Retention mechanisms in liquid chromatography. (NP&RP LC, HILIC)

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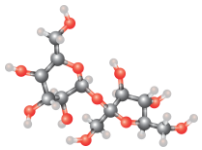
Foreword
analyte, mobile and stationnary phases in LC

The triple equilibrium in LC

The analyte must be soluble in the mobile phase



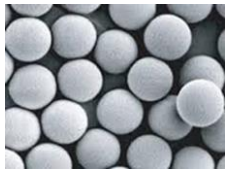
Analyte or solute

Solute-Stationary phase interactions

Solute-solvent interactions

Stationary phase

Packed in the column tube



Solvent-Stationary phase interactions

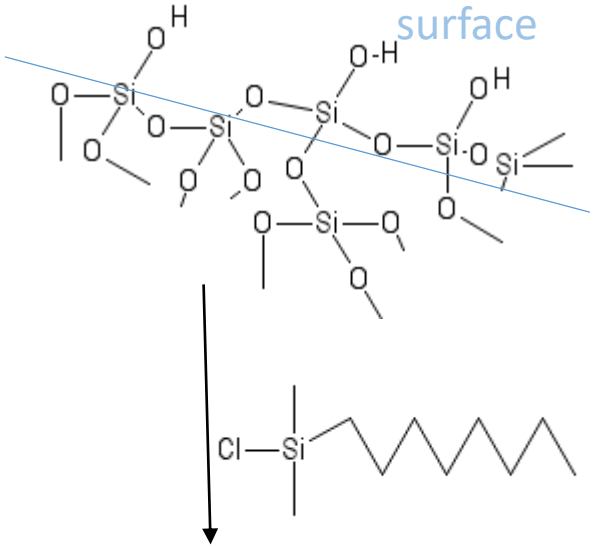
Mobile phase

Usually a mixture of 2 or 3 solvents.

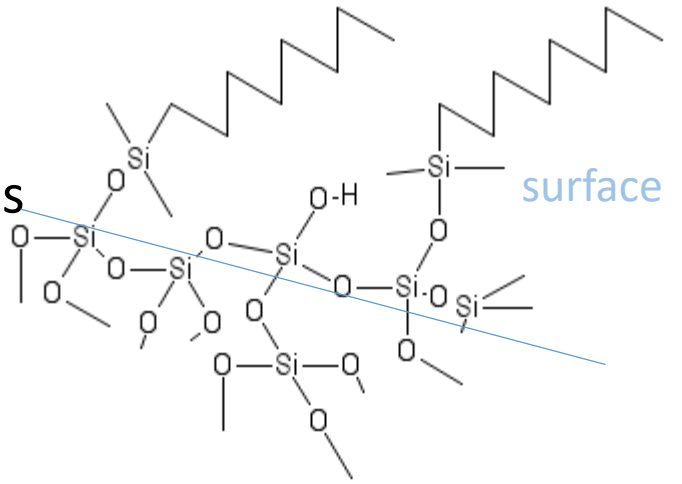
The stationary phase - mobile phase pair as well as the analyte - stationary phase interactions determine the retention mechanism.

The stationary phase

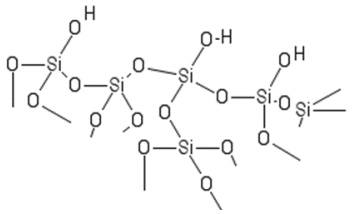
- Basic material = Silica gel = **bare silica**
  - orthosilicic acid [$Si(OH)_4$] polymer
  - Amorphous and **porous** silicon dioxyde
  - withstands high pressures
  - High specific area (up to 800 m^2/g)
  - Pore size and particle size depend on the method of preparation
  - Water **adsorbent** by hydrogen binding with **silanols**



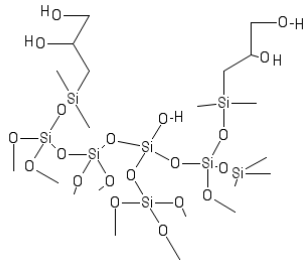
- **Grafted silica**:
  - Surface properties modified by grafting reactions of the silanol groups by various polar or non-polar organic silanes



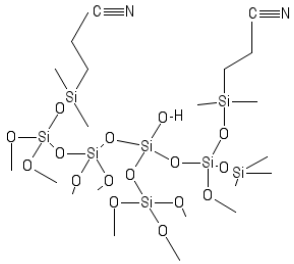
Stationary phase polarity



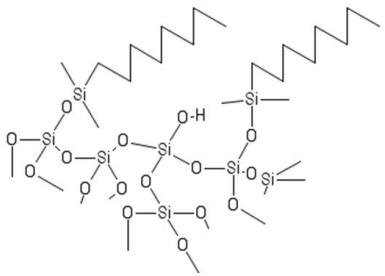
- Bare silica — highly polar adsorbent



- Propyl-diol or « diol » — polar



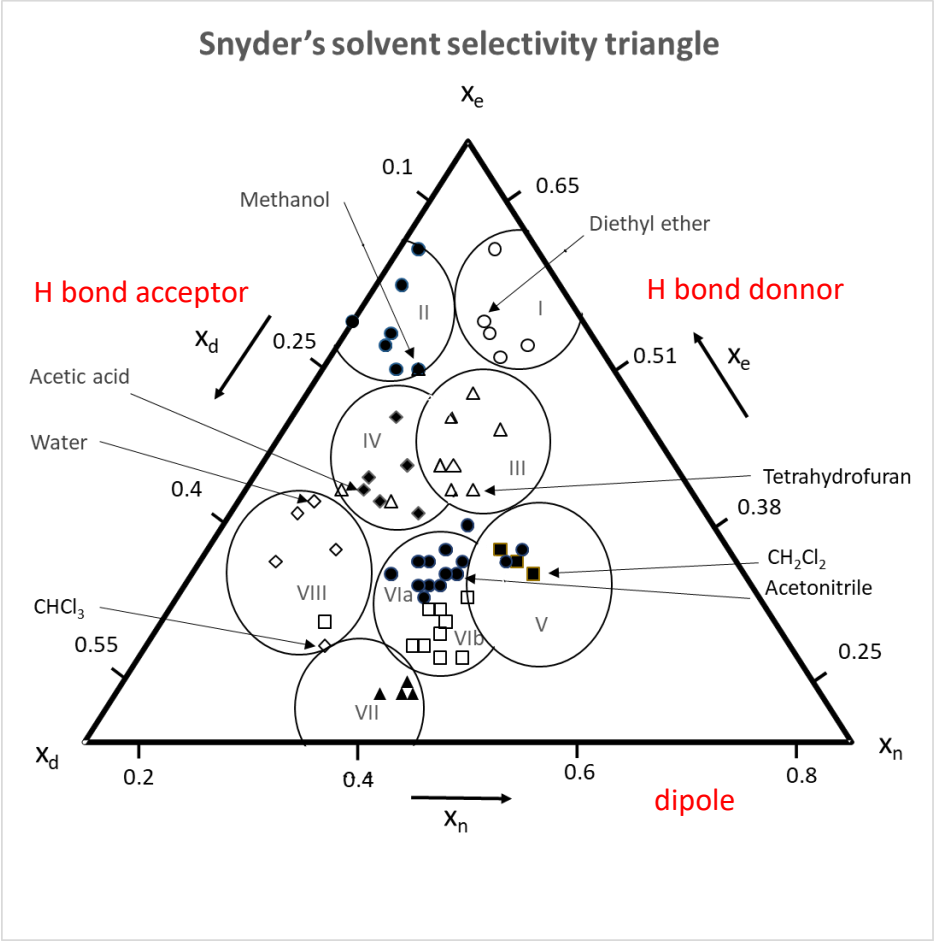
- Propyl-cyano or « cyano » — intermediate polarity



Alkyl (C8, C18) silica — non-polar

The mobile phase

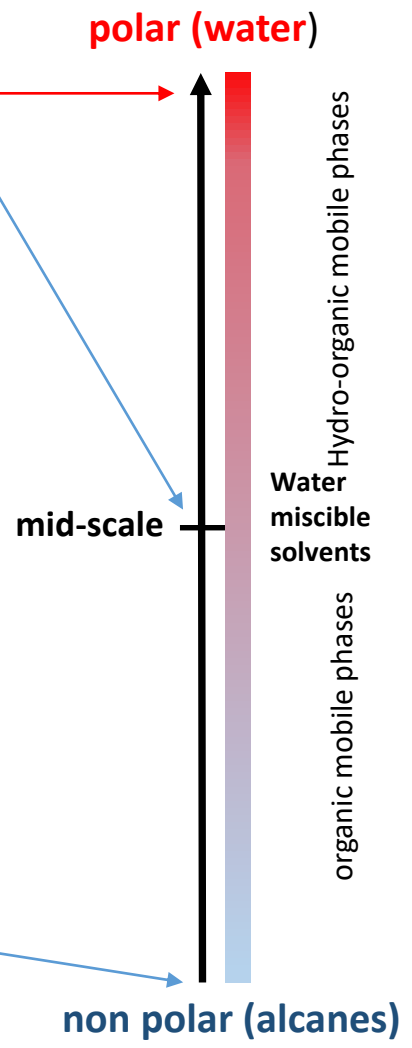
- Solute-solvent interactions involved in the solubilization process
  - H bonding (donnor/acceptor)
  - Dipoles interactions
  - Plus dispersion (London) forces
- overall solvation capability of solvents = solvent polarity
  - Like dissolves like
  - Oils dissolve in non polar solvents
  - Sugars dissolve in water and polar solvants (alcohols)

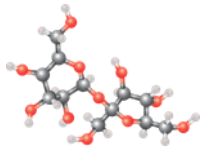


Solvent polarity scale

- P’ (Rohrschneider)
  - Calculated from the gaz-liquid partition coefficients ($k^{"}$) of 3 probe compounds (ethanol, dioxane, nitromethane) placed in a sealed vial containing the solvent to be characterized
  - $P' = \log(k_e^{"}) + \log(k_d^{"}) + \log(k_n^{"})$
- δ (Hildebrand)
  - square root of the cohesive energy density or amount of energy needed to completely remove unit volume of molecules from their neighbours to infinite separation (an ideal gas)
  - $\delta = \sqrt{E_S^V / V_S}$

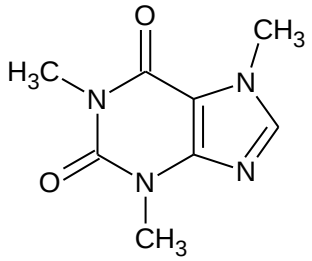
Solvent	δ cal½cm-3/2)	P'
Water	**21**	**10.2**
Methanol	12.9	5.1
Acetonitrile	11.8	5.8
Ethanol	11.2	4.3
Isopropyl alcohol	10.2	3.9
Dioxane	9.8	4.8
Dichloroethane	9.7	3.5
Dichloromethane	9.6	3.1
Acetone	9.4	5.1
Chloroform	9.3	4.1
THF	9.1	4
Toluene	8.9	2.4
Ethyl acetate	8.6	4.4
Cyclohexane	**8.2**	**-0.2**
n-Hexane	**7.3**	**0.1**



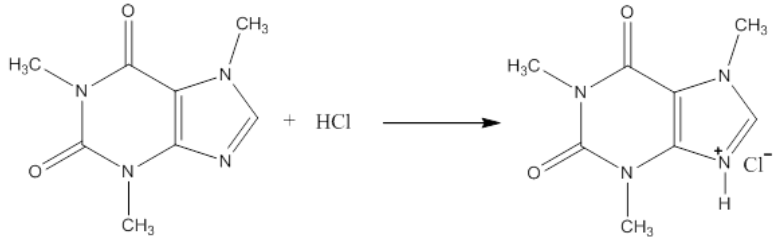


The analyte or solute

- Caffeine



- LogP = -0.07
- P octanol/water distribution coefficient (concentration ratio)
  - Polar or Hydrophilic compounds LogP < 0
  - Hydrophobic or non polar compounds LogP >0
  - Amphiphilic LogP ≈ 0
- Solubility in water is increased par addition of dilute HCl



1 g caffeine dissolves in
- 46 mL of water
- 66 mL of alcohol
- 50 mL acetone
- 530 mL ether

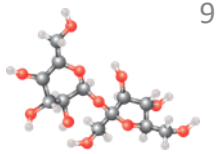
Ionizable compounds are more polar when ionized

Acidic compound

$$LogD = LogP + Log\left(\frac{1}{1+10^{pH-pKa}}\right)$$

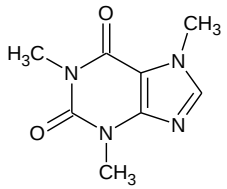
Basic compound

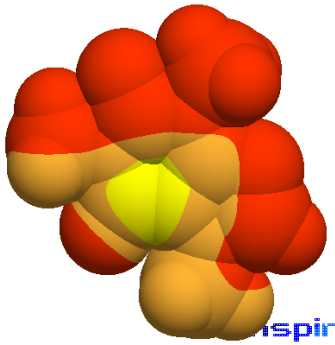
$$LogD = LogP + Log\left(\frac{1}{1+10^{pKa-pH}}\right)$$



LogP, MLP and polar groups

- Caffeine (Log P -0.07)

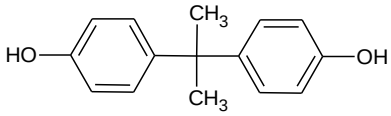


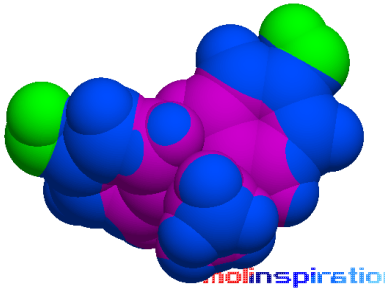


Molecules colored according to their Molecular Lipophilicy Potential.

LogP/LogD offers a global vision of solute polarity.

- Bisphenol A (LogP 3.3)

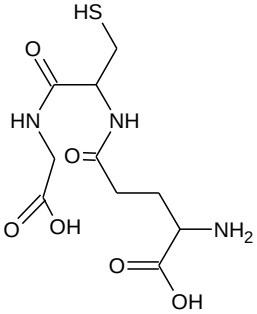


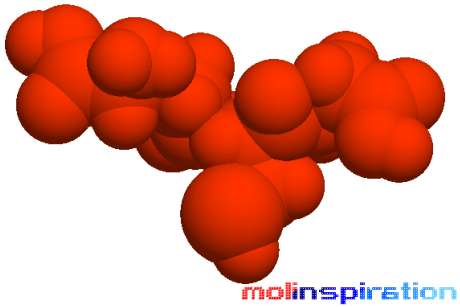


MLP describe how lipophilicity is distributed all over the different parts of a molecule.

