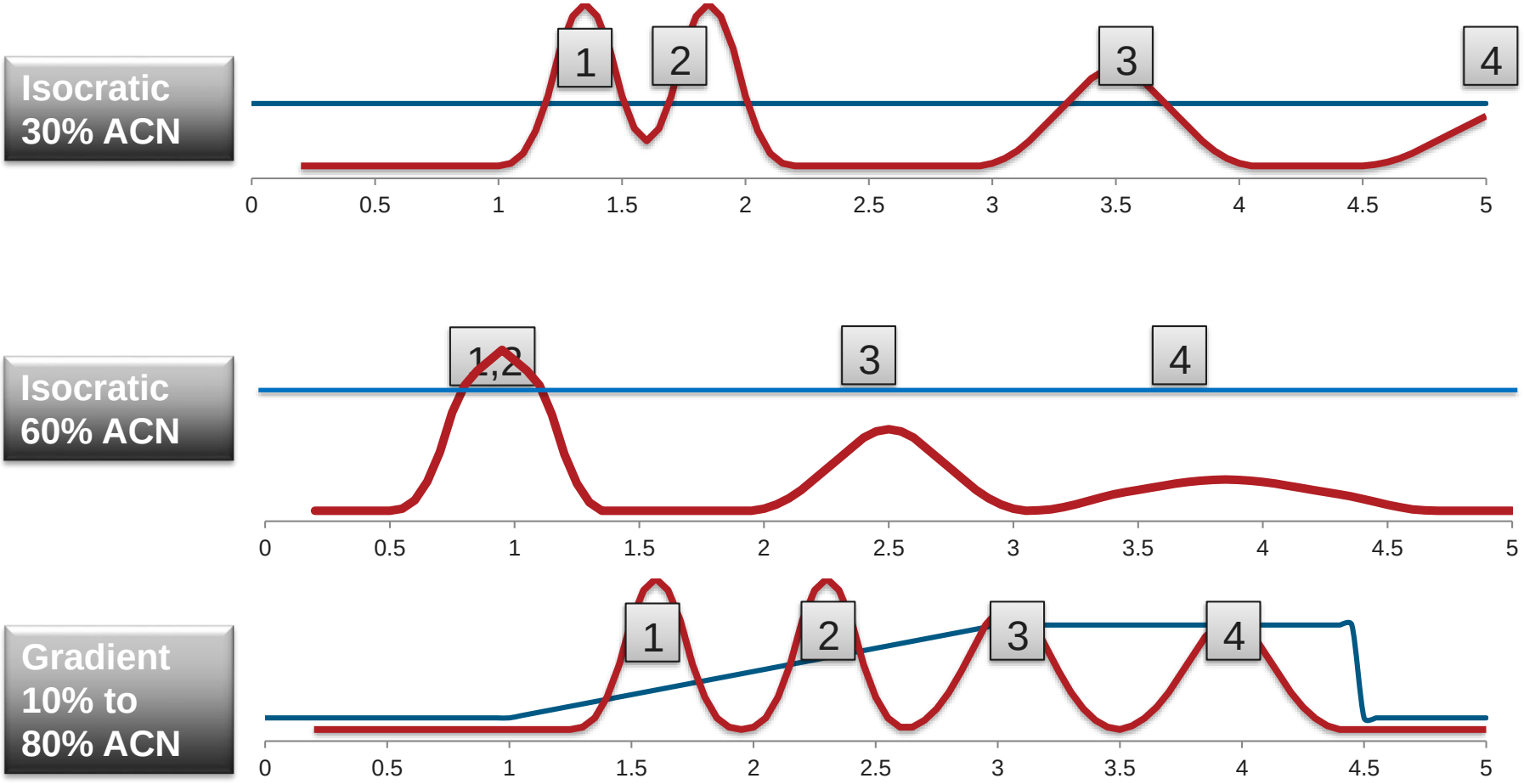
Disadvantages:
- Peak spreading
- Some compounds may not be eluted



