Chromatographic separations

Athena Kasselouri
Université Paris-Saclay

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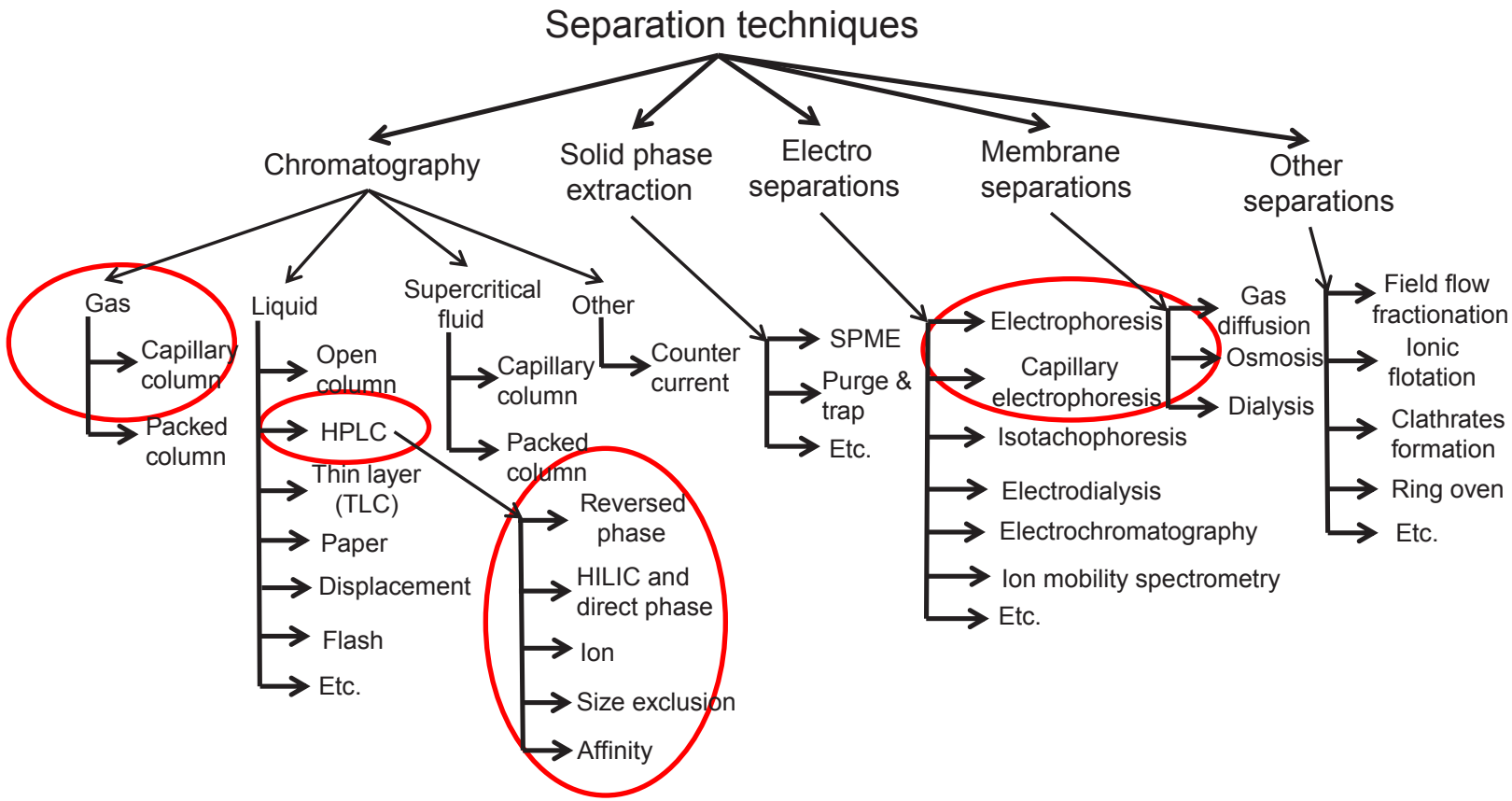
Analytical Techniques
containing a Separation Step



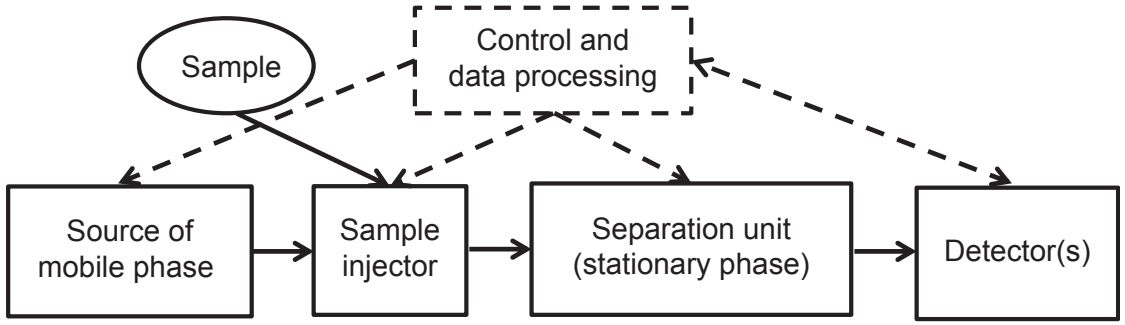
S. C. Moldoveanu, V. David, « Selection of the HPLC method in chemical analysis » Chap. 3, Elsevier Inc, 2017.

Analytical Techniques

containing a Separation Step

- Separation technique + detection
- <u>Not included :</u> distillation, sublimation, crystallization, precipitation

- Components of an analytical chromatographic instrument.

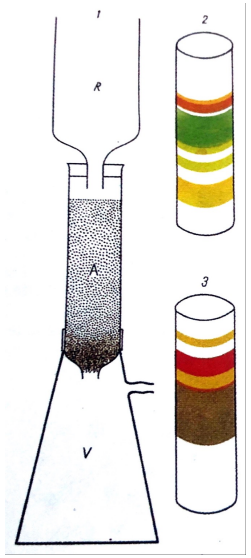


Introduction to chromatographic Separations

High Performance Liquid Chromatography
HPLC

- Half century of history …

History



Chromatography: χρώμα (colour) + γράφειν (writing)

1900: Russian botanist Mikhail Tswet invents chromatography to separate plant pigments

1941: Martin and Synge predict efficient separations for high pressure and small particle size

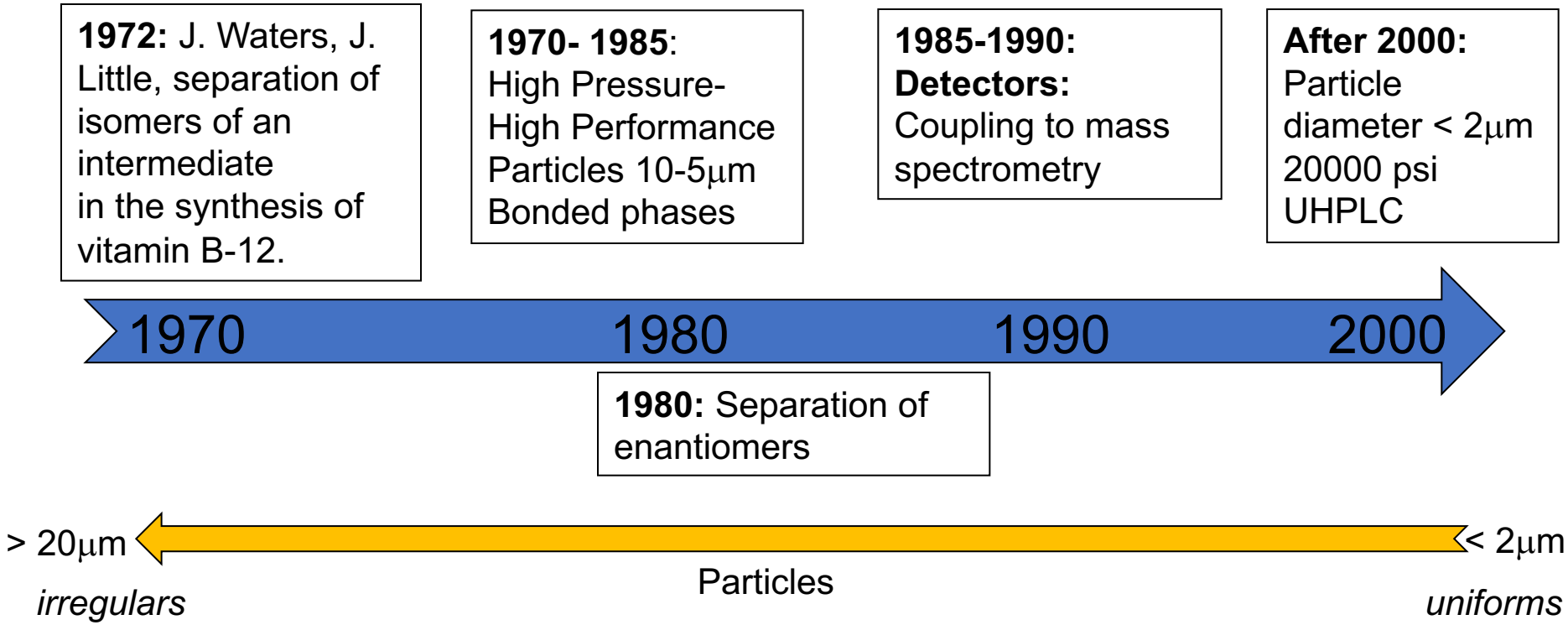
1966: C.Horváth and S. Lipsky published nucleotides separation by HPLC

1967: First commercial HPLC instrument (Waters)

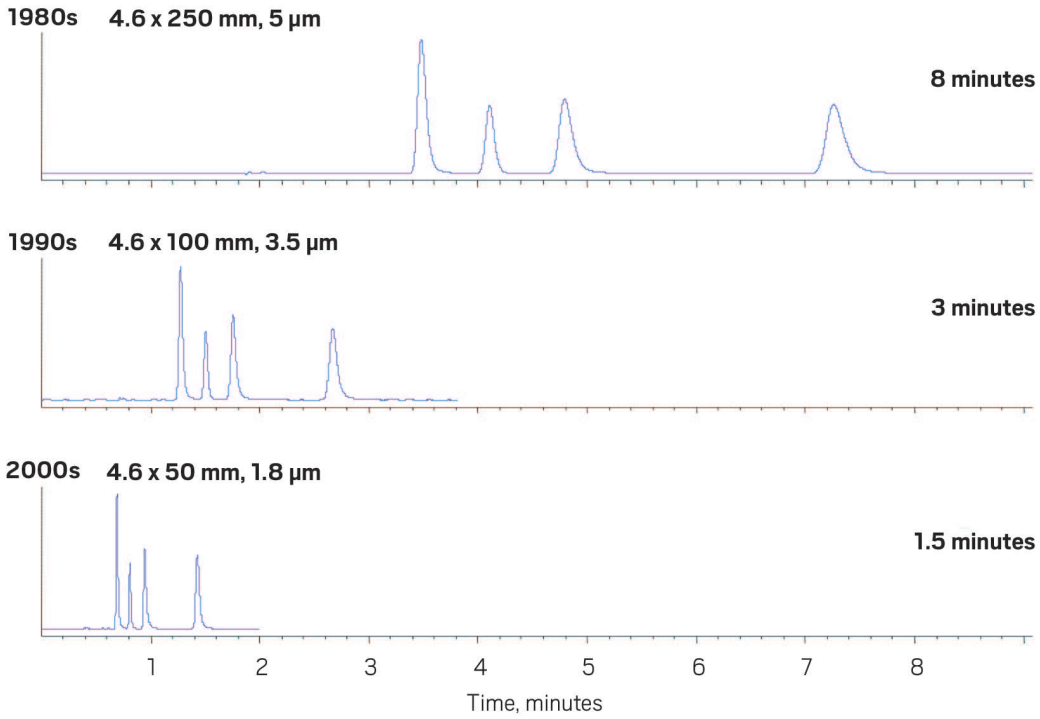
1900 1940 1960

History

HPLC: evolution rather than revolution



History



UHPLC

Compounds: 1-methylxanthine, 1,3-dimethyluric acid, 3,7 dimethylxanthine, and 1,7-dimethylxanthine. (in order of elution, left to right)