Polar groups or non polar areas of the molecules are important to explain molecular interactions.

- Glutathione (LogP -4.5)





Normal phase LC.

non polar (alcanes)

Non-polar to moderately polar mobile phase

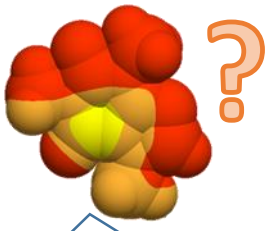
mid-scale

polar to moderately polar stationnary phase

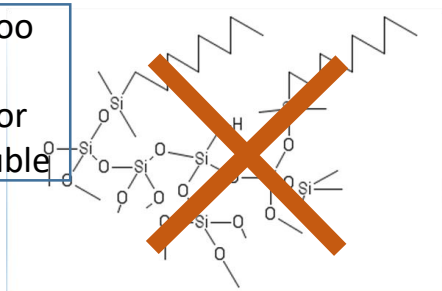
polar

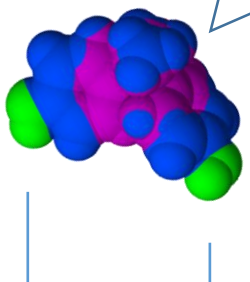
Analytes soluble in organic mobile phases

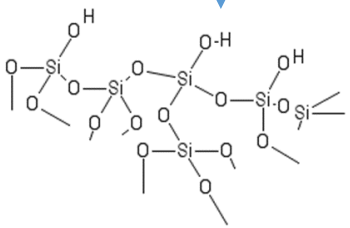
Interaction between the polar functional groups of the molecule and the polar moeities of the stationnary phase.

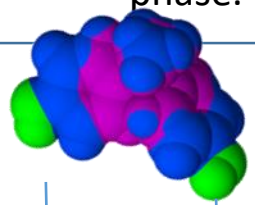


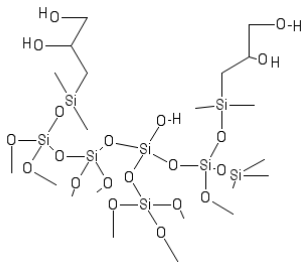
Perhaps too much retained or poorly soluble

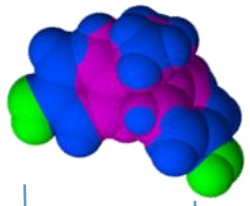


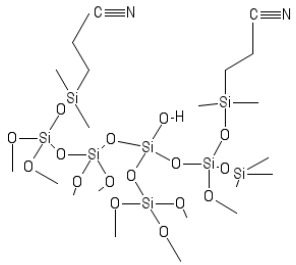












Reversed phase LC.

Polar (water) to moderately polar mobile phase

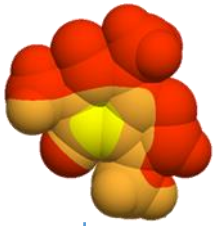
Polar (Water)

mid-scale

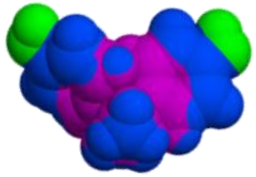
non polar

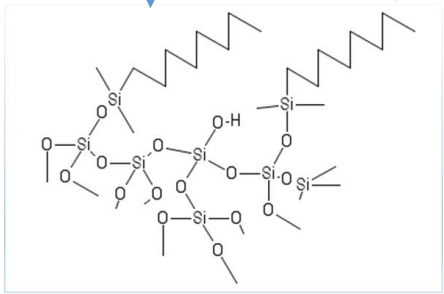
Non-polar stationnary phase

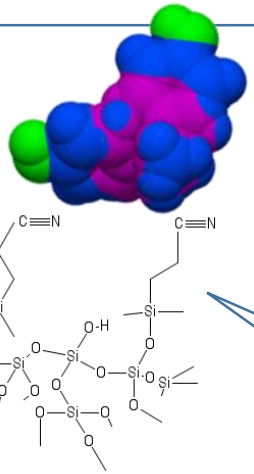
Analytes soluble in hydro-organic mobile phases



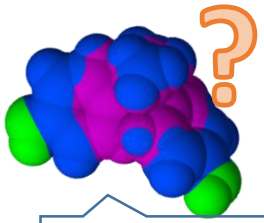
Interaction between the non polar area of molecules and the alkyl chains.



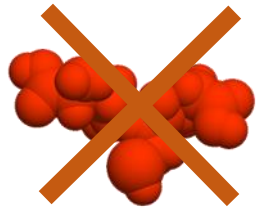


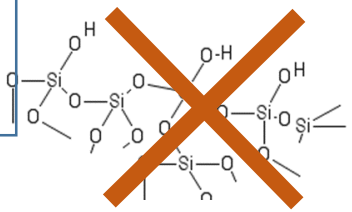


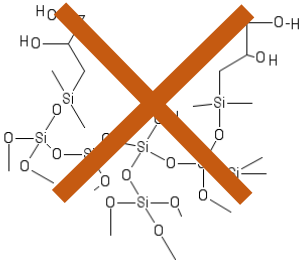
Cyano columns (mid-scale polarity) are usable in NP or RP LC



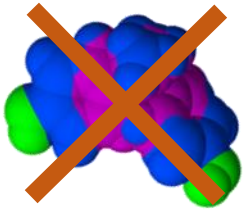
Perhaps too retained or poorly soluble

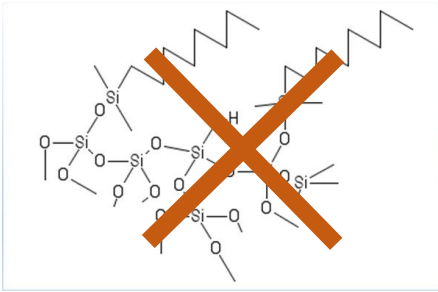






Hydrophilic Interaction Ch.





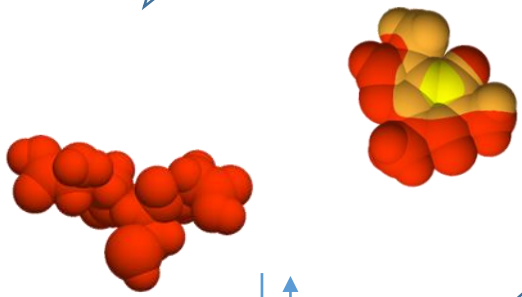
mid-scale

Water rich mobile phase

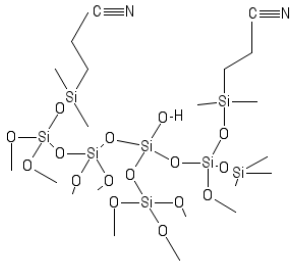
polar to moderately polar stationnary phase

polar

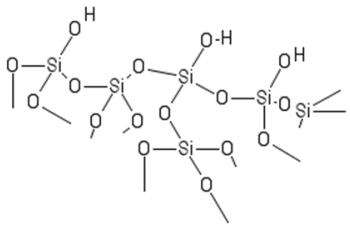
Water soluble analytes

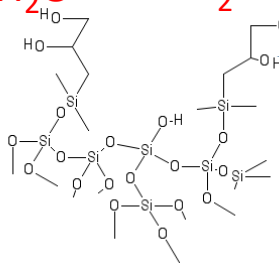


Partitioning between the mobile phase and the water layer (plus specific contributions of the polar groups of the St. Ph.).



H_2O H_2O H_2O H_2O





Water uptake by the stationnary phase.

More details on

Normal Phase chromatography

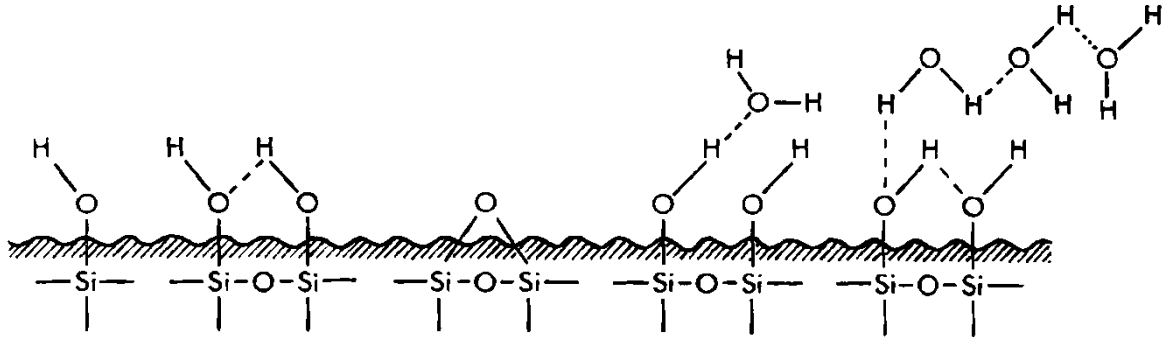
Adsorption (bare silica) and polar grafted phases

Normal Phase LC keypoints

- Sample is soluble in organic solvents mixtures
  - LogP > 1
- The mobile phase is non-polar or at least less polar than the St. Phase.
- The stationnary phase is polar
  - Bare silica (adsorbtion chromatography)
  - Polar grafted silica (diol, cyano, amino)

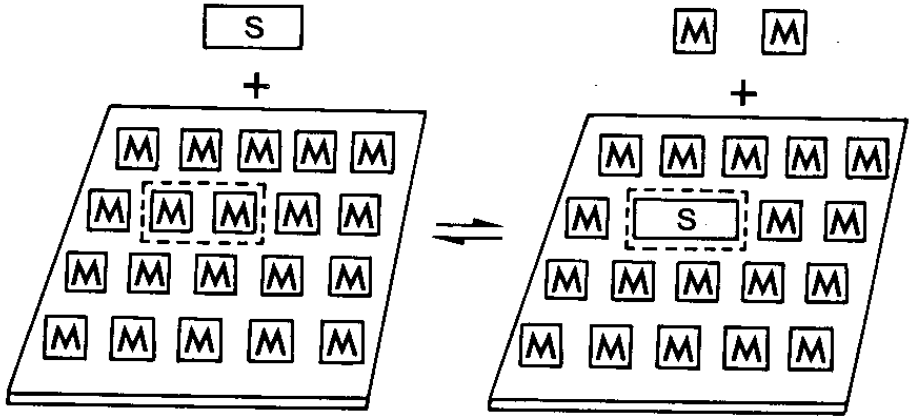
Silica gel (bare silica) properties

- Silica surface



- analytes interact with silanols
- the most energetic are the free silanols
- the activity decreases when they are involved in hydrogen bonds with other silanols or water molecules
- an anhydrous silica is said to be activated and deactivated when it is fully hydrated.

Adsorption equilibrium



The Solute dissolved in the mobile phase [S(in MP)] must displace several [n] solvent molecules of the mobile phase (MP) adsorbed on the stationary phase [M(adsorbed)] in order to take their place on the silica.

$$S_{(in\ MP)} + nM_{(adsorbed)} \rightleftarrows S_{(adsorbed)} + nM_{(in\ MP)}$$

- Adsorption is a competitive phenomenon
- a 1:1 stoichiometry is assumed
- the different chromatographic parameters are integrated into the Snyder-Soczewinski model.

$$\log(k) = \log(Va) + \beta^{*}[Eo - As.\varepsilon_0] + \log\left(\frac{Wa}{V_M}\right)$$

Snyder-Soczewinski model

k retention factor of the solute

Va volume of mobile phase adsorbed per unit mass of silica
Depends on the specific area of the silica particles

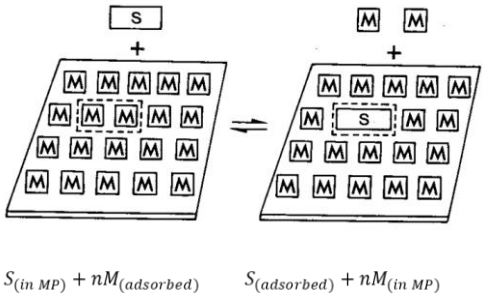
Wa mass of silica contained in the column
V_M dead volume of the column (also noted V_0)

$$\log(k)=\log(Va)+\beta^*[Eo-As.\varepsilon_0]+\log\left(\frac{Wa}{V_M}\right)$$

β* activity of the adsorbent :
β*=0 highly hydrated / deactivated silica
β*=1 highly activated silica, anhydrous

As surface occupied by one mole of solute on the silica

ε_o eluent strength of the solvent (and A_M surface occupied by the solvent)



$S_{(in\ MP)} + nM_{(adsorbed)}$ $S_{(adsorbed)} + nM_{(in\ MP)}$

E_o free energy of adsorption of solute molecules under standard conditions of activity (β*=1)

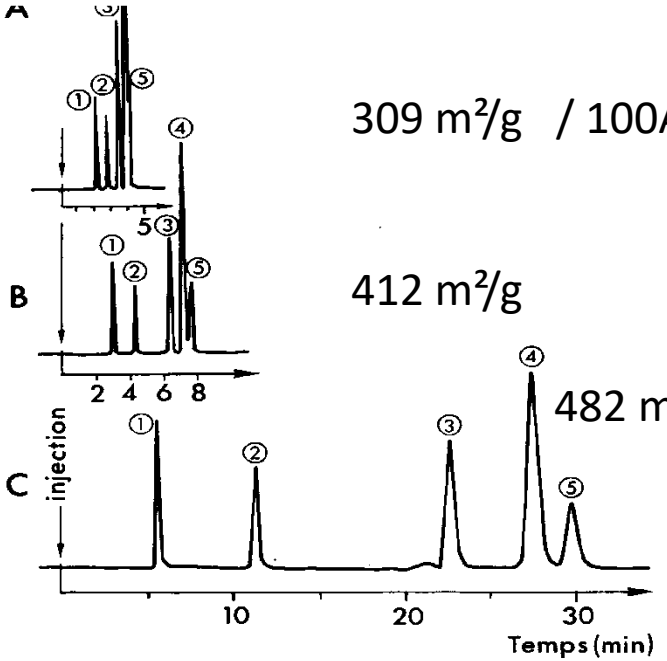
$$Eo = -\frac{\Delta G_S^0}{2{,}3.R.T}$$

$$\varepsilon_0 = \frac{\Delta G_M^0}{2{,}3.R.T.A_M}$$

Va and specific surface area

Va: the volume of mobile phase adsorbed per g of silica, is proportional to the **specific surface area** (m^2/g) of the support.

However, the increase of the specific surface area is to the detriment of the **pore diameter**: as well as the number of free silanols per unit of surface area.



309 m²/g / 100Å

412 m²/g

482 m²/g / 60 Å

FIG. VIII.6. — Influence de la surface spécifique d'une silice Sphérosil sur la séparation d'un mélange d'hydrocarbures aromatiques.

