CHROMATOGRAPHY
fundamentals, methods, instrumentation, trends

Zdeněk Šimek

Materials for individual study

Masaryk University in Brno
Faculty of Science
Research Centre for Toxic Compounds in the Environment

RECETOX

SEPARATION METHOD CATEGORISATION

1. Separations based on *distribution of sample components between two phases*

2. Separations based on *migration rates differences of sample components*
   a) through semi-permeable membrane
   b) in force field

membrane separations :
- ***ultrafiltration*** (hydrostatic pressure)
- ***reverse osmosis*** (hydrostatic pressure)
- ***dialysis*** (concentration differences on membrane sites)
- ***electrodialysis*** (electric potential differences)

force field:
- *electrophoresis*
- *thermal diffusion*
- *mass spectrometry*
- *ultracentrifugation*
- *gravitation*

SEPARATIONS BASED ON DISTRIBUTION OF SAMPLE COMPONENTS BETWEEN TWO PHASES

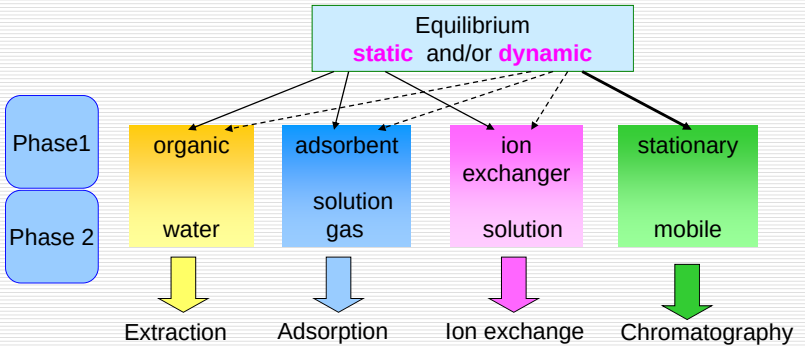
Types of interfaces	separation methods	abbreviation
gas - liquid	distillation **gas chromatography**	**GLC**
gas - solid	sublimation **gas chromatography** molecular sieve	**GSC**
liquid – liquid	extraction **liquid chromatography**	**LLC,** **SEC** (GPC)
liquid - solid	fractional crystallization precipitation **liquid chromatography** molecular sieve, inclusion	**LSC, IEC**

Crucial factor of sample component separation is

DISTRIBUTION CONSTANT

$$K_{D(A)} = \frac{(C_A)_1}{(C_A)_2}$$

measure of component affinity to contacted phases in equilibrium



SEPARATION REQUIREMENT → $K_{D(A)} \neq K_{D(B)} \neq K_{D(C)}$

separation factor $\alpha_{A,B}$
separation ratio
separation coefficient
elution ratio
retention ratio
relative volatility - distillation

$$\alpha_{A,B} = \frac{K_{D(A)}}{K_{D(B)}}$$

High α = easy separation : higher concentration of component A in phase 1 and component B in phase 2

Two extremes:

- ✓ $K_{D(A)}$= 6 $K_{D(B)}$ = 2 **α = 3** components A and B *easy penetrate* into phase 1
- ✓ $K_{D(A)}$= 0,6 $K_{D(B)}$ = 0,2 **α = 3** components A and B *remain mainly* in phase 2

CHROMATOGRAPHY

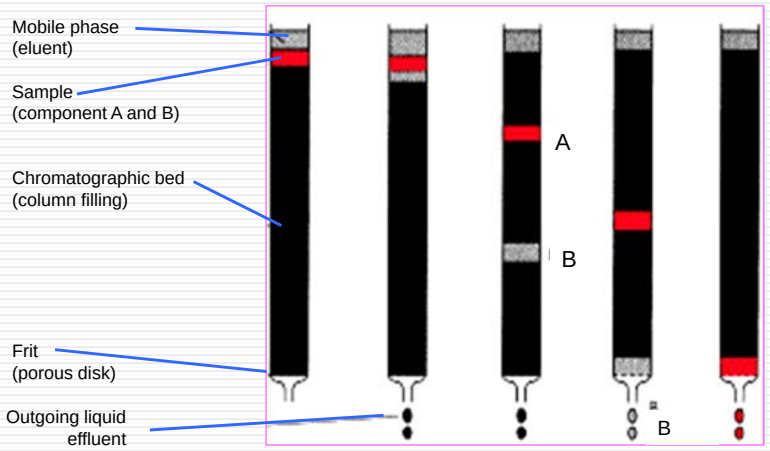
Definitions

Method of separation, identification and subsequent quantification of components more or less complex mixtures.
M.S. Cvet (1903-1906)

Stationary phase: $CaCO_3$
Sample: leaf pigments

Physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase, SP) while the other moves (mobile phase, MP or 'eluant') in a definite direction IUPAC (1993)

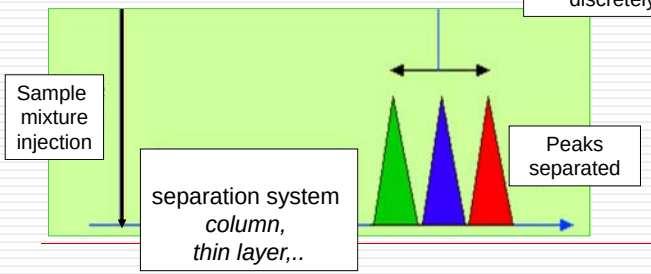
Common chromatographic arrangement



CHROMATOGRAPHY

Two basic processes occur in the column

1. The components are moved apart as a result of their relative affinities for the stationary phase
2. The spread (dispersion) of the zones (peaks) is constrained so that solutes can be eluted discretely



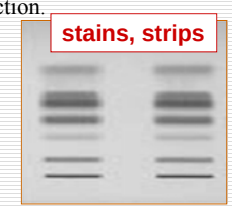
SEPARATION OF COMPONENTS IN CHROMATOGRAPHIC SYSTEM

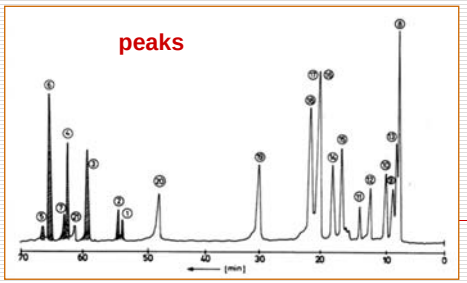
Components are moved through system in the mobile phase direction.

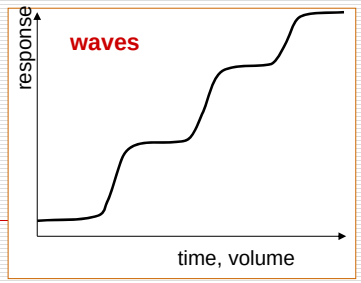
Result: chromatographic zones of separated components

Detection: - detector at the end of chromatographic system
- physical property of effluent and sample component

Record: **chromatogram: chromatographic zones**





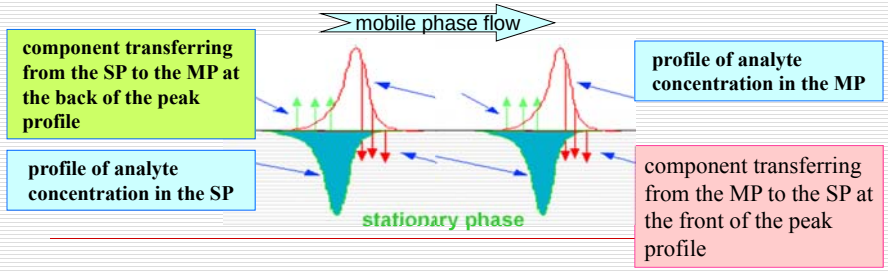


Chromatographic process

Chromatography takes for separation of components:

multiple repeated set up of components equilibrium between two phases

stationary (SP) and **mobile** (MP)



INTERACTIONS IN CHROMATOGRAPHIC SEPARATION SYSTEMS

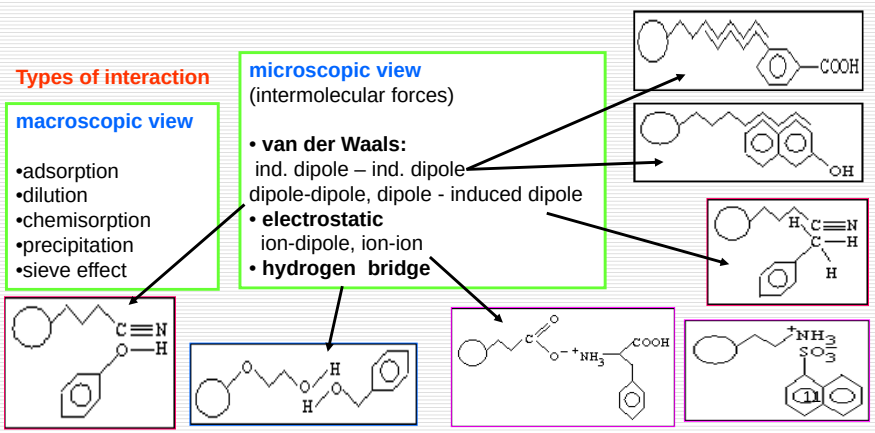
Equilibrium is created by various (qualitatively and quantitatively)

physical - chemical interaction between
- ✓component and mobile phase
- ✓component and stationary phase
- ✓ mobile and stationary phase

Types of interaction

macroscopic view
- •adsorption
- •dilution
- •chemisorption
- •precipitation
- •sieve effect

microscopic view
(intermolecular forces)
- **van der Waals:** ind. dipole – ind. dipole dipole-dipole, dipole - induced dipole
- **electrostatic** ion-dipole, ion-ion
- **hydrogen bridge**



INTERACTION with MOBILE PHASE
INTERACTION with STATIONARY PHASE

1. Adsorption - adsorption chromatography - stationary phase = adsorbent
- components are caught on the surface of stationary phase

a) physical adsorption – reversible, energy 0,3 -3 kJ/mol

Acting forces:

polar (specific)	*non-polar* (non-specific, dispersion)
between permanent dipoles *(columbic)* permanent dipole vs. induced dipole	between electroneutral molecules induced dipoles
alcohols, carboxylic acids sulfonic acids	aliphatic hydrocarbons aromatic hydrocarbons

b) chemisorption – irreversible retention of component part
energy comparable with energy of covalent or ionic bond 40 - 400 kJ/mol

characterisation of physical adsorption
ADSORPTION ISOTERM

effect of component concentration in environment on amount of adsorbed the component in equilibrium

Attention ! chromatography is dynamic process !!!!!!!!!

$C_S = D \cdot C_m$ — invalid for real systems

D - *distribution coefficient* ~ K_D

C_s, C_m – *concentration in SP, MP*

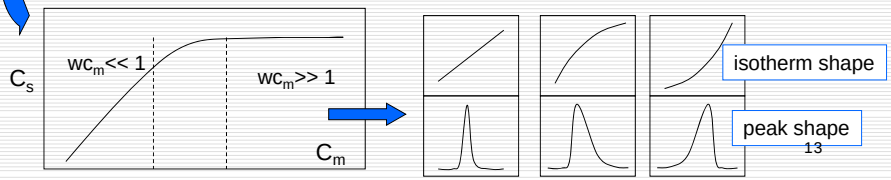
Freundlich: $C_S = k \cdot C_m^{1/n}$ — k,n - *constants*

Langmuir: $C_S = w \cdot z \cdot C_m / (1+wC_m)$

w - adsorption coefficient

z – number of free interaction sites on the surface

valid for chromatography



INTERACTION with MOBILE PHASE
INTERACTION with STATIONARY PHASE continue

2. Partition

dissolution in **two phases** → partition equilibrium creation
liquid bonded on the inert surface - **film of bonded stationary phase**

3. Precipitation

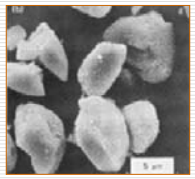
precipitant bonded on stationary phase
separation of component according to solubility product K_{sp}

4. Sieve effect

Inclusion:
ions of similar dimensions and same charge are caught in crystal lattice
K^+ in NH_4MgPO_4

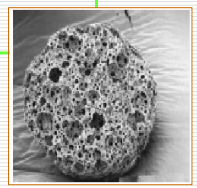
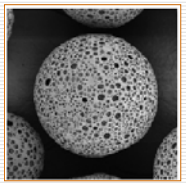
Occlusion
component, which is not a part of crystal lattice is caught in cavities
water in $AgNO_3$

STATIONARY AND MOBILE PHASE – general information



STACIONARY PHASE general: sorbent

-***solid particles*** diameter 1 - 100 (200, 300) μm
-***thin liquid layer*** on solid particles
-***thin liquid layer*** on inner wall of capillary

Arrangement: thin layer
tube - column
capillary - capillary column

MOBILE PHASE

- **gas**
- **supercritical fluid**
- **liquid**

density *[g.cm^{-3}]*	**viscosity** *[g.cm^{-1}. s^{-1}]*	**diffusion coefficient** *[cm^2 · s]*
0,001	0,0001	0,1
0,1 – 1	0,0001 – 0,001	0,001-0,0001
1	0,01	<0,00001

CHROMATOGRAPHIC METHOD CLASSIFICATION

1.According to mobile phase

Gas - gas chromatography - **GC**

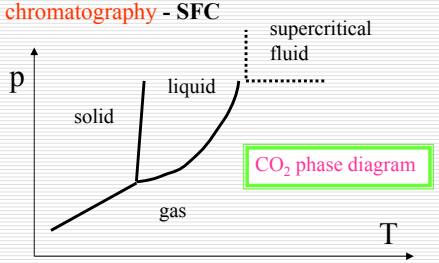
liquid - liquid chromatography - **LC**

Supercritical fluid – supercritical fluid chromatography - **SFC**

SFC:
- supercritical temperature and pressure

CO_2, SF_6, Xe, NH_3

Higher values of diffusion coefficients in comparison with similar liquids,
Lower viscosity



CRITICAL VALUES

Critical temperature - T_c:
only one phase exists in a system of liquid-gas over T_c
substance is in the fluid stage

Critical pressure-p_c:
Tlak needed to condensation of fluid substance at critical temperature

Fluid	T_c, °C	P_c, atm	d*
CO_2	31.3	72.9	0.96
N_2O	36.5	72.5	0.94
NH_3	132.5	112.5	0.40
n-C_5	196.6	33.3	0.51
n-C_4	152.0	37.5	0.50
CCl_2F_2	111.8	40.7	1.12
CHF_3	25.9	46.9	-----

*density in g/ml at 400 atm

How to prepare supercritical fluid

1. Substance in liquid form is exposed to temperature and pressure to form equilibrium with its vapour : two phase exist
2. Created system is closed in tube and temperature is increased over T_c to form only one phase disregarding the pressure

CHROMATOGRAPHIC METHOD CLASSIFICATION

2. According to stationary phase:

Solid: ***Adsorption chromatography***

Separation is based mainly on differences between the adsorption affinities of the sample components for the surface of an active solid.

GSC - ***gas adsorption chromatography***
LSC - ***liquid adsorption chromatography***

Liquid: ***Partition chromatography***

Separation is based mainly on differences between the solubility of the sample components in the stationary phase (gas chromatography), or on differences between the solubility of the components in the mobile and stationary phases (liquid chromatography).

GLC - ***gas partition chromatography*** liquid on support
LLC - ***liquid partition chromatography*** liquid on support – immiscible with MP

CHROMATOGRAPHIC METHOD CLASSIFICATION

2a . Special types

Exclusion chromatography
Separation is based mainly on exclusion effects, such as differences in molecular size and/or shape or in charge.

SEC - size exclusion chromatography may also be used when separation is based on molecular size.

GPC - gel permeation chromatography were used earlier to describe this process when the stationary phase is a swollen gel.

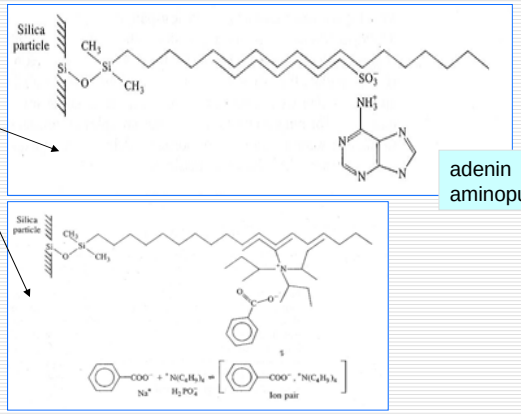
IEC - ion-exclusion chromatography is specifically used for the separation of ions in an aqueous phase.

Ion exchange chromatography, Ion chromatography - ***IC***
Separation is based mainly on differences in the ion exchange affinities of the sample components.
competition of analyte and mobile phase ions for ionic groups bonded on the stationary phase surface

Affinity chromatography - ***AFC***
Particular variant of chromatography in which the unique biological specificity of the analyte and ligand interaction is utilized for the separation.
„affinant" bonded on chromatographic support specific biological/biochemical interaction of antidote-antigen, enzyme-substrate, hormone-receptor

3. According to main separation mechanism

- adsorption
- partition
- ion exchange
- ion paired
- affinity
- gel permeation
- …….



4. According to experimental set-up

Column Chromatography
A separation technique in which the **stationary bed is within a tube.**

Packed Column: The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube or be concentrated on or along the inside tube wall leaving
Open-Tubular Column:
An open, unrestricted path for the mobile phase in the middle part of the tube

Planar Chromatography ***Open-Bed Chromatography.***
A separation technique in which the **stationary phase is present as or on a plane**.

Paper Chromatography, PC: The plane can be a paper, serving as such or impregnated by a substance as the stationary bed
Thin Layer Chromatography, TLC: Layer of solid particles spread on a support, e.g., a glass plate

5. According to method of component transport through separation system
≈ method of injection

Elution chromatography

A procedure in which the **mobile phase is continuously passed** through or along the chromatographic bed.
The sample is fed into the system as a finite slug.
Sample components are more retained as mobile phase components

A procedure in which the **mobile phase contains a compound** (the ***Displacer)*** **more strongly retained than the components** of the sample under examination.
The sample is fed into the system as a finite slug.
Mobile phase is more retained as sample components

Displacement chromatography

Frontal chromatography

A procedure in which the **sample** (liquid or gas) **is fed continuously** into the chromatographic bed. In frontal chromatography no additional mobile phase is used.
Sample solution act as „mobile phase"
Solvent is less retained as sample components

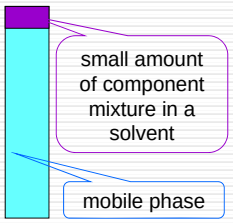
ELUTION CHROMATOGRAPHY

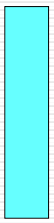
Step 1:
Sample injection into the flow of mobile phase

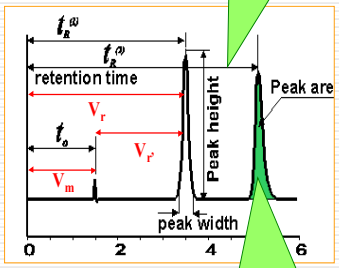
Step 2:
Component zone development in mobile phase flow

Step 3:
Component zone elution by mobile phase

Position of zone:
magnitude of interaction
identification of component







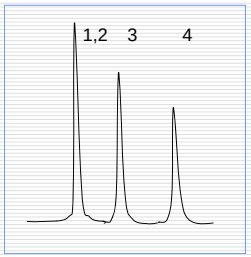
- elution of components in separated zones in the order of interaction magnitude with stationary phase
- components are segregated by clear mobile phase zone
- The best separation of sample components

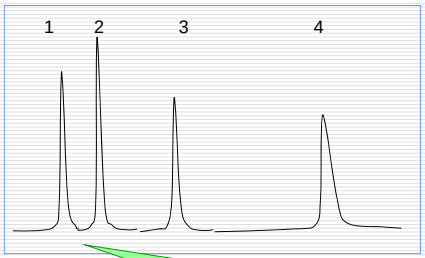
Area/height:
quantitative parameter

Eluted components dissolved in mobile phase

ELUTION:

isocratic - composition of the mobile phase **remains constant** during the elution process.
stepwise - composition of the mobile phase is **changed in steps** during a single chromatographic run.
gradient - composition of the mobile phase is **changed continuously** or stepwise during the elution process.





separation of amide C_1- C_4
MP: ethylacetate/heptane

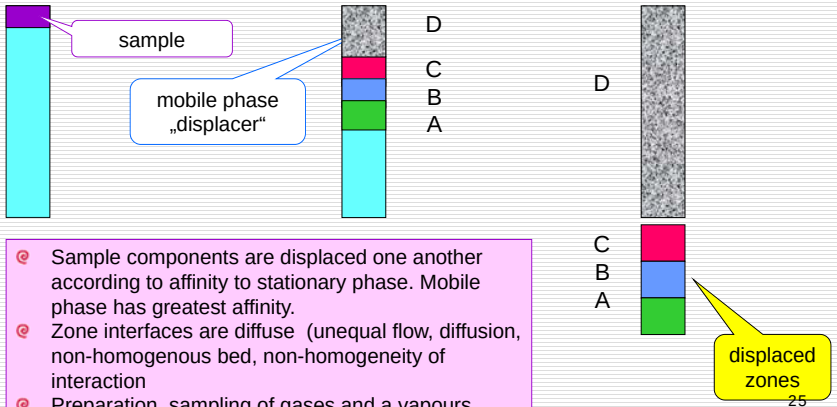
lower content of ethylacetate in heptane