C. H. Arnaud, C&EN, CEN.ACS.ORG, 2016, p. 29-33

General description

- Classification

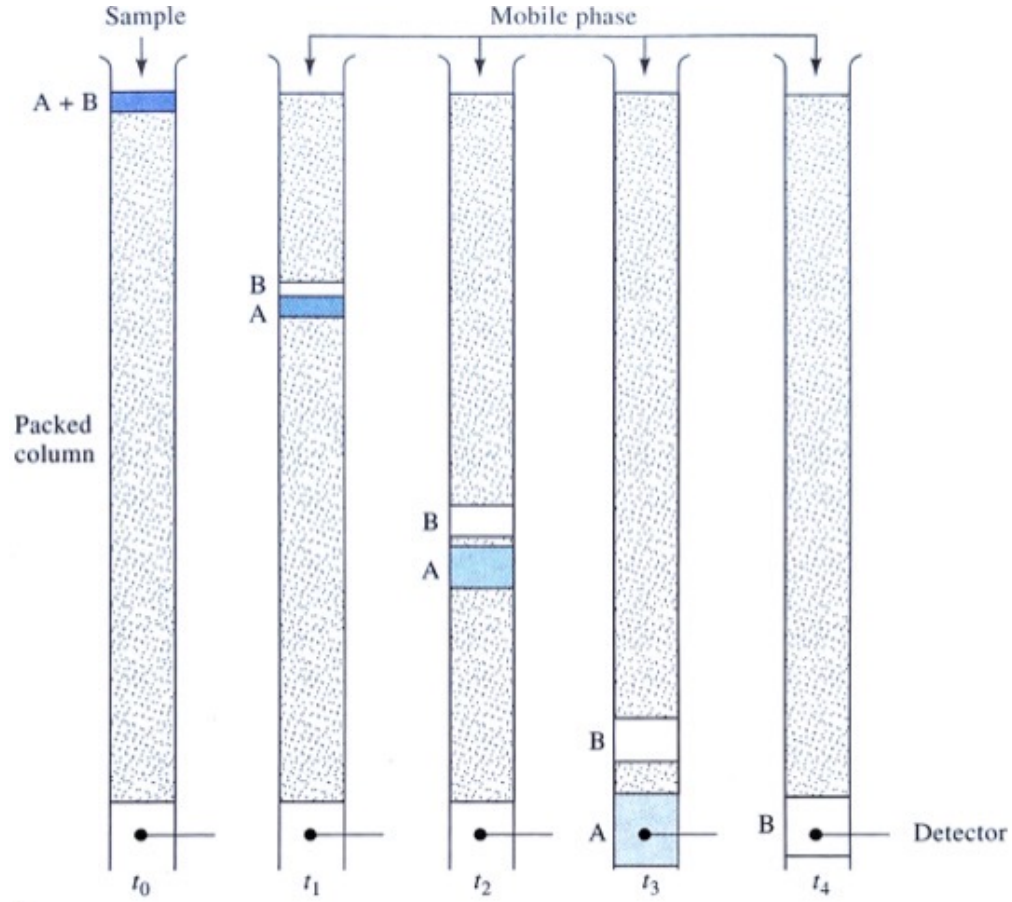
Disposition

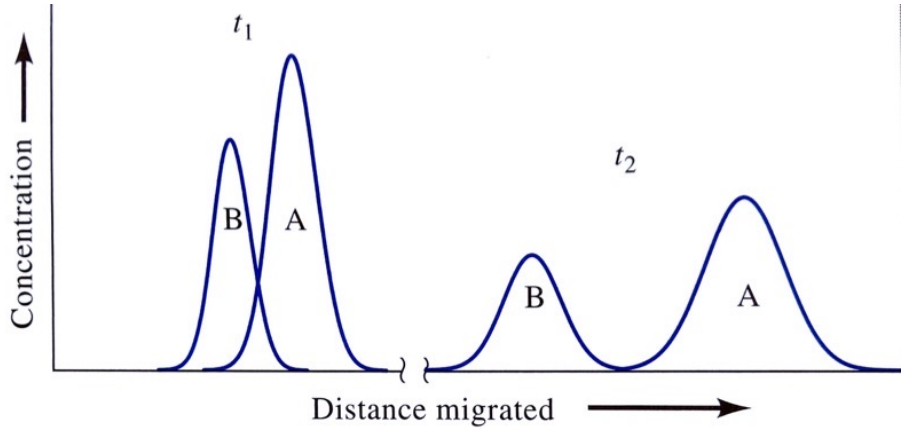
- Column chromatography
- Planar chromatography

Mobile phase

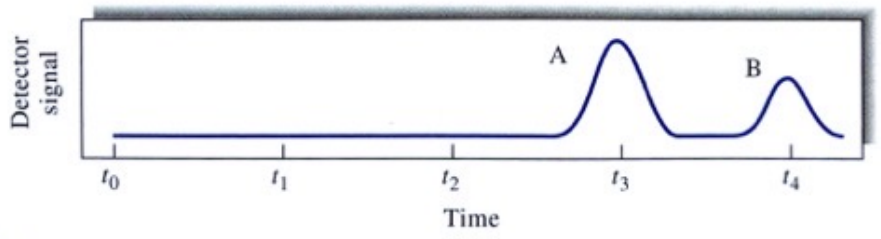
- Gaz chromatography
- Liquid chromatography
- Supercritical fluid chromatography

- Elution in Column Chromatography



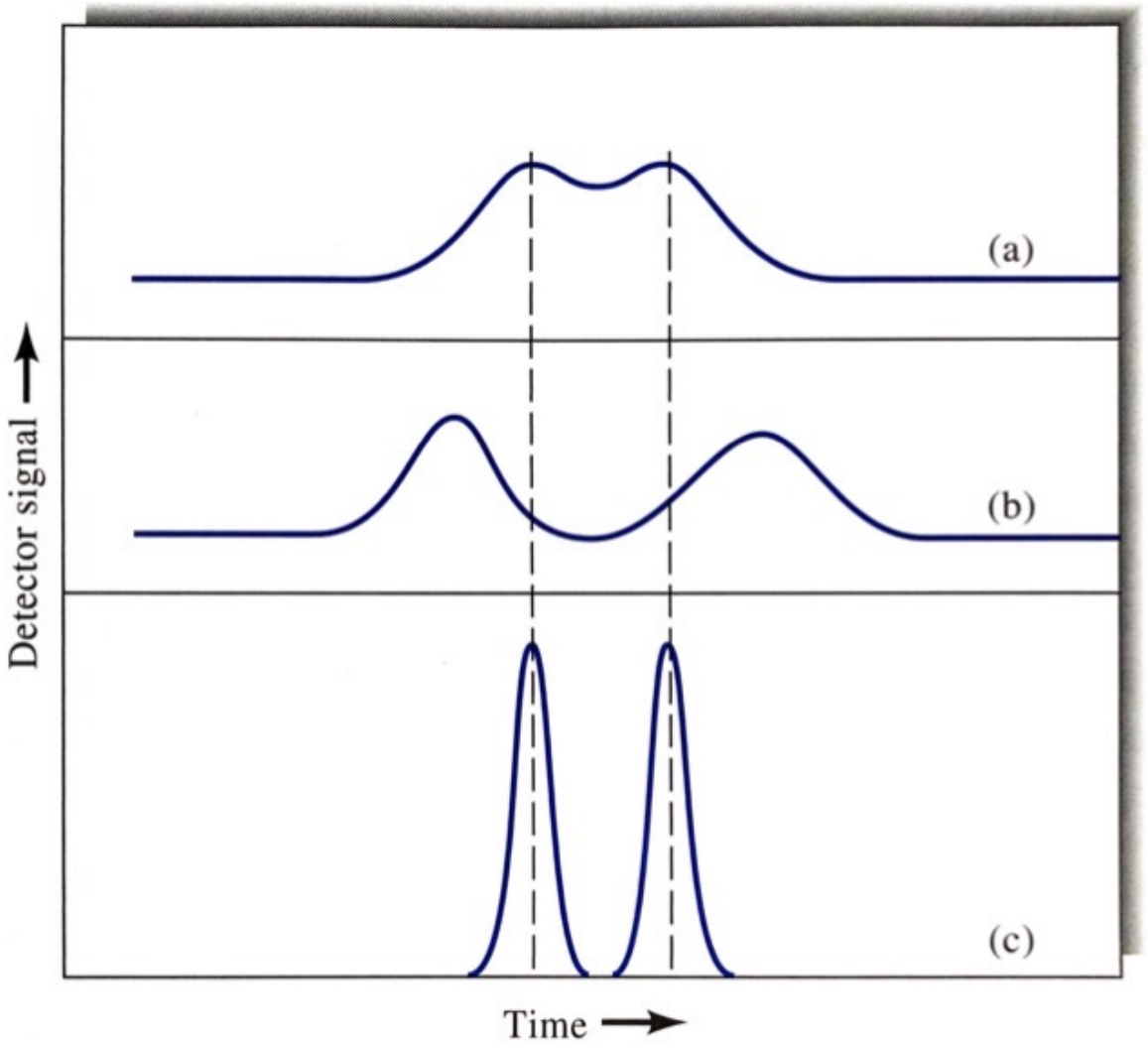


Solute dilution



Chromatogram

- Improving separation
  - Increase band separation
  - Decrease band width



D. A. Skoog, E. J. Holler, S.R. Crouch, « Principles of Instrumental Analysis », Chap. 26, 2017, Cengage Learning.

Migration rates of solutes

- Distribution constant K_A (or partition coefficient) of a solute A

$A_{mobile} \rightleftharpoons A_{stationary}$

$$K_A = \frac{(\alpha_A)_S}{(\alpha_A)_M} \Rightarrow K_A = \frac{C_S}{C_M} = \frac{n_S/V_S}{n_M/V_M}$$

Linear chromatography

Activity coefficient nearly unity

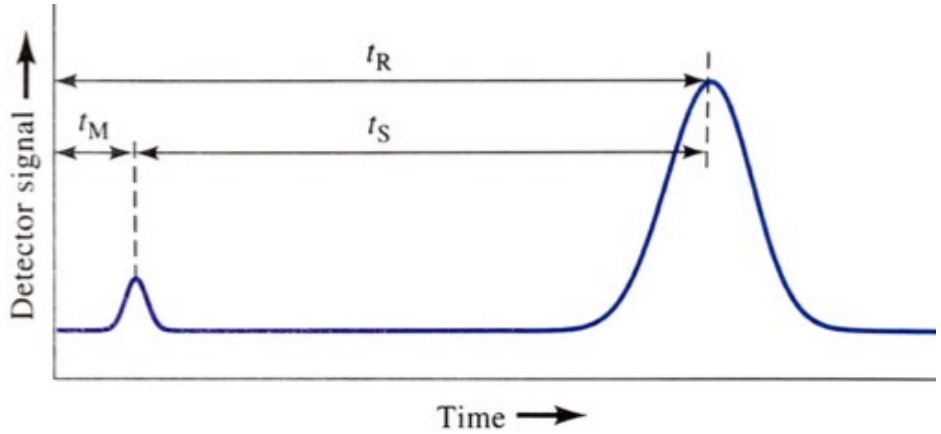
- C_S Concentration of A in the stationary phase
- C_M Concentration of A in the mobile phase
- n_S moles of A in the stationary phase
- n_M moles of A in the mobile phase
- V_S volume of stationary phase
- V_M volume of mobile phase