Colonne : longueur, 10 cm ; diamètre intérieur, 4 mm. Phase stationnaire : silice de type Sphérosil expérimental de 5,6 µm et de surfaces spécifiques variées :

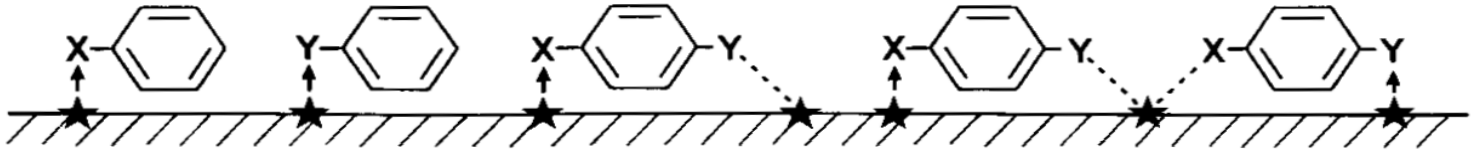
$$A = 470\ m^2.g^{-1}\ ;\quad B = 590\ m^2.g^{-1}\ ;\quad C = 900\ m^2.g^{-1}\ .$$

Phase mobile : hexane sec. Débit : 0,9 ml.min $^{-1}$. Température : ambiante. Échantillon : nature des solutés : 1 : toluène ; 2 : naphtalène ; 3 : biphényle ; 4 : anthracène ; 5 : phénanthrène (d'après [20]).

TABLEAU VIII.3. — Variation du nombre de sites OH actifs en fonction de la surface spécifique du support [3].

Nature du support	Surface spécifique $m^2.g^{-1}$	Nombre total de sites OH par nm^2	Nombre de sites actifs par nm^2 (OH libres)
Lichrosorb Si 100.....	309	4,6	2,95
Partisil 5.....	412	4,6	2,80
Lichrosorb Si 60.....	482	4,6	2,20

Adsorption energy E_0



$$E^0 = Q_X^0 + (1-f)\sum_{i \neq x} Q_i^0$$

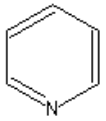
E_0 depends on the nature and the number of polar groups disponible to interact with silanols

Groupement	*X et Y =Ar.*	*X=Al, Y=Ar*	*X et Y = Al*
X-CH3	0.11		0.07
-C=	0.25	0.25	0.25
X-F	-0.15		1.54
X-O-Y	0.87	1.83	3.61
X-OH	4.20		5.60
X-NH2	5.10		8.00
C-CN	3.33		5.27
X-COOH	6.1		7.6

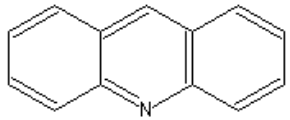
Steric hindrance & E_0:

$$E^0 = Q_X^0 + (1-f)\sum_{i \neq x} Q_i^0$$

Pyridine

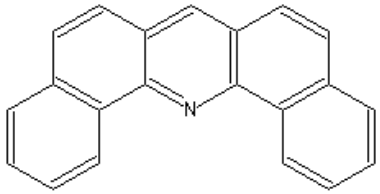


$Q^0_{-N=} = 6.10$



$Q^0_{-N=} = 5.30$

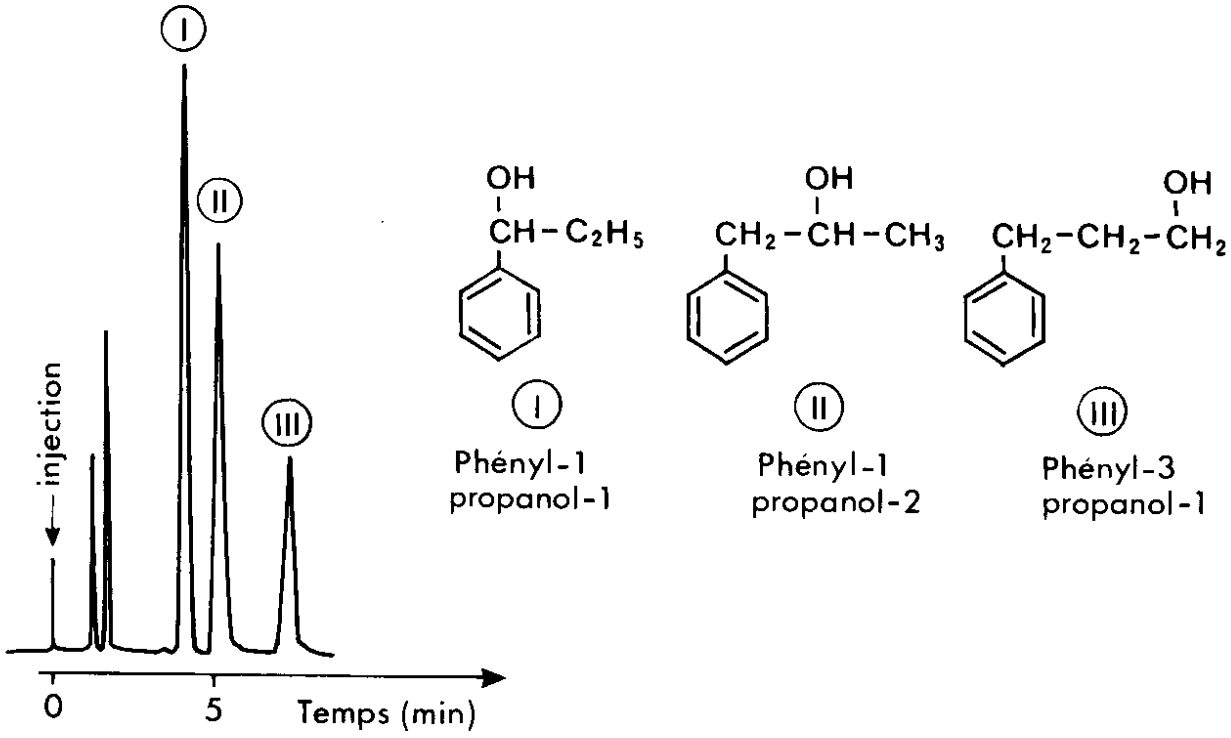
3,4,5,6 dibenzoacridine



$Q^0_{-N=} = 0.00$

Adsorption energy E_0

Influence of position isomerism for the separation of three phenyl-propanol solutes by adsorption chromatography



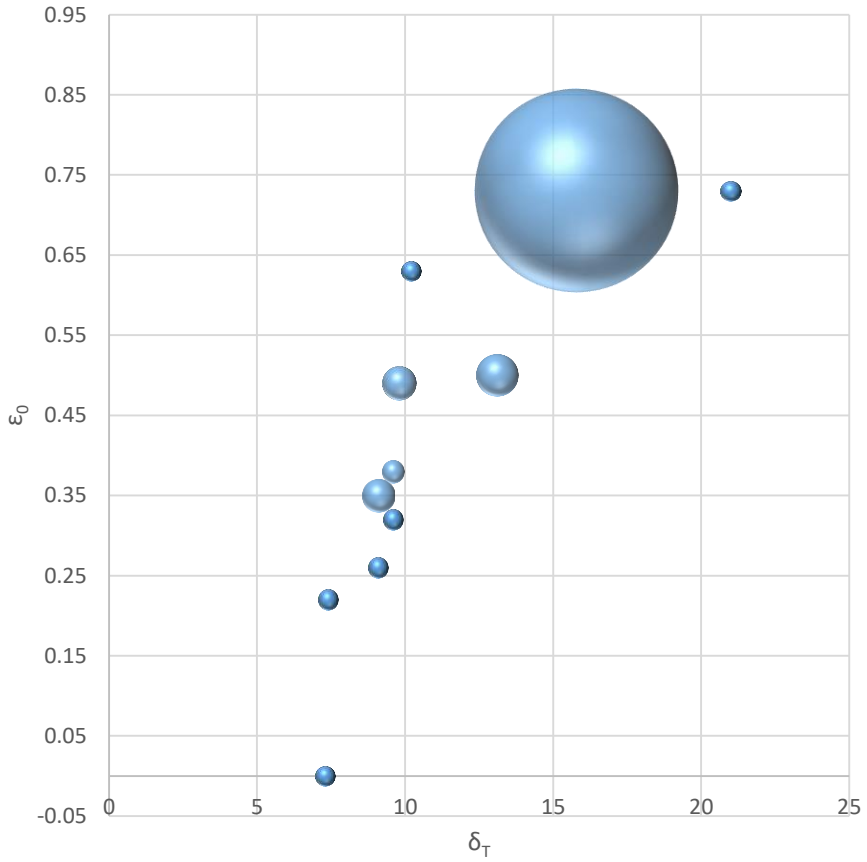
Séparation d'un mélange de phényl-propanols par chromatographie d'adsorption.

Eluting strength ε_0 is not polarity

	ε_0	δ_T	water %
Hexane	0	7.3	0.0005
Isopropylic ether	0.22	7.4	0.008
Chloroforme	0.26	9.1	0.005
Dichlorométhane	0.32	9.6	0.007
Tetrahydrofuran	0.35	9.1	0.13
Ethyl acetate	0.38	9.6	0.06
Dioxane	0.49	9.8	0.14
Acetonitrile	0.5	13.1	0.22
Isopropanol	0.63	10.2	
Methanol	0.73	15.8	5.2
Water	0.73	21	

The eluting strength and polarity are roughly correlated but not all solvents activate silica in the same way.

Controling the silica hydration is somewhat difficult



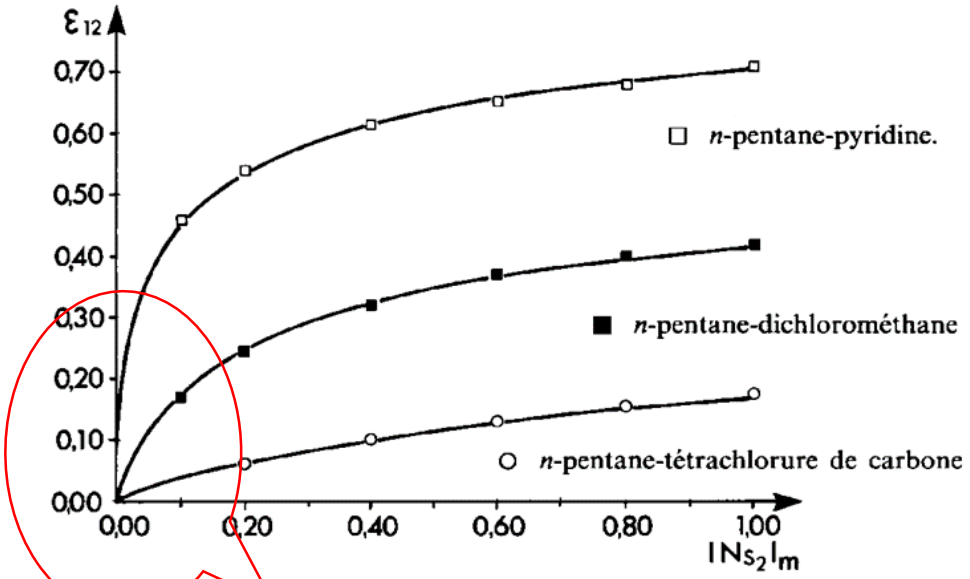
Eluting strength vs polarity
Size of the bubbles = water % in solvent

Eluting strength ε_0 of solvent mixtures

$$\varepsilon_{1+2} = \frac{\log\left[1+\left|N_{S2}\right|_m\left(10^{\beta^*.As(\varepsilon_2-\varepsilon_1)}-1\right)\right]}{\beta^*.As}$$

Solvent 2 stronger than 1 ($\varepsilon_2>\varepsilon_1$).

β^*	Adsorbant activity.
As	assumes the same surfaces for the two solvents
$\lvert N_{S2}\rvert_m$	Molar fraction of solvent 2 in the mobile phase

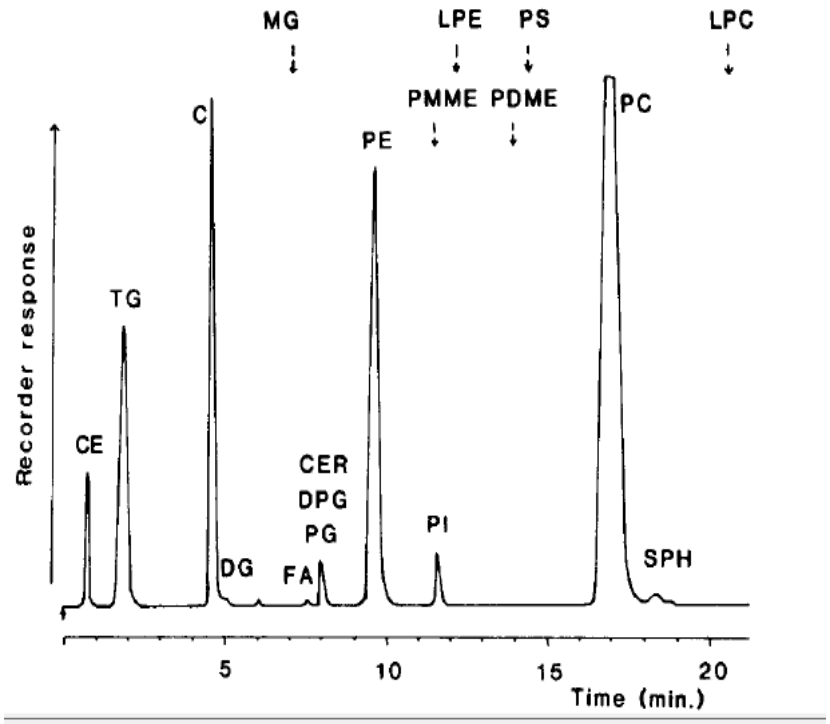


Violent increase of the eluting strength of the mobile phase when too much difference exists between the weak and the strong solvent.