Advantages:
- Higher resolution
- Larger range of components eluted

Disadvantages:
- Programing more complicated
- Column needs recovery time

Isocratic vs. Gradient Separation



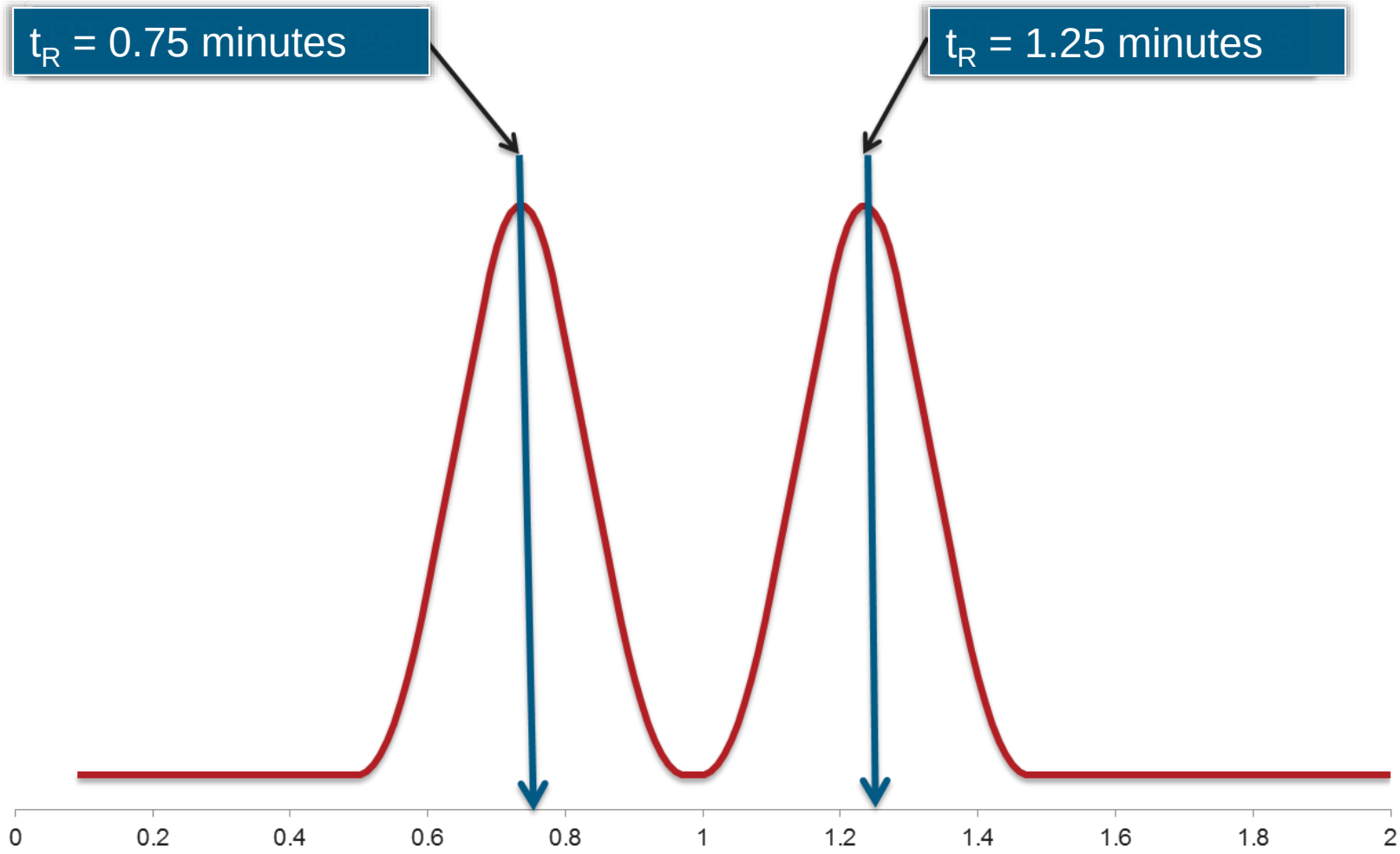
The gradient is reset to initial conditions to re-equilibrate post-analysis