Migration rates of solutes

- Retention time t_R

$$t_R = t_S + t_M$$

  - t_M void or dead time (for unretained species)
  - t_S time spend by solute in the stationary phase



- Migration rates

$$\bar{v} = \frac{L}{t_R} \qquad u = \frac{L}{t_M}$$

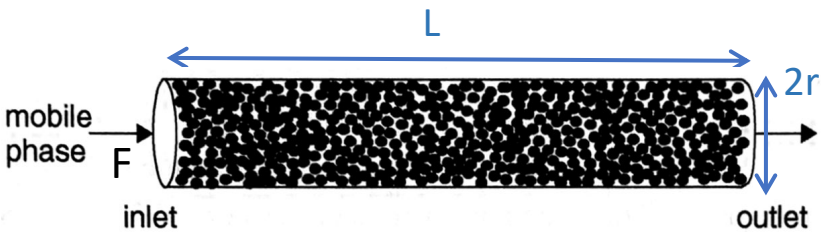
Solute Mobile phase

  - $\bar{v}$ rate of migration (cm/s) of the solute
  - u linear velocity of the mobile phase

$$\bar{v} = u \times \text{solute fraction of time at the mobile phase} \Rightarrow \bar{v} = u \times \frac{n_M}{n_M + n_S}$$

$$\Rightarrow \bar{v} = u \times \frac{C_M V_M}{C_M V_M + C_S V_S} = u \times \frac{1}{1 + (C_S V_S)/(C_M V_M)} = u \times \frac{1}{1 + K_A (V_S/V_M)}$$

Migration rates of solutes



$$\varepsilon = \frac{V_M}{V_C}$$

- ε porosity of packed column
- V_C column total volume
- F flow rate
- A cross sectional area = $\pi\ r^2$

$$F = u\ A\ \varepsilon = u\ \pi\ r^2 \varepsilon$$

- Retention factor k_A

$$k_A = \frac{n_S}{n_M} = \frac{C_S\ V_S}{C_M V_M} = K_A\ \frac{V_S}{V_M}$$

$$\bar{v} = u \times \frac{1}{1 + k_A} \Rightarrow \frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k_A} \Rightarrow \boxed{k_A = \frac{t_R - t_M}{t_M}}$$

Migration rates of solutes

- Selectivity factor α

$$\alpha = \frac{K_B}{K_A}$$

- K_B distribution constant of the more retained solute B
- K_A distribution constant of the less retained solute A

$$\alpha = \frac{k_B}{k_A}$$

$$\alpha > 1$$

- k_B , k_A the retention factors for B and A

Band broadening and column efficiency

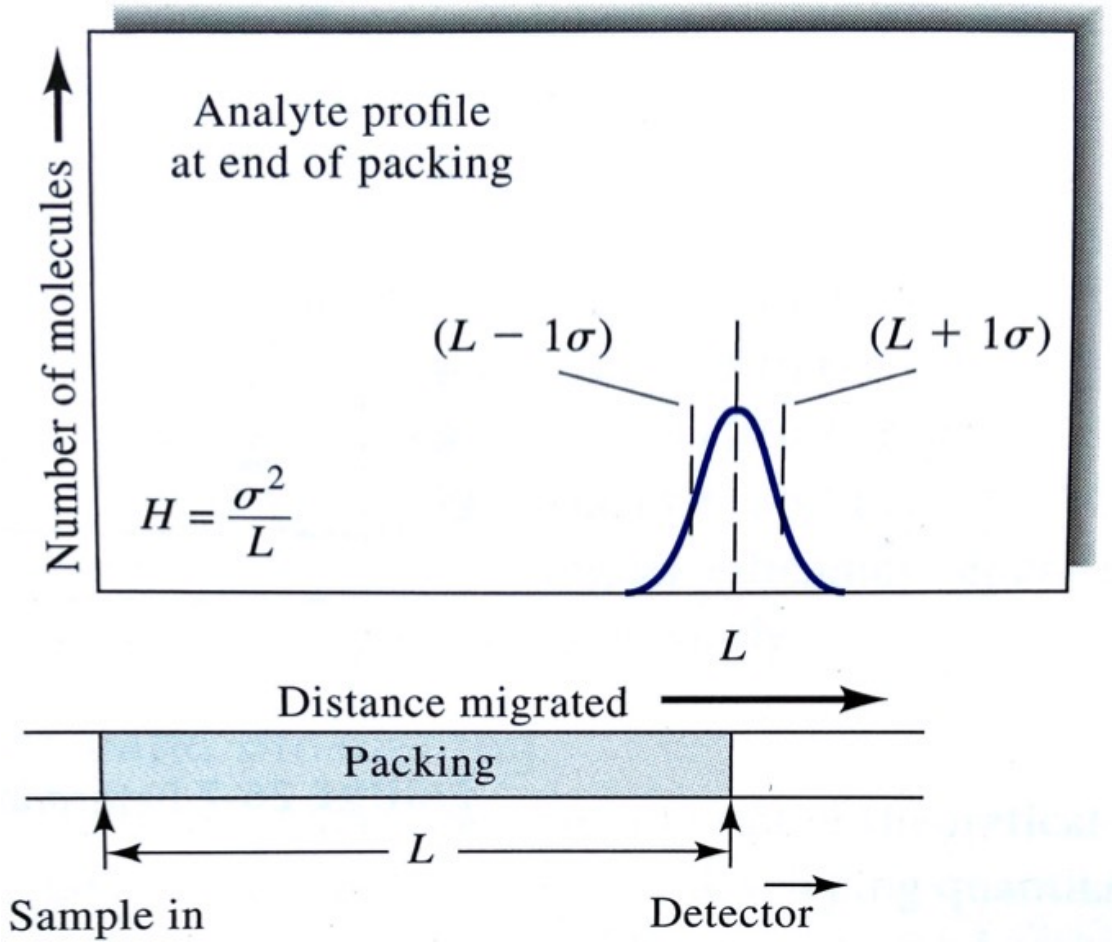
- Number of theoretical plates (N)

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

- Plate height (H)

$$H = \frac{\sigma^2}{L}$$

Plate theory

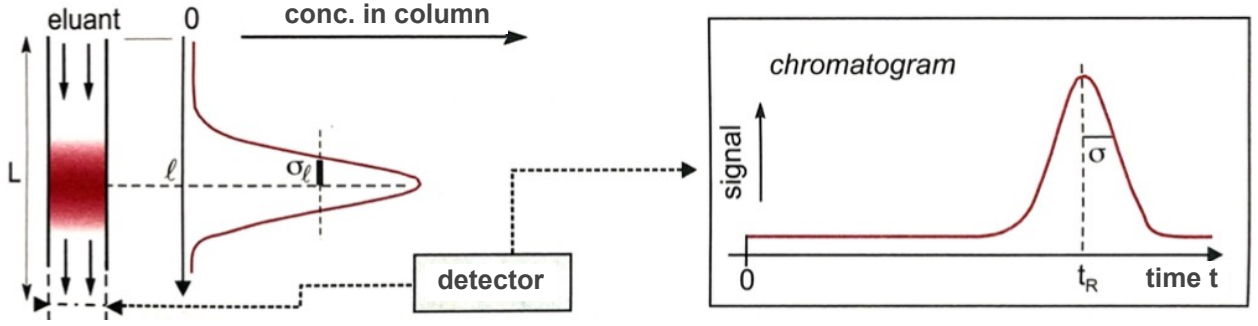


D. A. Skoog, E. J. Holler, S.R. Crouch, « Principles of Instrumental Analysis », Chap. 26, 2017, Cengage Learning.

- Experimental evaluation

$$\frac{\sigma_t}{t_R} = \frac{\sigma_L}{L} \Rightarrow \sigma_L = \frac{\sigma_t L}{t_R}$$

$$H = \frac{\sigma_t^2 L^2}{L\, t_R^2} = \frac{\sigma_t^2 L}{t_R^2} \Rightarrow N = \frac{L}{H} = \frac{t_R^2}{\sigma_t^2}$$



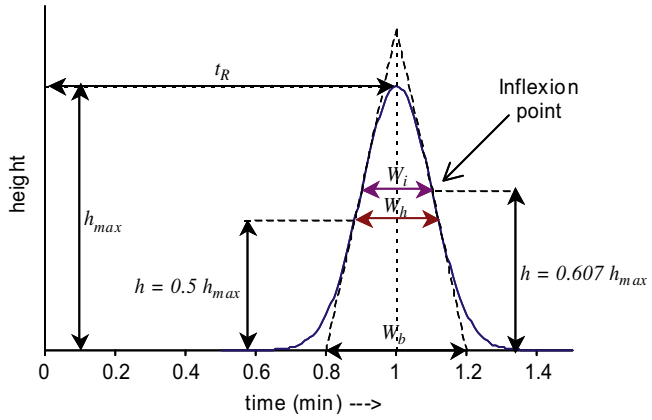
$$N = \left(\frac{t_R}{\sigma}\right)^2$$

$\sigma = 0{,}25\ W_b$

$\sigma = 0{,}425\ W_h$

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

$$N = 5{,}54\left(\frac{t_R}{w_h}\right)^2$$



Gaussian peak

F. Rouessac, A. Rouessac « Analyse chimique: Méthodes et techniques instrumentales », Dunod ed, 2019.

- Factors affecting band broadening

$$\sigma_T^2 = \sigma_C^2 + \sigma_{e.c}^2 = \sigma_C^2 + \sum_i \sigma_i^2$$

Variances: total column extra-column

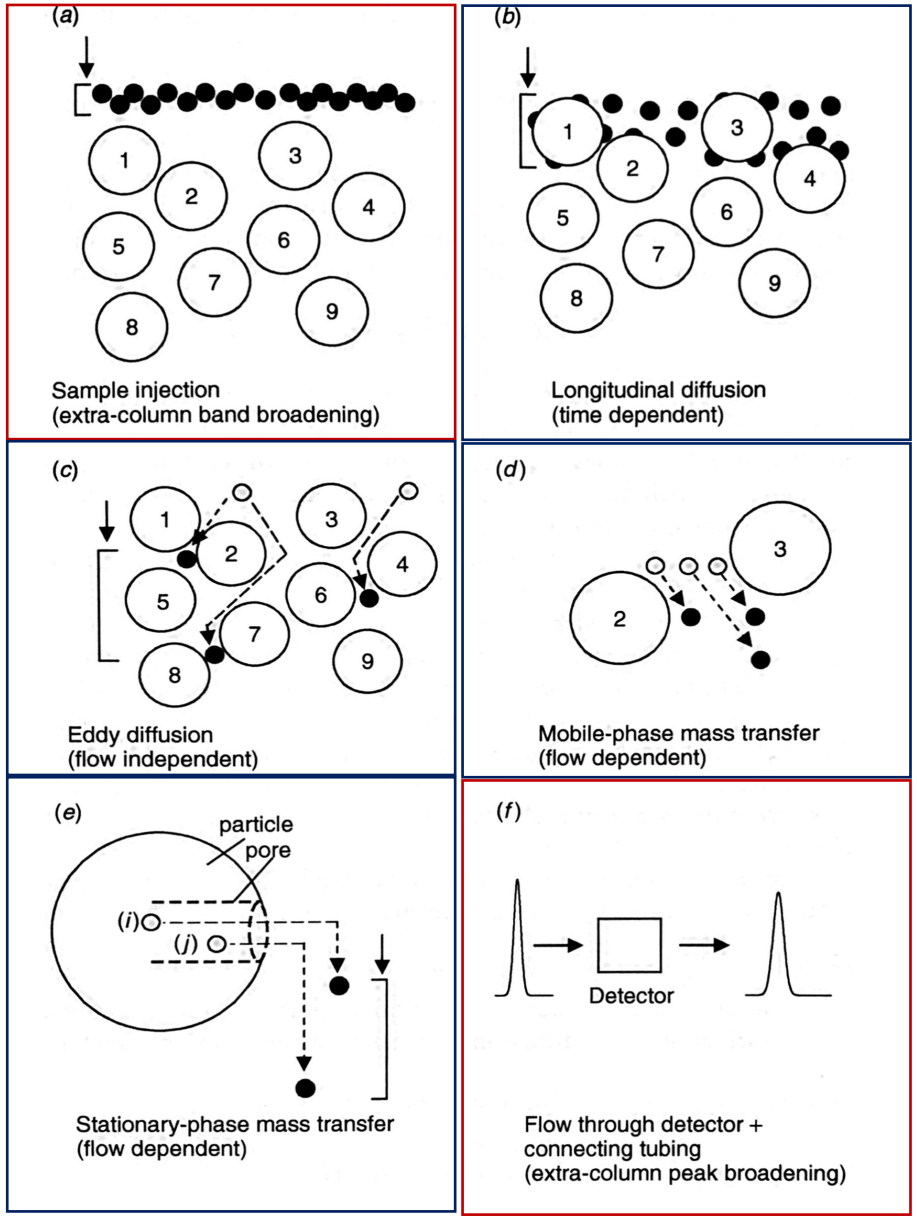
$$\sigma_C^2 = \sigma_A^2 + \sigma_B^2 + \sigma_{C_S}^2 + \sigma_{C_M}^2$$