3 or 4 way gradient elution is frequently used to increase gradualy ε_0

Example: lipid class analysis

Rat liver lipids



column: Spherisorb 3µm 150x5mm

TABLE 1. Ternary gradient elution system required for the elution of lipid classes and reactivation of the column

Time[a]	% Solvent A	B	C
min			
0	100		
1	100		
5	80	20	
5.1	42	52	6
20	32	52	16
20.1	30	70	
25	100		
30	100		

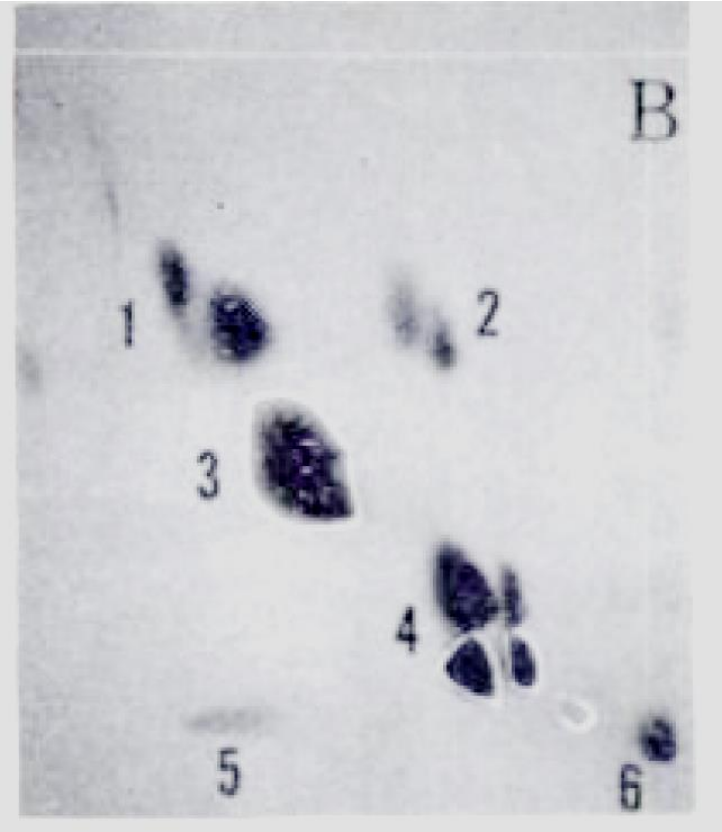
A Isooctane: THF 99:1 v/v

B Isopropanol:$CHCl_3$ 4:1 v/v

C Isopropanol: water 1:1 v/v

Rapid separation and quantification of lipid classes by high performance liquid chromatography and mass (light-scattering) detection. W W Christie, *1985 The Journal of Lipid Research, 26, 507-512.*

Two dimensions Thin Layer Chromatography.



(1) cerebrosides (2) sulfatides (3) PE
(4) PC, PS, SM, LPE (5) PA
(6) ganglisosides (starting spot)

- Rat brain lipids
- Silice G
- 1st dimension

  $CHCl_3$/MeOH/NH_4OH-
  65/35/4
- 2nd dimension

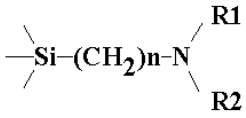
  CHCl3/ Acetone / Methanol / acetic acid/ water

  10/4/2/2/1

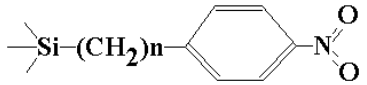
Quantitative analysis of lipid classes, O.S. Privett et al. *Am J Clin Nutr. 1971 vol. 24 no. 10 1265-1275*

NP with Polar grafted silica

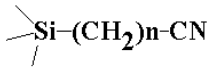
- Chemically modified silica with polar grafts
- Retention orders are similar to those encountered on silica.
- The solvents are the same as in adsorption chromatography.
- The retention is globally less important
- Water can be added to the mobile phase.



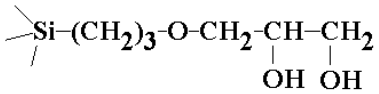
Amino



Nitro

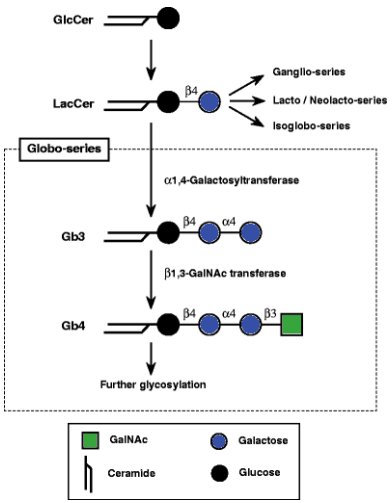


Cyano

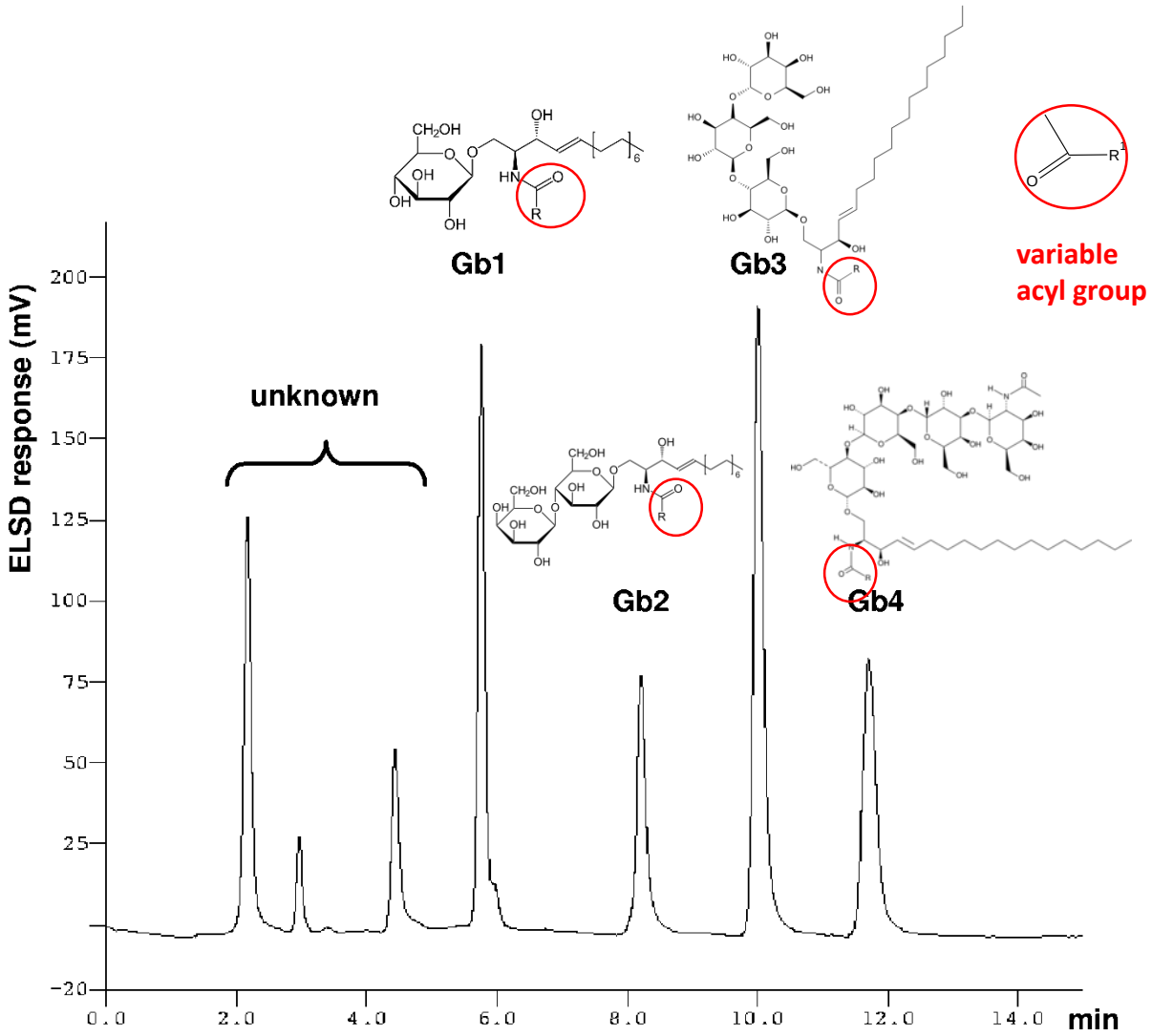


Diol

Example of glycolipid separation

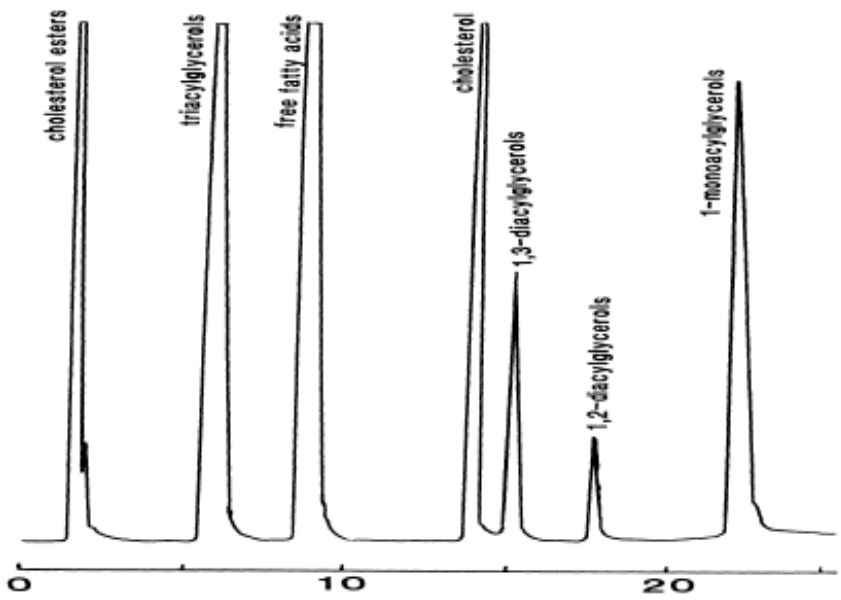


- The order of elution follows the number of sugar units on the glycosylceramides.
- Stationary phase:
- Polyvinyl alcohol grafted on silica
- Mobile phase: acetone:methanol gradient



Example Cyanopropyl St Phase

« neutral » lipids



Spherisorb S3CN stationnary phase

- Colonne (100x4.6)
- Gradient
  - Hexane to MTBE

Separation of non-polar lipids by high performance liquid chromatography on a cyanopropyl column
Ali H. El-Hamdy, W.W. Christie, Journal of High Resolution Chromatography, 16, (1993) Iss 1, pages 55–57

More details on

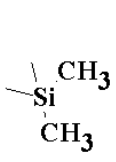
Reversed Phase chromatography

Reversed Phase LC Keypoints

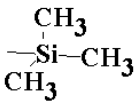
- Up to 70~80% of HPLC applications are RP-HPLC
- Stationary phase is non-polar
  - Most popular C18 grafted silica
- The mobile phase is polar
  - Consists of water to which a miscible organic solvent (methanol, acetonitrile or more rarely tetrahydrofuran) is added to accelerate elution.
  - The pH of the mobile phase can be modified
- For solutes with LogP/LogD in the -1~4 range

Stationnary phase structure (1)

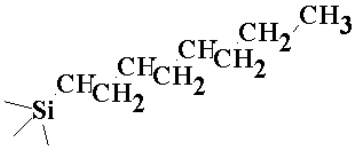
- generally alkyl grafts.
- most classical are C8 and C18



Dimethyl

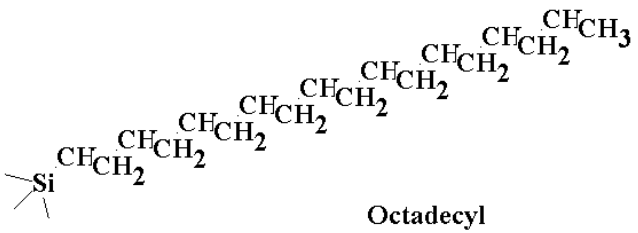


Trimethyl

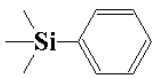


Octyl

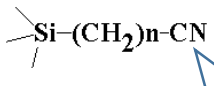
- Phenyl- and cyano
  - intermediate polarity
  - specific interactions



Octadecyl



Phenyl



Cyano

π electrons specific interactions

Strong dipole interactions

Stationnary phase structure (2)

- alkyl grafts.
- Differ by
  - Chain length
  - Phase chemistry
  - Bound density

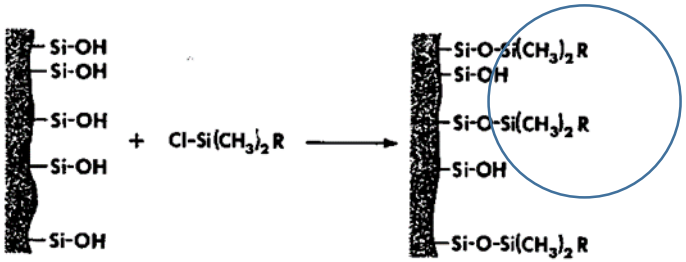


Figure 2 Synthesis scheme for monomeric surface modification of silica, using monofunctional silane reagents. (From Ref. 37.)

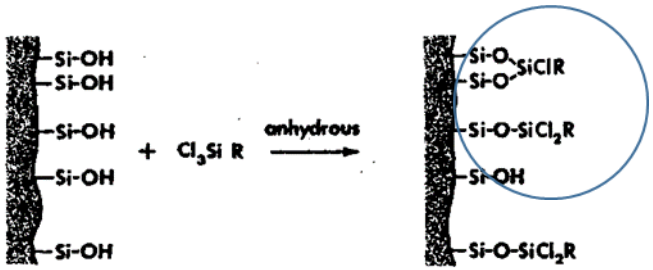


Figure 4 Synthesis scheme for monomeric surface modification of silica, using polyfunctional silane reagents (From Ref. 37.)

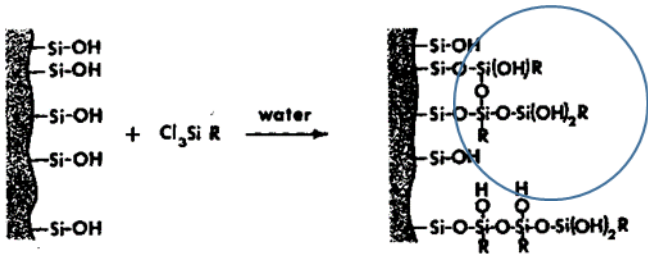
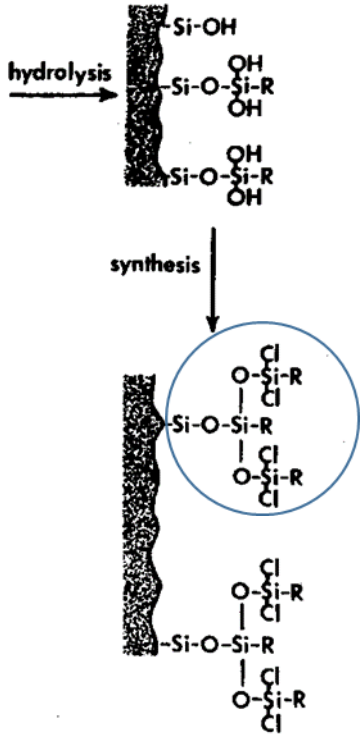


Figure 5 Synthesis scheme for polymeric surface modification of silica. Water is added either to the silica (*water preequilibration synthesis*) or to the reaction mixture (*water-slurry synthesis*). (From Ref. 37.)