Chromatographic Parameters

- Basic concepts
  - Retention time, t_R
  - Resolution, R_s

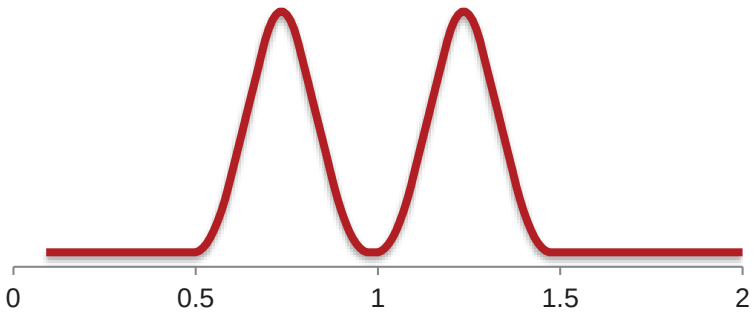
- Column variables that impact separation
  - Efficiency, N
  - Retention (Capacity), κ
  - Selectivity, α

Retention Time, t_R

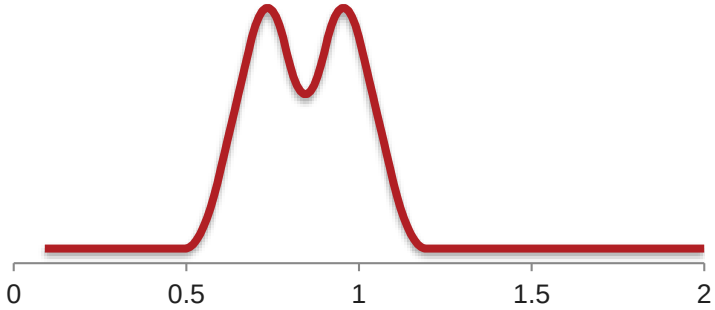


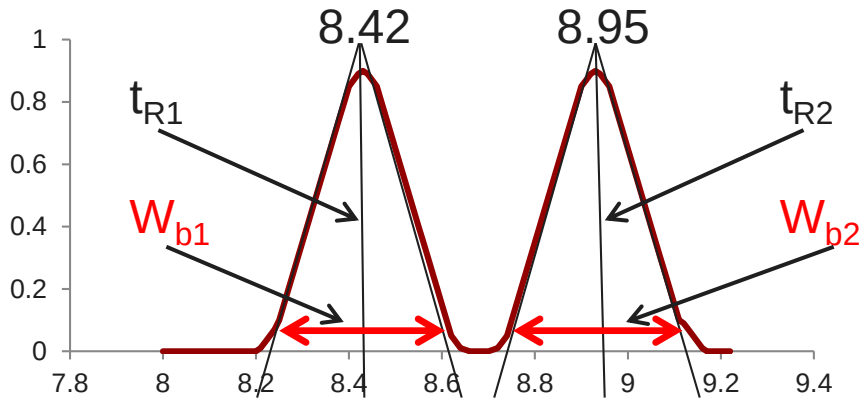
Resolution, R_s

Complete Resolution



Incomplete Resolution





Wb1 = Wb2 = 0.33

$$Rs = \frac{(t_{R2} - t_{R1})}{(W_{b1} + W_{b2})/2} = \frac{2(t_{R2} - t_{R1})}{W_{b1} + W_{b2}}$$