Eddy Diffusion

Longitudinal Diffusion

Stationary phase Mass transfer

Mobile phase Mass transfer

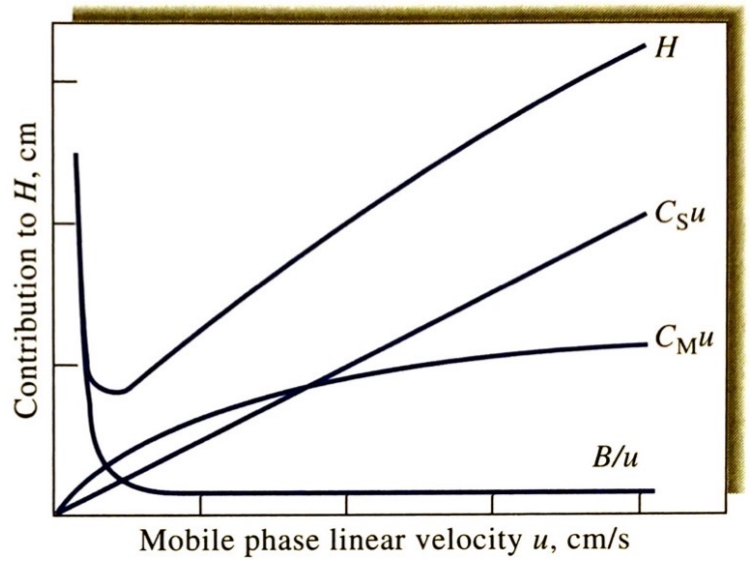


L.R. Snyder, J,.J. Kirkland, J. W. Dolan, « Introduction to Modern Liquid Chromatography », John Wiley &Sons Inc. 2010.

- Kinetic variables affecting column efficiency

Van Deemter plate height (H) equation

$$H = A + \frac{B}{u} + C_S\, u + C_M\, u$$



Multiple flow paths	$A = 2\lambda d_p$
Longitudinal diffusion	$\frac{B}{u} = \frac{2\gamma D_M}{u}$
Mass transfer from and to stationary phase	$C_S\, u = \frac{f(k) d_f^2}{D_S}\, u$
Mass transfer in mobile phase	$C_M\, u = \frac{f'(k) d_p^2}{D_M} u$

- u: linear velocity of mobile phase
- D_M: diffusion coefficient in mobile phase
- D_S: diffusion coefficient in stationary phase
- d_P: diameter of packing particles
- d_f: Thickness of liquid coating on stationary pahse
- λ and γ depend on quality of the packing

D. A. Skoog, E. J. Holler, S.R. Crouch, « Principles of Instrumental Analysis », Chap. 26 2017, Cengage Learning.

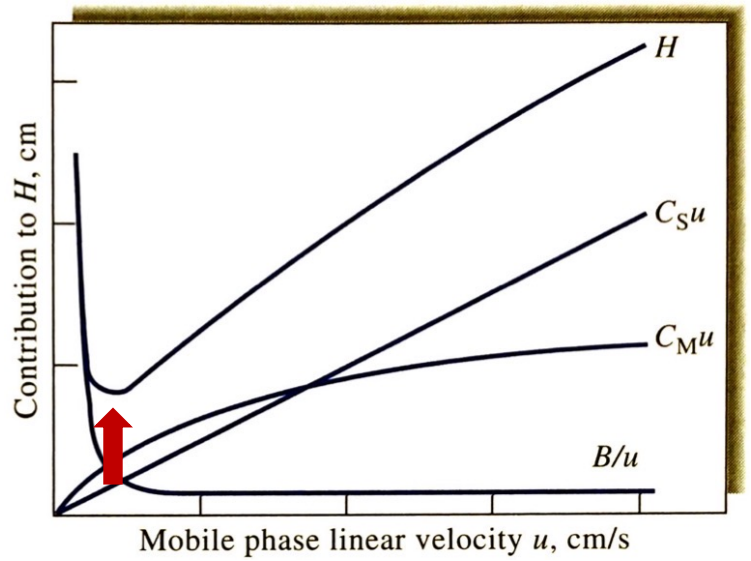
- Kinetic variables affecting column efficiency

Van Deemter plate height (H) equation

$$H = A + \frac{B}{u} + C_S\, u + C_M\, u$$

- A: multiple path effects (eddy coefficient)
- B: longitudinal diffusion coefficient
- C_S : mass transfer coefficient to and from stationary phase
- C_M : mass transfer coefficient in mobile phase

Rate theory



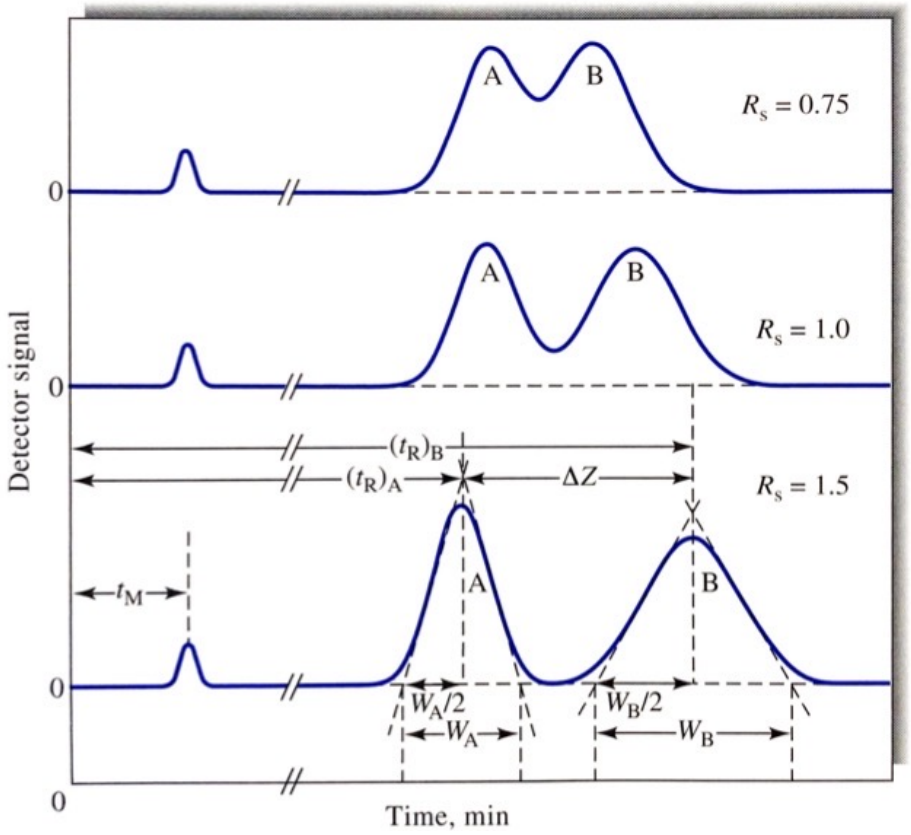
- u: linear velocity of mobile phase

Optimizing
Column efficiency

- Column resolution

$$R_S = \frac{\Delta Z}{\frac{w_{(A)}}{2} + \frac{w_{(B)}}{2}}$$

$$R_S = 2\,\frac{t_{R(B)} - t_{R(A)}}{w_{(A)} + w_{(B)}}$$



Overlap 4%

Overlap 0,3%

D. A. Skoog, E. J. Holler, S.R. Crouch, « Principles of Instrumental Analysis », Chap. 26, 2017, Cengage Learning.

- Effect of retention and selectivity on resolution

$$w_{(A)} = w_{(B)} \approx w \Longrightarrow R_S = \frac{t_{R(B)} - t_{R(A)}}{w} \Longrightarrow$$

$$R_S = \frac{t_{R(B)} - t_{R(A)}}{t_{R(B)}} \times \frac{\sqrt{N}}{4} \Longrightarrow R_S = \frac{k_B - k_A}{1 + k_B} \times \frac{\sqrt{N}}{4}$$

$$R_S = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_B}{1 + k_B}\right)$$

$$N = 16R_S^2 \left(\frac{\alpha}{\alpha - 1}\right)^2 \left(\frac{1 + k_B}{k_B}\right)^2$$

$$\bar{v}_B = \frac{L}{t_{R(B)}} \Longrightarrow t_{R(B)} = \frac{NH(1 + k_B)}{u}$$

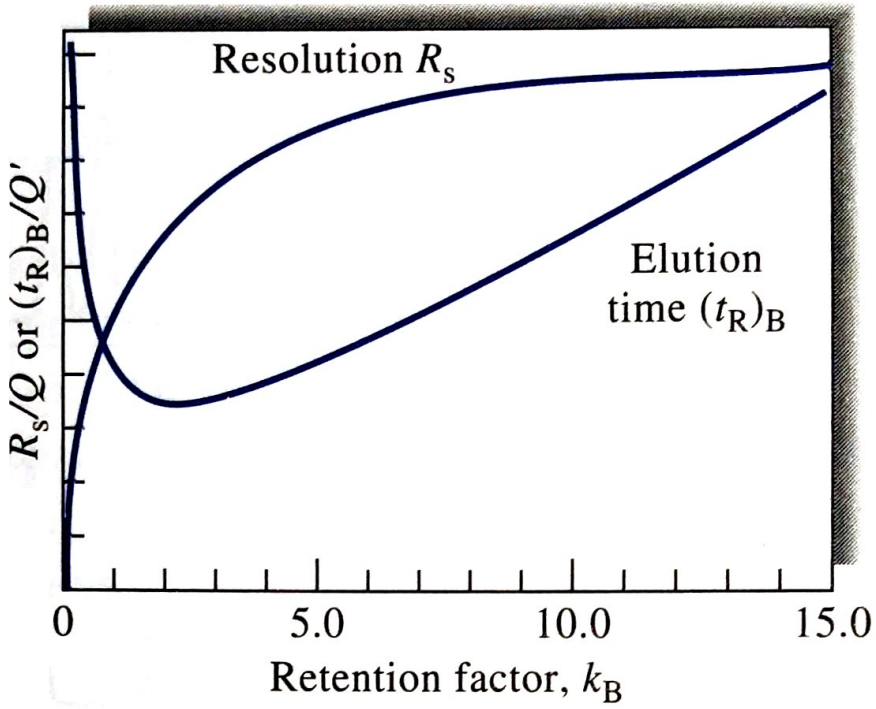
$$t_{R(B)} = \frac{16R_S^2 H}{u}\left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{(1 + k_B)^3}{k_B^2}$$