St. Phase structure and retention

Solute retention increases with increasing alkyl chain length and/or chain density

Effect of alkyl graft length

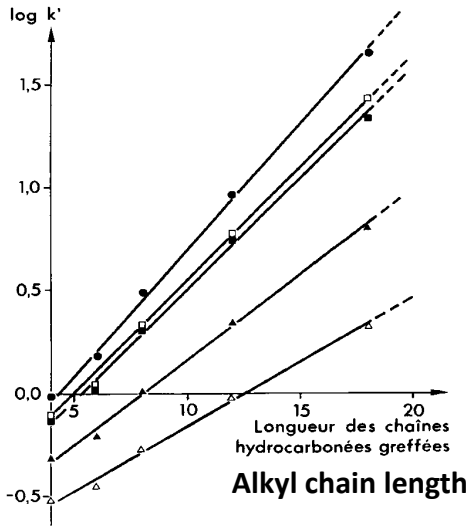


FIG. IX.17. — Variation du logarithme du facteur de capacité k' d'hydrocarbures polyaromatiques en fonction de la longueur de la chaîne greffée dans le cas d'une silice (Partisil 5 µm) ayant 2,1 µmol.m^{-2} de greffons.

Phase mobile : méthanol-eau (70 : 30 v/v) ; autres conditions identiques à celles de la figure IX.15. △ benzène ; ▲ naphtalène ; ■ phénanthrène ; □ anthracène ; ● pyrène.

Effect of alkyl graft density

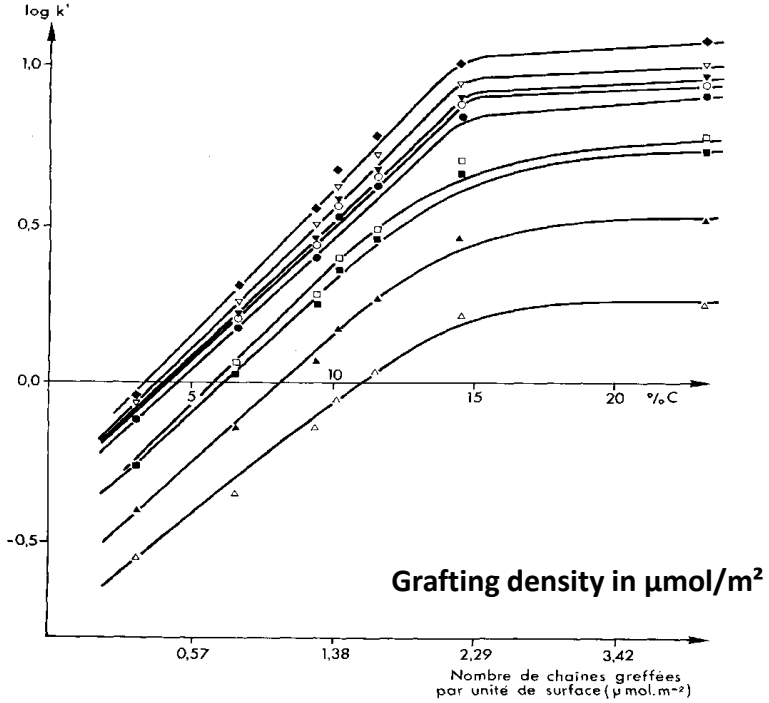
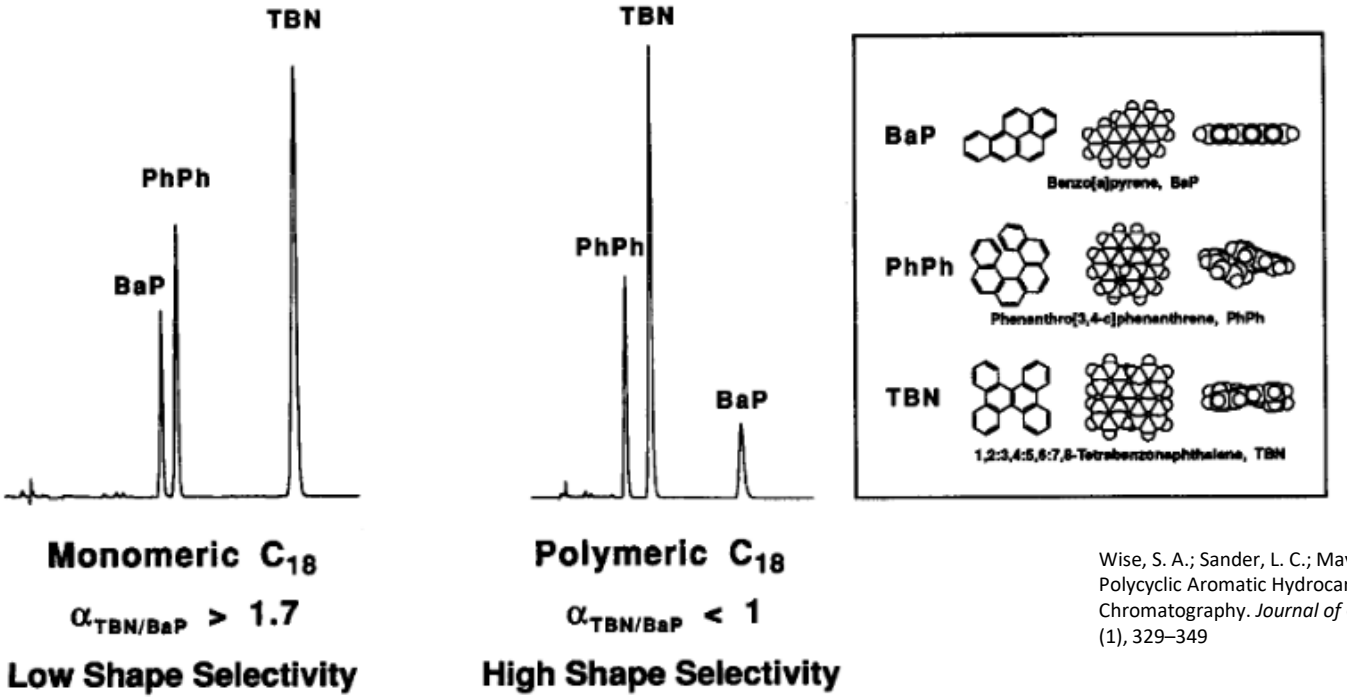


FIG. IX.15. — Variation du logarithme du facteur de capacité k' d'hydrocarbures monoaromatiques substitués en fonction du pourcentage en carbone de la phase stationnaire (Partisil 5 µm greffé C_{18}) et du nombre de greffons par unité de surface.

Colonne : longueur, 15 cm ; diamètre intérieur, 4,8 mm (1/4"). Phase mobile : méthanol-eau (60 : 40 v/v) ; débit 0,82 ml.min^{-1}. Détection : absorptiométrie dans l'ultra-violet à 254 nm. △ benzène ; ▲ toluène ; ■ *o*-xylène ; □ *m*-xylène ; ● éthyl-2 toluène ; ○ éthyl-3 toluène ; ▼ éthyl-4 toluène ; ▽ triméthyl-1,3,5 benzène ; ◆ diéthyl-1,2 benzène.

St. Phase structure and selectivity



Wise, S. A.; Sander, L. C.; May, W. E. Determination of Polycyclic Aromatic Hydrocarbons by Liquid Chromatography. *Journal of Chromatography A* **1993**, *642* (1), 329–349

Fig. 3. Separation of SRM 869, Column Selectivity Test Mixture for Liquid Chromatography, on a polymeric and a monomeric C_{18} stationary phase. Structures of the three components in the mixture are illustrated in the box. Chromatographic conditions: mobile phase isocratic at 85% acetonitrile in water at 2 ml/min; UV detection at 254 nm.

- The stationnary phase structure also influence selectivity

Solute-stationnary phase interactions

- Solute-St. Phase interactions cannot be explained by H-bonds and van der Waals forces.
- H-bonds and van der Waals forces explain solute-solvent interactions.
- The hydrophobic repulsion of the solute on the alkyl chains is the primary cause of retention.
- Water and polar solvents self-organize around the solutes and grafts to minimize entropy of the solute-stationary phase system in contact with the mobile phase.

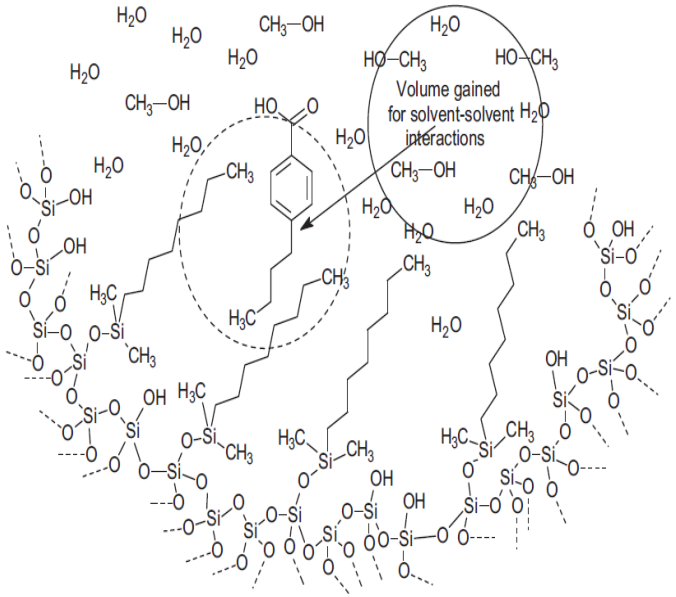


FIGURE 5.1.3 Schematic diagram picturing the "repulsion" of a hydrophobic molecule from a polar solvent and its "acceptance" by the hydrophobic phase at the interface between a mobile phase H_2O/CH_3OH and a C8 bonded phase.

As a consequence, there is no unique and widely accepted retention model in RP-HPLC

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Empirical retention model.

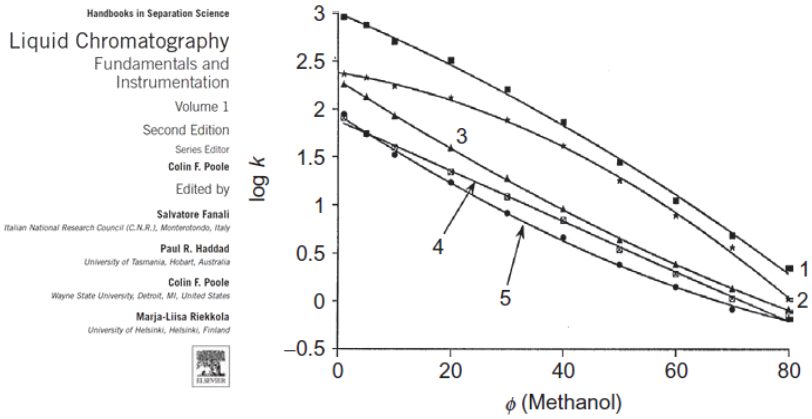


FIG. 4.1

Plot of the logarithm of the retention factor (log *k*) as a function of the volume fraction of methanol (×100) in RPLC for a silica-based octadecylsiloxane bonded stationary phase. Solute identification: 1=naphthalene; 2=bromobenzene; 3=acetophenone; 4=2-phenylethanol; and 5=benzamide.

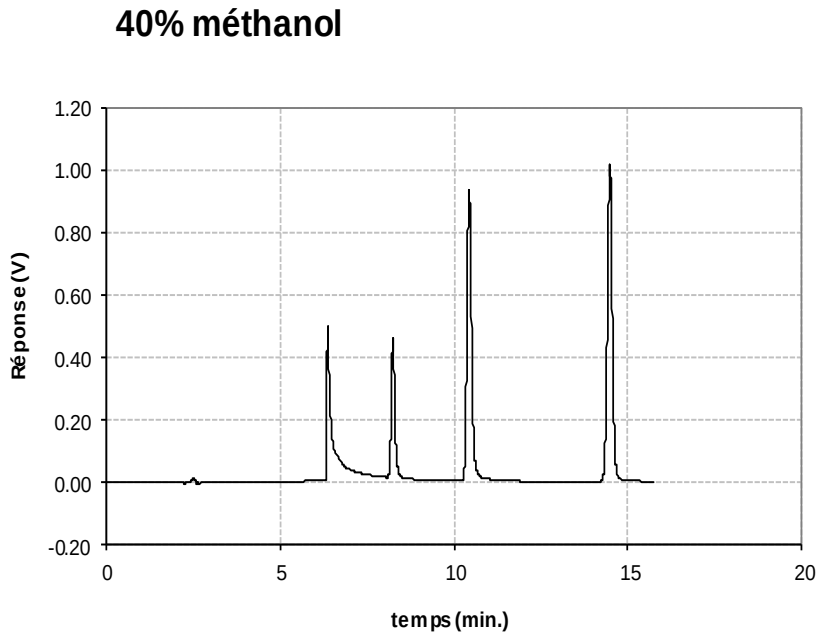
Reproduced with permission from Poole CF, Poole SK. Column selectivity from the perspective of the solvation parameter model. J Chromatogr A 2002;965:263–9. Copyright Elsevier Science Publishers.

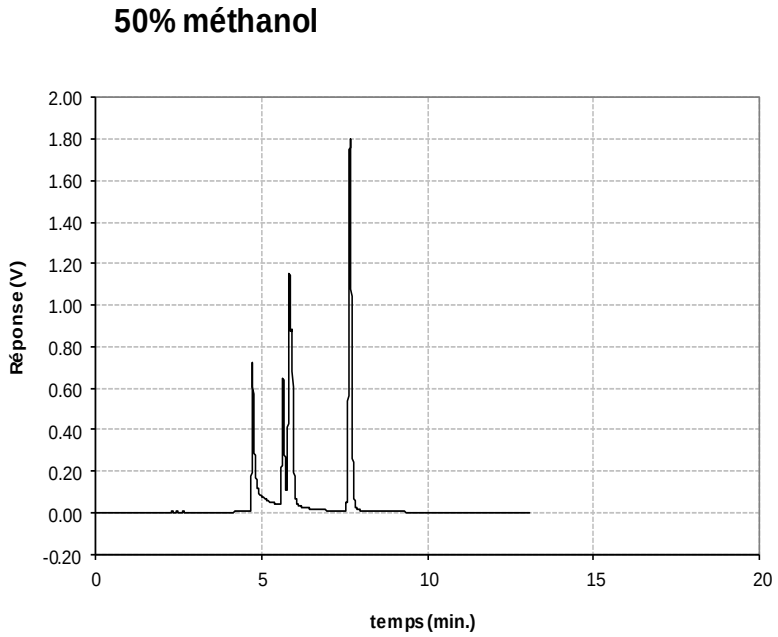
The log-linear decrease of the retention factor as a function of the quantity (volume fraction φ_{org}) of (strong) organic solvent in the mobile phase leads to the following equation:

$$\ln(k_i) = Lnkw - S.\varphi_{org}$$

- Lnkw: estimate (projection) of the log. retention factor in a mobile phase without organic modifier. Lnkw strongly correlates with Log(P)
- S (Slope) influence of the organic modifier on retention.