$$\cong \frac{2(t_{R2} - t_{R1})}{2W_{b2}}$$

W = the width of a peak

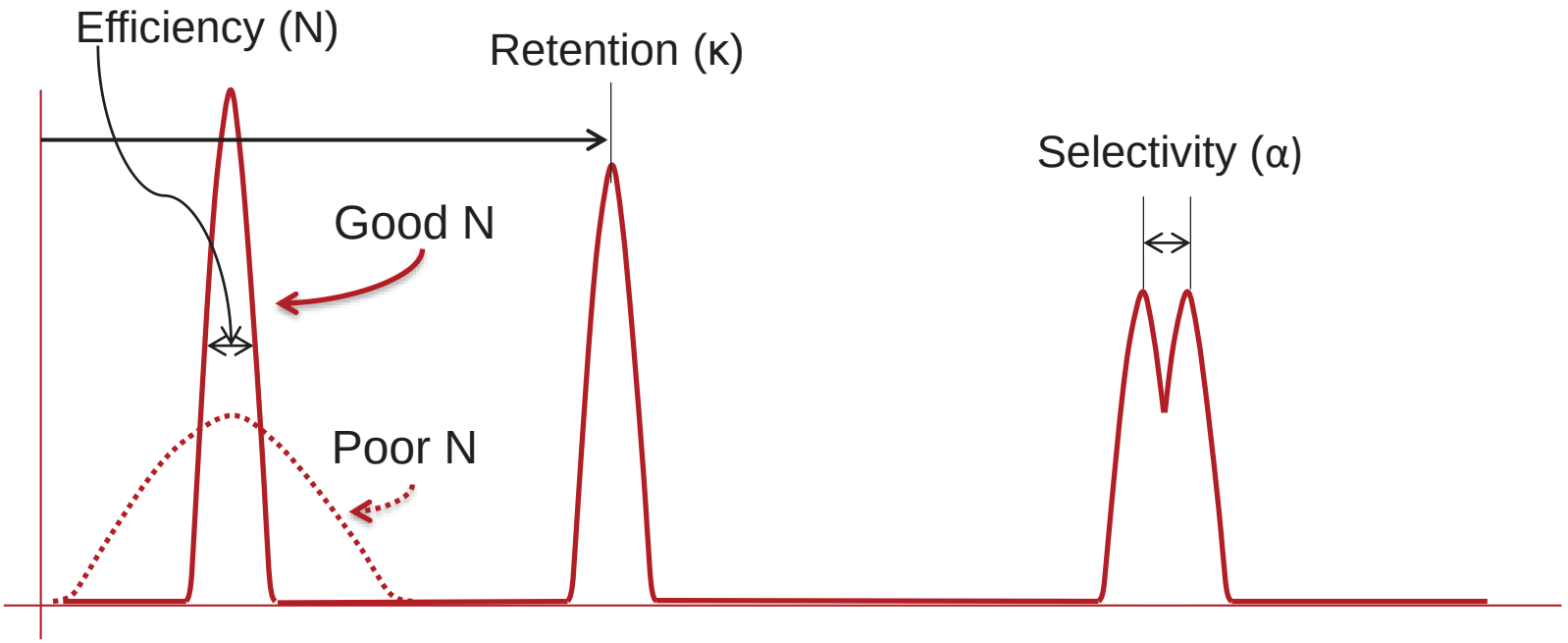
$R_s \geq 1.5 \rightarrow$ two peaks are baseline resolved; the signal returns to baseline before the response for the second analyte starts

$$Rs \cong \frac{2(8.95 - 8.42)}{2 * 0.33} = \frac{2 * 0.53}{0.66} \cong 1.6$$

Three Major Chromatogram Factors Impacting Resolution, R_s

$$R_s = \frac{1}{4}\sqrt{N} \times \frac{k}{1+k} \times \frac{\alpha - 1}{\alpha}$$