- Optimisation of separation

$$R_S = \underbrace{\frac{\sqrt{N}}{4}\left(\frac{\alpha-1}{\alpha}\right)\left(\frac{k_B}{1+k_B}\right)}_{Q}$$

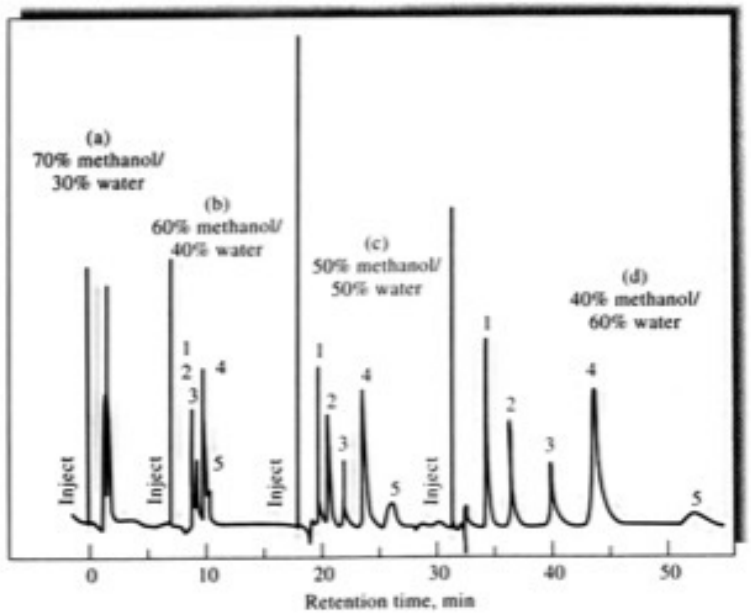
$$t_{R(B)} = \underbrace{\frac{16R_S^2H}{u}\left(\frac{\alpha}{\alpha-1}\right)^2\frac{(1+k_B)^3}{k_B^2}}_{Q'}$$

For Q and Q' constant:



Optimum
1 < retention factor < 5

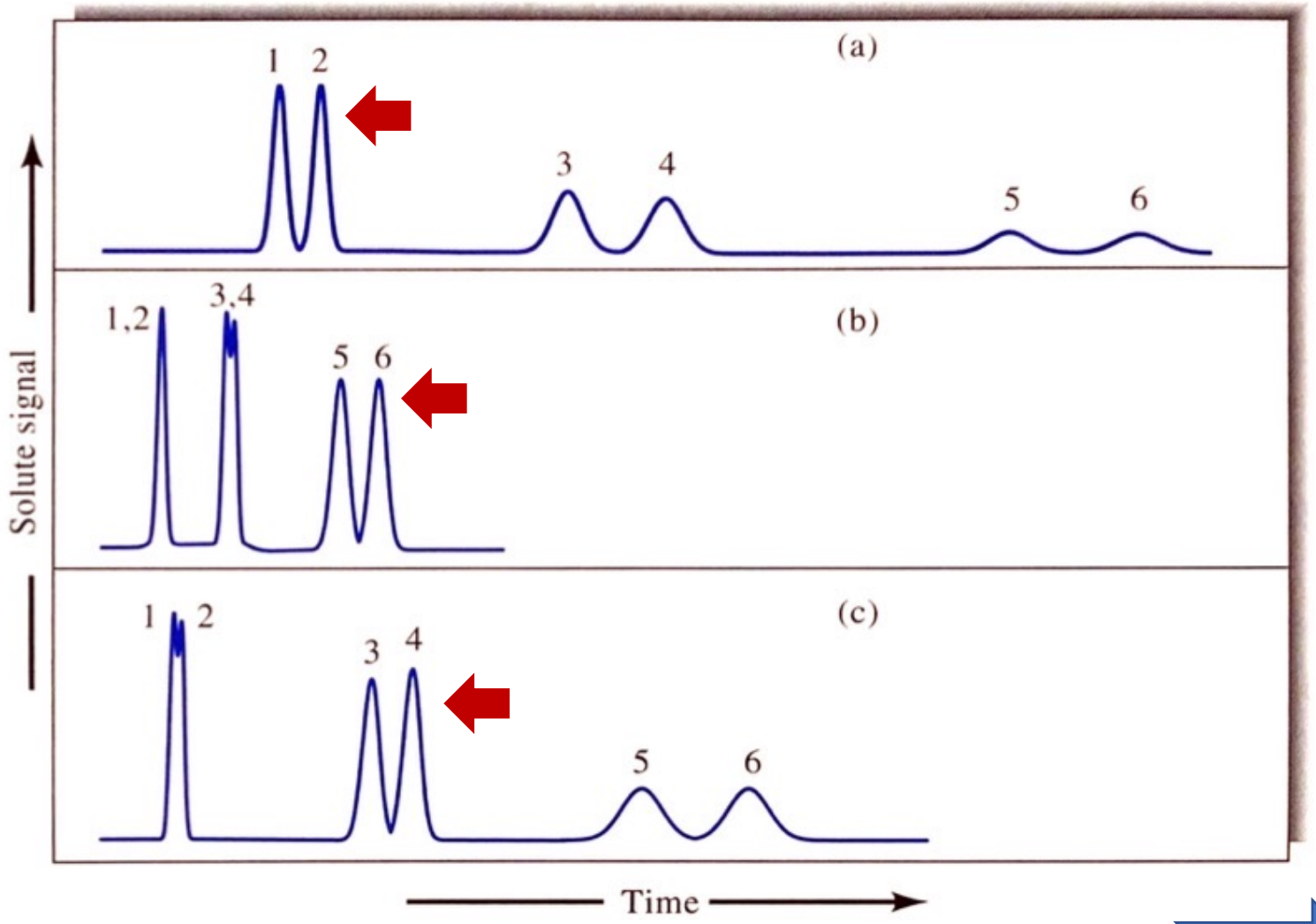
Example of k variation



D. A. Skoog, E. J. Holler, S.R. Crouch, « Principles of Instrumental Analysis », Chap. 26 2017, Cengage Learning.

The general elution problem

LC: Isocratic elution



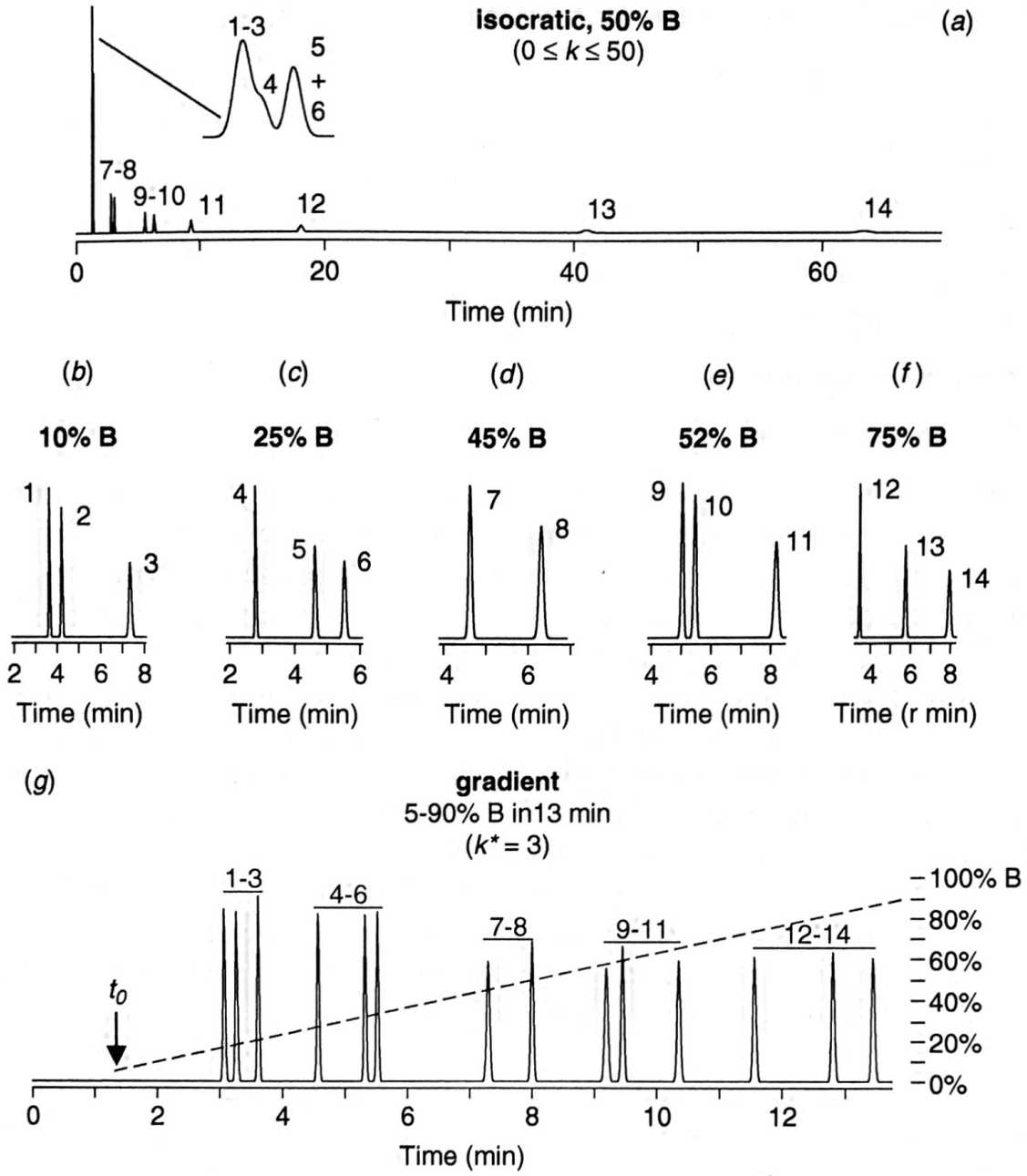
$1 < k_1, k_2 < 5$

$1 < k_5, k_6 < 5$

$1 < k_3, k_4 < 5$

<u>For optimal elution of all compounds</u>
LC: Gradient elution mode
GC: Temperature programming

D. A. Skoog, E. J. Holler, S.R. Crouch, « Principles of Instrumental Analysis », Chap. 26, 2017, Cengage Learning.



Example: Analysis of 14 toxicology standards by RP-LC
Column C18; eluent: Acetonitrile (B)/buffer pH=2,5

Isocratic elution (fixed eluent composition):
1-6 poor resolution
13-14 extensive retention

Separate analysis
Adequate eluent compositions
for each group of compounds
(k≈3)

Gradient elution (continuous change in eluent composition)
Adequate retention for all compounds in a single run (k*=3)

L.R. Snyder, J,.J. Kirkland, J. W. Dolan, « Introduction to Modern Liquid Chromatography », John Wiley &Sons Inc. 2010.