DISPLACEMENT CHROMATOGRAPHY

Step 1:
Sample injection

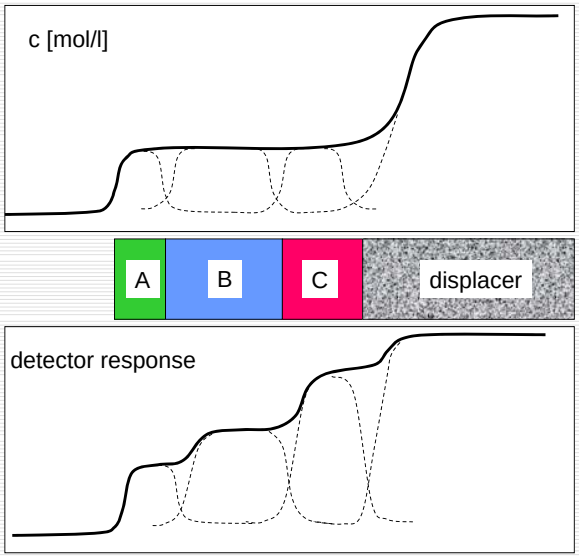
Step 2:
Component zone development in mobile phase flow

Step 3:
Component zone displacement by mobile phase



- Sample components are displaced one another according to affinity to stationary phase. Mobile phase has greatest affinity.
- Zone interfaces are diffuse (unequal flow, diffusion, non-homogenous bed, non-homogeneity of interaction
- Preparation, sampling of gases and a vapours

DISPLACEMENT CHROMATOGRAPHY



Result 1:
A, A+B, B, B+C, C, C+D, D

Inserted displacers
X,Y,Z
affinity between A, B, C
volatile

Result 2:
A, A+X, X, X+B, B, B+Y, Y, Y+C, C, C+Z, Z, Z+D, D

X,Y,Z evaporate, distil

FRONTAL CHROMATOGRAPHY

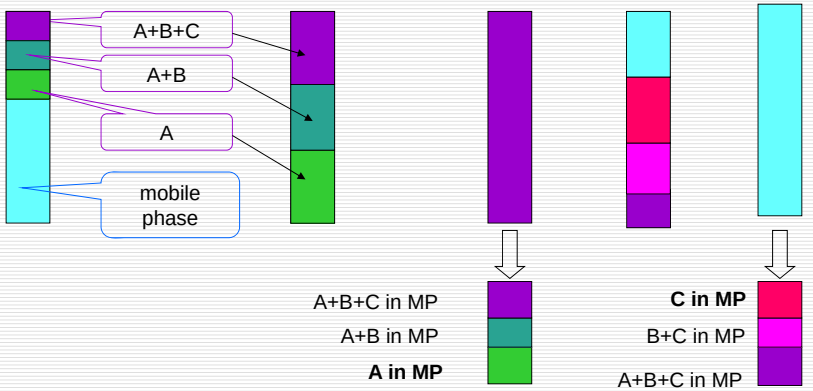
Step 1:
Continuous feed of component mixture in MP

Step 2:
Component zone A perforation

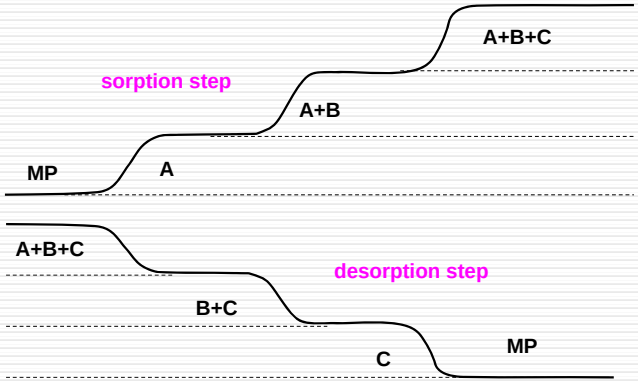
Step 3:
Saturation of bed (all components)

Step 4:
Feed of clear MP

Step 5:
Elution of all components



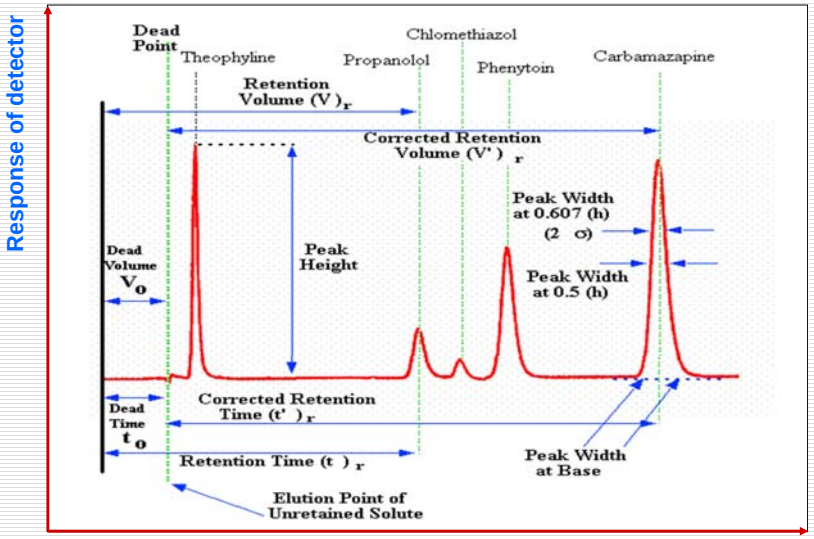
FRONTAL CHROMATOGRAPHY



- clear only: first component (A with lowest affinity to SP)
  last component (C with highest affinity to SP)
- other components in mixtures
- quantity – area defined by integration curve

Example of a result of chromatographic analysis

CHROMATOGRAM



SELECTED TERMS RELATED to THE CHROMATOGRAPHIC PROCESS and THE THEORY OF CHROMATOGRAPHY continued

t_R - **retention time of compound retained in the system**

compound moves slowly than mobile phase

d_R - retention distance

t_M - **retention time of compound not retained in the system**

dead retention time , hold-up time

compound moves as quickly as mobile phase

F_m - **flow rate** of mobile phase - [cm^3/ s, ml/min, µl/ min]

the volume of mobile phase passing through the column in unit time.

V_M - **hold-up volume, dead volume** = $F_m . t_M$

often equal to V_m – volume of mobile phase in column, the sample injector, the detector, and connectors..

V_R - **retention volume of retained compound** = $F_m . t_R$

L - **length of chromatographic bed , column length**

u - **mobile phase velocity,** linear velocity $u = L / t_M$ [cm / s]

u_A - retained compound A velocity = $L / t_{R,A}$

SELECTED TERMS RELATED to THE CHROMATOGRAPHIC PROCESS and THE THEORY OF CHROMATOGRAPHY

„Equilibrium" (component distribution between two phases)

$A_{mobile\ phase} \Leftrightarrow A_{stationary\ phase}$

Distribution constant - crucial factor of separation

- ratio of total concentrations of component A in two phases

Generally: $K_{D,A} = \frac{(c_A)_1}{(c_A)_2} = \frac{(n_A)_1 . V_2}{(n_A)_2 . V_1} = \frac{(n_A)_{SP} . V_{MP}}{(n_A)_{MP} . V_{SP}}$ ***chromatography***

$c = \frac{n}{V}$ $\frac{(n_A)_O . V_W}{(n_A)_W . V_O}$ ***extraction***

K_D doesn't reflect how components are shared in two phases !!!!

Better:

retention factor k (k_A for component A)

Partition Ratio
Capacity Ratio
Capacity Factor
Mass distribution Ratio
found in literature

$$k_A = \frac{(n_A)_1}{(n_A)_2} = \frac{(c_A)_1 . V_1}{(c_A)_2 . V_2} = K_{D,A} \frac{V_1}{V_2} = K_{D,A} \frac{V_{SP}}{V_{MP}}$$

Meaning of **k**

- **measure of the time the sample component resides** in the stationary phase relative to the time it resides in the mobile phase
- **how much longer** a sample component is retarded by stationary phase than it would take to travel through column with velocity of mobile phase
- **equilibrium ratio** of component amount in SP and MP
- **migration speed of analyte in chromatographic bed**
- **comparison of components interactions** in MP and SP (K_D requires V_{MP} a V_{SP})

ATTENTION!!!!!

Chromatographic bed, Chromatographic column is dynamic system

it is impossible to reach equilibrium in particular step of component transportation

TIME can be observed only,

time witch molecule spends in both phases during transport through chromatographic system.

TIME depends on interaction with mobile and stationary phase

different interaction

⇒ different time spent in column

⇒ different time spent between inlet and outlet of chromatographic system

RETENTION TIME t_R

R_F - retardation factor

The fraction of the sample component in the mobile phase at equilibrium; it is related to the retention factor and other fundamental chromatography terms

$$R_F = \frac{u_A}{u} = \frac{(n_A)_m}{(n_A)_m + (n_A)_s}$$

$n = m / M_r$

$(n_A)_s \to 0$ analyte molecule all the time in MP = no retention $\to R_F \to 1{,}0$

$(n_A)_m \to 0$ analyte molecule remains in SP $\to R_F \to 0$

R_F – measure of presence probability of compound A in MP

Relation between R_F a k ?????

$k_A = (n_A)_s / (n_A)_m$

$$R_{F(A)} = \frac{(n_A)_s / k_A}{(n_A)_s / k_A + (n_A)_s} = \frac{1}{1 + k_A}$$

HOW TO OBTAIN k ???

$$u_A = u \cdot R_F = \frac{u}{1+k}$$

$$\frac{L}{t_{R,A}} = \frac{L}{t_M (1+k)}$$

$$\frac{1}{t_{R,A}} = \frac{1}{t_M (1+k)}$$

$$t_M + t_M \cdot k = t_{R,A}$$

$$k_A = \frac{t_{R,A} - t_M}{t_M} = \frac{t_{R,A}}{t_M} - 1 = \frac{V_{R,A}}{V_M} - 1 = \frac{V_{R,A} - V_M}{V_M} = \frac{V'_{R,A}}{V_M} = \frac{t'_R}{t_M}$$

$V_R - V_M = V'_R$ - **adjusted retention volume**

$t_R - t_M = t'_R$ - **adjusted retention time**

k_A can be obtained from chromatogram !!!!!!!

- velocity ratio of analyte and mobile phase
- ratio of times spent in chromatographic system for retained and unretained solute

If $V_M = V_m$

$$\frac{V_{R,A} - V_M}{V_M} = K_D \frac{V_S}{V_M}$$

$$V_R = V_M + K_D V_S$$

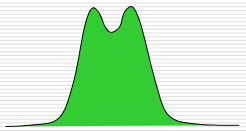
$$V'_R = K_D V_S$$

Retention volume depends on volume of stationary phase

ADJUSTMENT OF SEPARATION PROPERTIES

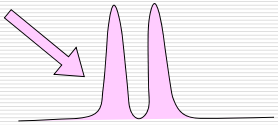
Quality of separation of sample components

Suitable combination of retention times difference and zone width of separated components

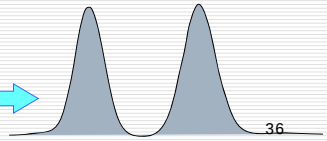


Insufficient zone resolution

Influence of zone width (transport of analyte through column)
kinetic aspect of separation



Influence of magnitude of interaction of components with MP and SP (migration velocity)
thermodynamic aspect of separation

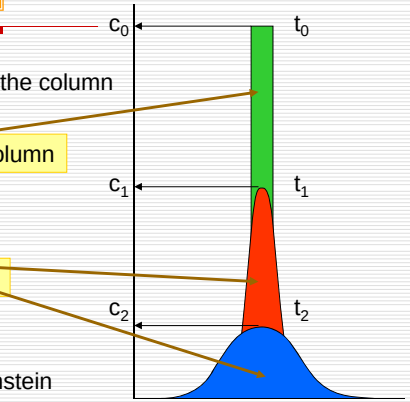


KINETIC ASPECT OF SEPARATION

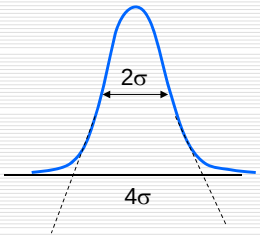
Zone broadening
during movement of component through the column

Injection of analyte into the middle of column

Zone broadening caused by diffusion



σ – standard deviation



$\sigma^2 = 2Dt$ Einstein

σ^2 – square of distance covered by component molecule in time t ~ *standard deviation*

peak width in the inflection points= 2 **σ**

the segment of the peak base intercepted by tangents drawn to the inflection points on either side of the peak = 4 **σ**

EFFICIENCY OF CHROMATOGRAPHIC SEPARATION
Efficiency of chromatographic column

Ability of system to separate sample components to independent zones of individual component during elution.

Measure of efficiency – peak width ~**σ**

Expression – Number of theoretical plate N

Plate - part of chromatographic bed or column
makes possible to establish dynamic equilibrium between component fractions in mobile and stationary phase.
The faster equilibrium, the higher plate number

From theory of chromatographic plate:

$$N = \left(\frac{t_R}{\sigma_t}\right)^2 = \left(\frac{V_R}{\sigma_V}\right)^2 = \left(\frac{L}{\sigma_L}\right)^2$$

σ_V, σ_t, σ_L
standard deviations in consistent units

$\sigma^2 = 2\ D.\ t$

Higher **σ** ⇒ lower number of separated components in time
lower separation efficiency

HOW TO CALCULATE EFFICIENCY OF CHROMATOGRAPHIC SEPARATION?

Peak width measurement:

a) peak-width at base

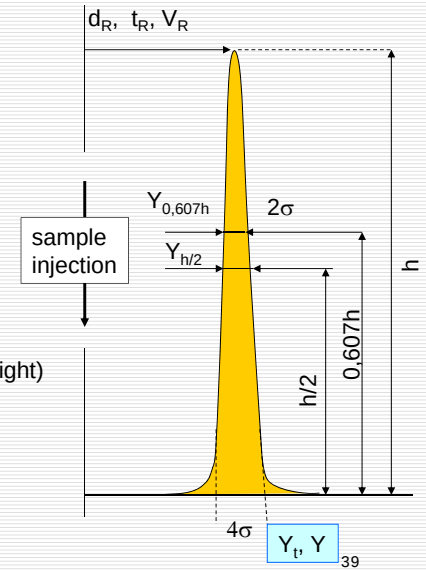
$$N = 16\left(\frac{t_R}{Y_t}\right)^2 = \left(\frac{d_R}{Y}\right)^2 \qquad Y = 4\sigma$$

b) peak-width at half height $Y_{h/2}$:

$$N = 5{,}545\left(\frac{d_R}{Y_{h/2}}\right)^2 \qquad Y = 2{,}355\sigma$$

c) peak-width at inflection points (at 0,607 height)

$$N = 4\left(\frac{d_R}{Y_{0,607}}\right)^2 \qquad Y = 2\sigma$$



d_R – retention distance
N - depends on retention time
peculiar to individual component

Effective plate number - N_{eff}

A number indicative of column efficiency calculated by using the adjusted retention volume (time) instead of total retention volume (time).

$$N_{eff} = 16\left(\frac{t_R'}{Y_t}\right)^2 = 16\left(\frac{d_R - d_M}{Y}\right)^2 = n\left(\frac{t_R - t_M}{t_R}\right)^2$$

N is usually calculated for one meter of column
10 000 plate / meter

Comparison of efficiency of different columns:

Height Equivalent to One Plate **H** *plate height*

$$H = \frac{L}{N} = \frac{LY_t^2}{16t_R^2} = \frac{\sigma^2}{L}$$

[μm]

Why is zone (peak) broadened ????

1. Theory of chromatographic plate – *unable to describe separation correctly*

2. Dynamic theory - van Deemter

Four factors causing zone broadening:

1. **eddy diffusion** in mobile phase - H_F

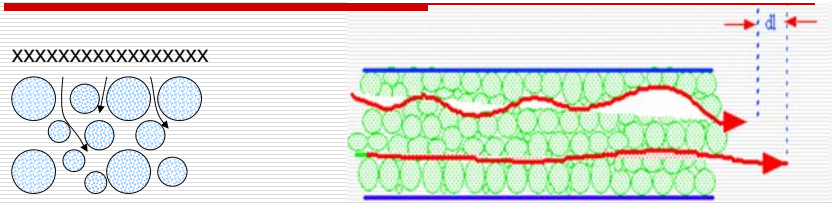
2. **molecular (longitudinal) diffusion** in mobile phase - H_L

3. **resistance to mass transfer in the stationary phase** - H_S

4. **resistance to mass transfer in the mobile phase** - H_M

$H = H_F + H_L + H_S + H_M$

H_F : the eddy diffusion



- Different speed of sample particles v in broad and narrow „canals“
- High number of streamlines, different local speed

Eddy diffusion is proportional to
dimension and shape of stationary phase particles

Lower particle: higher bed homogeneity
⇓
lower differences in „canals“

Flow-round: different for spherical particles and irregular

Zone dispersion σ^2 during chromatographic process involving high number of random steps ,

generally $\sigma^2 = l^2.n$

l = average length of random steps
n = number of random steps

- speed of moving zone **u**
- molecule remains time t_e in streamline with speed **u***
- deviation from midpoint of zone $t_e(u^*-u)$ - length of random step
- difference u*-u is proportional to average speed

- number of steps $n = L/t_e u$
- $L/t_e u$ is proportional to particle diameter d_p

$H_F = 2\lambda d_p$

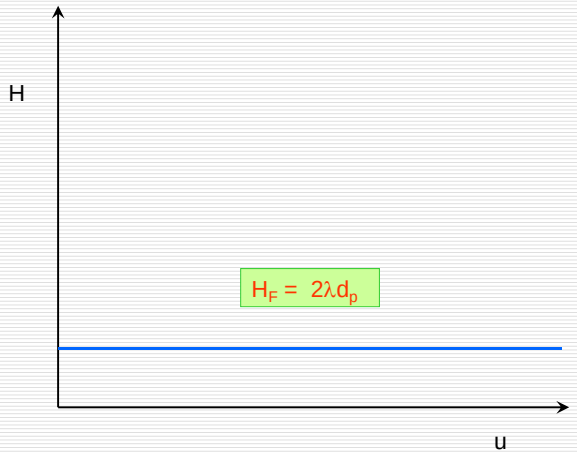
λ - geometrical factor
d_p - diameter of sorbent particle

$l = (u^*-u)\, t_e$
$u^*-u = \alpha u$
$n = L/ut_e = L/\beta d_p$

α,β = constants of proportionality

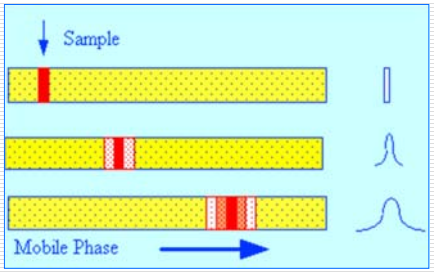
$\sigma_L^{\,2} = \alpha^2\beta\, d_p L = 2\lambda d_p L$

The eddy diffusion is not proportional to linear velocity of mobile phase
⇓
$H_F \sim u \rightarrow$ parallel line with x-axis



H_L : molecular diffusion

- at both sides of zones
- transport of molecules from higher concentration area to lower concentration area



diffusion proceeds:
- in the direction of mobile phase flow and in opposite direction
- only in longitudinal axis of column

Fick's law: $\frac{dn}{dt} = -D\frac{dc}{dx}$

Einstein's law $\sigma^2 = 2D_m t_M$

total time of molecule diffusion in mobile phase:
- independent on retention,
- identical with hold-up time

σ^2 - square of distance covered by component molecule in time t ~ *standard deviation*

$u = L / t_M$

$\sigma^2 = 2D_m L / u$ diffusion in „free" mobile phase (capillary column)

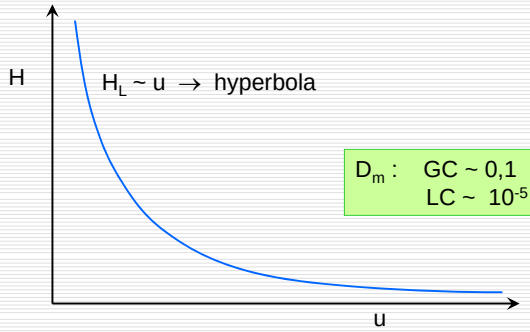
$H = \sigma^2 / L$

$H_L = 2 D_m / u$

$H_L = 2\psi D_m / u$ packed column

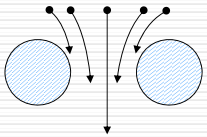
ψ - factor expressed impossibility of „free diffusion in columns proportional to quality of bed (canal shape,...)

packed



D_m : GC ~ 0,1 (1,0) $cm^2 . s^{-1}$
LC ~ 10^{-5} $cm^2 . s^{-1}$ ← can be vanished

H_M : mass transfer in mobile phase

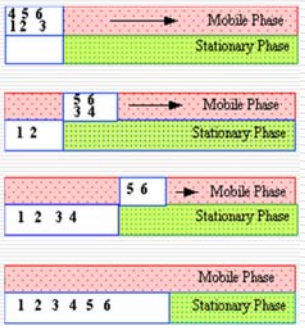


Different speed near surface or capillary wall

$$H_M = \frac{\omega d_p^2}{D_m} u$$

ω - factor dependent on packing type ~ 1,3

H_M ~ u → line

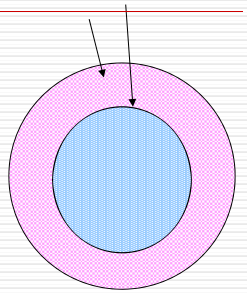


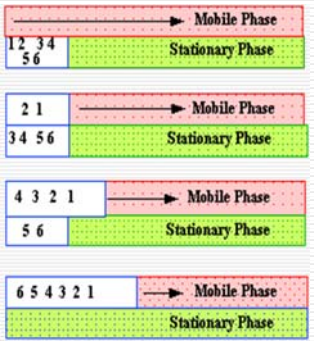
Different speed of sample molecules in MP towards to SP surface

H_S : mass transfer in stationary phase

Molecules penetrate into layer of SP and back.
Molecule entering deeper falls behind molecule entering only under surface.