Mobile phase strength in RP-LC

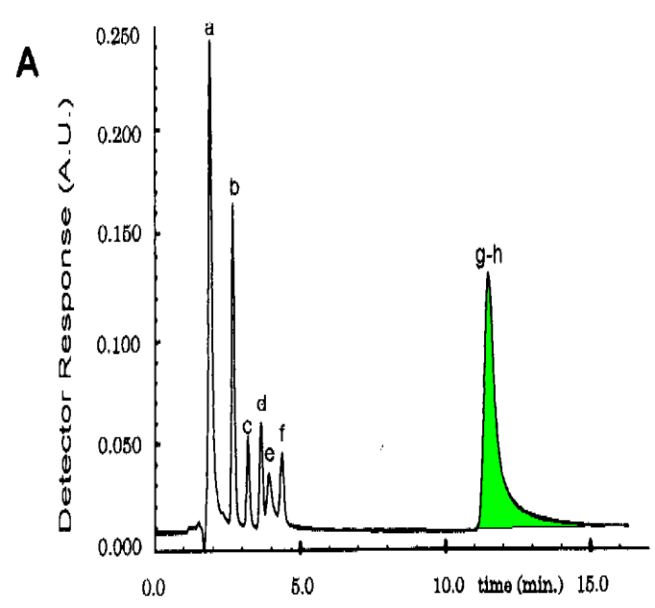




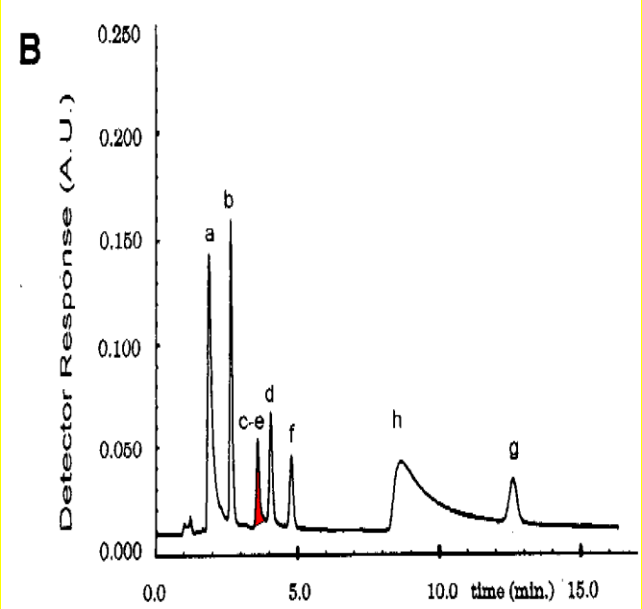
- An increase of the % of organic modifier decrease mobile phase polarity and decrease retention
- Empirical rule: +10% → retention times /2

Mobile phase solvent Selectivity

Methanol:water 73:27



Acétonitrile:water 60:40



- Preferential solute-solvent interactions influence separation selectivity.

Mobile phase pH Selectivity

Depending on the mobile phase pH, the retention factor of a weak acid AH is intermediate between

- k_0 the retention factor of the non ionized AH form
- k_{-1} the retention factor of the ionized, less retained, A^- form

$$k = \frac{k_0 + k_{-1}\dfrac{K_{a_m}}{[H^+]_m}}{1 + \dfrac{K_{a_m}}{[H^+]_m}}$$

- A similar equation can be derived for weak bases.

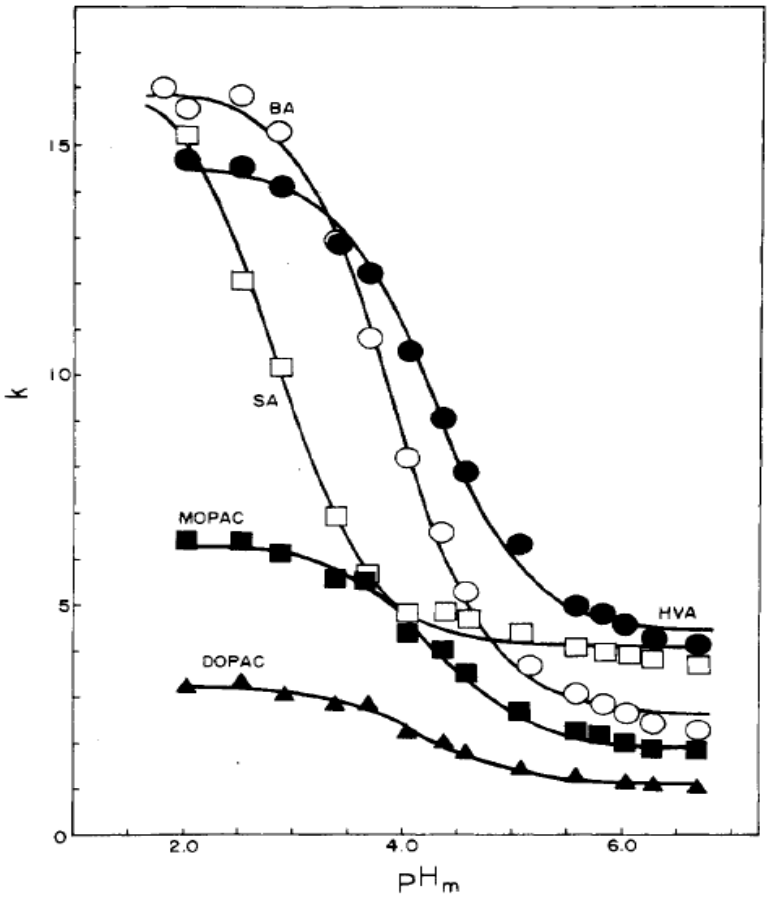


Figure 6. Plots of the capacity factor vs. the pH of the eluent for monoprotic acids

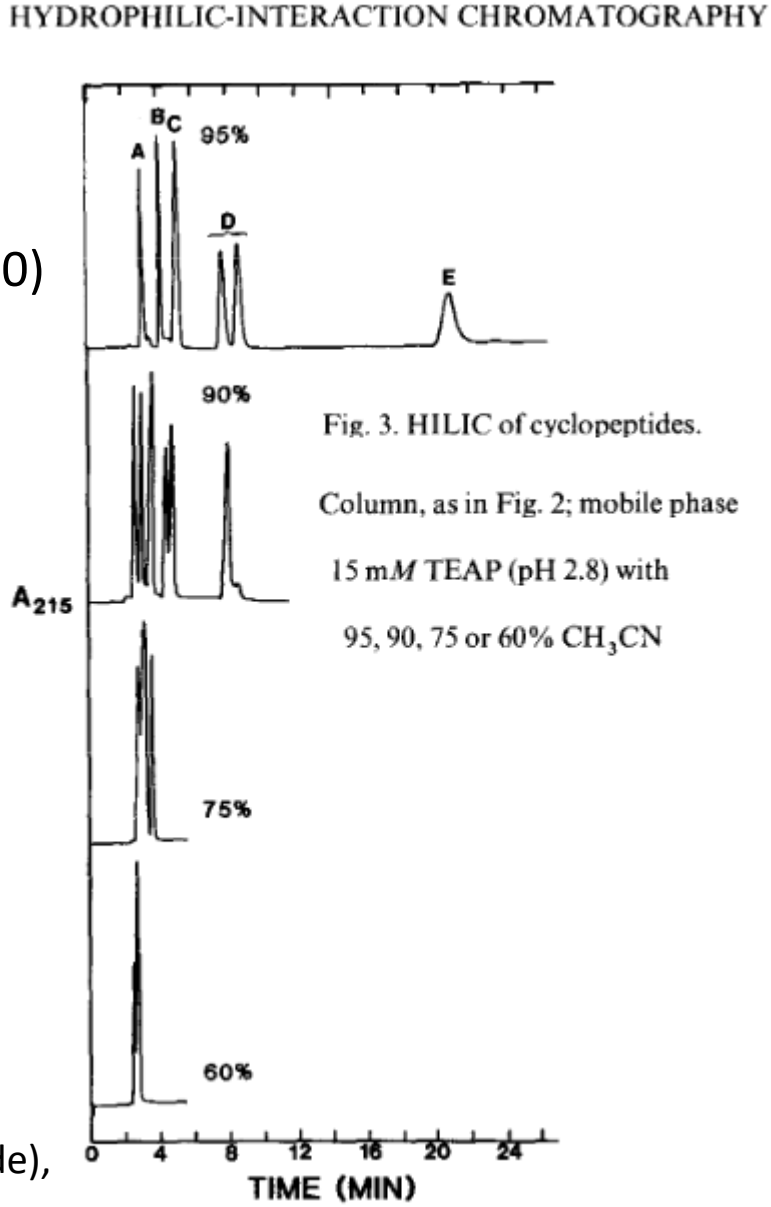
Column, Partisil 1025 ODS; eluent, 1.0 M Na_2SO_4 in 0.05 M phosphate buffers;

More details on

Hydrophilic Interaction Chromatography

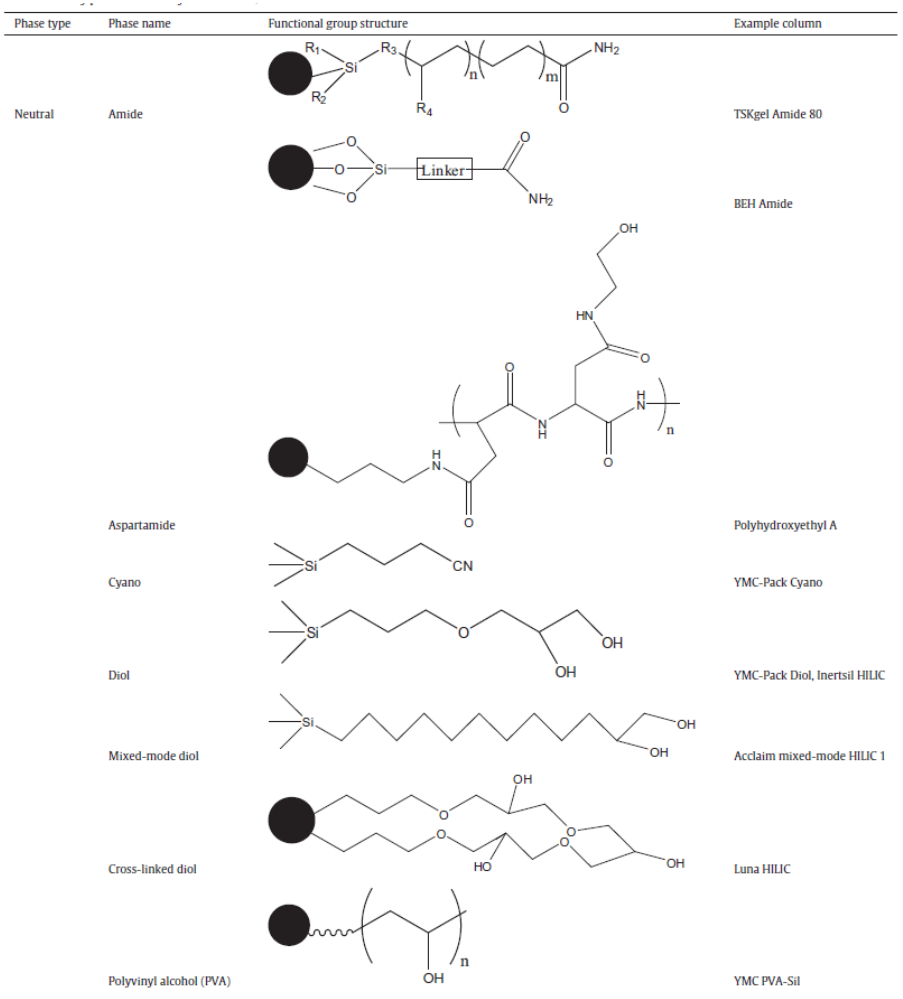
HILIC Keypoints

- Introduced by Alpert (J. Chromatography, 1990)
- Addresses (very) polar (water-soluble) compounds (LogP/LogD < 1)
- Hydro-organic mobile phases (acetonitrile-water)
- Avoids the use of ion exchange for ionized analytes
- Polar stationary phase "normal phase type"
  - bare silica
  - « Neutral » phases: amide, cyano, diol
    - non-ionizable at pH 3~7
  - "charged" phases:
    - Positive ionization: amino, imidazole, triazole
    - Negative ionization: poly(2-sulfoetlaspartamide), poly(aspatic acid), sulfones
    - Zwiterionics: sulfobetaine, phosphocholine,



Alpert, A. J. Hydrophilic-Interaction Chromatography for the Separation of Peptides, Nucleic Acids and Other Polar Compounds. *Journal of Chromatography A* **1990**, *499*, 177–196.