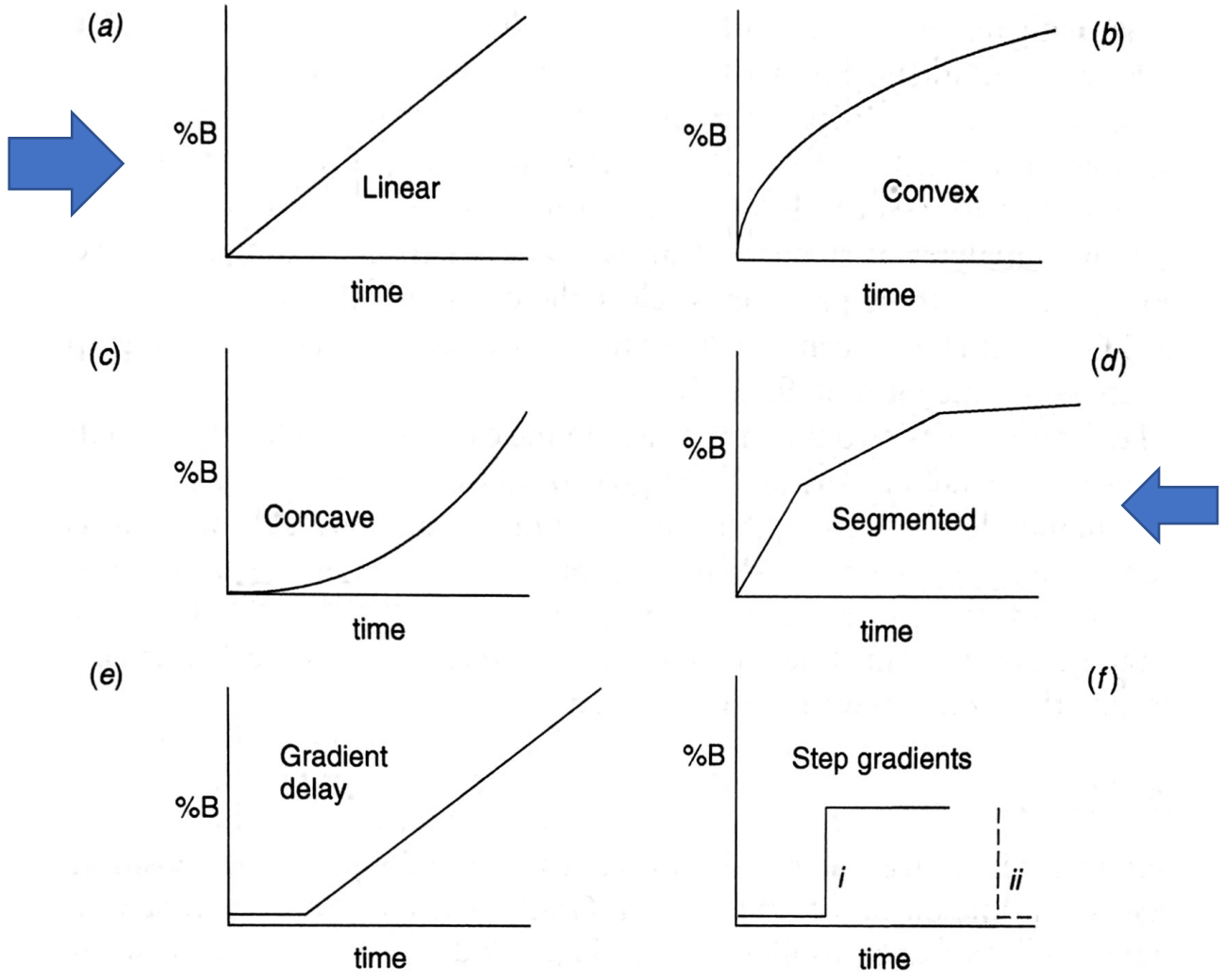
- Gradient elution

REASONS OF USE

- Retention range that exceeds $1 \leq k \leq 10$ (main reason)

- Gradient elution

Gradient shape



L.R. Snyder, J,.J. Kirkland, J. W. Dolan, « Introduction to Modern Liquid Chromatography », John Wiley &Sons Inc. 2010.

Applications of chromatographic methods

Qualitative analysis

- Based on the retention time of the solute
- Limited structural information, depending on detector type
- Presence or absence of a compound in a mixture
- Performs separation of the solute before identification

Quantitative analysis

- Major chromatography application
- Comparison of peak area or peak height with one or more standards
- Peak areas are more frequently used as independent from band broadening

High Performance Liquid Chromatography (HPLC)

Column efficiency in LC

- Band broadening as described

Intra-column

$$H = A + \frac{B}{u} + C_S\, u + C_M\, u$$

Extra –column (tubing)

$$H_{ex} = \frac{\pi\, r^2 u}{24\, D_M}$$

- r: radius of the tube

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- d_P: diameter of packing particles
- d_f: Thickness of liquid coating on stationary phase
- λ and γ depend on quality of the packing

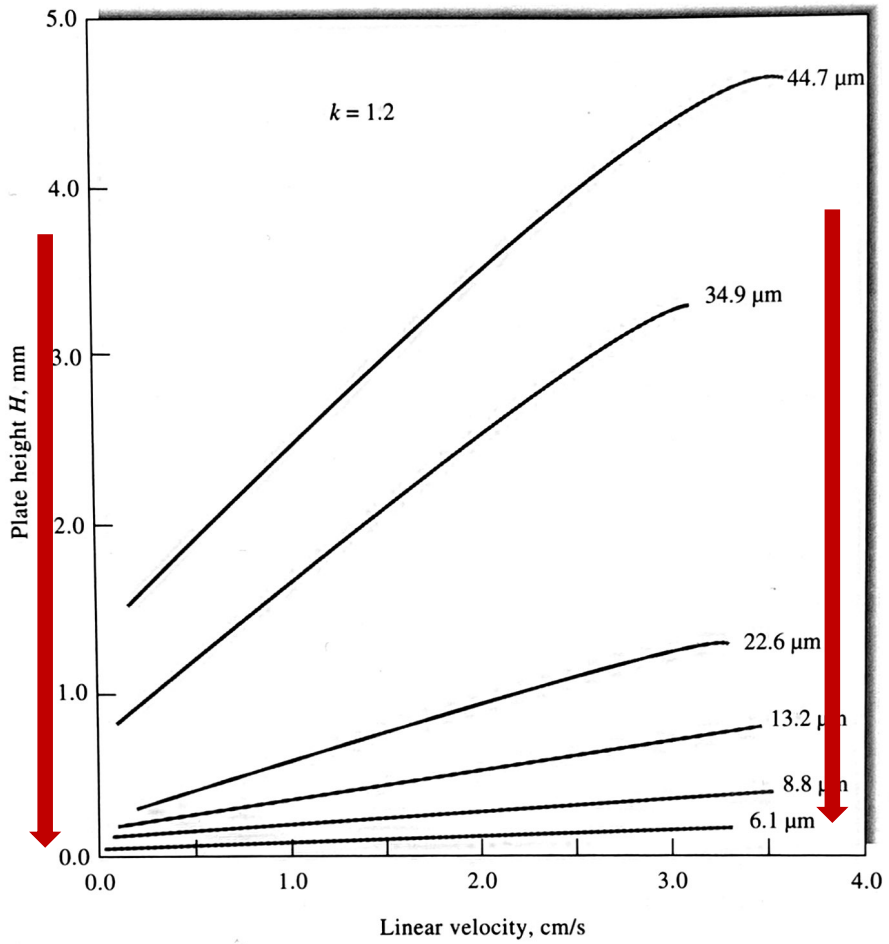
- Importance of particle size

Mass transfer in mobile phase

$$C_M \sim d_p^2$$

Particle size decrease

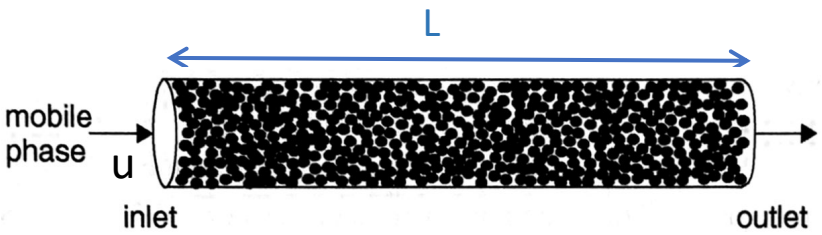
Plate height decrease



Experimentally

D. A. Skoog, E. J. Holler, S.R. Crouch, « Principles of Instrumental Analysis », Chap. 28, 2017, Cengage Learning.

- Column backpressure (ΔP)



$$\Delta P = \frac{\eta\, u\, \Phi_r L}{d_p^2}$$ *Darcy equation*

- η is the mobile phase viscosity
- L is column length
- d_p is the diameter of the particles
- Φ_r is a column flow resistance factor

Small particles

High performance

High pressure

Intermolecular interactions affecting elution

- Intermolecular interactions

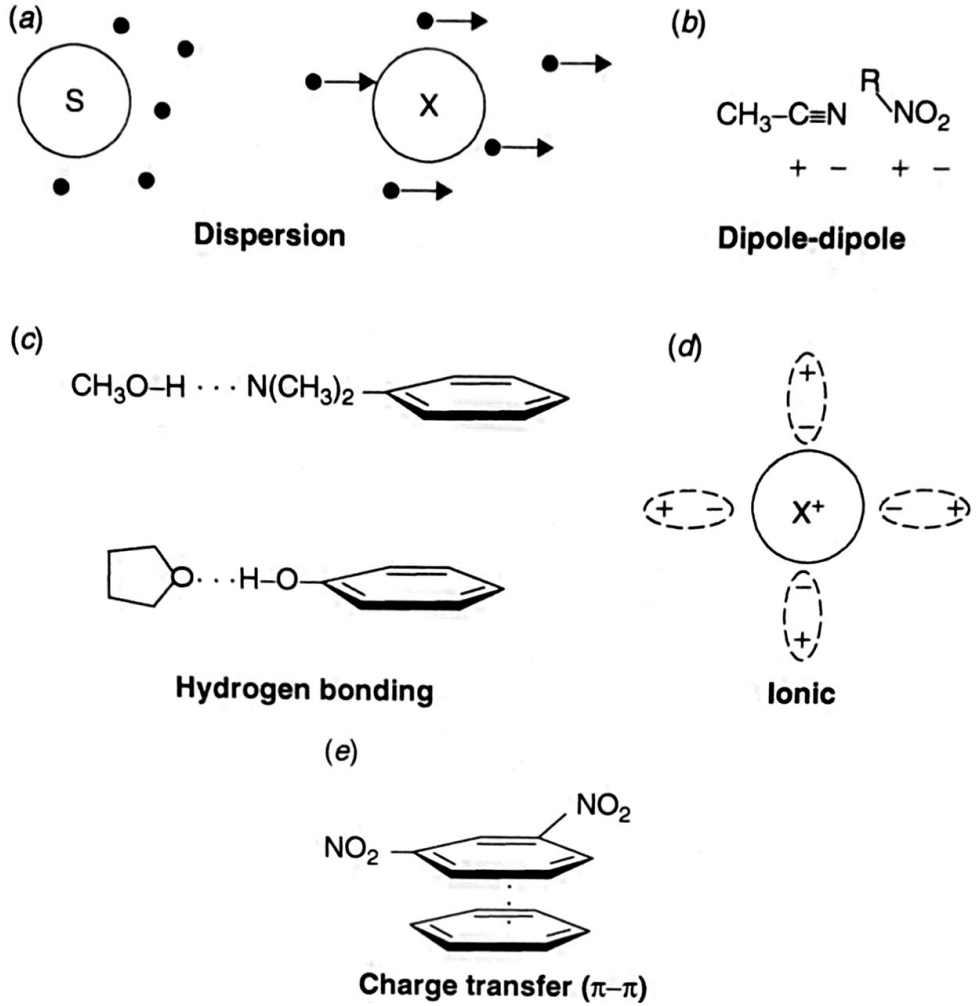
(a) Dispersion: Nonspecific and universal, contribute to hydrophobic interactions

(b) Dipole-dipole: between molecules that both have a permanent dipole moment (electrostatic interactions), depends on dipole moments of interacting groups

(c) Hydrogen bonding: between a proton donor (solute of solvent) and a proton acceptor (solute or solvent).

(d) Ionic (coulombic) interactions: interactions of an ion with surroundings molecules of a polarizable solvent or with other ions. Increase with solvent dielectric constant

(e) Charge transfer: between aromatic or unsaturated species



L.R. Snyder, J,.J. Kirkland, J. W. Dolan, « Introduction to Modern Liquid Chromatography », John Wiley &Sons Inc. 2010.