Sample zone in MP runs faster than zone in SP





$$H_S = \frac{q d_f^2 u}{D_S} \frac{k}{(k+1)^2}$$

q - configuration factor – packing geometry
d_f - stationary phase width
D_S - diffusion coefficient of molecule in SP
H_S ~ u → line

TOTAL EFFICIENCY OF CHROMATOGRAPHIC SYSTEM

$$H = H_F + H_L + H_S + H_M$$

$$H = 2\lambda d_p + \frac{2\psi D_m}{u} + \frac{qd_f^2 u}{D_S}\frac{k}{(k+1)^2} + \frac{\omega d_p^2 u}{D_m}$$

A B/u Cu

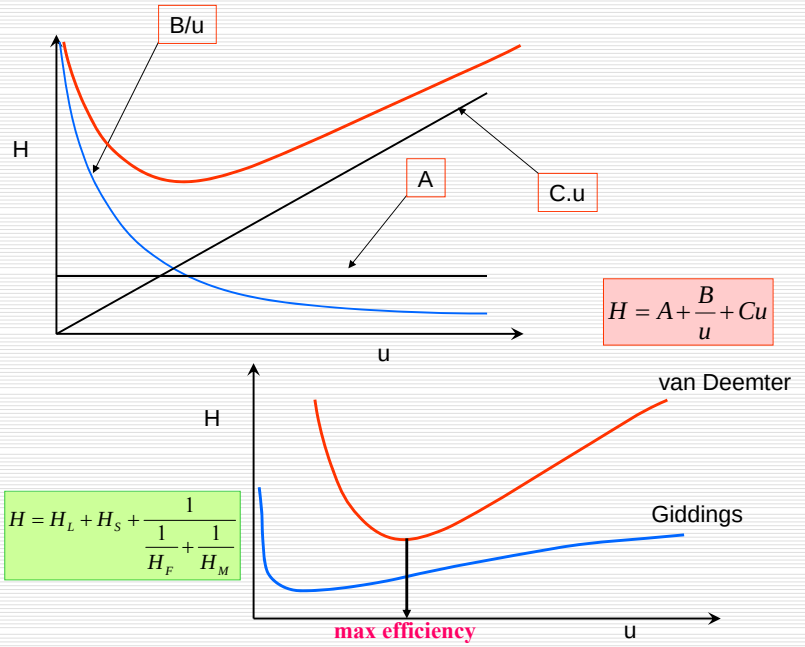
$$H = A + \frac{B}{u} + Cu$$

for GC van Deemter

High D_m in gas phase → low H_M

$$H = H_L + H_S + \frac{1}{\frac{1}{H_F} + \frac{1}{H_M}}$$

for LC Giddings



$$H = 2\lambda d_p + \frac{2\psi D_m}{u} + \frac{qd_f^2 u}{D_S}\frac{k}{(k+1)^2} + \frac{\omega d_p^2 u}{D_m}$$

$$H = A + \frac{B}{u} + Cu$$

GC
A: open tubular column → eliminated
B: significant owing to high diffusion coefficients (high diffusion in gas phase)
C: thin films → lower
H: ~ 0.1 mm

LC (HPLC)
A: packed columns, high-homogenous particles, high pressure → lower
B: significant owing to relatively high diffusion coefficients in liquid phase (lower than in GC)
C: thin films → lower
H: ~ 0.1 mm

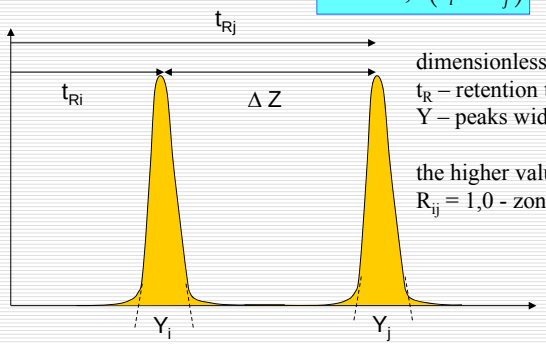
CE
A: open tubular column → eliminated
B: significant owing to relatively high diffusion coefficients in liquid phase
C: only one „phase“ , no equation between phases → eliminated
H: ~ 0.001 mm

PEAK RESOLUTION

Measure of relative separation of two adjacent zones - peaks

$$R_{ij} = \frac{t_{R_j} - t_{R_i}}{0{,}5(Y_i + Y_j)}$$

$$R_{ij} = \frac{2\Delta Z}{Y_i + Y_j}$$

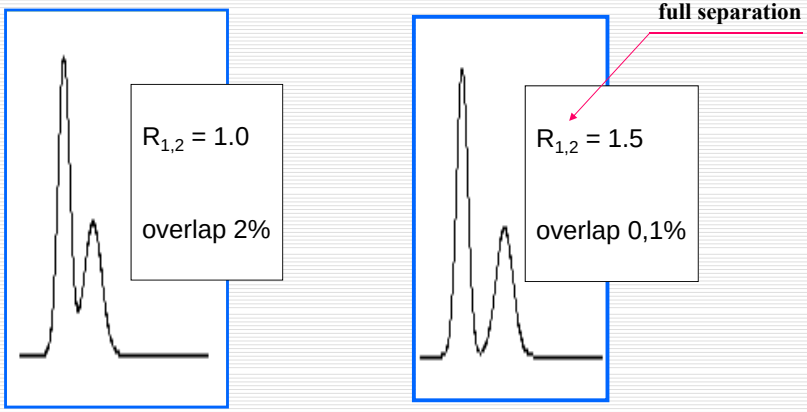


dimensionless number
t_R – retention times
Y – peaks width on base.

the higher value R_{ij} , the better separation
R_{ij} = 1,0 - zones overlap by 2%

PEAK RESOLUTION

Measure of relative separation of two adjacent zones - peaks



Effect of chromatographic parameters on resolution

- difference of elution times ~ thermodynamics of separation
- zones (peaks) width ~ kinetics of separation

$$R_{ij} = \frac{t_{R_j} - t_{R_i}}{0,5(Y_i + Y_j)}$$

$$t_R = t_M (1 + k)$$

$$n = 16 \left(\frac{t_R}{Y_t} \right)^2$$

$$Y_t = \frac{4}{\sqrt{n}} t_R$$

$$R_{ij} = \sqrt{n} \frac{t_M (k_j - k_i)}{4 t_{R_i}}$$

If $Y_2 = Y_1$

k_i - retention factor (capacity ratio) of component ***i***

α - k_i / k_j - separation factor

$$R_{ij} = \frac{\sqrt{n}}{4} \frac{(k_j - k_i)}{(1 + k_i)}$$

$$R_{ij} = \frac{\sqrt{n}}{4} (\alpha - 1) \frac{k_i}{1 + k_i}$$

$$R_{ij} = \frac{\sqrt{n}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \frac{1 + k_j}{k_j}$$

THREE INDEPENDENT TERMS AFFECTING RESOLUTION

$$R_{ij} = \frac{\sqrt{n}}{4} (\alpha - 1) \frac{k_i}{1 + k_i}$$

KINETIC TERM

can be changed by:

- mobile phase velocity
- column length
- sorbent particle diameter
- diffusion coefficients

THERMODYNAMIC TERM

can be changed by:

- change of MP and/or SP

CAPACITIVE TERM

can be changed by:

- amount SP in the column
- temperature
- change of MP and/or SP

GAS CHROMATOGRAPHY

Mobile phase: gas – inert – transport of components – carrier gas
hydrogen, nitrogen, helium, argon, CO_2
according to type of detection, influence on separation efficiency

Carrier gas	D_G (30 °C)	η (50 °C)	η (150 °C)
H_2	0.277	94	112
He	0.248	208	249
N_2	0.073	188	227
Ar	0.059	242	296
CO_2	0.059	162	206

Diffusion coefficients D_G ($cm^2 s^{-1}$) of n-octane and viscosity η (μP) of most used carrier gases

H_2

disadvantage: explosive in mixture of air - Attention

advantage: fast separation (low analysis time), separation of strong retained components

Sample components to be separated, main application:

- gas mixtures
- volatile organic compounds b.p. < 400°C
  (requirement for sample conversion into gas phase)

Interaction with stationary phase:

- Adsorption gas chromatography *GSC* — gas-solid phase
  gases, liquids (low M_r)
- Partition gas chromatography *GLC* — film of non-volatile liquid on the surface of solid carrier

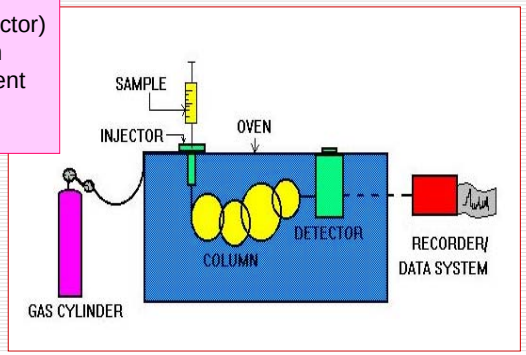
Elution:

- isocratic — constant temperature
- gradient — variable temperature

INSTRUMENTATION in Gas Chromatography

Gas Chromatograph Components

- carrier gas source
- sample introduction (injector)
- chromatographic column
- thermostated compartment
- detector
- data station



INSTRUMENTATION in Gas Chromatography

Sources of carrier gas:
gas cylinders
generators (molecular sieves)

couplings, connections :
metal capillary, ideal gas tightness

gas purifying :
moisture, low molecular hydrocarbons and oxygen removing

flow regulation :
a) mechanical regulators
b) electronic regulators

gas flow : 1 - 100 ml/min., ± 1 - 2 % accuracy
± 0.2 % repeatability of set value

TEMPERATURE MODES

Isocratic GC - Isotherm

Two basic characteristics:

retention data ≅ number of methylated groups (-CH_2-)
(vapour pressure, boiling point)

≅ $1/T_c$ (log t_R = f ($1/T_c$)
linear dependence of homologous series
(n-alkanes, n-alkylbenzenes, …)

T_c – *column temperature*

disadvantages :

- ☐ it is not possible to find temperature suitable for separation of components with b.p. difference than 100 °C
- ☐ poor separation of early eluted peaks
- ☐ poor delectability of later eluted peaks (*broadened peaks*)

Gradient GC – *Temperature programming*

- analytes retention times decreasing in samples with broad range of boiling points
- improving of delectability
- large volumes of injected samples
- ***optimum:*** temperature slightly over average b.p. of sample components

temperature stability: ± 0,1°C, change: about 1°C, temperature range: up to 450°C

INJECTORS for GC

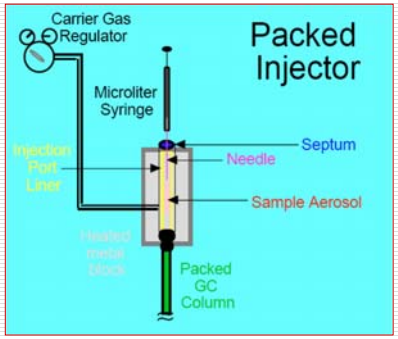
Function:
- ✓ Sample injection on the column beginning as a narrow zone
- ✓ Conversion of sample into the gas phase
- ✓ Mixing of sample and carrier gas ahead of column entry

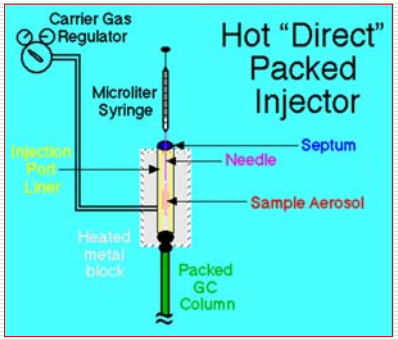
Requirements (ideal) :
- ➢ injection without decreasing of separation efficiency
- ➢ injection without temperature degradation and sample adsorption
- ➢ injection without discrimination according to b. p, polarity or M_r
- ➢ injection with total recovery of all sample components

Injection devices
depend on sample state
gases - gastight syringes, injection valves (volume up to 1 ml)
liquids - syringes, autosamplers (volume 0.5 - 5 µl)

Methods of sample injection

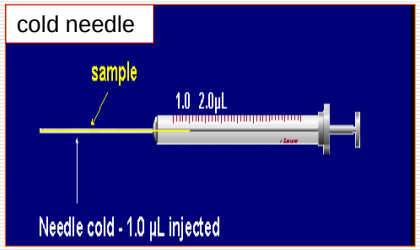
- ❑ over the column inlet – packed column
- ❑ on-column – capillary columns





Syringe injection methods

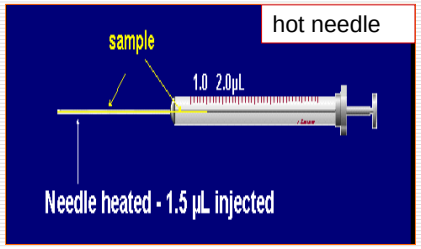
cold needle



Fast injection with
minimum delay in injector space
following fast extraction from injector.

Liquid remains in needle.

hot needle



Needle remains in hot injector space approx. 5 sec before sample injection.

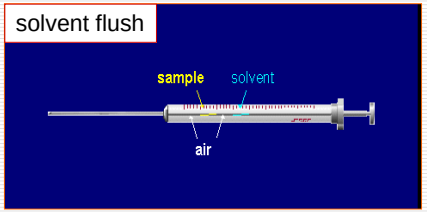
Samples with high boiling point of components.

Hot needle increases evaporation speed.

Injection of sample in needle and syringe cylinder.

Syringe injection methods

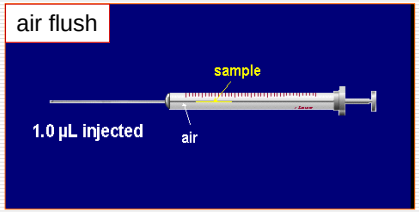
solvent flush



Small amount of solvent is full field into the syringe, following by air, sample and again air.

Polar compounds absorbable on glass and/or needle

air flush



Syringe is full field more than required volume and tip into required volume.

Liquid sample is retracted in to the syringe.

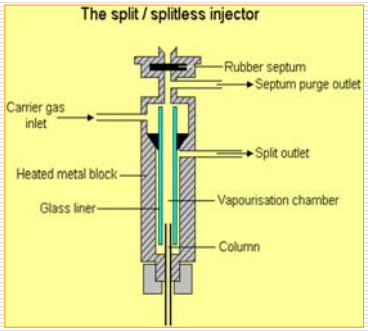
Sample is fast injected in to the GC and piston is released

Syringe is fast taken out the injector.

Complete sample injection, low loss of volatile compounds, excellent repeatability

Methods of sample injection into capillary columns

SPLIT – *flow splitter*
- high number of components
- < 10% of sample

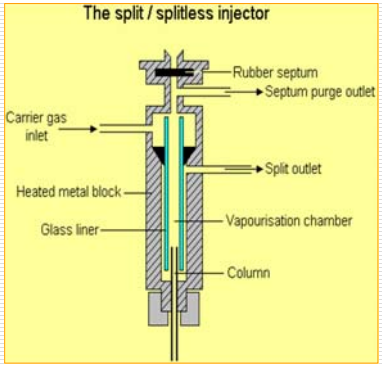


Use :
- qualitative analysis at high chromatographic resolution
- less for quantitative analysis

Disadvantages :
- discrimination of compounds with higher b.p.
- loss of injected sample according to split opening

SPLIT LESS – *without flow splitter*
- diluted samples
- approx. 80% of sample

Splitter closed 1 - 2 minutes, sample enters column any longer



injection ways :
- into hot column
- into cold column (temperature 20 - 30 °C lower, than b.p. of solvent used)

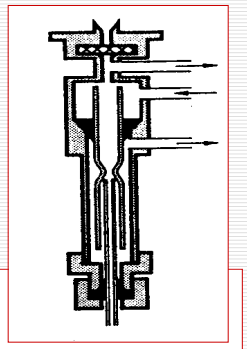
advantages :
- analysis of diluted samples without preconcentration (the whole sample is injected into a column)
- analysis of impure samples (change of injection liner and retention gap)

ON COLUMN INJECTOR
- direct into capillary column
under b.p. of solvent, thermolabile components

injection ways

- direct on column (micro syringe with thin needle,
- injection into short column with i.d. 0.53 mm)

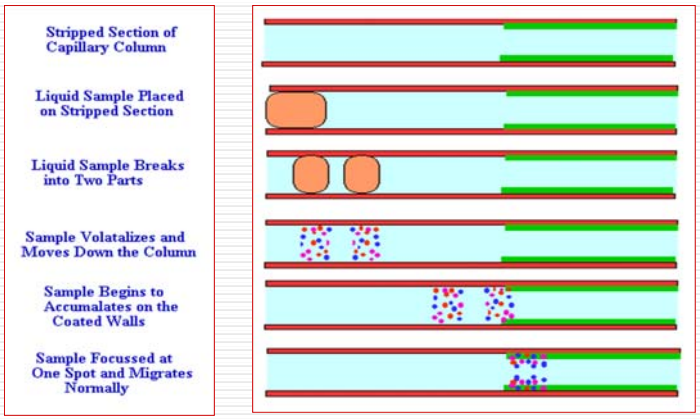
temperature 20 - 30 °C lower, than b.p. of solvent used



difficulties:
- Sample could be broken into separated parts.
- Bubbles generated et the inlet of column separate sample into different pars of column depending on way of solvent evaporation
- Zone with different concentration are formed, each behaves as individual injection.
- Chromatogram with more peaks of the same component.

Retention gap

reduction of the length of submerged zone in capillary column
(deactivated fused silica capillary, 1 - 10 m)



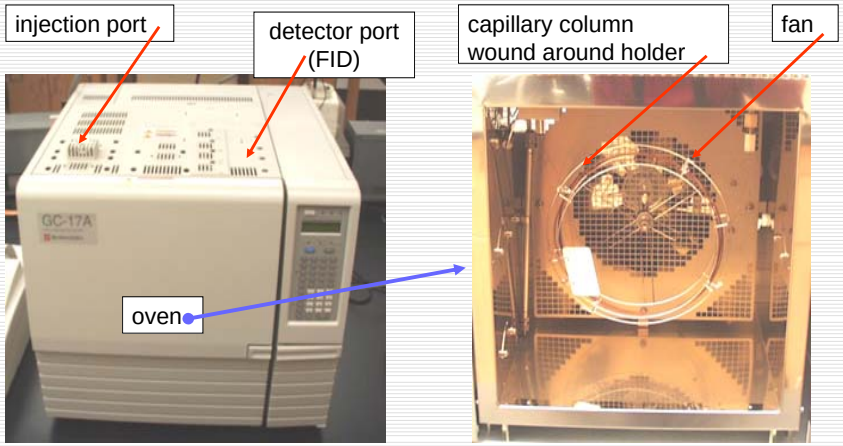
PTV INJECTOR (programmed temperature vaporization)

➢Control of temperature of injector body with liner packed with chromatography bed.
➢advantage combination of split, split less and on-column injectors.

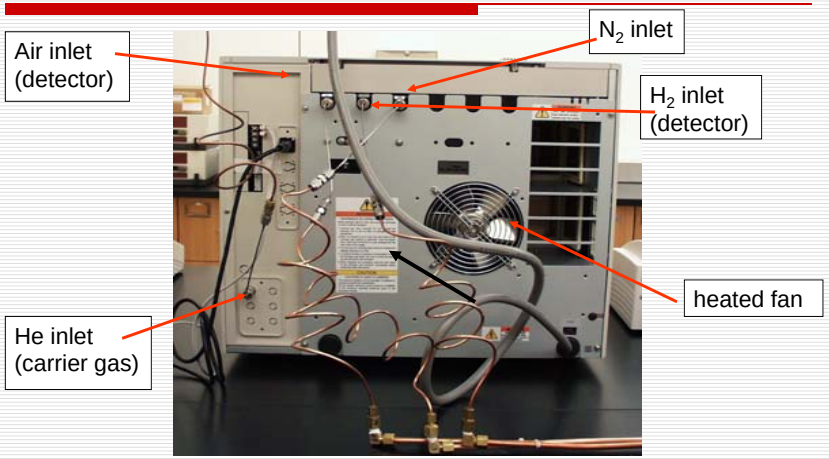
advantages

- minimum discrimination caused by injection from needle of microinjector
- minimum discrimination according to b.p. of analytes
- not necessary to use special needle as for on column injection
- large volume injection
- elimination of solvent and low molecular compounds before analysis
- retention of non-volatile compounds in injection liner
- high repeatability of retention times and peaks areas.