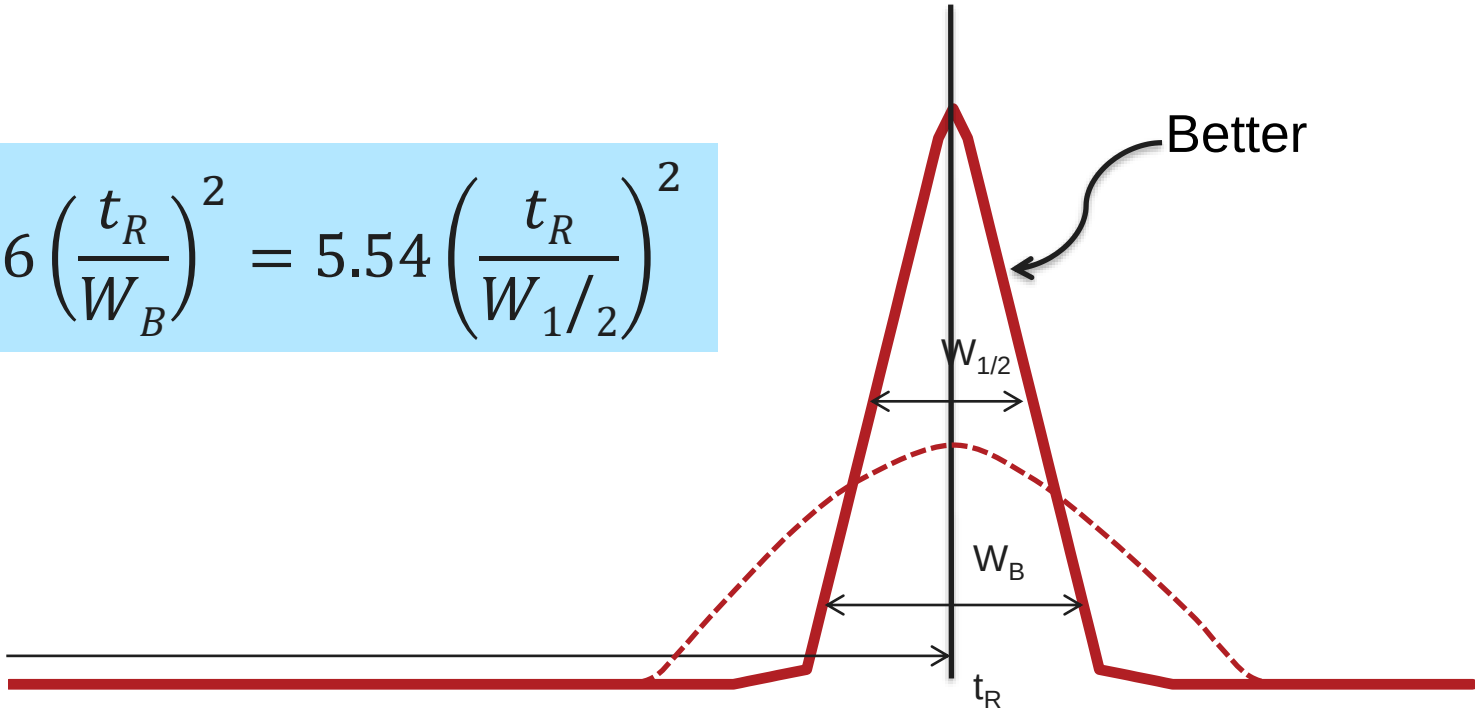
Efficiency Retention Selectivity



Efficiency, N

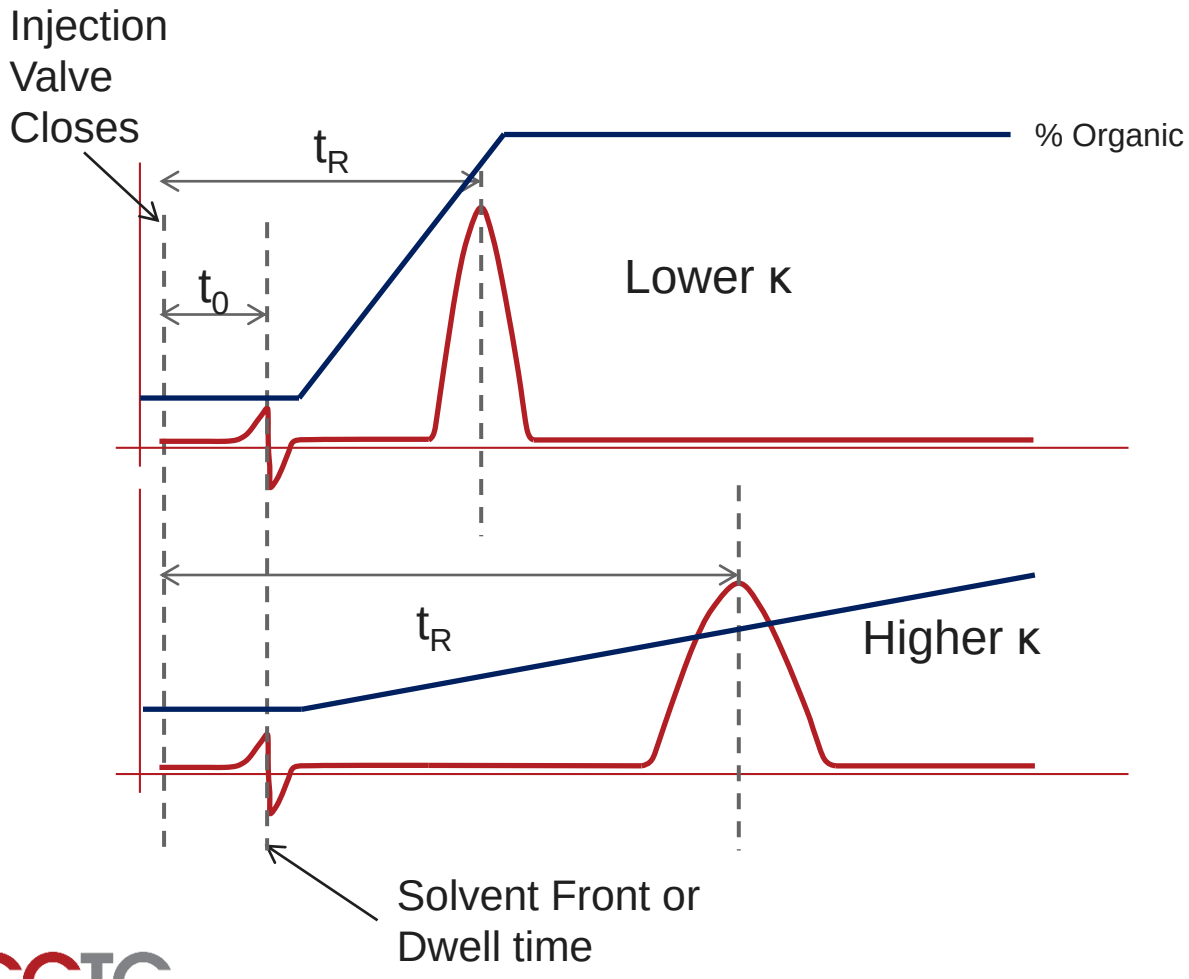
The ability of a column to produce narrow peaks