• solvent properties

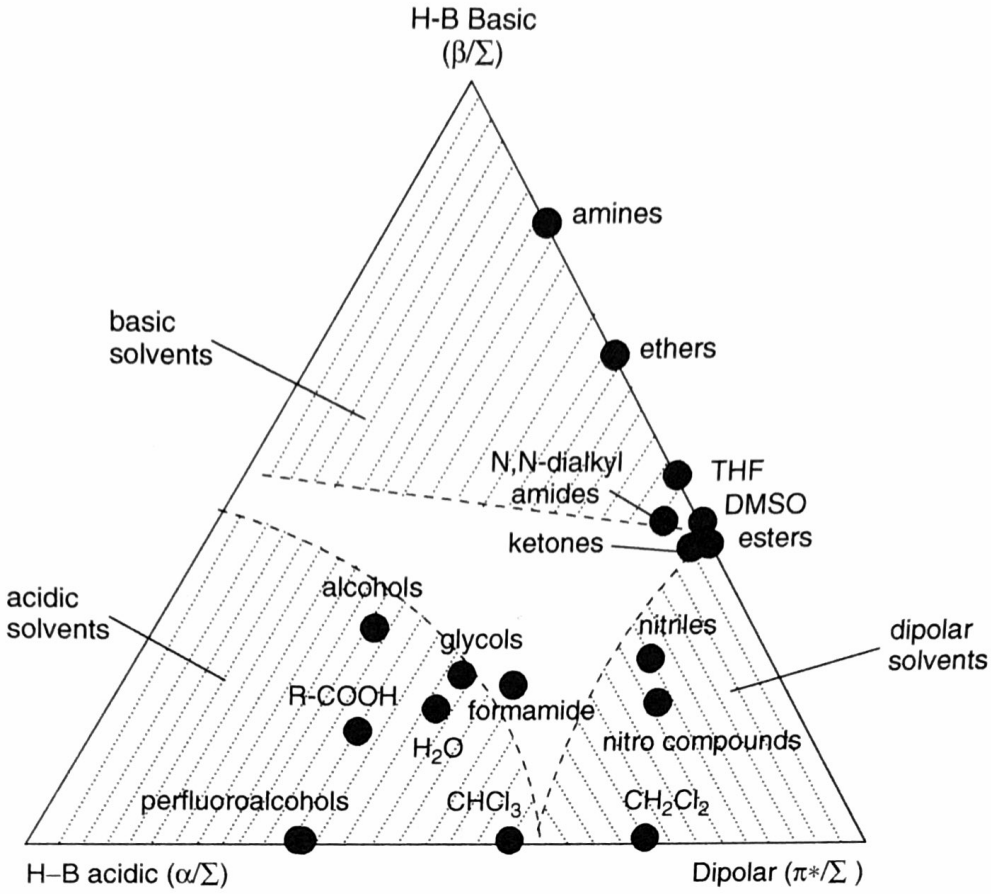
	Normalized Selectivity				
Solvent	H-B Acidity α/Σ	H-B Bacicity β/Σ	Dipolarity π^*/Σ	P’	ε
Acetic acid	0,54	0,15	0,31	6	6,2
Acetonitrile	0,15	0,25	0,6	5,8	37,5
Alkanes	0	0	0	1	1,9
Chloroform	0,43	0	0,57	4,1	4,8
Dimethylsulfoxide	0	0,43	0,57	7,2	4,7
Ethanol	0,39	0,36	0,25	4,3	24,6
Ethylacetate	0	0,45	0,55	4,4	6
Ethylene chloride	0	0	1	3,5	10,4
Methanol	0,43	0,29	0,28	5,1	32,7
Methylene chloride	0,27	0	0,73	3,1	8,9
Methyl-t-butyl ether	0	~0.6	~0.4	2,4	~4
Nitromethane	0,17	0,19	0,64	6	35,9
Propanol (n- or iso)	0,36	0,4	0,24	3,9	6
Tetrahydrofurane	0	0,49	0,51	4,0	7,6
Triethylamine	0	0,84	0,16	1,9	2,4
Water	0,43	0,18	0,45	10,2	80

Σ: sum of α,β and π^*

ε: Dielectric constant

P’: Polarity index

- Solvent selectivity characteristics



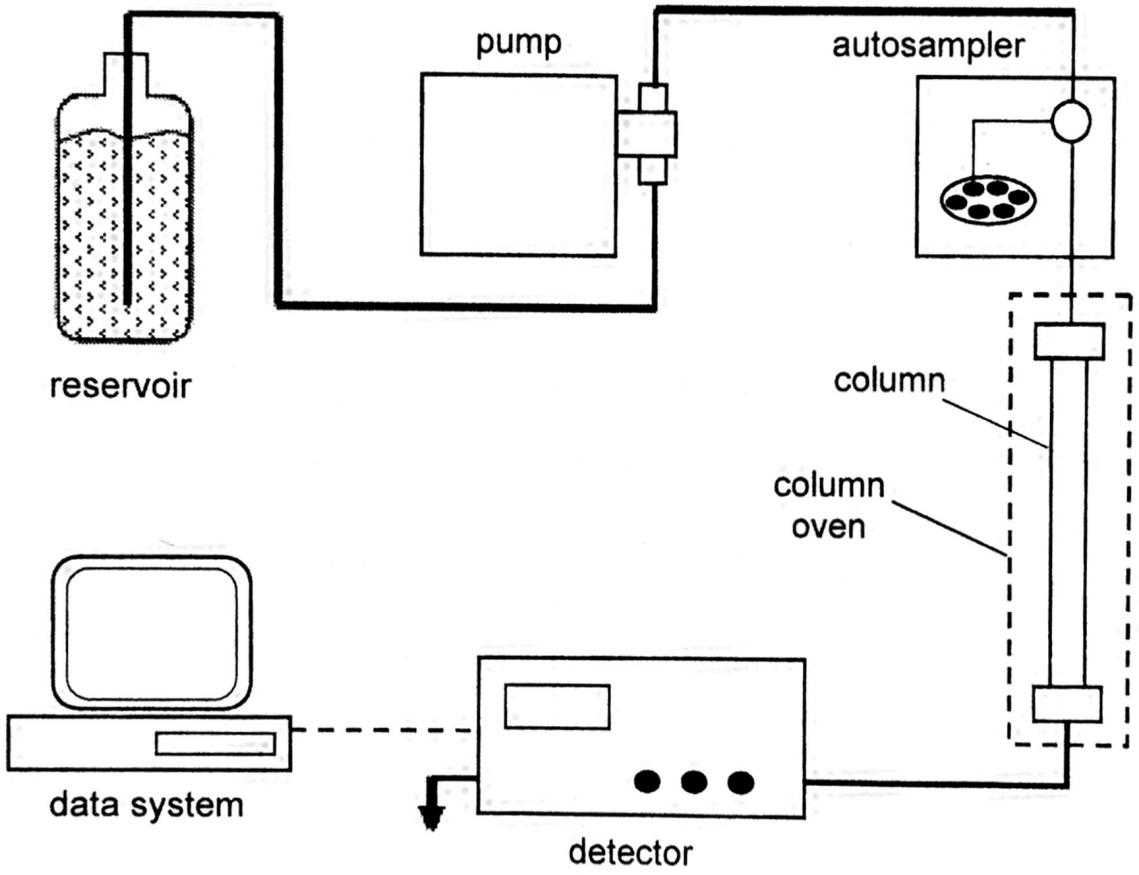
Polar interactions of nonionic solvents

Solvent-selectivity triangle

Control method selectivity

L.R. Snyder, J,.J. Kirkland, J. W. Dolan, « Introduction to Modern Liquid Chromatography », John Wiley &Sons Inc. 2010.

Instrumentation



Solvents and reservoirs

- Particle free mobile phase
- Degassing

Tubing

- Low pressure 100psi 1/8-in o.d
- High- pressure 7000 psi 1/16-in o.d.

Pumping systems

- Routine operation: 2000-3000psi

L.R. Snyder, J,.J. Kirkland, J. W. Dolan, « Introduction to Modern Liquid Chromatography », John Wiley &Sons Inc. 2010.

Instrumentation

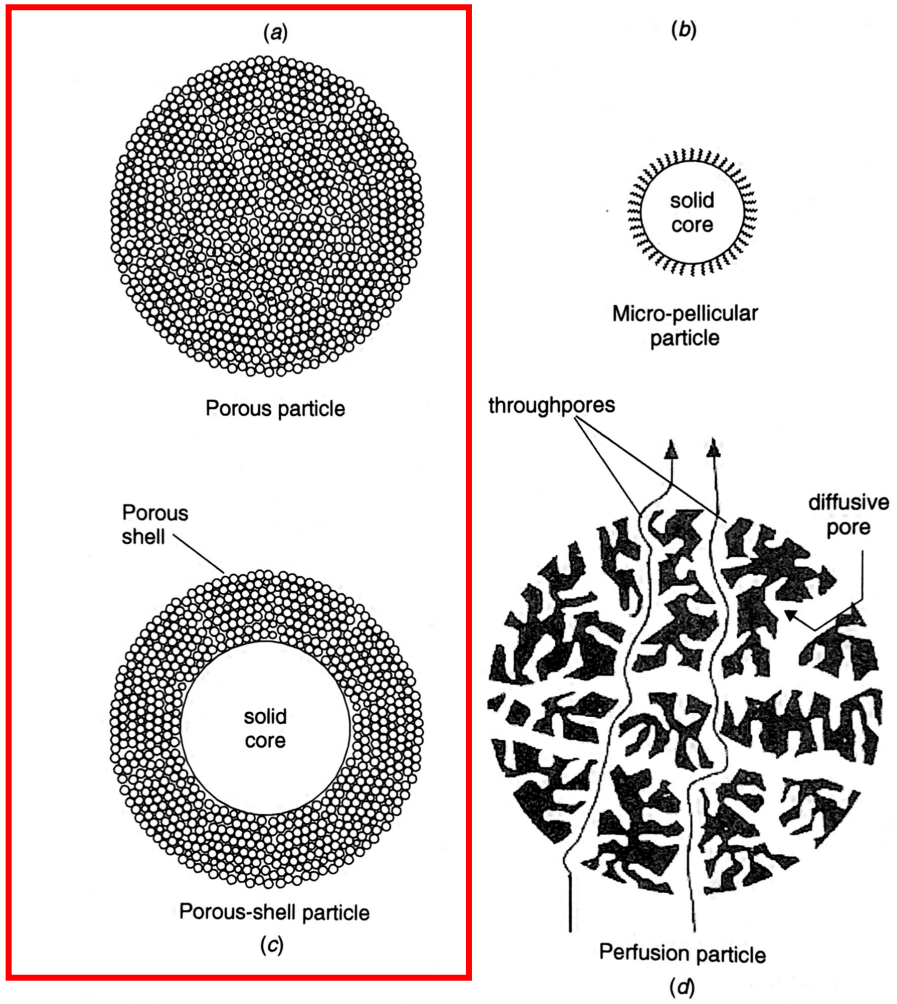
Columns

- Stainless steel tubing
- 5 - 25cm long; Inside diameter 3-5mm
- 4-5 μm particles
- 40000-70000 plates/meter
- Support: silica the most used
  - High mechanical strength
  - High values of N
  - Can be bonded with various ligands
  - Unaffected by eluent composition
  - Pressure up to 15000 psi
  - Limited to pH<8 (hybrid packings for pH>8)

Chromatographic columns

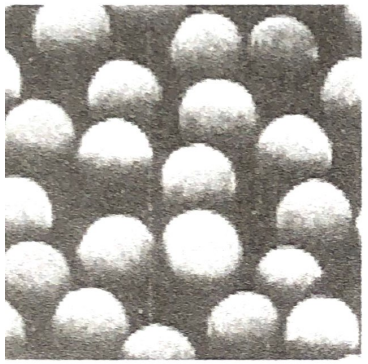
Separation unit

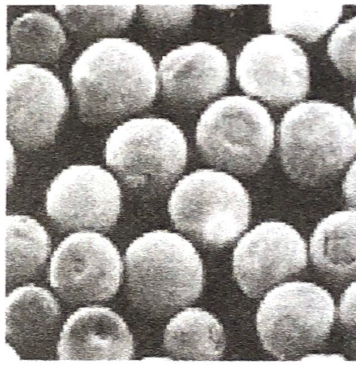
Packings used in LC: particle packing



Particles types

- Totally porous:
  - the most used
- Pellicular:
  - macromolecule analysis
  - lower surface area-rapid elution
- Superficially porous particles
  - Higher N than totally porous
  - Faster analysis than totally porous
  - Higher retention than pellicular
- Perfusion particles
  - Large pores connected to smaller
  - Advantaged for large molecules at high flow rates


Zorbax-PSM-300


Nucleosil-7-300

L.R. Snyder, J,.J. Kirkland, J. W. Dolan, « Introduction to Modern Liquid Chromatography », John Wiley &Sons Inc. 2010.