GAS CHROMATOGRAPH



GAS CHROMATOGRAPH



COLUMN FOR GC / STATIONARY PHASES

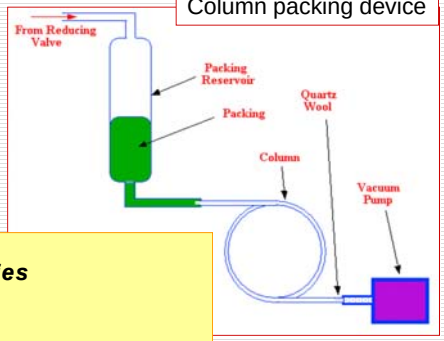
PACKED COLUMNS:

tubes:	Al, Cu, Ni, stainless steel, glass, 2-6 mm, 1-5 m
particles:	0,13 až 0,40 mm
adsorbents *(GSC):*	silica gel, alumina, active carbon, molecular sieve, porapak (styren-divinylbenzen copolymer)
bonded phases *(GLC):*	non-volatile, chemically inert liquids siloxane, polyethylenglykole, esters, hydrocarbons (squalan, $C_{30}H_{62}$),silicone
inert carriers*:*	siliceous earth, modified siliceous earth

Properties of packed columns:
worse resolution
higher volume of stationary phase, preparative purposes
low-boiling components, less retained gases

Chromatographic carriers for stationary phases

Column packing device



Porous / nonporous particles of suitable chromatographic properties

Particles:
- 0.5 - 0.1 mm
- narrow distribution of particle diameter
- low specific surface (1 - 7 $m^2.g^{-1}$)
  suppression of adsorption
- sufficient porosity (0.1 - 1 $ml.g^{-1}$)
  good wetting by liquid SP

Properties:
- inert
  chemical reactivity – peak deformation or disappearance
  specific adsorption – peak tailing

Chromatographic carriers for liquid stationary phases Sorbents

Graphitized active carbon *(heated to 3000 K)*
non-porous, non-specific, high inert material
adsorbent and solid carrier
Carbopack , Carbotrap, Spherosil, Spherocarb

Silica gels
particles based on silicon dioxide
activity depends on amount of adsorbed water
Porasil, Chromosil, Chromosorb T, Chromosorb G, Supelcoport

Activated alumina
particles based on aluminium oxide

Siliceous earth

Siliceous earth micro amorphous silicate rock (formation) treated by calcinations at 1000 °C, sintered particles separated according to size

Molecular sieves
aluminosilicate, zeolite

Graphitized molecular sieves
separation of permanent gases and low molecular organic compound
Carbosieve, Carboxen, Supelcarb

Polymers
STY-DVB, Acryl ester, Polystyrene, EVB-DVB, ACN-DVB
Chromosorb (polar copolymer), Durapak, Porapak, Tenax

Teflon PTFE

Liquid stationary phases for GC

requirements:

- ***low volatility*** (vapor pressure 1 - 10 Pa)
- ***suitable chemical composition*** (from non-polar to polar phase)
- ***good solubility of separated components*** in the liquid phase
- ***different solubility of separated components***
- ***no chemical reaction*** with analytes

- High molecular hydrocarbons
- Perfluorinated alkanes
- Polysiloxanes
- Polyethylenglykoles
- Polyfenylethers, Phtalates,
- Liquid crystals

Liquid stationary phases for GC

	Type	Product name	Temperature °C	Use	Properties
High molecular hydrocarbons	Non-polar	Squalan Apiezon	< 300	Hydrocarbons Non-polar compounds	easy oxidizable
Perfluorinated alkanes	Polar Fluorinated alkylesters	Fomblin Fluorad	< 250	Halogens Amines Phenols Carboxylic acids	High reactivity Low temperature stability
Polysiloxanes	- R_2SiO – Variable polarity	OV SE	< 350	Non polar-polar	High temperature stability easy oxidizable
Polyethylenglykoles $HO(CH_2CH_2O)_n CH_2CH_2OH$	Polar	Carbowax Superox	<220 < 300	oxygen compounds Alcohols	Low temperature stability easy degradable

Selected commercially available polysiloxane phases

Mark	Type	Structure
OV-1	Dimethylsiloxane	CH_3
OV101	Dimethylsiloxane	CH_3
OV-7	Phenylmethyldimethylsiloxane	C_6H_5 (20%)
OV-17	Phenylmethylsiloxane	C_6H_5 (50%)
OV-25	Phenylmethyldiphenylsiloxane	C_6H_5 (75%)
OV-210	Trifluoropropylmethylsiloxane	$CH_2CH_2CF_3$ (50%)
OV-225	Cyanopropylmethylsiloxane	C_6H_5(25%)
OV-275	Dicyanoalkylsiloxane	C_3H_6CN(20%)

Liquid crystals

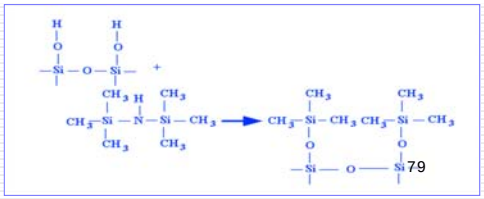
- polar liquids
- strong columbic interaction of ions with analyte
- positional isomers

Cation	Anion
Tetrabutylamonium	Perfluorooktansulfonate
	4-toluensulfonate
	Tetrafluoroborate
	Picrate
Tetrapentylamonium	4-toluensulfonate
Tributylbenzylphosphonium	Chloride
Ethypyridinium	Bromide

Bonded liquid phases

carrier	stacionary phase (w/w)	polarity	temperature limit (°C)	use
Porasil C	3-hydroxypropionitrile (3%)	middle	135	hydrocarbons, aromates
Porasil C	Carbowax 400 (7.86%)	non-polar	175	alcohols
Porasil C	n-Octanol	polar	175	alcohols, hydrocarbons
Porasil S	Carbowax400 (16.75%)	non-polar	230	hydrocarbons
Porasil S	Carbowax 4000	polar	230	aromates, chloraromtes
Porasil F	Carbowax 400 (1.41%)	non-polar	230	waxes,steroids, PAHs

- Porasil C (100 m^2/g, pores 30 nm)
- Porasil S (300 m^2/g)
- Porasil F (10 m^2/g, pores 300 nm)

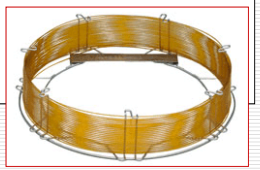


CAPILLARY COLUMNS

- capillaries with low inner diameter wetted by thin layer of liquid
- efficiency 100 times higher than packed columns.

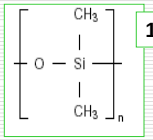
Marcel Golay 1957

- glass, fused silica, organic polymers (PAD, PES, PTFE, FEP)
- metals (stainless steel, Ni, Al, Cu)
- i. d. 100 – 530 μm (700)
- length 15 – 100 m
- layer 0.1 - 10 μm

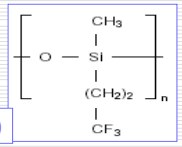


According to stationary phase immobilization

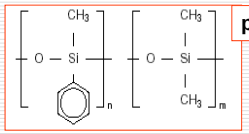
- classical capillary columns
  disperse forces-bonded stationary phase,
  permanent dipoles, induced dipoles, hydrogen bonds, ….
- capillary column with bonded phase
  SP chemically bonded to inner wall of capillary column.



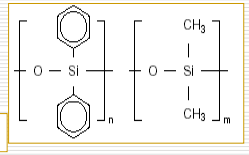
100% dimethylpolysiloxane



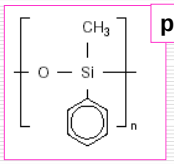
trifluoropropyl-methylpolysiloxane (trifluoropropyl)



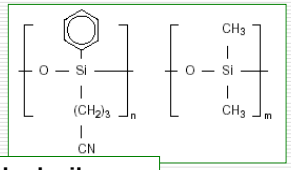
phenyl –dimethylpolysiloxane



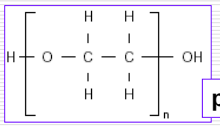
diphenyl –dimethylpolysiloxane



phenylmethylpolysiloxane



cyanopropyl-phenyl –dimethylpolysiloxane



polyethylene glycol

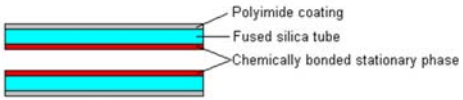
CAPILLARY COLUMNS with NON- FILLED FREE SPACE

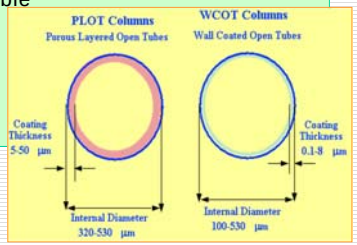
PLOT ***(Porous Layer Open Tubular*** column***)***
thin layer of solid sorbent (10 µm) on inner wall

WCOT ***(Wall Coated Open Tubular*** column***)***
thin film (0.01 - 1 µm) of SP created directly on inner surface of column,
small inner diameter (narrow bore) - pod 0,1 mm I.D.
conventional diameter - 0.32 - 0.15 mm I.D.
big inner diameter (wide bore) - 0.53 mm I.D.

FSOT ***(Fused Silica Open Tubular*** column***)***
- type of WCOT, thinner film, smaller diameter
- rigidity increased using polyimide laser, flexible
- low reactivity

SCOT (***Support Coated Open Tubular*** column***)***
thin layer of carrier (1 - 5 µm) wetted by SP on inner wall of capillary





CAPILLARY COLUMNS FILLED in WHOLE VOLUME

Advantage:
column capacity improving

packed column with floating and irregular packing

capillary tube drawn from tube field by bulk chromatography carrier followed by wetting of stationary phase

packed column with regularly loaded bad

capillary packed in ultrasonic bath using inert gas

DEACTIVATION

elimination of ***negative effect of silanol groups*** from inner surface of capillary

silylation
aliphatic or cyclic silylation dyes

polycondensation
reaction of silanol groups with deposited thin layer film of Carbowax or silicone stationary phase at high temperature

esterification
reaction of silanol groups with aliphatic alcohols C4 - C10 and tetraethylglycole at high temperature

polyimide layer
layer of polyimide (1 - 5 µm) on the inner surface of capillary

Retention time in gas chromatography

- Gas compressibility - change of mobile phase volume
- Decry's law for liquid flow trough nonporous bed

$$u = -\frac{B_o}{\varepsilon_o \eta}\frac{dp}{dz}$$

Bo - specific permeability constant
ε_o - antiparticle porosity
η - liquid viscosity
dp/dz – fall in pressure in the flow direction

$$u = \left(\frac{B_o}{\varepsilon_o \eta L}\right)(p_i - p_o)$$

p_i a p_o - absolute pressure at inlet and outlet
L - column length

pro GC

$$u(p_o) = \left(\frac{B_o}{\varepsilon_o \eta L}\right)\frac{(p_i^2 - p_o^2)}{2\,p_o}$$

$u(p_o)$ – velocity at pressure **p_o**,

$$u(\bar{p}) = \left(\frac{B_o}{\varepsilon_o \eta L}\right)\frac{(p_i^2 - p_o^2)}{2\,\bar{p}} = \frac{u(p_o)p_o}{\bar{p}} = u(p_o)j$$

$$\bar{p} = \frac{2}{3}\frac{(p_i^3 - p_o^3)}{(p_i^2 - p_o^2)}$$

$\bar{p}$ - middle pressure in column
$u(\bar{p})$ - velocity at middle pressure
j - James-Martin's compressibility factor

$$j = \frac{3\left[\left(\frac{p_i}{p_o}\right)^2 - 1\right]}{2\left[\left(\frac{p_i}{p_o}\right)^3 - 1\right]}$$

$$t_R = \frac{L(1+k)}{u} = \frac{L(1+k)}{u(p_0)\,j}$$

Hold-up volume
Dead volume

retention volume - corrected to 0°C
- correlated to → mass unit (m_s) of stationary phase V_g
- correlated to → surface unit (S) of adsorbent V_S

$$V_g = \frac{j(t_R - t_M)F_m.273}{m_s.T_c}\left[ml\,.g^{-1}\right]$$

$$V_S = \frac{j(t_R - t_M)F_m.273}{S.T_c}\left[ml\,.g\,.m^{-2}\right]$$

Values of specific retention volumes (times) affected by experimental errors:

V_g – dependent on accurate mass determination of used SP
loss of liquid SP as o result of volatility

Detectors - classification

according to the basis of response:

concentration-sensitive - response depends on change of sample concentration in detector (g/ml) ***TCD, ECD PID***

mass-flow-sensitive - response depends on mass component in detector (g/s) ***FID, TID, FPD***,

according to detector selectivity

universal - response to every component in the effluent except the mobile phase
selective - response to a related group of sample component in the effluent
specific - response to a single component or to a limited number of components having similar chemical characteristics

according to detection principle

ionization of molecules	(FID, TID, PID, ECD, HID)
bulk physical properties of molecules	(TCD)
optic properties of molecules	(FPD)
electrochemical properties of molecules	(HECD)

Detectors - requirements

- high sensitivity
- low limit of detection
- good stability and repeatability of signal
- low noise and drift of signal
- fast response independent on flow of MP
- linear response over several orders

A – peak area
F – flow through detector
w – sample amount

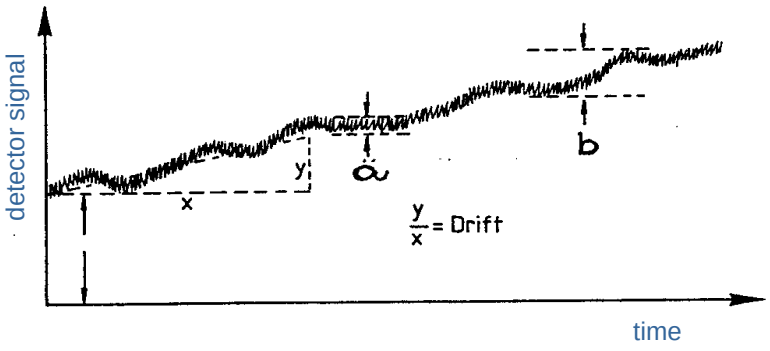
sensitivity – slope of calibration curve

S = A . F/ w (detector with concentration response)
S = A / w (detector with mass response)

Limit of detection - the lowest concentration or amount of detected compound which caused signal magnitude three times higher than magnitude of detector noise

Signal noise - changes of output signal (base line) not caused by eluted sample component

a) *short-time* - frequency higher than eluted peak frequency
b) *long-time* - frequency similar to eluted peak frequency



Signal drift – frequency lower than frequency of eluted peaks

Linear dynamic response range of detector

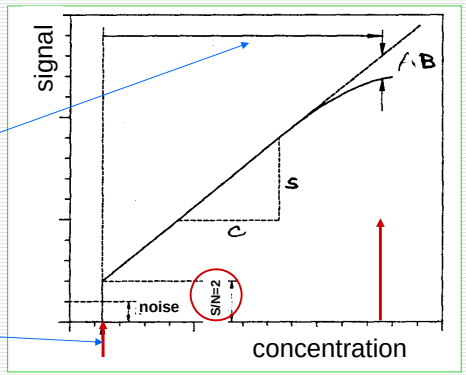
Range of concentration or mass flow of analyte with linear response

Upper limit

five percent deviation of signal from linearity

Lower limit

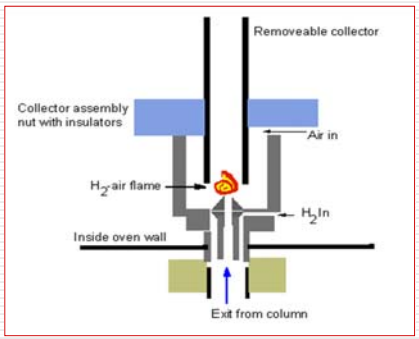
peak height equal to double height of signal/noise ratio



Flame ionization detector - FID

working principle

- ✓ hydrogen-air flame between two electrodes
- ✓ difference in potentials ≈ 500 V
- ✓ burning of organic compounds – ion generation
- ✓ ions increase flame conductivity
- ✓ generated current equal to concentration of organic compound in carrier gas



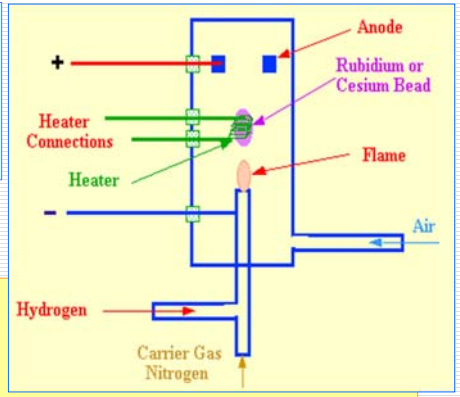
- organic compounds detection
- limit of detection (up to 10^{-11} mol)
- linear dynamic range (up to 10^{7}).
- low sensitivity for :
  inert gases,
  hydrogen, nitrogen, oxygen,
  chlorine, ammonia,
  sulphane, water, CO_2
- small hold-up volume

Termionic ionization detector - TID

Alkali-flame ionisation detector - AFID *(FID with alkali metal)*

Nitrogen phosphorus detector – NPD

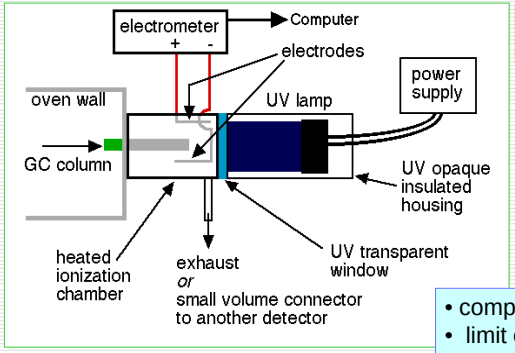
- selective for N, P, S, B and halogenated compounds
- pesticide, drugs
- selected metals (Sb, As, Sn, Pb)
- limit of detection (10^{-13} - 10^{-14} mol)
- linear dynamic range (10^5)



High noise
Low signal stability

working principle
- ✓ similar to FID detector
- ✓ carrier gases N_2, O_2, air, air + H_2
- ✓ alkali metal salt (Rb_2SO_4) in bead on platinum wire or ceramic ring
- ✓ difference i potentials ≈ 180 V
- ✓ plasma production in bead ≈ 800°C ⇒ high amount of ions, high current

Photoionization detector - **PID**

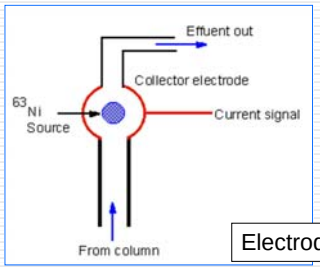


$R + h\nu \longrightarrow R^+ + e^-$

- compounds absorbed UV radiation
- limit of detection (10^{-14} mol)
- linear dynamic range (10^7).

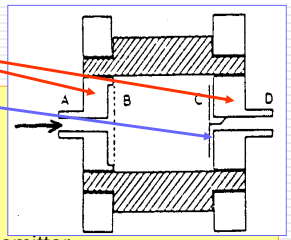
working principle
- ✓ absorption of radiation emitted from UV lamp
- ✓ ion current detection in ionization chamber
- ✓ non-destructive detector

Electron-capture detector - **ECD**



- detection of compounds generated stabile ions: *alkylhalogenide, carbonyle compounds, nitrile, organometalic compounds, water vapour compounds with P and S, NO_2, oxygen, ozone*
- halogenated compounds
- limit of detection (10^{-15} mol)
- linear dynamic range (10^5)

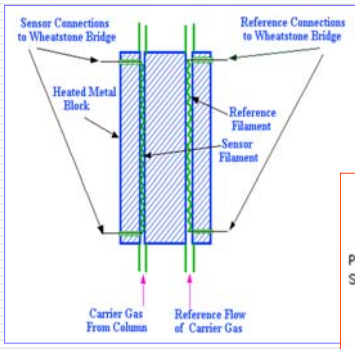
Electrodes (anode, cathode)



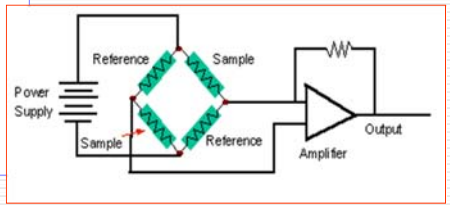
β-emitter

working principle
- ✓ carrier gases N_2, H_2, He
- ✓ proportional counter for measurement of intensity of radiation
- ✓ beta-source (^{63}Ni, ^{3}H on Pt or Ti layer).
- ✓ ionization of carrier gas caused by electron from β –emitter
- ✓ strong production of „thermal electrons“.
- ✓ absorption of thermal electrons during collision with positive charged particles
- ✓ decreasing of number of negative charged particles → current decrease

Thermal conductivity detector -**TCD** (catarometer)

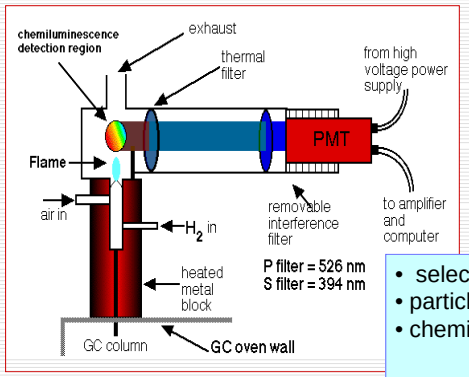


- organic and inorganic compounds
- non-hydrocarbons gases
- low-molecular hydrocarbons
- limit of detection (10^{-8} mol)
- linear dynamic range (10^4)



working principle
- ✓ thermal conductivity changes of carrier gas outgoing from column
- ✓ heated Pt, W, Au fibre
- ✓ measurement of temperature changes of sensor (thermistor, metal fibre)

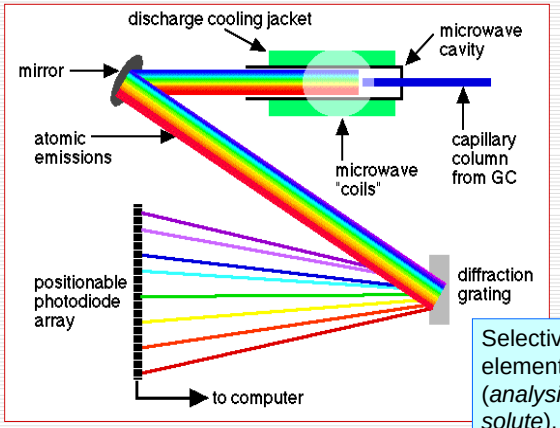
Flame photometric detector - FPD



- selective for compounds with S or P
- particles S_2 or POH
- chemiluminiscence blue (394nm) green (526nm). compounds N, halogens, B, Se, Ge
- limit of detection (10^{-13} g P/s, 10^{-12} g S/s)
- linear dynamic range (10^4).