$$N = 16\left(\frac{t_R}{W_B}\right)^2 = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2$$



Determination of Retention Factor (Capacity), κ

How well any one analyte is retained

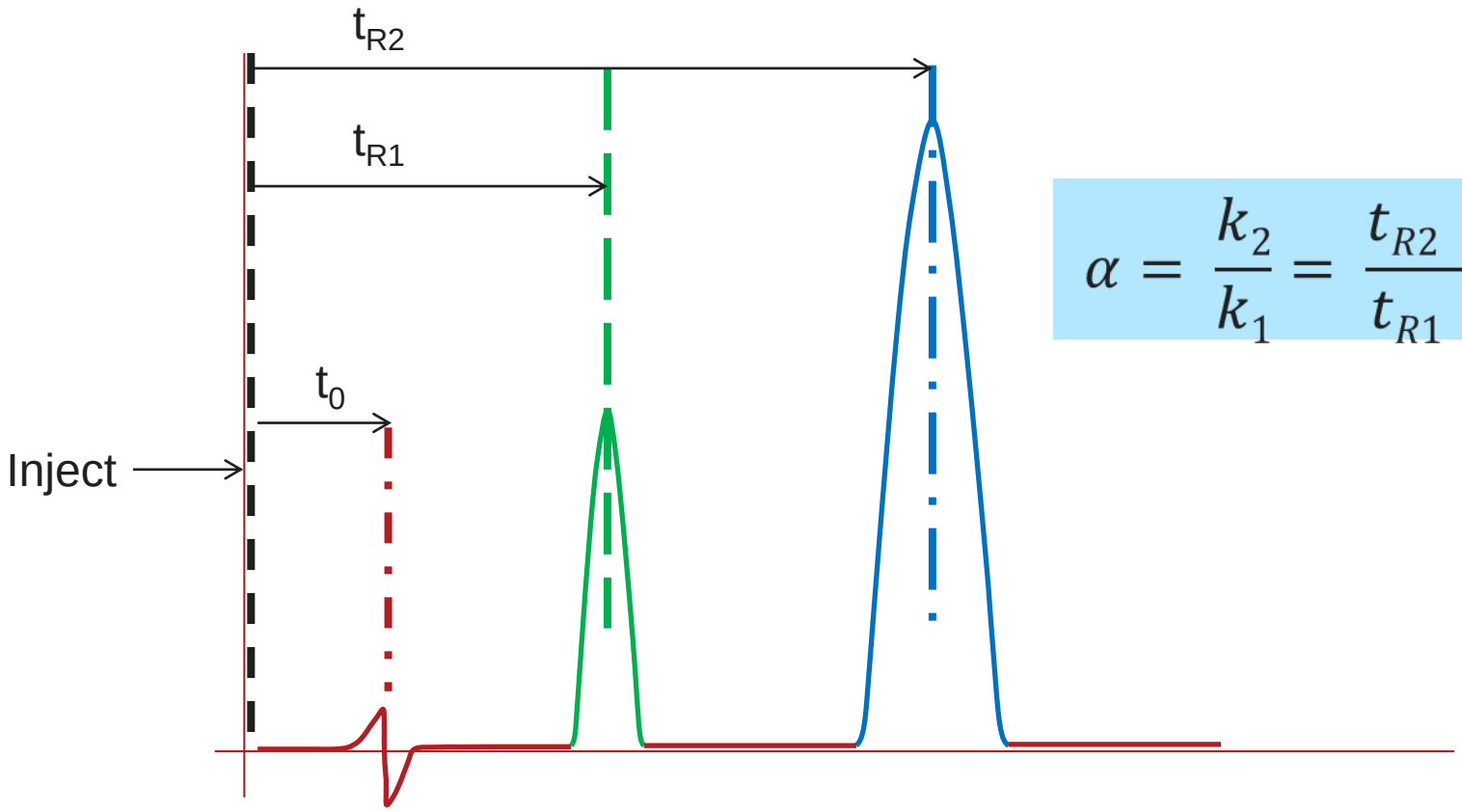


$$\kappa = \frac{t_R - t_0}{t_0}$$

The blue line illustrates the gradient profile in a reverse phase separation showing the difference in retention time for the ***same analyte*** resulting from a different gradient.

Selectivity (Separation) Factor, α

The ability to distinguish between species being separated



$$\alpha = \frac{k_2}{k_1} = \frac{t_{R2} - t_0}{t_{R1} - t_0}$$

Parameter Summary

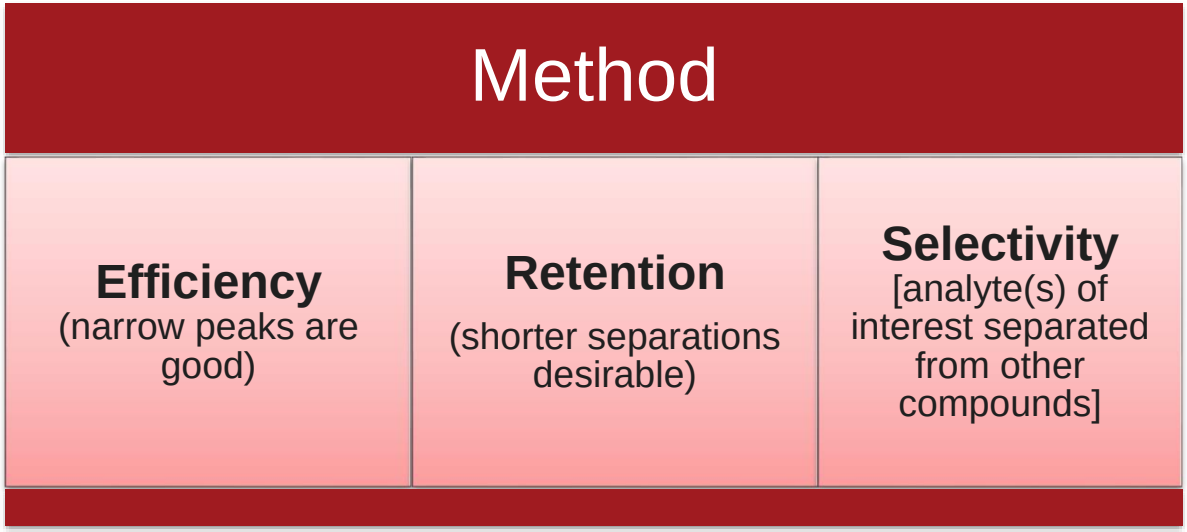
Parameter	N (Efficiency)	κ (Capacity)	α (Selectivity)
% Organic Solvent	●	↑↑	↑
Organic Solvent Choice	●	↑	↑↑
Column Type	●	↑	↑↑
Column Length	↑↑	●	●
Particle Size	↑↑	●	●
Mobile Phase pH *	↑	↑↑	↑↑
Buffer Concentration *	●	↑	↑
Ion-pair Reagent Concentration *	↑	↑↑	↑↑
Flow Rate	↑	●	●

↑↑ Large effect ↑ Small effect ● Little to no effect

Dark arrows indicate parameters commonly used to control N, κ or α.
Light arrows indicate parameters not commonly used for control
* Most applicable to acidic or basic analytes

Chromatography Method Development

- Chromatography method development is a balance between efficiency, retention, and selectivity
- Goal: obtain an adequate separation within the desired timeframe



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Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

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