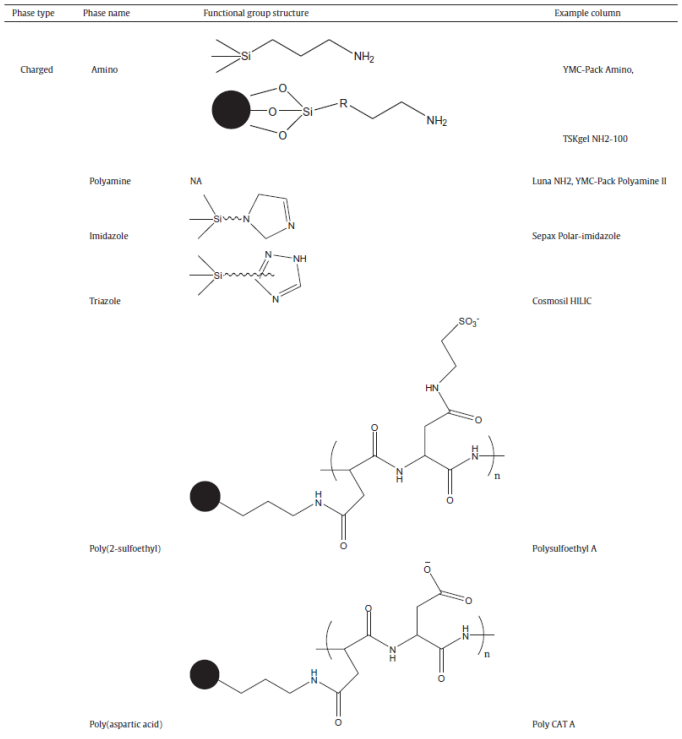
« Neutral » stationnary phases

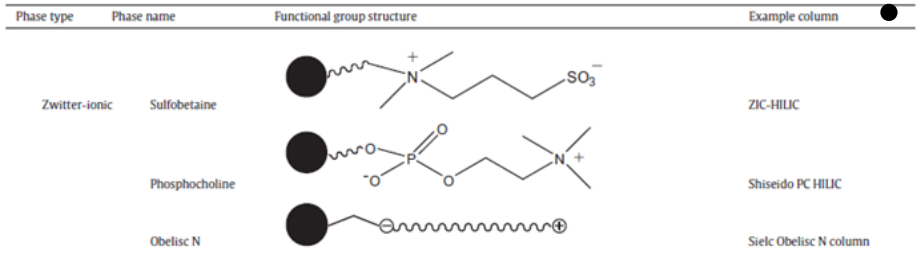


- Polar functional groups
- **Amide** phase: The most classical
- **Aspartamide** phase: created specifically for HILIC
- **Cyano** phases: not very retentive, no H-bound donor character
- **Diols** phases: Hydrophilic character modulated by the length of the linker
- **Polyvinyl alcohol** and cross-linked diols are more stable

Y. Guo et S. Gaiki, « Retention and selectivity of stationary phases for hydrophilic interaction chromatography », Journal of Chromatography A, vol. 1218, n° 35, p. 5920-5938, sept. 2011.

« Charged » Stationnary phases





- Ionized phases at pH 3~7
- Positive charges
  - **NH2** amine
  - **Imidazoles and triazoles**, new phases developed for HILIC, improved stability
- Negative charges
  - Sufonates and carboxyates
  - Ion exchange phases used in HILIC
  - bare silica
- Zwitterionic
  - Initially developed for the analysis of ionized compounds
  - Differs by the accessibility of the charge (+) or (-)

Y. Guo et S. Gaiki, « Retention and selectivity of stationary phases for hydrophilic interaction chromatography », Journal of Chromatography A, vol. 1218, n° 35, p. 5920-5938, sept. 2011.

Retention mechanism

- Complex and not fully elucidated
  - **Partition** of the solute between the mobile phase and an aqueous phase immobilized on the surface of the stationary phase
  - **Solute/St. Phase Interactions**
    - Adsorption, and/or
    - ion interactions, and/or
    - hydrophobic

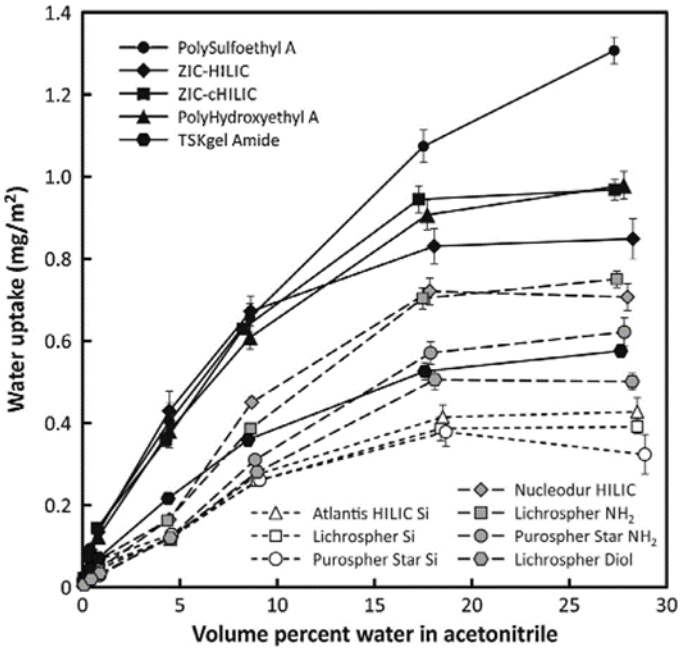


Fig. 2. Water uptake isotherms of the tested HILIC materials obtained by equilibration with water followed by coulometric Karl Fischer determinations of water in the supernatant liquid. Filled black symbols with full lines denote polymeric grafted phases, filled gray symbols with dashed lines denote monomeric grafted phases, and open white symbols with dotted lines denote unmodified silica. The mobile phase contained only acetonitrile and water without any buffers or additives.

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Retention mechanism and St. Phase selectivity

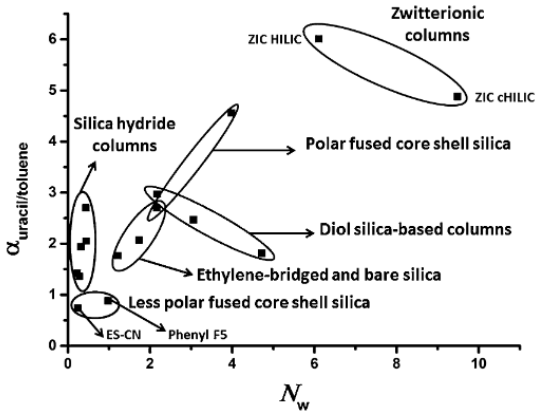


Fig. 8. The effect of the equivalent number of the monomolecular water layers, N_w, on the selectivity factor, related to the retention of uracil and toluene, $\alpha_{uracil/toluene}$.

J. Soukup, P. Jandera / J. Chromatogr. A 1374 (2014) 102–111

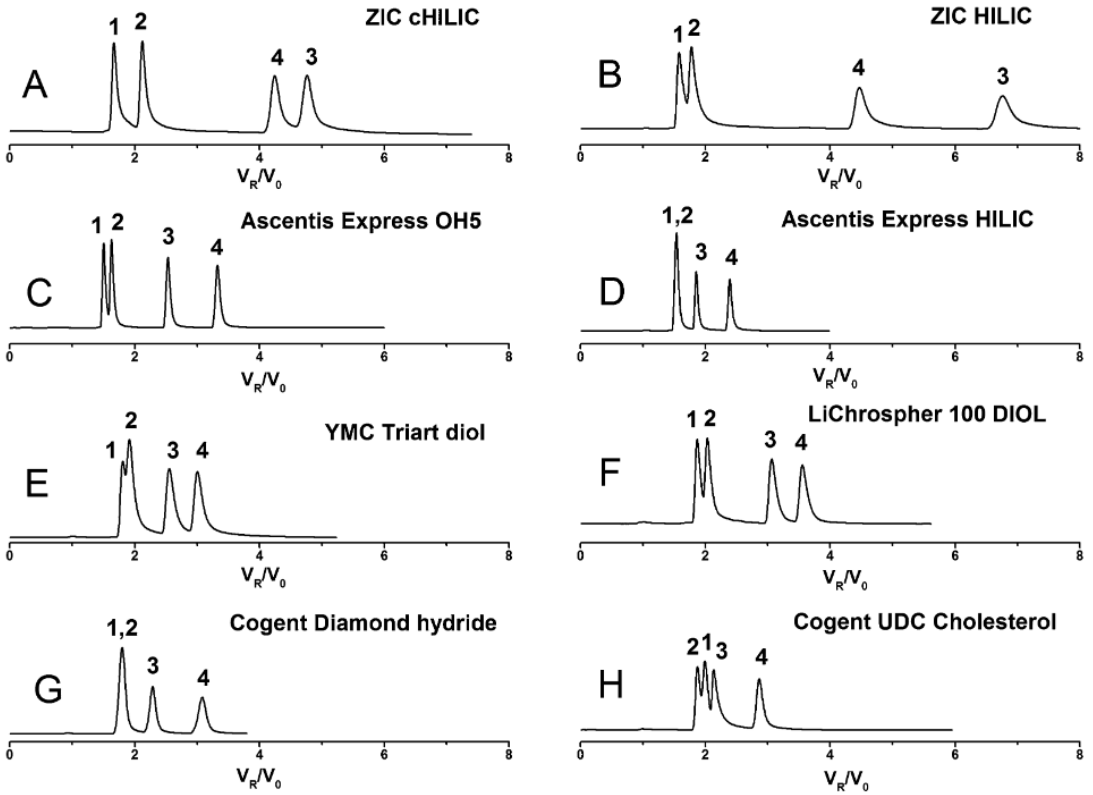
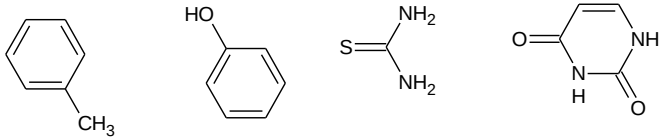


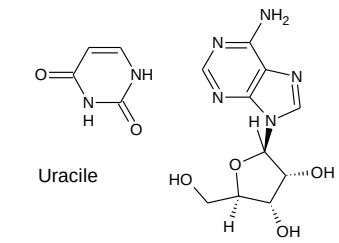
Fig. 7. Examples of separations of 1—toluene, 2—phenol, 3–thiourea and 4—uracil on zwitterionic sulfobetaine (A, B), core shell (C, D), diol (E, F) and hydrosilated silica (G, H) stationary phases. Experimental conditions: mobile phase 5/95 water/acetonitrile, λ = 210 nm, temperature set at 40 °C, injection volume 3 μL, concentration of standards 30 μg/mL.

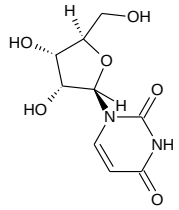
1. Toluene
2. Phenol
3. Thiourea
4. uracil

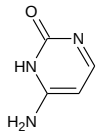


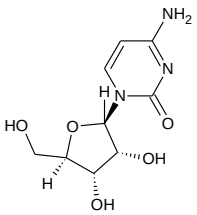
J. Soukup, P. Jandera / J. Chromatogr. A 1374 (2014) 102–111

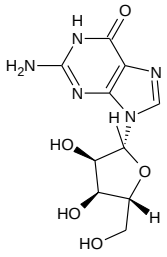
Stationnary Phases selectivity (1)

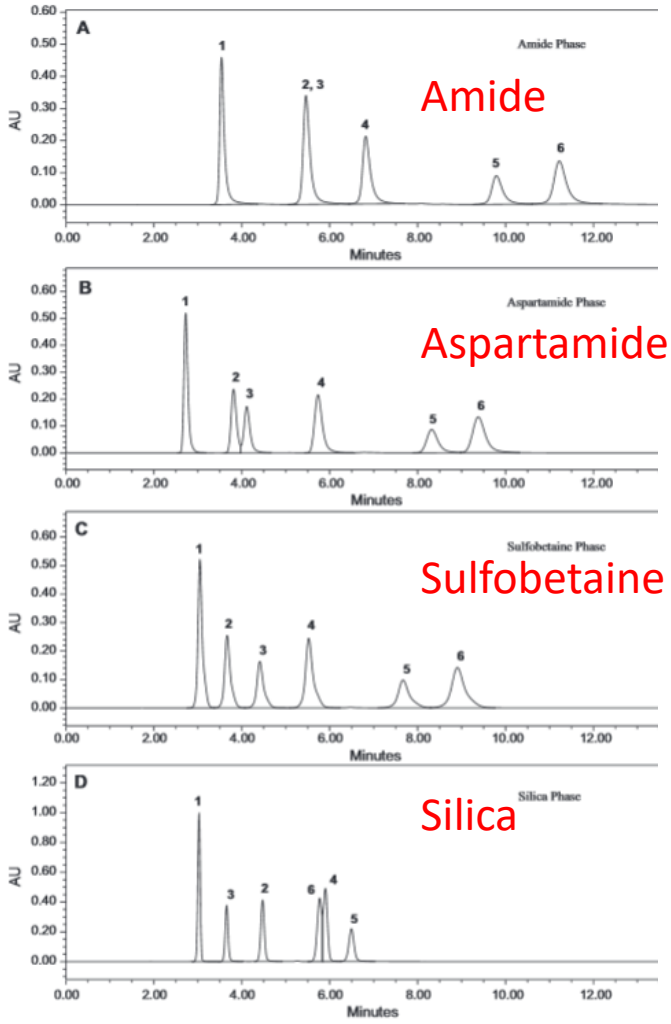


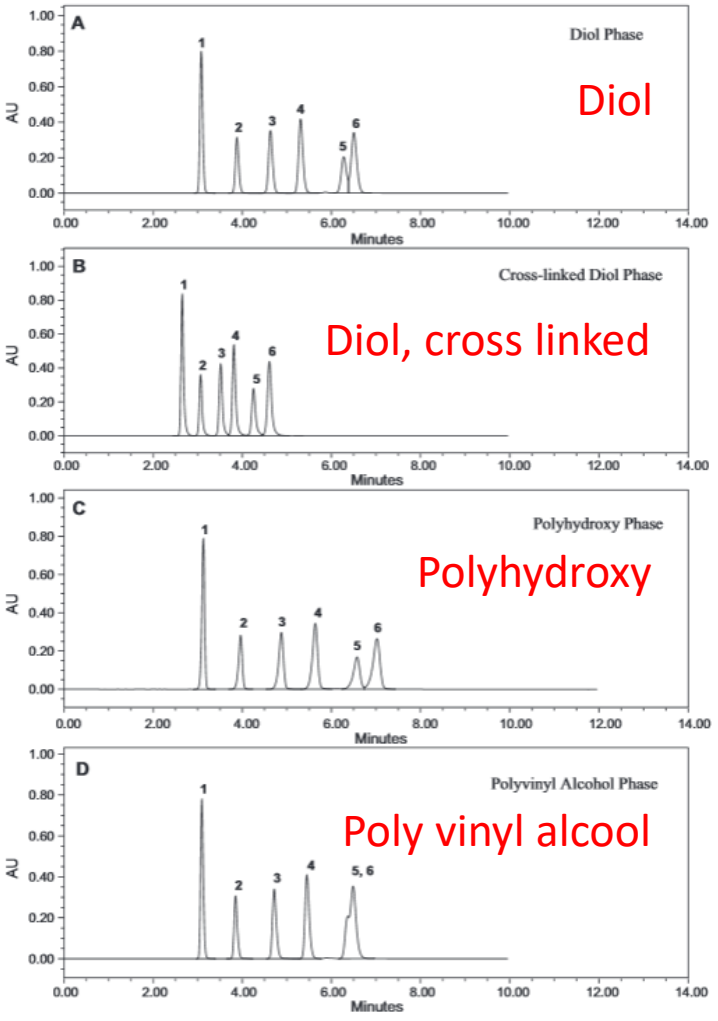












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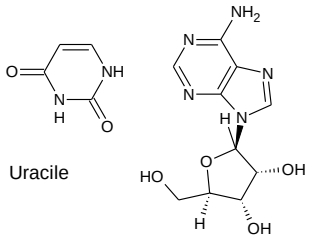
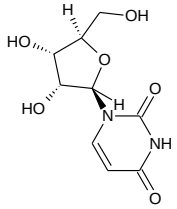
Stationnary Phases selectivity (2)