working principle
- ✓ similar construction as FID,
- ✓ separated optical system and photomultiplier
- ✓ excitation of molecules in flame
- ✓ emission of specific irradiation

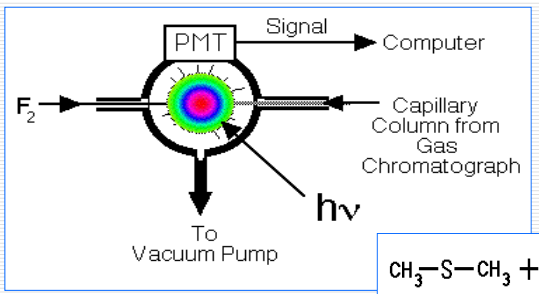
Atomic emission detector - AED

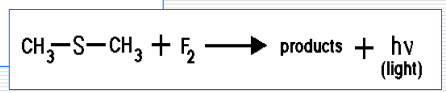


Selective detection of elements in organic molecules (*analysis of elements in solute*).

working principle
- ✓plasma source – excitation of atoms (C, H, D, O, N, S, P and halogens)
- ✓detection of emitted irradiation

Chemiluminiscence detector – CLD



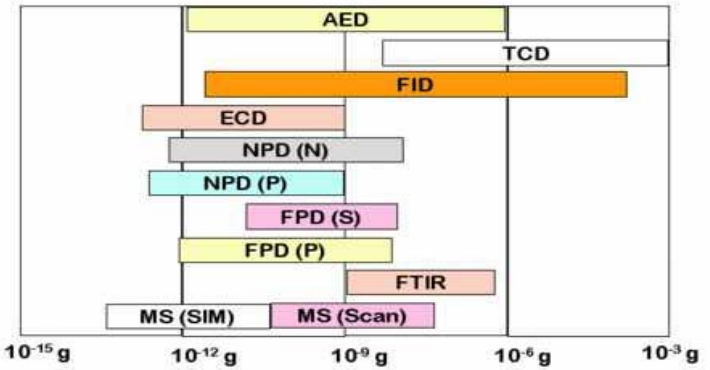


working principle
chemically produced vibration- or electron- excited particles
excited particles emit photons

Comparison of most used GC detectors

Detector type	Signal generation	Signal production	Advantages	Disadvantages	Limit of detection (g analyte /ml carrier gas)
TCD	thermal conductivity of gaseous analyte	change of electrical resistance of filament in stream of analyte	universal, simple, wide dynamic range, non-destructive number of compounds	low sensitivity	10^{-8} (10-100 ppm)
FID	ionization of analyte in H_2/air flame	current of ions	high sensitivity, wide dynamic range, broad applicability	destructive	10^{-13}
TID	ionization of analyte in H_2/air flame	current of ions	selective for org. P or N		10^{-13} Not for N a P
ECD	decreasing of ionisation of carrier gas caused by radioactive source	current of ions decreased in the presence of organic molecule	selective for org. compound with electronegative function groups, non-destructive, high sensitivity	narrow dynamic range	10^{-15}
MS	ionization of analyte	ions of analyte, separation according to mass/charge ratio	universal. complex mixture of organic compound, speed, high sensitivity, identification of compounds	price	10^{-12}

Detectors for GC
range of applicability



LIQUID CHROMATOGRAPHY

Differences in comparison with GC:

- compressibility of mobile phase is not considered
- lower influence of temperature on retention characteristics
- **active role of mobile phase**
- **85 % compounds are** non-volatile , bad volatile, unstable

„Classical" LC:
Open system, large-size particles > 100 μm, MP flow controlled by gravitation
time consumed separation – a number of hours
fractions analyzed separately Cl^-, Br^-,J^-
low efficiency

GC theory → LC theory → technique for faster LC separation → **HPLC**
HIGH PERFORMANCE LC

Result: **low particles 3-10μm 30-90 thousand plates per meter**
- homogenous filling – narrow particle distribution
- homogenous film of stationary phase

$H = H_F + H_L + H_S + H_M$

$$H = 2\lambda d_p + \frac{2\psi D_m}{u} + \frac{q d_f^2 u}{D_S}\frac{k}{(k+1)^2} + \frac{\omega d_p^2 u}{D_m}$$

$$H = A + \frac{B}{u} + Cu$$

$<d_p$: lower eddy diffusion: **< A**
$<D_m$: lower molecular diffusion in the liquid: **< B**
$> u, <d_f, >D_s$: faster mass transport between phases : **< C**

$$H = H_L + H_S + \frac{1}{\frac{1}{H_F} + \frac{1}{H_M}}$$

better for HPLC - Giddings

CHOMATOGRAPHIC SYSTEMS IN LIQUID CHROMATOGRAPHY

according to dominant mechanisms of interaction of sample components with SP and MP

LSC - liquid **adsorption** chromatography

LLC - liquid **partition** chromatography

SEC - **size exclusion** chromatography
GPC - *gel permeation chromatography*

IC - **ion** chromatography

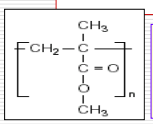
LSC - Liquid adsorption chromatography

Stationary phases for LSC - solid particles

particle character / particle property	*irregular fully porous*	*spherical fully porous*	*spherical surface porous*
specific surface	100-500 m^2/g	100-500 m^2/g	5-15 m^2/g
capacity	high	high	low
permeability	lower	high	high
efficiency	lower	high	high
area of applicability	preparative	analytical	analytical
price	low	high	high

Basic material for SP: - polar adsorbents - silica gel
- aluminium oxide

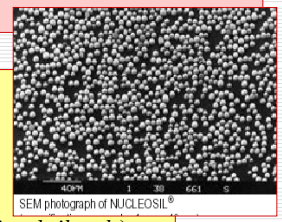
Two forms: wide pores > 10 nm surface area: 100 - 500 m^2/g
narrow pores < 10 nm > 500 m^2/g



Polymeric packing
- stabile in broad range of pH: 2 - 12
- HEMA

Alumina (aluminium oxide):

- surface - hydroxyl groups and electron-acceptor centres
  - strong electrostatic field, creation of induced dipoles
- pH = 8 - 11 - separation of weak acidic compounds from neutral
  - strong acids – chemisorption (*no requested phenomenon)*
- activation - 400 °C 6 - 16 hod, activity control with water addition
- capacity - lower than silica gel



Silica gel:

- surface - hydroxyl (silanol) groups
  - creation of H-bridges
- pH ≥ 8 - chemically labile
- pH 3 – 5 - strong retention of basic compounds
  (*protonated basic compounds + dissociated silanols*)
- activation - 180°C 3 hour

Adsorption of analytes **increases with** increasing surface activity and area
decreases with increasing mobile phase polarity
depends on geometrical distribution of functional groups

MAGNITUDE of ANALYTES POLAR INTERACTIONS and ADSORBENT
depends on analytes polarity and MP polarity

Retention order of compounds on polar adsorbents

Aliphatic hydrocarbons
Aromatic hydrocarbons
Halogenated compounds
Ethers
Tertiary amines
Nitryl
Nitro compounds
Esters of carboxylic acids

depends on type of substituent

Ketones
Aldehydes
Primary amines
Amides of carboxylic acids
Alcohols
Phenols
Carboxylic acids
Sulfonic acids

Mobile phase for LSC
Organic solvent and/or water
⇒ different polarity
MP polarity must be lower than SP polarity

NORMAL PHASE CHROMATOGRAPHY SYSTEMS
NPLC, NP-HPLC

Polarity of MP for LSC

Heptane
Pentane
Cyclohexane
Benzene
Ethyl ether
Dichloromethane
Acetone
2-propanole
water

LLC - Liquid partition chromatography

- separation of analytes between two immiscible phases (liquids)
analytes penetration through phase interface into whole space of SP – **absorption**
- similar as extraction

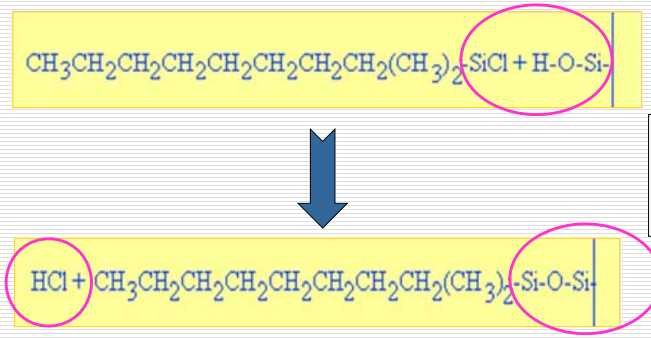
Advantage over LSC:
- retention depends on stationary phase amount
$$k = K_D V_S / V_m$$
- immobilization of different amount of SP

Liquid stationary phases:

- **mechanically immobilized** on suitable carrier (silica gel) – physically bonded
  low solubility in mobile phase → saturation column

- **chemically bonded** on reactive carrier
  - insoluble in mobile phase
  - possible use of gradient elution
  - possible temperature changes

Bonds with surface silanols



≡ Si - O - C
≡ Si - O - Si - C
≡ Si - C
≡ Si - N - C

Chemically bonded stationary phases

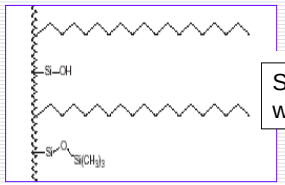
1. No polar, hydrophobic hydro carbonic

2. Polar

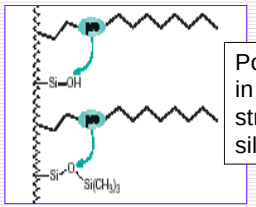
Octadecyl, base deactivated polymer modification $-(CH_2)_{17}-CH_3$	C_{18}
Octadecyl, endcapped $-(CH_2)_{17}-CH_3$	C_{18} ec
Octyl, endcapped $-(CH_2)_7-CH_3$	C_8ec
Octyl, not endcapped $-(CH_2)_7-CH_3$	C_8
Phenyl, endcapped $-(CH_2)_3-C_6H_5$	
Phenyl $-(CH_2)_3-C_6H_5$	
Butyl $-(CH_2)_3-CH_3$	C_4
Propyl	
Dimethyl $-(CH_3)_2$	C_2

Cyano (Nitrile) $-(CH_2)_3-CN$	
Nitro $-(CH_2)_3-C_6H_4-NO_2$	
Diol $-(CH_2)_3-O-CH_2-CH(OH)-CH_2(OH)$	
Amino $-(CH_2)_3-NH_2$	
Dimethylamino $-(CH_2)_3-N(CH_3)_2$	
Sulphonic acid $-(CH_2)_3-C_6H_4-SO_3Na$	SA
quaternary ammonium groups $-(CH_2)_3-C_6H_4-CH_2-N^+(CH_3)_3Cl^-$	SB

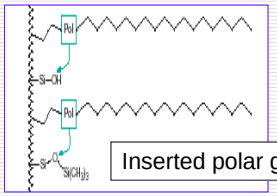
„ENDCAPPING“



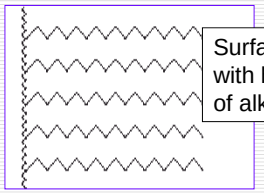
Surface of silica gel covered with SP with free silanol groups



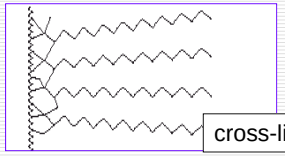
Polar protective group in carbon chain strongly interact with silanol groups



Inserted polar group



Surface coverage with high density of alkyl groups



cross-linked carbon skeleton

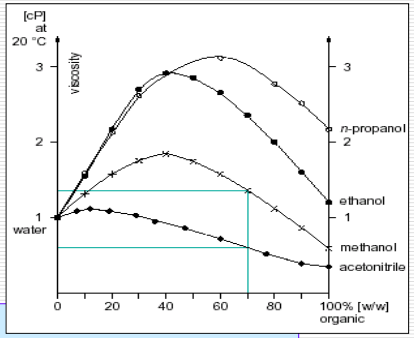
REVERSED PHASE CHROMATOGRAPHY SYSTEMS

SP – non-polar
- long hydrocarbon chain
usually C_{18}, C_8, C_4

MP- polar
- acetonitrile
- tetrahydrofuran
- dioxane
- diethylether
- methanol
- propanol

mixture with water: decreasing of high elution strength of organic solvents

MP viscosity changes as a function of organic/water ratio backpressure



Modification of mobile phase properties:
change of pH: increasing/decreasing of solute ionisation and a polar groups at the surface of stationary phase
increasing pH: - higher retention of basic sample components
- lower retention of acid sample components

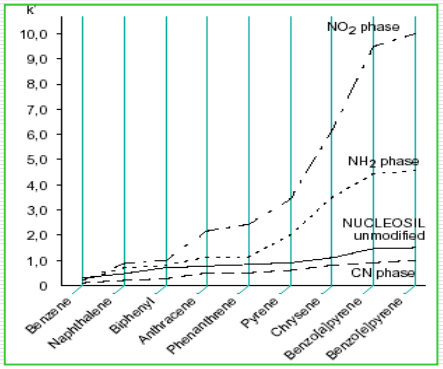
Reversed phase chromatography systems

Analytes separated in reversed phase systems

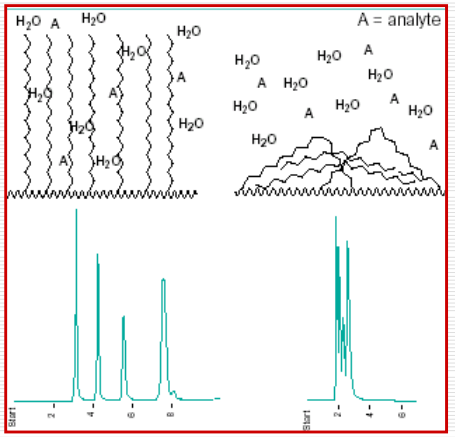
n - alkanes
aromatics
halogenated hydrocarbons
ethers
nitro compounds
esters
amines
amides
acids
sulfonic acids

increase of retention

Effect of SP surface modification on the retention of aromatic hydrocarbons



Effect of higher water content > 95% in MP



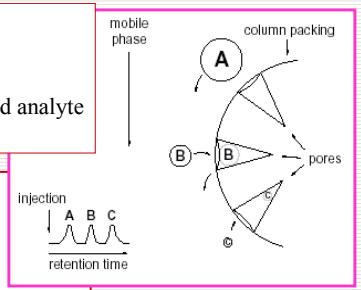
- plainly visible deterioration of **column efficiency**
- non-polar alkyl chains lose their **"brush-type-structure"**
- drastically decrease of **retention times and resolution**

SEC - Size exclusion chromatography

- separation according to size of molecules
- mechanical separation according to particle hydrodynamic diameter

SP: particles with high number of pores
- defined pore size
- pores filed by MP
- no interaction with MP and analyte

MP: good dissolution of analyte

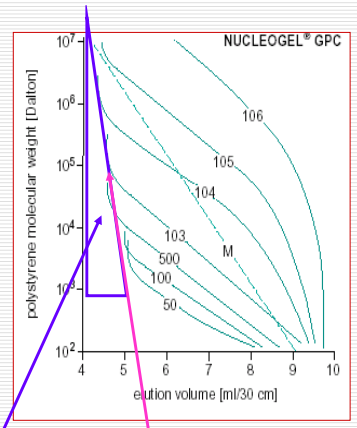


Small molecules - diffusion into the SP
- highest retention

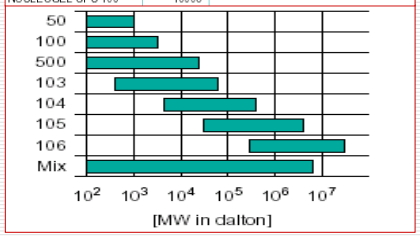
Middle and large - only widest pores

Larger than pore diameter - no retention
- $M_{r,\,min}$ - ***exclusion limit***

$V_R' = A - B \log M_{r,A}$



Column type	Exclusion limit [kdalton]	Application
NUCLEOGEL GPC 50	2	low molecular weight organics
NUCLEOGEL GPC 100	4	oligomers, oils
NUCLEOGEL GPC 500	25	low molecular weight polymers
NUCLEOGEL GPC 103	60	low molecular weight polymers
NUCLEOGEL GPC 104	500	polymers up to 500 kDalton
NUCLEOGEL GPC 105	4000	molecular weight distribution of polymers
NUCLEOGEL GPC 106	10000	



Exclusion limit: - pores accessible for molecules from certain size $K_D = 0$
Total exclusion: - size area over exclusion limit

Total penetration: - pores accessible for all molecules $K_D = 1$

Materials for GPC (SEC)

Gels:

hard	- aero gels, inorganic materials, **non-swelled**	porous glass
semi-hard, soft	- xero gels, organic materials, **swelled**	Sephadex
hybrid	- MP change causes only low volume changes	Spheron

Hydrophilic:

cross-linked dextran	
(epichlorhydrin)	Sephadex
(N,N -methylbisacrylamid)	Sephacryl Sepharose
glycolmethacrylate	Spheron
polyamides	

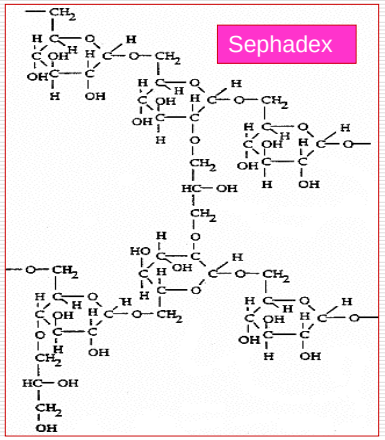
Hydrophobic:

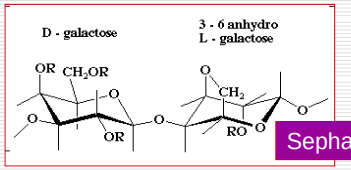
PS/DVB Nucleogel, Supelcogel

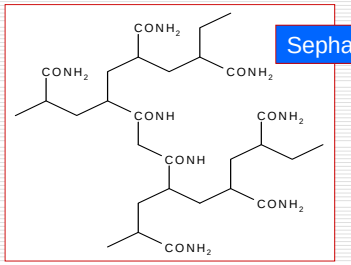
acrylate, polyvinylacetate

- stabile polymers with high pressure stability
- minimum changes in volume caused
- by MP polarity change
- suitable for different temperatures

Materials for GPC (SEC)



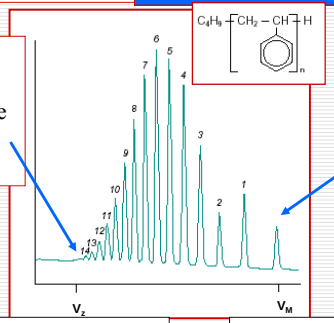




GPC application

molecule mass determination
biochemical analysis of macromolecules
oligomeric compounds

Chromatogram of styrene oligomers n =1-14



Excluded molecules

elution volume ~ volume of MP in interparticle space $\mathbf{V_z}$

Styrene monomer

size of small molecule equal to molecule MP elution volume ~ hold-up volume $\mathbf{V_M}$

SP pore volume
$V_M - V_z = V_p$

exclusion volume

hold-up volume

IC – Ion chromatography

separation of ions and charged particles

SP: Ion exchangers:
chemically bonded ion groups on the surface of inorganic carrier (silica gel) or organic carrier (PS/DVB)

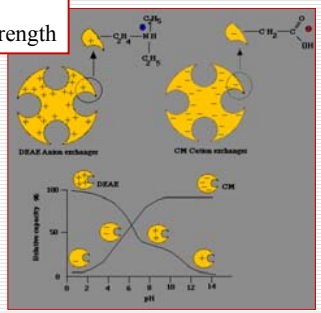
MP:
aqueous solution of salt of different pH and ionic strength

IC modes:

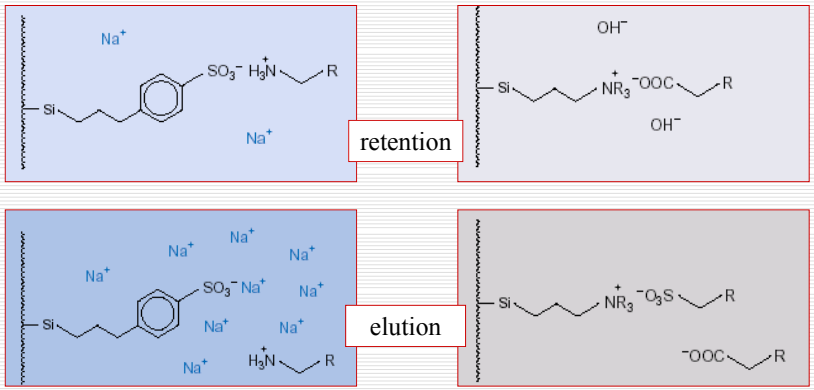
1. common ion exchange:
Sample and MP ions competition for ionic sites on the SP surface

$Na^+ + \sim\sim SO_3H \leftrightarrow \sim\sim SO_3Na + H^+$

MP: acid (diluted HCl) $t_R = f(pH, I, c_{H+})$



Ion chromatography interaction scheme



IC modes (continue)

2. Acid-base reaction

separation of weak acids

~~ $CH_2N^+(CH_3)_3OH^-$ + CH_3COOH ↔ ~~ $CH_2N^+(CH_3)_3CH_3COO^-$ + H_2O

3. Ligand exchange

Competition of sample and MP components for metal on SP surface

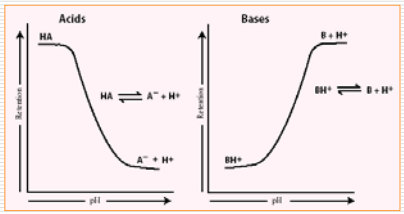
SP	ion exchanger with bonded metal element
MP	ligand created complex compound with bonded metal
Sample	creates similar complex as MP component

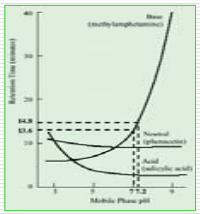
Solutes retentions

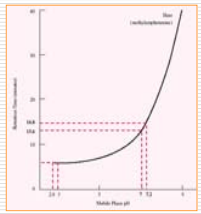
ratio of complexes constants of bonded metal with MP and sample ligand

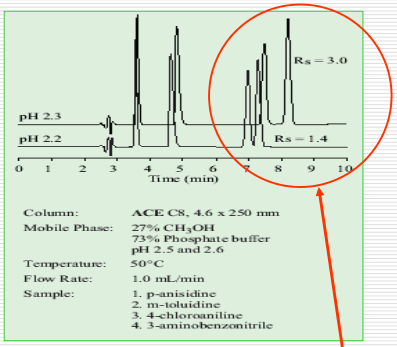
Separation of amino acids - bonded metals: Cu, Ni
- mobile phase ligand: NH_3
- sample ligand: NH_2 – groups of amino acids

Effect of pH mobile phase change on basic, neutral and acidic compounds retention in reversed phase system



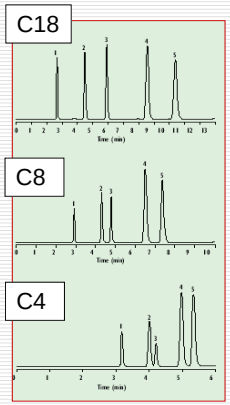






Resolution changes
R = 3,0 → 1,4
caused by 0,1 pH change

The choice of suitable bonded phase



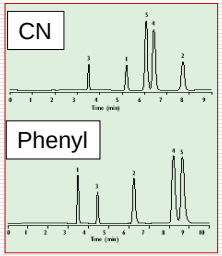
Rules

Retention times are changed with the chains length of bonded phase.