Packings used in LC: particle packing

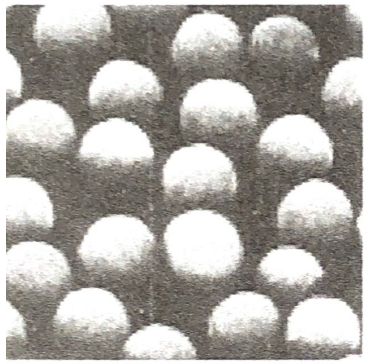
Particle size

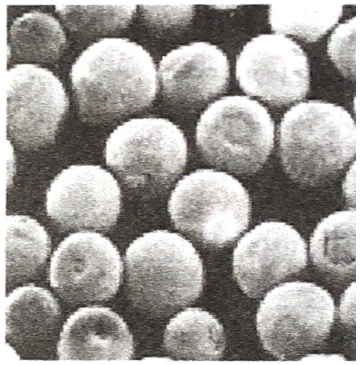
- particle diameter decrease: performance increase, rapid analysis
- 1980-2000: 5μm the most used
- >2000: 3μm or smaller
    - very high pressures up to 15000psi
    - new pumping systems

Pores size

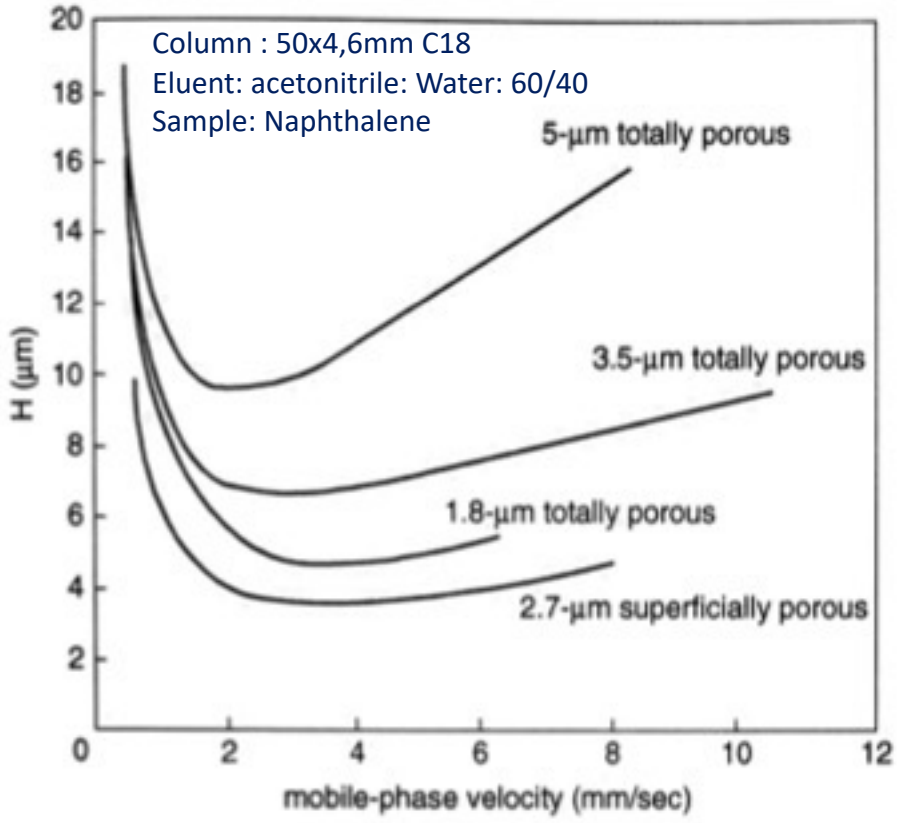
- Pore diameter increase: surface area decrease, performance decrease
- 8-12nm: small molecules <10000 Da, surface area: 150-400m^2/g (porous particles)
- 30nm: large molecules >10000 Da, surface area: 5-150m^2/g (perfusion particles)

Interstitial volume (space between particles):
40% of the column volume

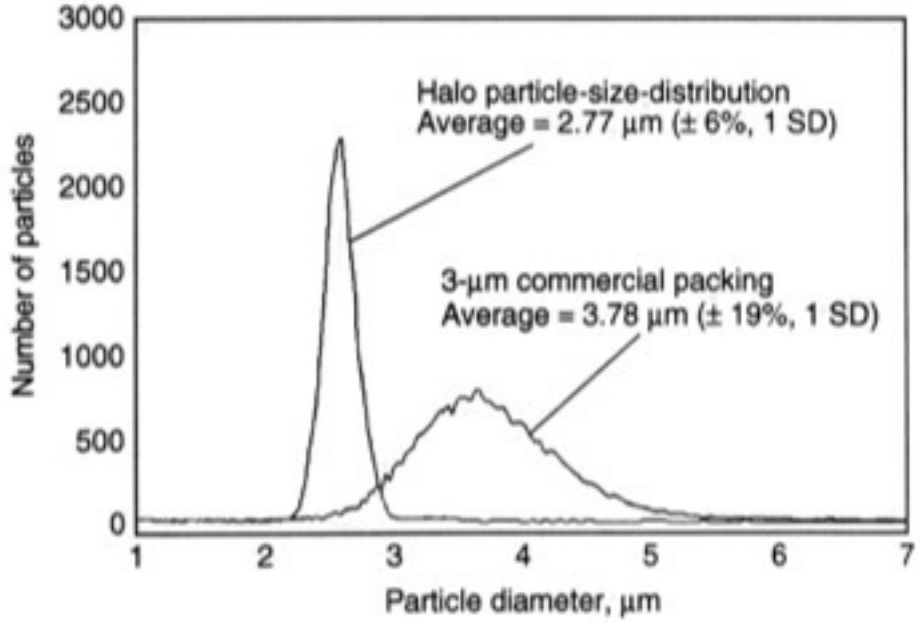

Zorbax-PSM-300


Nucleosil-7-300

Packings used in LC: particle packing



- Comparison of performance
- Totally porous (H/dp)minimum=2
- Superficially porous: (H/dp)minimum=1,5



- Size distribution
- Narrower size distribution: higher N
- Narrower size distribution for superficially porous and better packing → lower H

Detectors

- UV-visible absorption detectors
- Fluorescence detectors
- Electrochemical detectors
- Refractive index detectors
- Evaporative light-scattering detector (ELSD)
- Corona-discharge detectors (CAD)
- Mass spectrometry detectors

- HPLC-UHPLC

The basic diagram of an UHPLC system is the same as for the HPLC but:

	HPLC	UHPLC
Injection volumes	1 - 25 µL	0,1-1 µL
Particles diameter	3-5 µm	1,7-1,8 µm
Internal diameter of the column	3-10 mm	2,1 mm
Flow rate	0,3-2 mL/min	0,1-0,6 mL/min
Pressures up to	400 bar (6000 psi)	1030 bar (15000 psi)

Transition from HPLC to UHPLC

- Columns with very small particles and reduced i.d.
- Pumps that deliver higher pressures
- More precise injection systems
- Sensitive detectors with smaller dead volumes and high sampling frequency
- Small i.d. tubing and generally reduce void volumes

HPLC-UHPLC

- Fast-HPLC

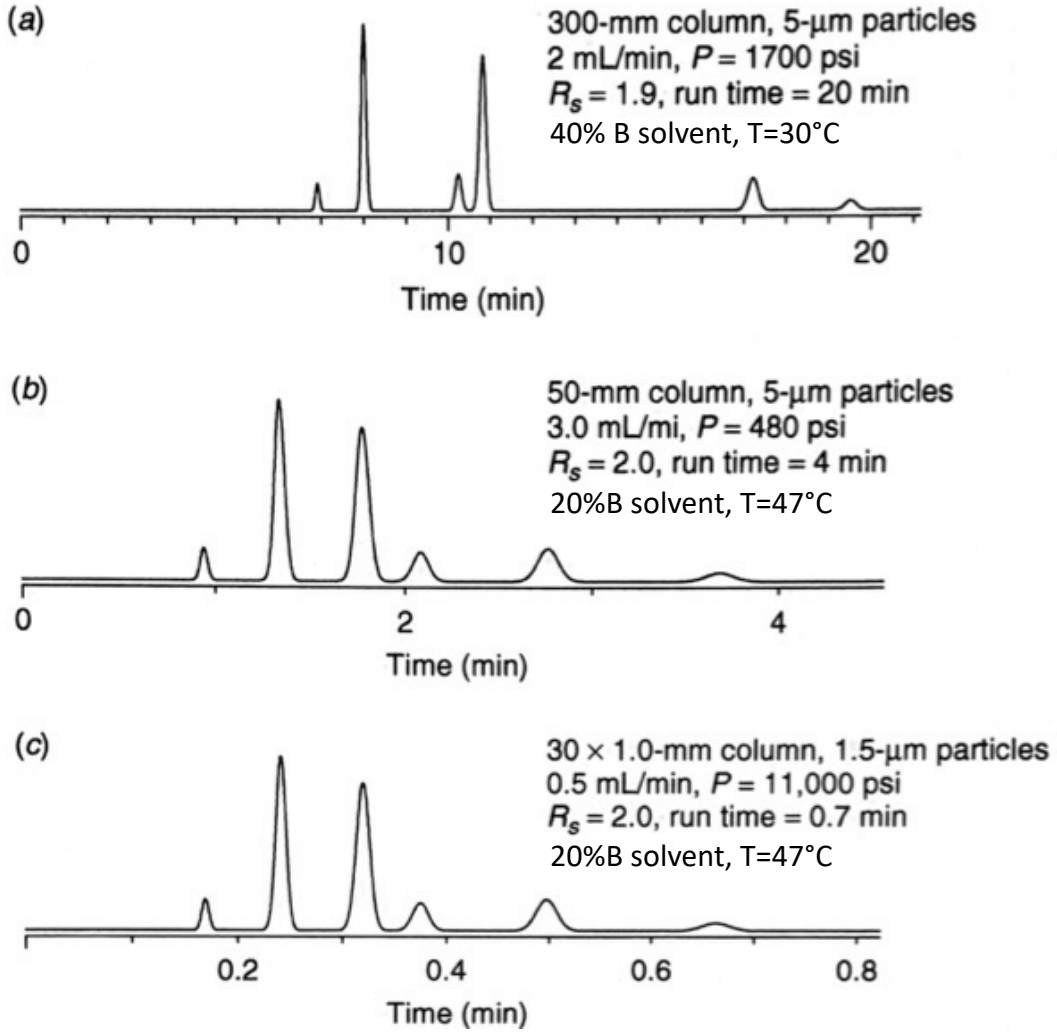
After optimization of k and α, resolution and run time depends on N (column). Fast separation can be obtained by:

- High flow rates
- Short columns
- Small particules

Small particules + short columns + high pressures

Fast separations without loss of N (or resolution)

Ultra-High Pressure Liquid Chromatography (U-HPLC)



(a) to (b): via optimization of α (changing mobile phase and temperature), decrease of L and increase of F

(b) to (c): HPLC to UHPLC