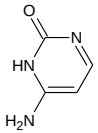


Uracile

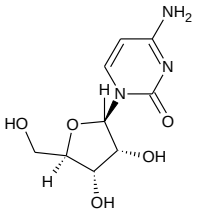
Adenosine



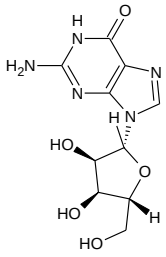
Uridine



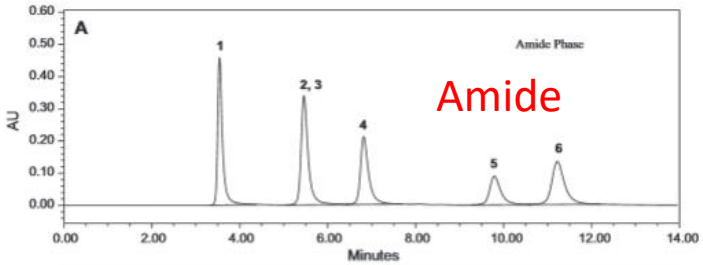
cytosine

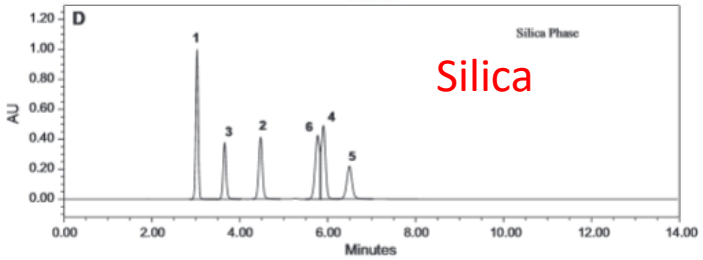


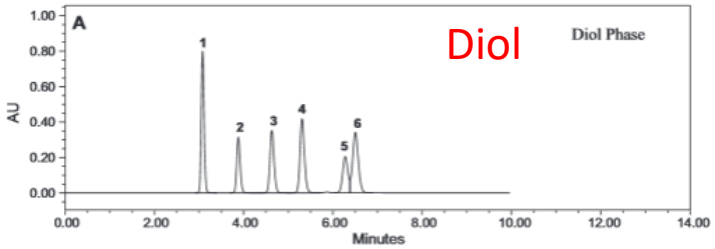
cytidine

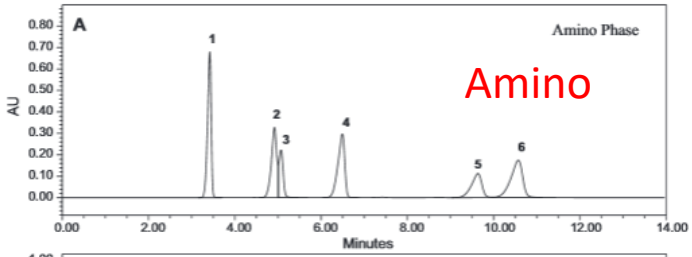


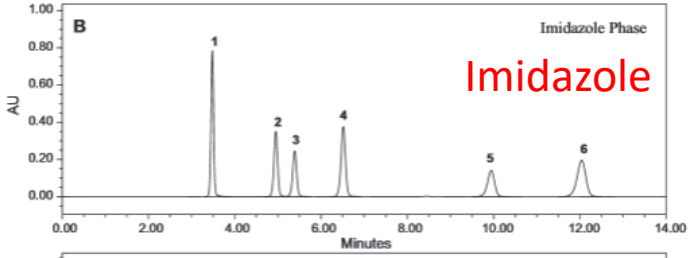
guanosine

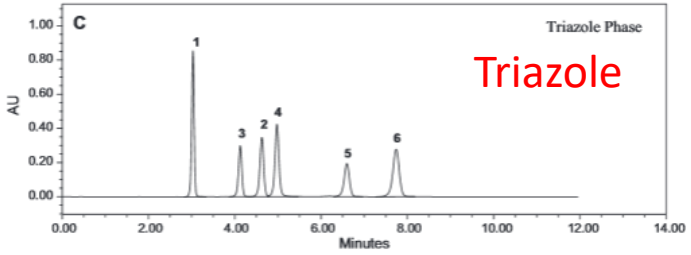












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solvents effect in HILIC

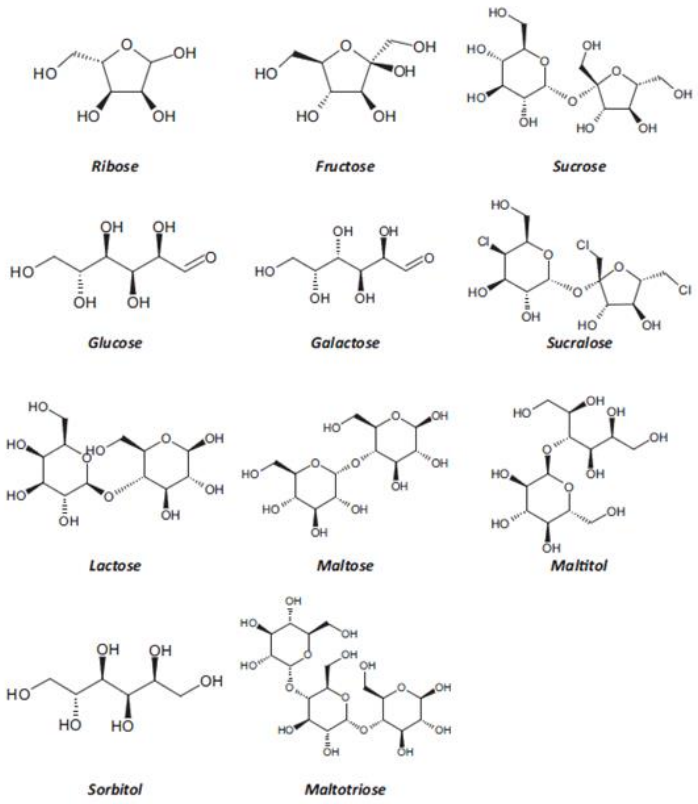

Fig. 1. Chemical structures of the analytes used in this study.

Shodex Asahipak NH2P-50 4E (250 mm long × 4.6 mm wide) packed with 5 µm aminated porous (10 nm) polyvinylalcohol particles, was purchased from Phenomenex

J.P. Hutchinson et al. / Analytica Chimica Acta *(2012)*

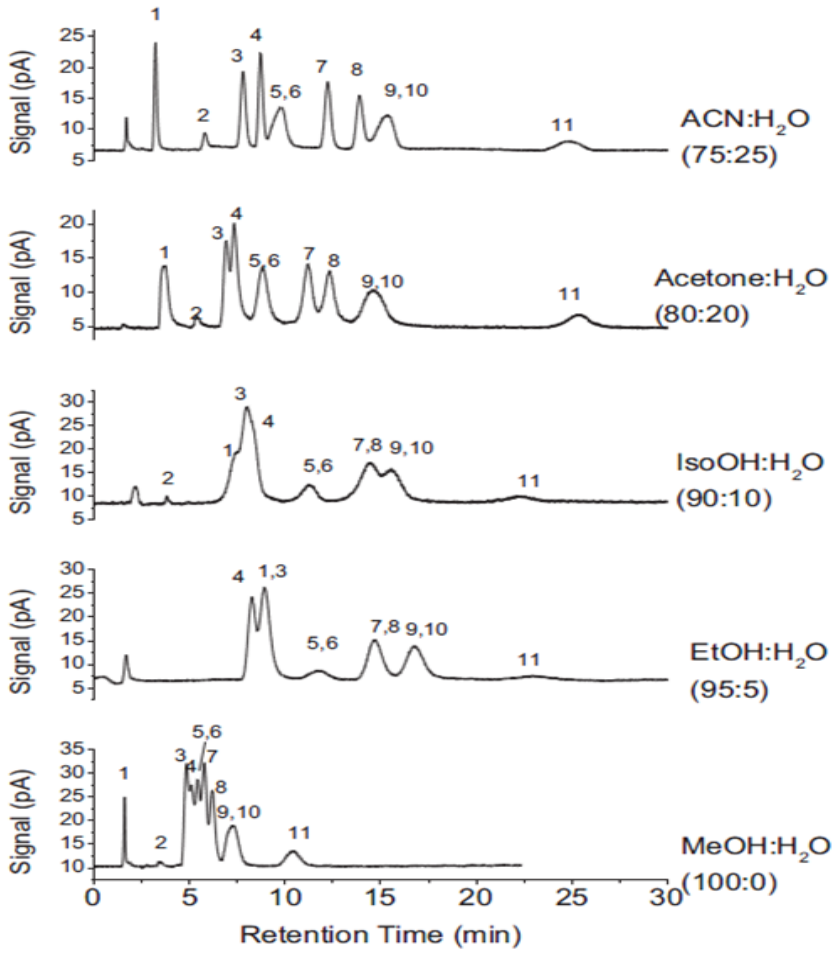

Fig. 3. Comparison of HILIC isocratic separations of 11 sugars and sugar alcohols achieved using different organic modifiers in the mobile phase with Corona CAD detection. *Conditions*: the sample contained 0.1 mg mL^{-1} (1) sucralose, (2) ribose, (3) fructose, (4) sorbitol, (5) glucose, (6) galactose, (7) sucrose, (8) maltitol, (9) maltose, (10) lactose, and (11) maltotriose. All other conditions as in Section 2.

pH effect in HILIC

ACETYLSALICYLIC ACID

pKa=3.5

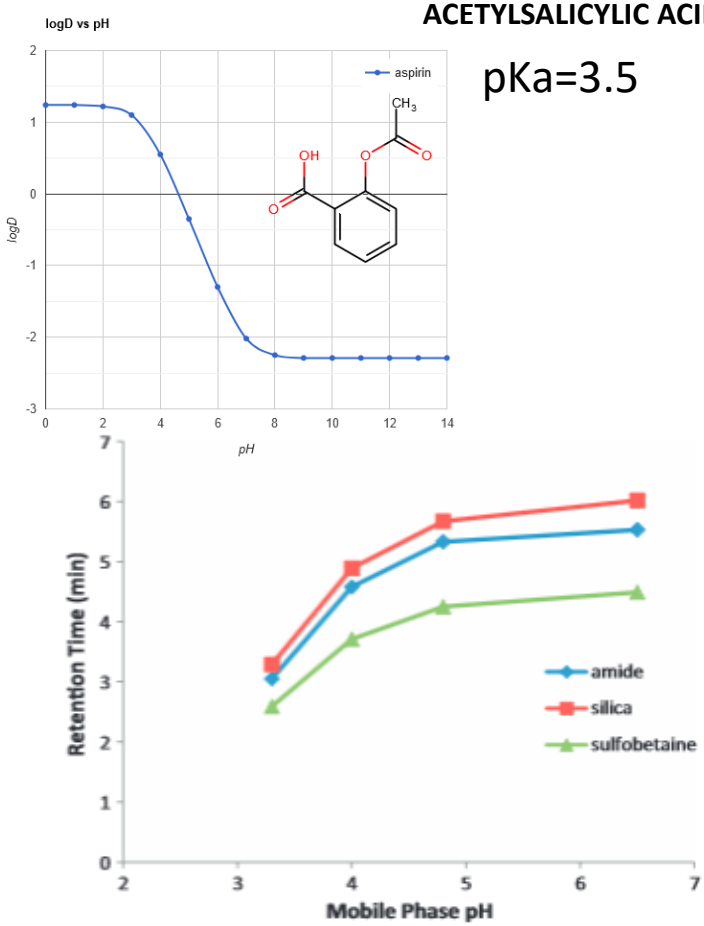

Fig. 15. The effect of mobile phase pH on the retention of acetylsalicylic acid on (♦) amide, (■) silica, and (▲) sulfobetaine phase. Mobile phase, acetonitrile/water (90/10, v/v) containing 10 mM ammonium formate. Mobile phase pH is the pH values of ammonium formate solutions. Column temperature 30 °C.
Adapted from Ref. [66] with permission.

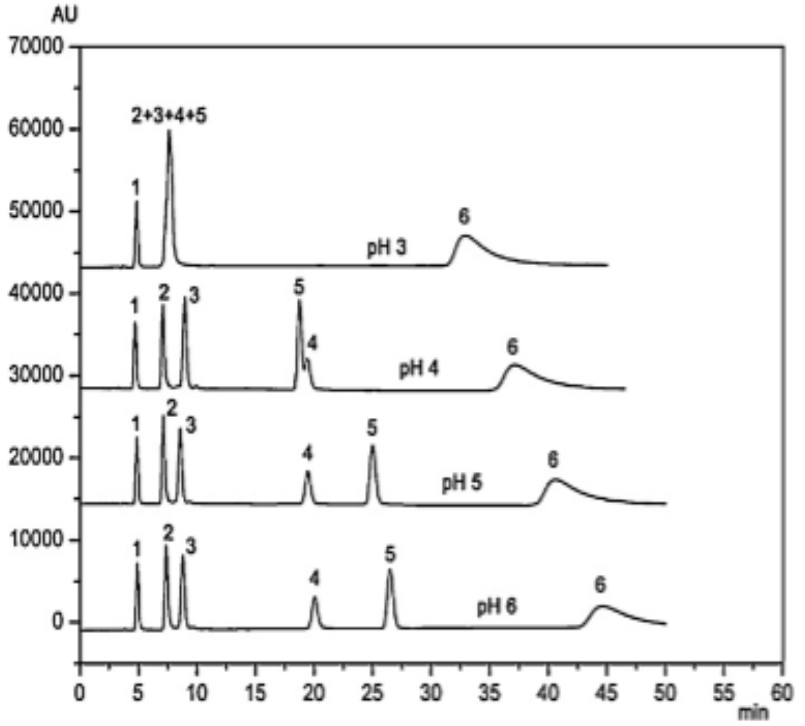

Fig. 16. The effect of pH on separation of selected water soluble vitamins on a diol phase (Inertsil HILIC, 150 mm × 4.6 mm ID, 5 μm particle size). Mobile phase: ACN/water (90/10, v/v) containing ammonium acetate 10 mM, with the aqueous buffer adjusted at various pH values. Flow rate 0.6 mL/min and column temperature 25 °C. Peaks: (1) nicotinamide, (2) pyridoxine, (3) riboflavin, (4) nicotinic acid, (5) L-ascorbic acid and (6) thiamine.
Adapted from Ref. [36] with permission.

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