Retention order of main compounds on „aliphatic" SP (C18, C8, C4) is the same.

The change of elution order or resolution of zones can be better realised using phenyl or CN phase than mobile phase change.

Higher resolution can be achieved on SP with low particle diameter.

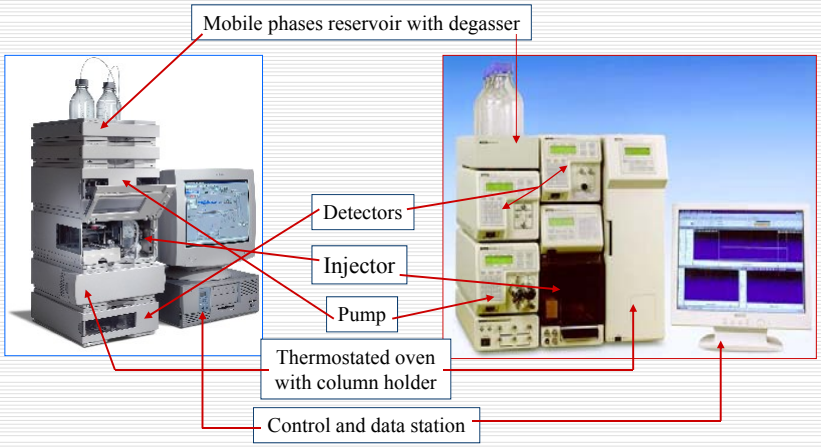


Column:
ACE 250 x 4.6 mm i.d., 5 µm

Mobile phase:
20% 25 mM KH_2PO_4, pH6
80% MeOH
Flow rate: 1.0 mL/min
Temperature: 22°C

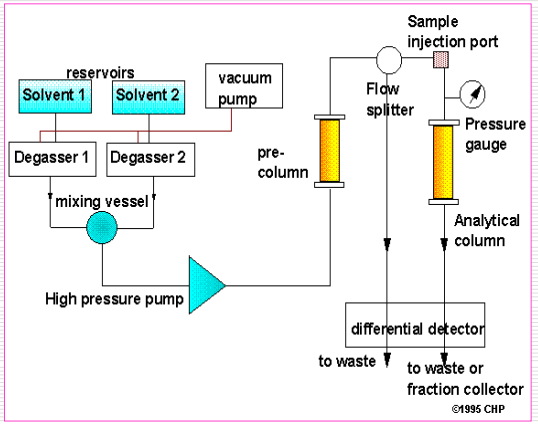
1. Norephedrine, 2. Norteiptiline, 3. Toluene, 4. Imipramine, 5. Amitriptyline

INSTRUMENTATION in COLUMN LIQUID CHROMATOGRAPHY



Instrumentation in column liquid chromatography (continue)

Scheme of liquid chromatograph



Instrumentation in column liquid chromatography (continue)

1. Mobile phase pumps

common analytical flow range: 0,1 - 10 ml/min flow precision < 2%

types:

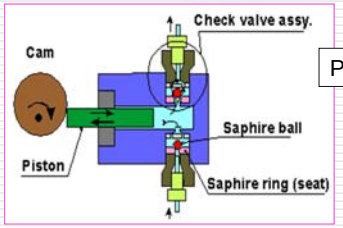
pulsed	- piston
	- membrane
non-pulsed	- linear „syringe“

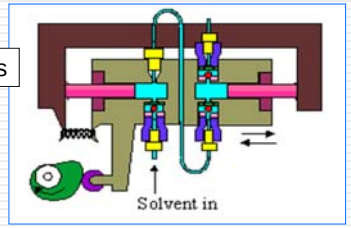
pump accessories

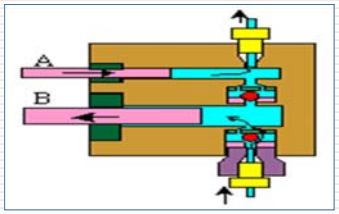
pulse dampener	- capillary resistor
	- two (or more) pump coupling
	- programmed change of piston speed
gradient programmer	- two linear syringes
	- continual mixing of two or more component of MP

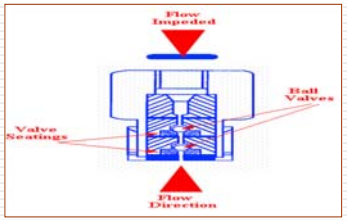
Instrumentation in column liquid chromatography (continue)

Piston pumps



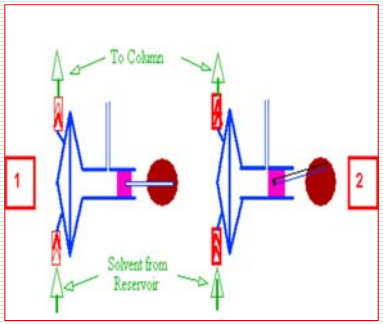




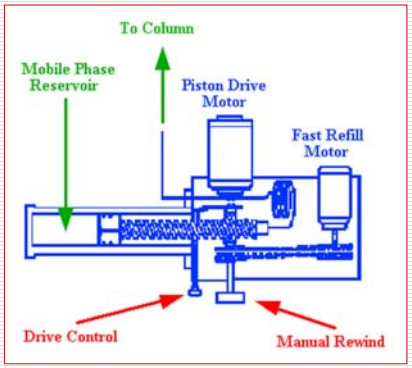


Instrumentation in column liquid chromatography (continue)

Membrane pump

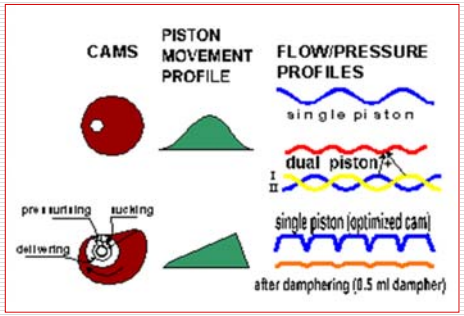


Syringe pump

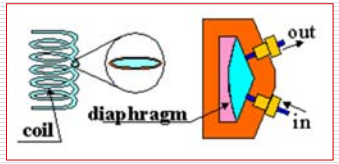


Instrumentation in column liquid chromatography (continue)

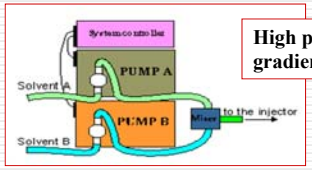
Flow/pressure profile



Pulse dampener

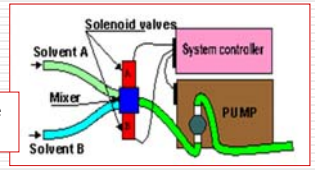


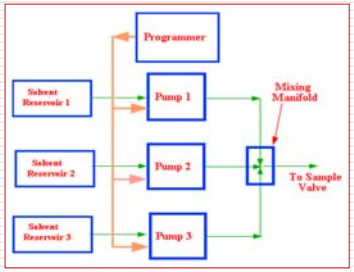
Instrumentation in column liquid chromatography (continue)

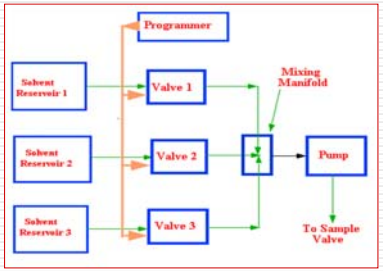


High pressure gradient

Low pressure gradient





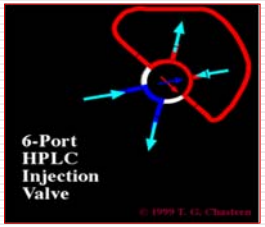


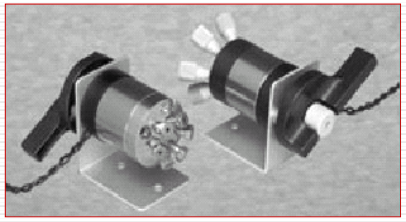
Instrumentation in column liquid chromatography (continue)

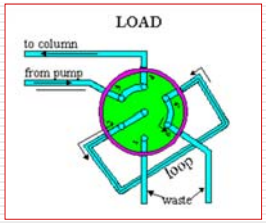
2. Sample injection

direct injection - septum, onto the MP flow
- stop flow
- *poor reproducibility*

injection valve
automatic injection

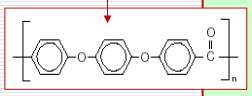






Instrumentation in column liquid chromatography (continue)

3. Columns
metals
stainless steel
glass
PEEK



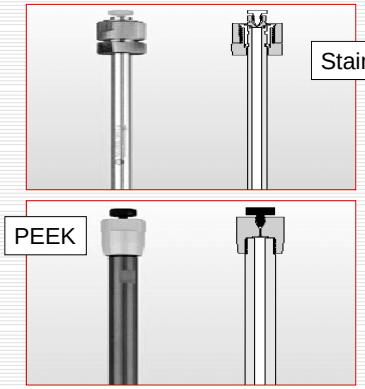
Type, proportions (according to application)

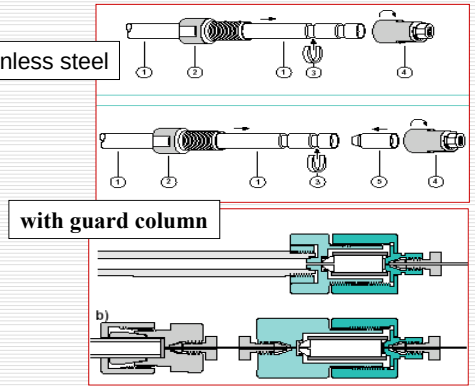
	diameter [mm]	length [mm]
Preparative	> 50	500 - 2000
	10 - 50	250 - 1000
Semi preparative	8 - 10	150 - 500
„Classical“ (SEC, GPC, IC)	6 - 10	150 - 1000
Analytical	2 - 6(4,6)	50 - 300
Micro columns	0,5 - 2	50 - 1000
Capillary columns	≤ 0,3	100 - 1000

packed
open tubular
with SP chemically bonded on the capillary wall

Instrumentation in column liquid chromatography (continue)

Construction details of selected column for analytical LC





Instrumentation in column liquid chromatography (continue)

4. Detectors

- ***universal***
- ***selective***

Types

1. *Optical*	- photometric - fluorescence - refractometric
2. *Electrochemical*	- voltammetric - conductimetric
3. *Mass*	

Requirements
- fast, linear concentration response
- high sensitivity
- low noise
- minimal effect of change pressure, MP flow and temperature
- minimal contribution to peak broadening
- gradient elution

DETECTORS for HPLC

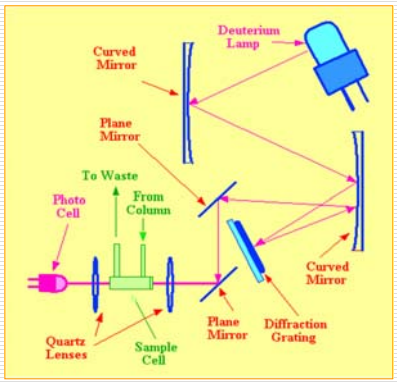
Photometric detector

UV - VIS detector:

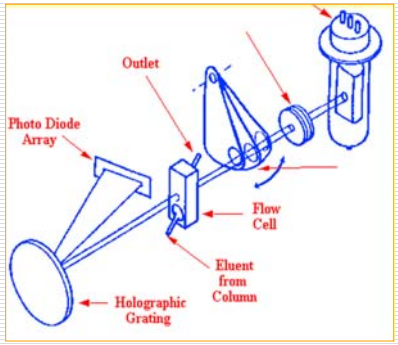
- fixed wave length: 254 nm
- filter detector : 254, 280, 313, 340, 365, 405, 436, 546 nm
- continuously changed wave length : 190-400 (600) nm
- fast record of optical spectrum: DAD - Diode Array Detector
  photodiodes
  simultaneous detection and quantification at different λ
  simultaneously obtained chromatogram and optical spectrum

DETECTORS for HPLC (continue)

Scheme of UV-VIS detector



Scheme of diode array detector - DAD



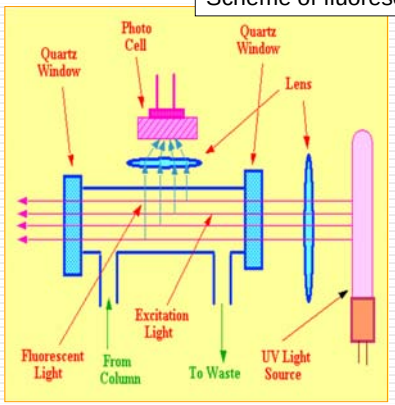
DETECTORS for HPLC (continue)

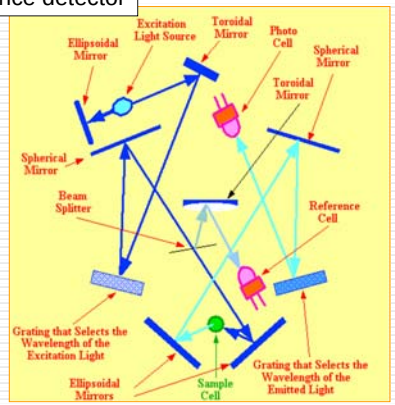
Absorbance maximum wave length for compounds with selected groups

	chromophor	wave length [nm]	absorption coefficient
acetylide	-C=C	175-180	6,000
aldehyde	-CHO	210	1,500
aminee	$-NH_2$	195	2,800
azo group	-N=N-	285-400	3-25
bromidee	-Br	208	300
carboxyle	-COOH	200-210	50 - 70
disulphide	-S-S-	194	5,500
ester	-COOR	205	50
ether	-O-	185	1,000
ketone	>C=O	195	1,000
nitrate	$-ONO_2$	270	12
nitrile	-C=N	160	-
nitrite	-ONO	220 - 230	1000-2000
nitro group	$-NO_2$	210	high

DETECTORS for HPLC (continue)

Scheme of fluorescence detector





DETECTORS for HPLC (continue)

Voltammetric (amperometric) detector:

- oxidizable / reducible compounds
- current is recorded according to impressed voltage between working polarizable and auxiliary electrode
- property: good MP conductivity

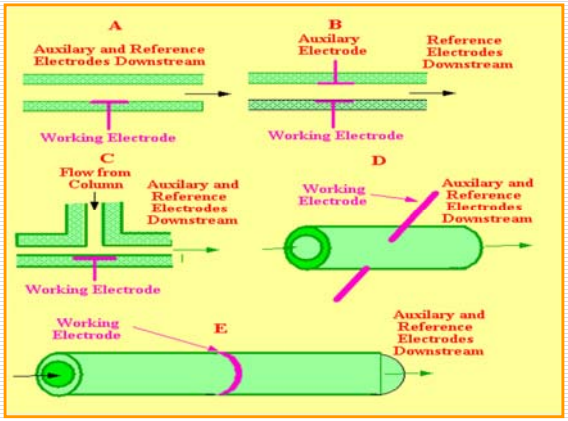
Phenoles, thioles, peroxides, aromatic amines, ketones aldehydes, nitrocompounds, nitriles, esters

Conductivity detector

- ionic compounds
- dominant in IC
- two electrodes connected to alternating current

DETECTORS for HPLC (continue)

Electrode lay-out in electrochemical detectors

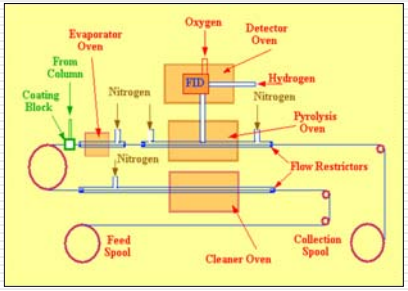


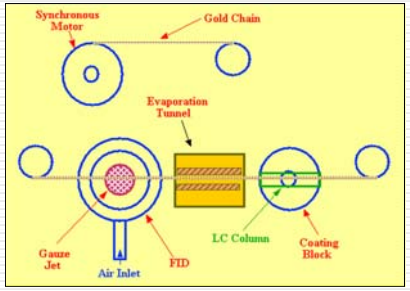
Comparison of selected detectors for HPLC

Detector	*limit of detection g/ml*	*dynamic range*	*gradient application*	*temperature effect*
photometric	10^{-9}	10^{4}	yes	low
fluorescence	10^{-10}	10^{3}	yes	low
refractometric	10^{-6}	10^{4}	no	high
voltammetric	10^{-10}	10^{4}	no	low
conductivity	10^{-8}	10^{4}	no	low

DETECTORS for HPLC (continue)

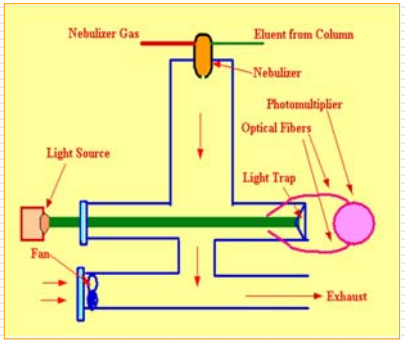
Transport detector





DETECTORS for HPLC (continue)

Evaporative Light Scattering Detector (ELSD)



working principle

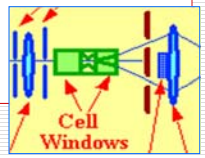
- ✓effluent from column is dispersed by spray into small droplet
- ✓droplets are evaporated and leave an analyte into the form of fine particles suspended in a gas
- ✓suspended particles absorb radiation of primary source
- ✓absorbed radiation is proportional to component amount

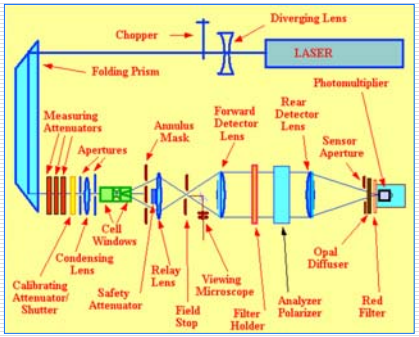
DETECTORS for HPLC (continue)

Low Angle Laser Light Scattering (LALLS) Detector

working principle

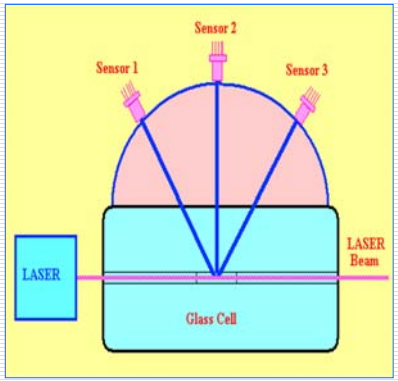
- Laser beam goes through set of optical elements and is focused on inlet cell window of detector
- Ring form mask situated between outlet cell window of detector and transferring lens selects beams dispersed on eluate particles under small angle and prevents primary laser beams to enter photomultiplier
- Dispersed beams are focused to photomultiplier





DETECTORS for HPLC (continue)

Multiple Angle Laser Light Scattering (MALLS) Detector



working principle

- dispersed irradiation is collected under more angels than LALLS (till 16, not in the direction of primary beam)
- significant decrease of effect of beam dispersed by effluent impurities
- recording of relation of mean quadratic value of molecule diameter and molecular ass

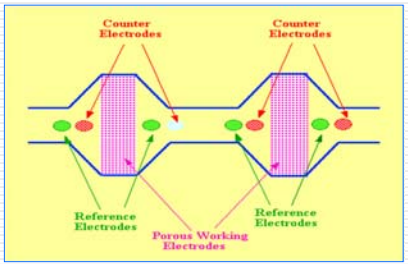
DETECTORS for HPLC (continue)

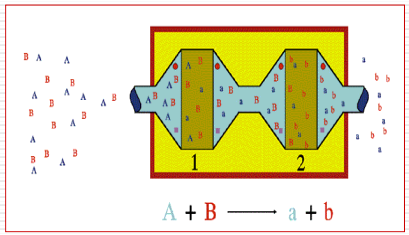
Multi–Electrode Array Detector

working principle

Porous large surface, permeable for mobile phase

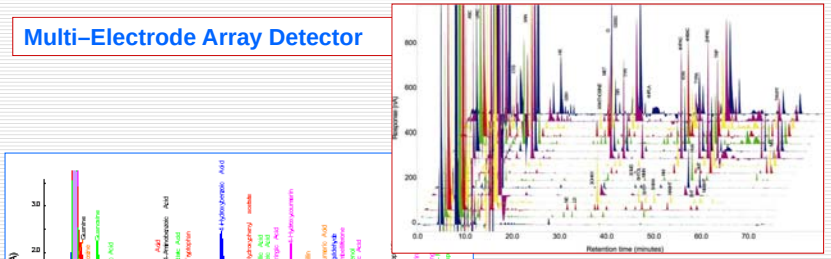
Record of coulometric response of electrochemical reaction on electrode





DETECTORS for HPLC (continue)

Multi–Electrode Array Detector



- ✓ *Limits of detection – femtomoles*
- ✓ *3D resolution of co-eluted peaks*
- ✓ *gradient elution*

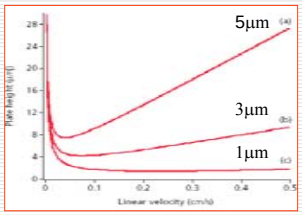
Trends in preparation of packing of HPLC column

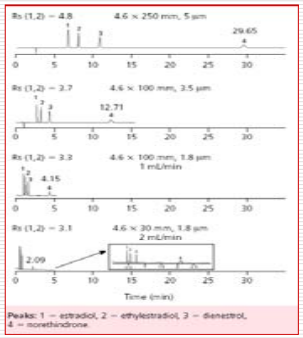
efficiency
separation velocity
chemical/pressure stability
miniaturization

Lower particles - d_p

$$\Delta P = \frac{\Phi \eta L u}{d_p^2}$$

- 10 μm → 5 μm → 3 μm → 2 μm → < 2 μm
- decreased time of transport of analyte into and from pores
- efficiency increasing
- increasing of permeability of chromatographic bed
- increasing of pressure → *special pump construction and injection requirements (max 450 bar → 5000 bar)*
- decreasing of analysis time
- **UPLC** ultra-high pressure liquid chromatography
- **UHPLC** ultra-high performance liquid chromatography





column length 25 cm
viscosity MF 1,0 cP

1000psi = 70 bar

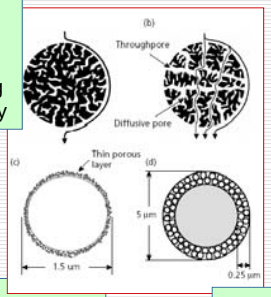
d_p (μm)	ΔP (psi)	Theoretical plates	Ret. time (min)
5.0	210	25 000	35
3.0	1000	42 000	21
1.5	8000	83 000	10.5
1.0	26 000	125 000	7
0.75	62 000	166 000	5

Trends in preparation of packing of HPLC column - continue

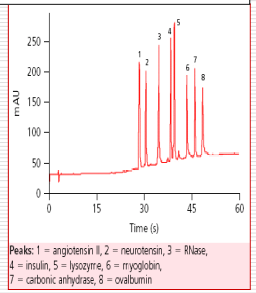
„Perfusion" packing

diffusion pores
through-hole pores

- flow of MP through particles
- mass transport speed increasing
- narrower zones, higher efficiency



Separation of peptides and proteins on surface porous particles
C 18 75 x 2.1 mm i.d.



Non-porous particles

non-porous silica gel (NPS)
non-porous resin (NPR)
d_p 1,5 – 2,5 μm

- high mass transport speed and separation
- low capacity of column

Surface porous particles – thin layer

d_p 5 μm

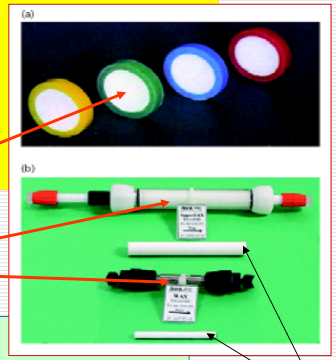
- separation of bio molecules
- high mass transport speed
- acceptable column capacity
- good recovery

Monolithic packing

Column packing created as „uninterrupted" homogenous porous phase

Types:

- agglomeration of polyacrylamide particles
- polyacrylamide block
- agglomeration of micro particulate silica gel bed
- PS-DVB block
- silica gel rod
- membrane of different types



a) disk monolithic column
b) cylindrical monolithic columns in glass and stainless steel shell

monolithic cylinders

Silica gel rods:

- through-hole pores, diffusion pores, to be modified (C18,...)
- efficiency equal to columns with particles 3-5 μm
- pressure drop 30-40% in comparison with 5 μm particle packing →
- column coupling for higher efficiency

Polymeric monolithic columns

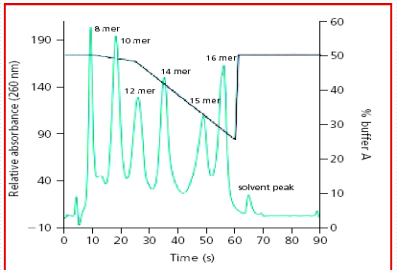
- „uninterrupted" cross-linked porous polymer
- polymethylacrylte, metylacrylat copolymer, PS-DVB

Separation of oligonucleotide on polymeric monolithic column CIM DEAE

ion exchanger
poly(glycidylmethacrylate-ethylenglycol) dimethacrylate

disk: ø16 mm, thickness 3mm

F_m = 6 ml/min



Number of Bases	5'–3' Sequence	Short Name
8	CCA TGT CT	8 mer
10	CTC CAT GTC T	10 mer
12	AGG TCC ATG TCT	12 mer
14	CGA CGT CCA TGT CT	14 mer
15	CCG AGG TCC ATG TCT	15 mer
16	GCCG AGG TCC ATG TCT	16 mer

Non-silica gel stationary phases

•***PS-DVB cross-linked copolymers***
lower efficiency than silica gel,
high resistance against pH

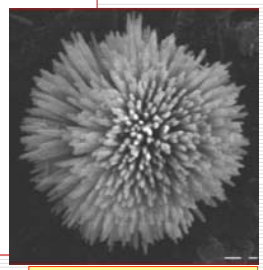
Hydroxyapatite
interaction with sensitive bio molecules

•***Zirkonia (ZrO_2)***
high stability against high pressure and temperature,
resistant against high pH (to pH 14)
surface without silanols, contain centres Lewis acidic centres
strong affinity to Lewis base (hydroxyl, phosphate, fluoride,..)

•***Graphitised carbon***

Silica gel packing with very low content of trace metals

•decreasing of interaction of metal with separated compounds
•effect on acidity of residual silanols



hydroxyapatite particle

Hybrid inorganic-organic stationary phase

• polymerization of tetrachloro- or tetraethoxysilane
creation of silica gel polymer with silanols on the surface

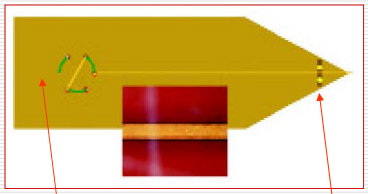
•triethoxysilane connected with ethylene
$(RO)_3SiCH_2CH_2Si(OR)_3$
high pH stability

Future trends in LC

- ***Lower particles of*** silica gel packing (max 3 mm) in short columns (7,5-15 cm) (LC/MS).
- ***Columns with low dimensions*** with inner diameter lower than 100 µm with low particles (proteomics, LC/MS).
- ***Monoliths*** in small diameter columns.
- ***Monoliths*** in 100 mm column diameter created *in situ*
- ***TiO_2*** particles
- ***Chip column*** „lab-on-a-chip“, column „packing“ created directly on inner capillary column wall electroosmotically generated liquid flow - see CEC capillary electrochromatography

Scheme of LC column on chip and part of packed capillary

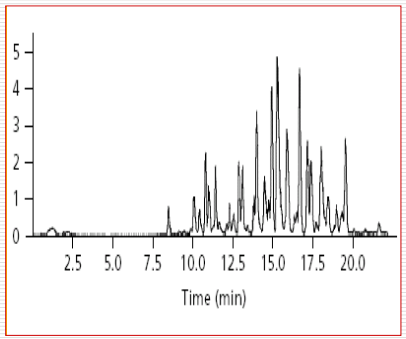
Column dimensions:
40 x 0,075 x 0,050 mm 5µm particles



detection

inner part of injection valve

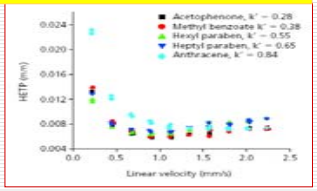
Analysis of BSA digest on „ChipLC column“



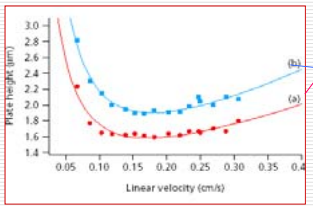
Column format trends in HPLC

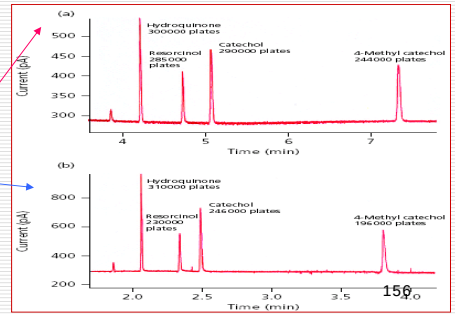
Description	Dimension	Approx. typical flow-rate (velocity 1–10 mm/s)
Open tubular liquid chromatography	< 25 µm i.d.	< 25 nL/min
Nanobore column HPLC	25 µm ≤ i.d. ≤ 100 µm	25–4000 nL/min
Capillary column HPLC	100 µm ≤ i.d. ≤ 1 mm	0.4–200 µL/min
Microbore column HPLC	1 mm ≤ i.d. ≤ 2.1 mm	50–1000 µL/min
Narrow(small)-bore column HPLC	2.1 mm < i.d. < 4 mm	0.3–3.0 mL/min
Normal-bore column HPLC	4 mm ≤ i.d. ≤ 5 mm	1.0–10.0 mL/min
Semipreparative column HPLC	5 mm < i.d. ≤ 10 mm	5.0–40 mL/min
Preparative column HPLC	i.d. > 10 mm	> 20 mL/min

Efficiency of capillary columns
HETP corresponds to particle size
150 x 0,5 mm 3,7 µm



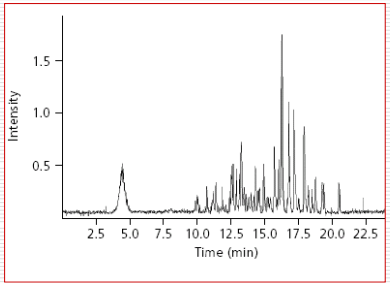
Separation on capillary column packed with micron particles
370 x 0,030 mm i.d. 1µm





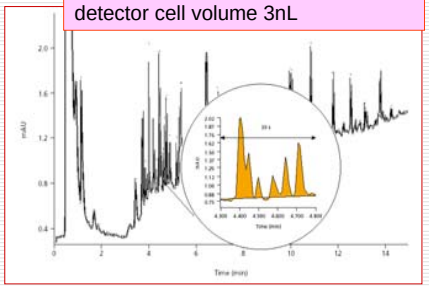
Other separation examples

Separation of peptides on nanoflow HPLC column
C 18 150 x 0,075 x 0,050 3,5 μm
injection 100 fmol 1 μl

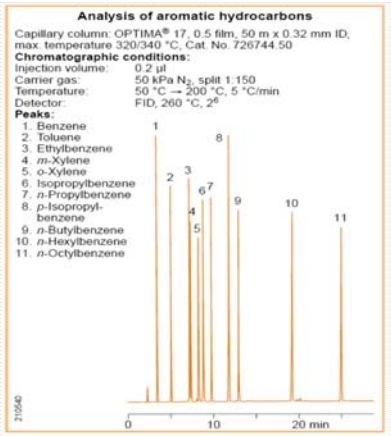


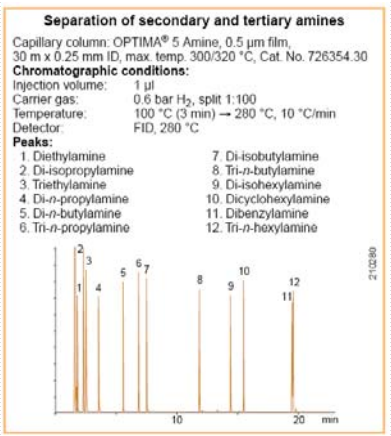
Separation of mixture of BSA digest, myoglobine a alfa-lactalbumine on monolithic capillary column

PS/DVB monolith 50 x 0,2 mm
F_m = 2,5 μl/min
detector cell volume 3nL



Selected examples of chromatographic separations

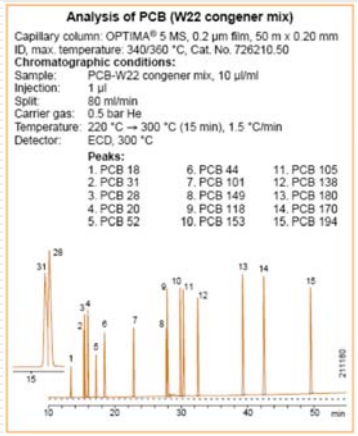


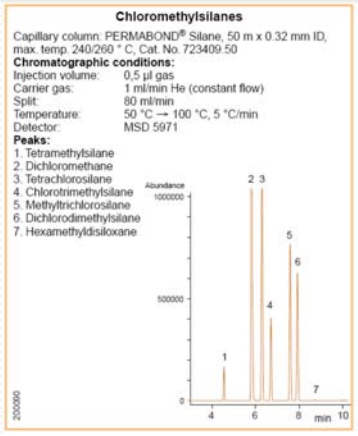


Optima 17 - Phenylmethylpolysiloxane

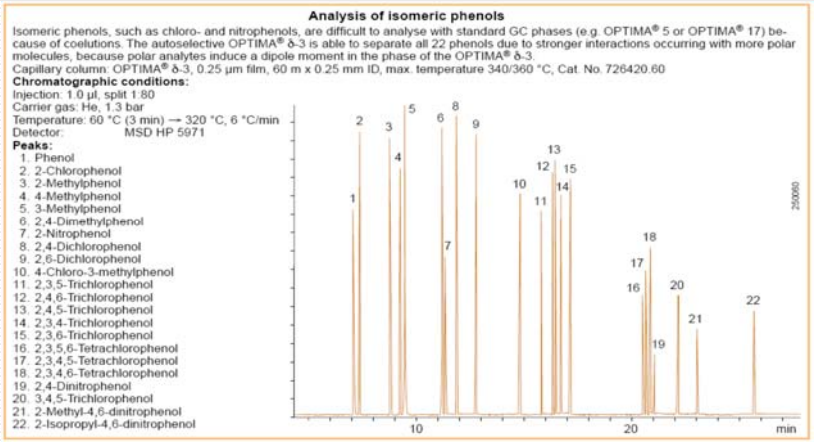
Optima 5 – 5% diphenyl-95% dimethylpolysiloxane

Selected examples of chromatographic separations

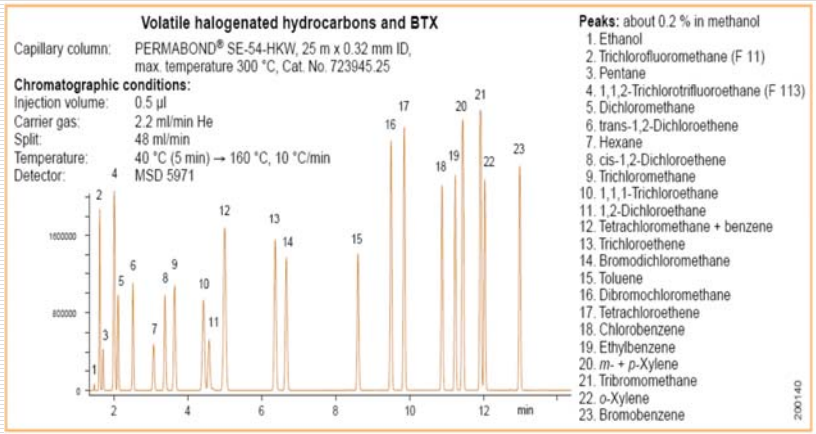




Selected examples of chromatographic separations

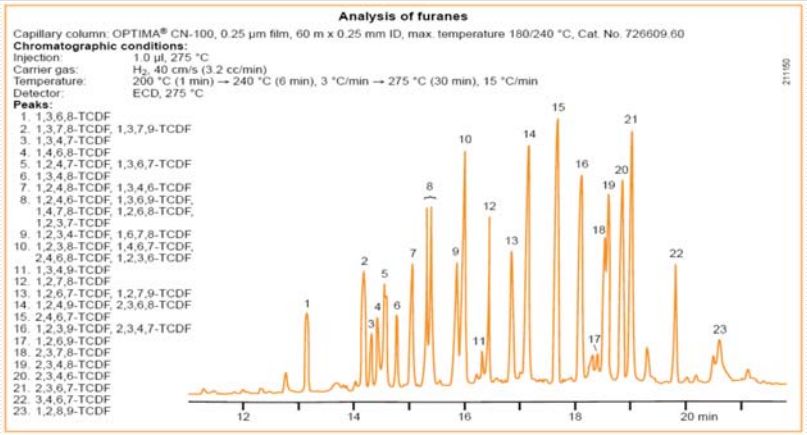


Selected examples of chromatographic separations

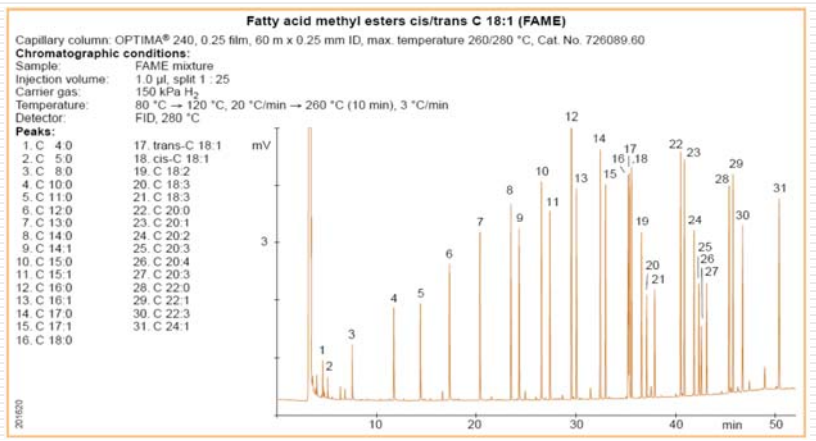


Permabond SE-54-HKW –polysiloxane type

Selected examples of chromatographic separations

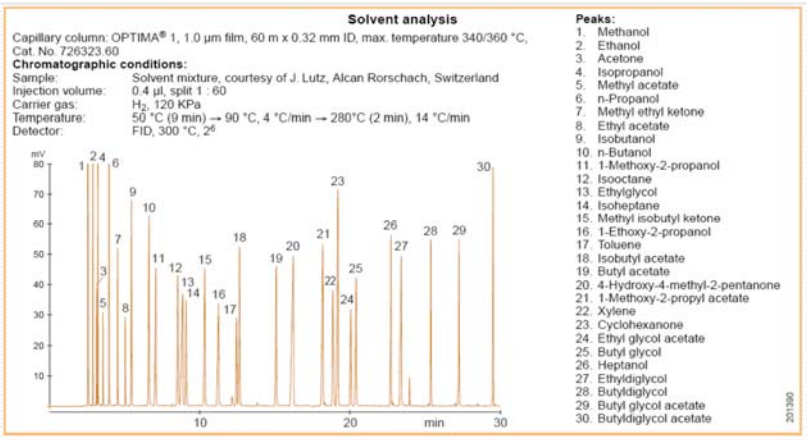


Selected examples of chromatographic separations



Optima 240 – 33%cyanopropyl-methyl-67%dimethylpolysiloxane

Selected examples of chromatographic separations



Optima 1 - dimethylpolysiloxane

Selected examples of chromatographic separations

Separation of organochlorine pesticides (EPA 8081)

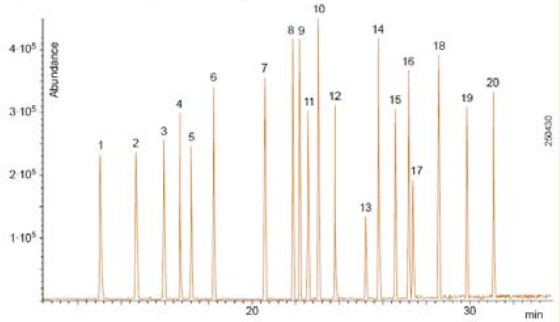
Capillary column: OPTIMA® δ-6, 0.2 µm film, 50 m x 0.2 mm ID, max. temperature 340/360 °C, Cat. No. 726465.50

Chromatographic conditions:

Sample: EPA 8081 organochlorine pesticide calibration mix (Restek), 200 µg/ml each in toluene : hexane (1 : 1)
Injection volume: 1 µl, Split 1: 30
Carrier gas: 2.0 bar He
Temperature: 180 °C → 300 °C (10 min), 4.0 °C/min
Detector: MSD HP 5971

Peaks:

1. α-BHC
2. γ-BHC (lindane)
3. β-BHC
4. Heptachlor
5. δ-BHC
6. Aldrin
7. Heptachlor epoxide
8. γ-Chlordane
9. α-Chlordane
10. Endosulfan I
11. 4,4'-DDE
12. Dieldrin
13. Endrin
14. 4,4'-DDD
15. Endosulfan II
16. 4,4'-DDT
17. Endrin aldehyde
18. Endosulfan sulphate
19. Methoxychlor
20. Endrin ketone



Optima delta-6: síťovaný mrthyl-phenyl-polysiloxan

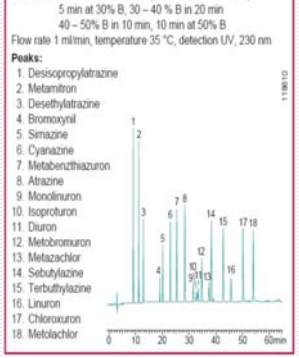
Selected examples of chromatographic separations

Separation of pesticides

Column: EC 250/4 NUCLEODUR® 100-3 C_8 ec
Eluents: A) water (1% acetic acid), B) acetonitrile
Gradient: 10 – 25% B in 10 min, 25 – 30% B in 10 min, 5 min at 30% B, 30 – 40 % B in 20 min 40 – 50% B in 10 min, 10 min at 50% B
Flow rate 1 ml/min, temperature 35 °C, detection UV, 230 nm

Peaks:

1. Desisopropylatrazine
2. Metamitron
3. Desethylatrazine
4. Bromoxynil
5. Simazine
6. Cyanazine
7. Metabenzthiazuron
8. Atrazine
9. Monolinuron
10. Isoproturon
11. Diuron
12. Metobromuron
13. Metazachlor
14. Sebutylazine
15. Terbuthylazine
16. Linuron
17. Chloroxuron
18. Metolachlor



Separation of organochlorine pesticides (EPA 8081)

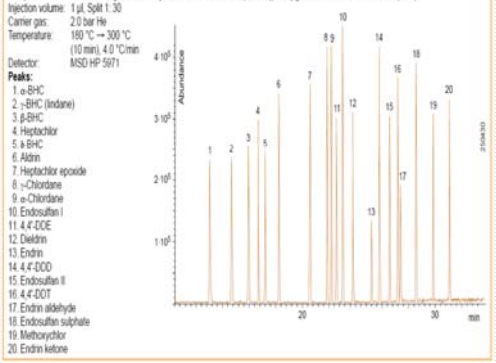
Capillary column: OPTIMA® δ-6, 0.2 µm film, 50 m x 0.2 mm ID, max. temperature 340/360 °C, Cat. No. 726465.50

Chromatographic conditions:

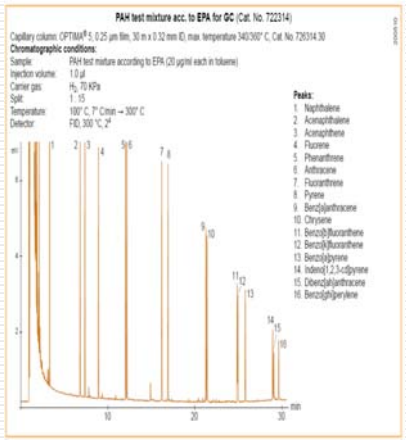
Sample: EPA 8081 organochlorine pesticide calibration mix (Restek), 200 µg/ml each in toluene : hexane (1 : 1)
Injection volume: 1 µl, Split 1: 30
Carrier gas: 2.0 bar He
Temperature: 180 °C → 300 °C (10 min), 4.0 °C/min
Detector: MSD HP 5971

Peaks:

1. α-BHC
2. γ-BHC (lindane)
3. β-BHC
4. Heptachlor
5. δ-BHC
6. Aldrin
7. Heptachlor epoxide
8. γ-Chlordane
9. α-Chlordane
10. Endosulfan I
11. 4,4'-DDE
12. Dieldrin
13. Endrin
14. 4,4'-DDD
15. Endosulfan II
16. 4,4'-DDT
17. Endrin aldehyde
18. Endosulfan sulphate
19. Methoxychlor
20. Endrin ketone



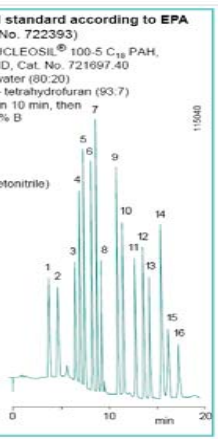
Selected examples of chromatographic separations



Separation of the PAH standard according to EPA (Cat. No. 722393)

Column: CC 150/4 NUCLEOSIL® 100-5 C_{18} PAH, 150 x 4 mm ID, Cat. No. 721697.40
Eluent A: Methanol – water (80:20)
Eluent B: Acetonitrile – tetrahydrofuran (93:7)
Gradient: 0 – 100% B in 10 min, then 5 min at 100% B
Flow rate: 1 ml/min
Pressure: 140 bar
Temperature: 20 °C
Detection: UV, 260 nm
Peaks: (10 µg/ml each in acetonitrile)

1. Naphthalene
2. Acenaphthylene
3. Acenaphthene
4. Fluorene
5. Phenantrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benz[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benzo[a]pyrene
14. Dibenz[ah]anthracene
15. Benzo[ghi]perylene
16. Indeno[1,2,3-cd]pyrene



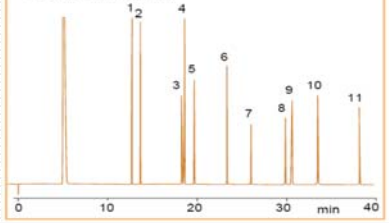
Optima 5 – 5%diphenyl-95% dimethylpolysiloxane

Selected examples of chromatographic separations

Phenol test mixture (Cat. No. 722315)

Capillary column: FS-SE-54, 0.35 µm film, 50 m x 0.25 mm ID, max. temperature 300 °C, Cat. No. 733623.50
Injection volume: 1 µl
Carrier gas: 0.95 bar N_2
Split: about 1 : 40
Temperature: 80 °C → 260 °C, 5 °C/min
Detector: FID 300 °C, 2⁴
Peaks:

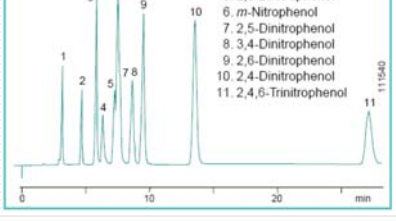
1. Phenol
2. 2-Chlorophenol
3. 2-Nitrophenol
4. 2,4-Dimethylphenol
5. 2,4-Dichlorophenol
6. 4-Chloro-3-methylphenol
7. 2,4,6-Trichlorophenol
8. 2,4-Dinitrophenol
9. 4-Nitrophenol
10. 2-Methyl-4,6-dinitrophenol
11. Pentachlorophenol



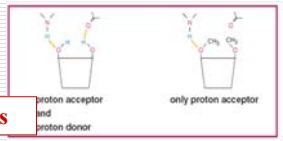
Separation of phenols

Column: CC 250/4 NUCLEOSIL® 100-5 PROTECT I, 250 x 4 mm ID, Cat. No. 721150.40
Eluent: acetonitrile – 25 mM KH_2PO_4 pH 7 (50:50, v/v)
Flow rate: 1 ml/min
Temperature: 20 °C
Detection: UV, 235 nm
Peaks:

1. *p*-Benzoquinone
2. Phenol
3. *o*-Nitrophenol
4. *p*-Nitrophenol
5. 2,3-Dinitrophenol
6. *m*-Nitrophenol
7. 2,5-Dinitrophenol
8. 3,4-Dinitrophenol
9. 2,6-Dinitrophenol
10. 2,4-Dinitrophenol
11. 2,4,6-Trinitrophenol

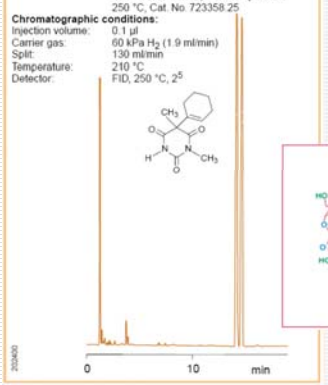


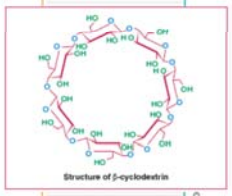
Selected examples of chromatographic separations



Enantiomer separation of hexobarbital (TFA)

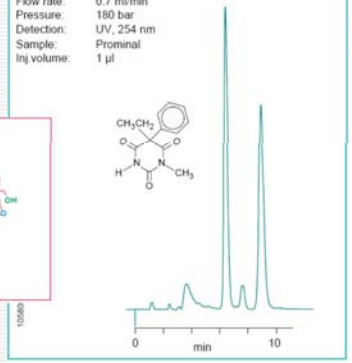
Capillary column: HYDRODEX® β-3P, 25 m x 0.25 mm ID, max. temperature 250 °C, Cat. No. 723358.25
Chromatographic conditions:
Injection volume: 0.1 µl
Carrier gas: 60 kPa H_2 (1.9 ml/min)
Split: 130 ml/min
Temperature: 210 °C
Detector: FID, 250 °C, 2^5





Enantiomer separation of mephobarbital (prominal)

Column: EC 200/4 NUCLEODEX® β-PM; 200 x 4 mm ID; Cat. No. 720125.40
Eluent: Methanol / 0.1% TEAA pH 4.0 (55 : 45, v/v)
Flow rate: 0.7 ml/min
Pressure: 180 bar
Detection: UV, 254 nm
Sample: Prominal
Inj.volume: 1 µl

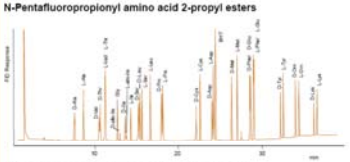


Selected examples of chromatographic separations

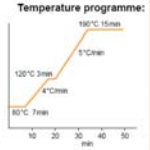
Enantiomer separation of amino acid derivatives

Capillary column: PERMABOND® L-CHIRASIL-VAL, 25 m x 0.25 mm ID, max. temperature 190 °C, Cat. No. 723730.25
Chromatographic conditions:
Injection volume: 0.5 µl
Carrier gas: 0.45 bar H_2, split 1 : 30
Detector: FID, 250 °C, AT 3

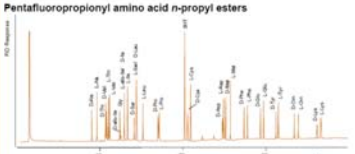
N-Pentafluoropropionyl amino acid 2-propyl esters



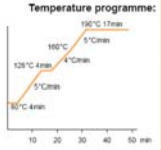
Temperature programme:



Pentafluoropropionyl amino acid *n*-propyl esters

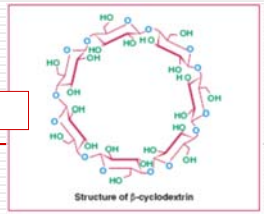


Temperature programme:



Chromatograms courtesy of Priv. Doz. Dr. W. Brückner, Dipl. Lebensmittel-Chemiker M. Hausch, Inst. f. Food Technology, University Hohenheim, Stuttgart, Germany, 1988

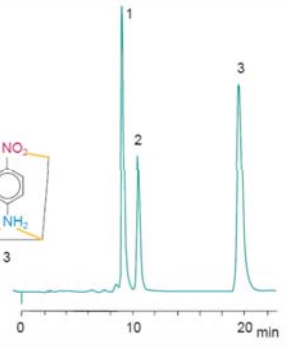
Selected examples of chromatographic separations



Separation of positional isomers of nitroaniline

Column: EC 200/4 NUCLEODEX β-OH, 200 x 4 mm ID, Cat. No. 720124.40
Eluent: methanol / 0.1% triethylammonium acetate pH 4.0, 50 : 50 (v/v)
Flow rate: 0.7 ml/min
Pressure: 180 bar
Detection: UV, 254 nm

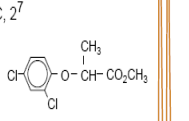
Peaks
(sample volume 1 µl):
1. *m*-Nitroaniline
2. *o*-Nitroaniline
3. *p*-Nitroaniline



Selected examples of chromatographic separations

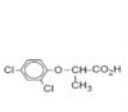
Enantiomer separation of dichlorprop methyl ester

Capillary column: HYDRODEX® β-3P, 25 m x 0.25 mm ID, max. temperature 250 °C, Cat. No. 723358.25
Chromatographic conditions:
Injection volume: 0.1 µl (~1% in methylene chloride)
Carrier gas: 60 kPa H_2 (1.9 ml/min)
Split: 130 ml/min
Temperature: 160 °C
Detector: FID, 250 °C, 2^7



Enantiomer separation of dichlorprop

Column: CC 200/4 NUCLEODEX® α-PM, Cat. No. 721463.40
Sample volume: 1 µl
Eluent: Methanol / 50 mM NaH_2PO_4 pH 3,0 (65:35, v/v)
Flow rate: 0,7 ml/min
Pressure: 170 bar
Temperature: 20 °C
Detection: UV, 230 nm



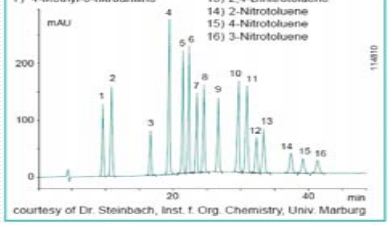
Selected examples of chromatographic separations

Analysis of nitroaromatic compounds (explosives)

Column CC 250/3 NUCLEOSIL® 120-3 C_{18}, 250 x 3 mm ID, Cat. No. 721 666.30; eluents: A) methanol, B) water; gradient 35 – 55% A in 12 min, then 40 min isocratic, finally 100% A; flow rate 0.35 ml/min; temperature 30 °C, detection DAD, 230 nm; injection volume 40 µl

Peaks:

1) 2,6-Diamino-4-nitrotoluene
2) 2,4-Diamino-4-nitrotoluene
3) Hexogene (Hexahydro-1,3,5-trinitro-1,3,5-triazine)
4) 1,3,5-Trinitrobenzene
5) 2-Methyl-3-nitroaniline
6) 2-Methyl-5-nitroaniline
7) 4-Methyl-3-nitroaniline
8) 1,3-Dinitrobenzene
9) 2,4,6-Trinitrotoluene
10) 4-Amino-2,6-dinitrotoluene
11) 2-Amino-4,6-dinitrotoluene
12) 2,6-Dinitrotoluene
13) 2,4-Dinitrotoluene
14) 2-Nitrotoluene
15) 4-Nitrotoluene
16) 3-Nitrotoluene

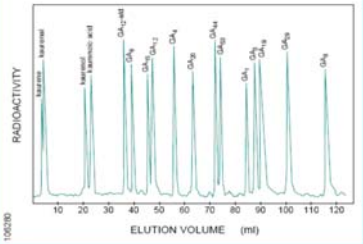


courtesy of Dr. Steinbach, Inst. f. Org. Chemistry, Univ. Marburg

Separation of gibberellins by normal phase HPLC

P.C. Odén et al. J. Chromatogr. **464** (1989) 195 – 200

Column: CC 125/4.6 NUCLEOSIL® 100-5 NO_2, 125 x 4.6 mm ID, Cat. No. 721864.46
Eluent A: *n*-Heptane half saturated with 1 M formic acid
Eluent B: Ethyl acetate with 1% water and 0.5% formic acid
Gradient: 100% A – 100% B in 60 min
Flow rate: 2 ml/min
Detection: Radioactivity



Selected examples of chromatographic separations

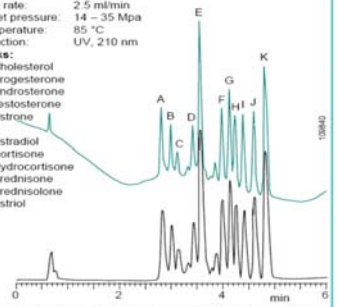
SFC separation of steroids

F. Vérillon et al. LC · GC int. **7** (1994) 710 – 716

Column: CC 250/2 NUCLEOSIL® 100-7 OH (Diol), 250 x 2 mm ID, Cat. No. 721877.20
Eluent: CO_2 with 1 – 12% methanol
Flow rate: 2.5 ml/min
Outlet pressure: 14 – 35 Mpa
Temperature: 85 °C
Detection: UV, 210 nm

Peaks:

A) Cholesterol
B) Progesterone
C) Androsterone
D) Testosterone
E) Estrone
F) Estradiol
G) Cortisone
H) Hydrocortisone
I) Prednisone
J) Prednisolone
K) Estriol



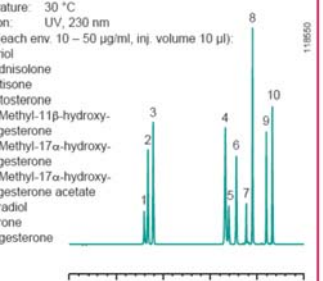
The hollow in the upper trace is caused by instantaneous detection of the pressure gradient and delayed detection of the methanol gradient. The lower trace shows a software-corrected chromatogram.

Steroids

Column: EC 125/4 NUCLEODUR® 100-5 C_8 ec Cat. No. 760701.40
Eluents: A) water, B) methanol
Gradient: 1 min at 20% B, 20 – 35% B in 10 min, 3 min at 35% B, 35 – 60% B in 6 min, 5 min at 60% B
Flow rate: 1 ml/min
Temperature: 30 °C
Detection: UV, 230 nm

Peaks (each env. 10 – 50 µg/ml, inj. volume 10 µl):

1. Estriol
2. Prednisolone
3. Cortisone
4. Testosterone
5. 6α-Methyl-11β-hydroxy-progesterone
6. 6α-Methyl-17α-hydroxy-progesterone
7. 6α-Methyl-17α-hydroxy-progesterone acetate
8. Estradiol
9. Estrone
10. Progesterone



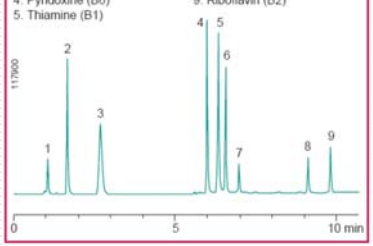
Selected examples of chromatographic separations

Separation of water-soluble vitamins

Column: EC 125/4 NUCLEODUR® 100-5 C_{18} ec
Eluents: A: 10 mM KH_2PO_4, pH 7.0; B: acetonitrile
Gradient: 2.5 min 100% A, then in 6 min to 20% A, 2.5 min 20% A, then in 1 min to 100% A
Flow rate: 1.0 ml/min
Detection: UV, 215 nm
Temperature: 30 °C
Inj. volume: 10 µl

Peaks:

1. Ascorbic acid (C)
2. Nicotinic acid (B)
3. *p*-Aminobenzoic acid (H1)
4. Pyridoxine (B6)
5. Thiamine (B1)
6. Folic acid
7. Biotin (H)
8. Cyanocobalamine (B12)
9. Riboflavin (B2)

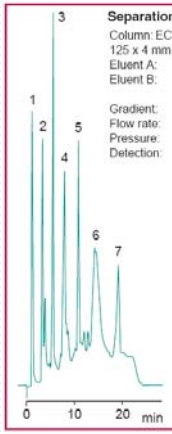


Separation of protein standards

Column: EC 125/4 NUCLEOSIL® 4000-7 PEI, 125 x 4 mm ID, Cat. No. 720402.40
Eluent A: 2 mM TRIS/acetate pH 8.0
Eluent B: 20 mM TRIS/acetate pH 8.0 + 1.5 M KCl
Gradient: linear 0 – 40% B in 20 min
Flow rate: 1 ml/min
Pressure: 76 bar
Detection: UV, 280 nm

Peaks: (Volume 20 µl)

1. Catalase
2. Myoglobin
3. α-Amylase
4. Transferrin
5. α-Lactalbumin
6. Glucose oxidase
7. Soybean trypsin inhibitor



Polyethylene imine on silica strongly basic polymer anion exchanger

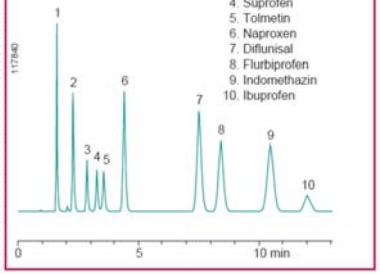
Selected examples of chromatographic separations

Separation of anti-inflammatory drugs

Column: EC 250/4 NUCLEODUR® C_{18} Gravity, 5 µm
Eluent: acetonitrile / 20 mM KH_2PO_4, pH 2.5 (45:55, v/v)
Flowrate: 1,0 ml/min
Temperature: 22 °C
Detection: UV, 230 nm
Inj. volume: 5 µl

Peaks:

1. Acetylsalicylic acid
2. Sulindac
3. Piroxicam
4. Suprofen
5. Tolmetin
6. Naproxen
7. Diflunisal
8. Flurbiprofen
9. Indomethazin
10. Ibuprofen



Separation of isomeric antiinflammatory drugs

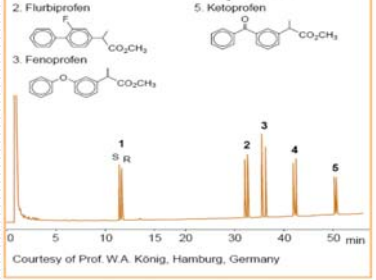
Capillary column: HYDRODEX® β-6-TBDM, 25 m x 0.25 mm ID, max. temperature 250 °C, Cat. No. 723381.25

Chromatographic conditions:

Carrier gas: He
Temperature: 135 °C → 200 °C, 1 °C/min
Detector: FID

Peaks:

1. Ibuprofen
2. Flurbiprofen
3. Fenoprofen
4. Naproxen
5. Ketoprofen



Courtesy of Prof. W.A. König, Hamburg, Germany