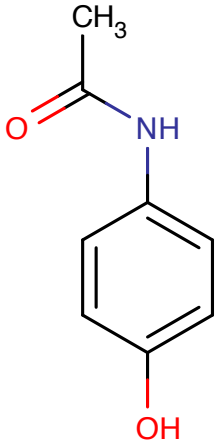
TU09
ANALYTICAL SCIENCES 1

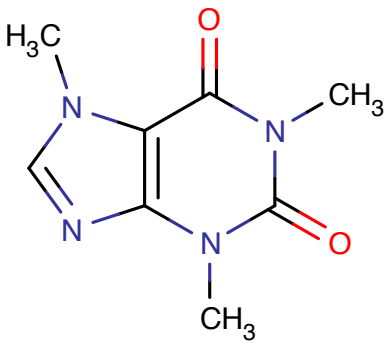
Tutorial 1:exercices
Liquid Chromatography

Analgesics to be analyzed



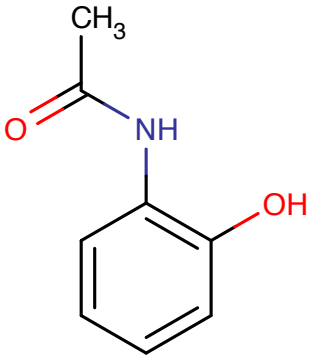
1. Paracetamol

logP=0,91



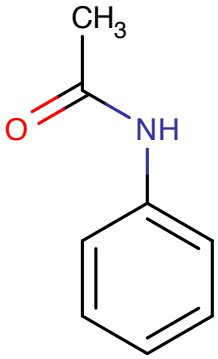
2. Caffeine

logP=-0,55



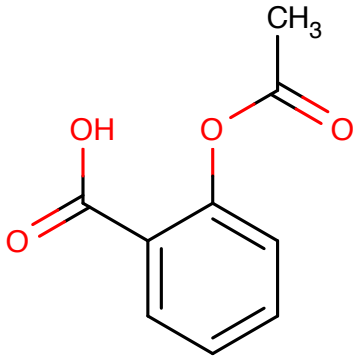
3. 2-acetamidophenol

logP=0,91



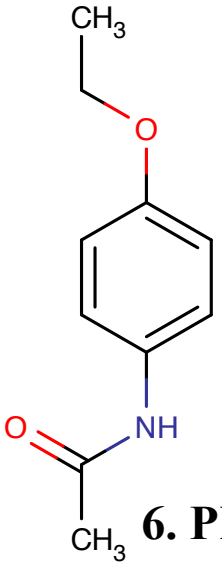
4. Acetanilide

logP=1,21



5. Acetylsalicylic acid

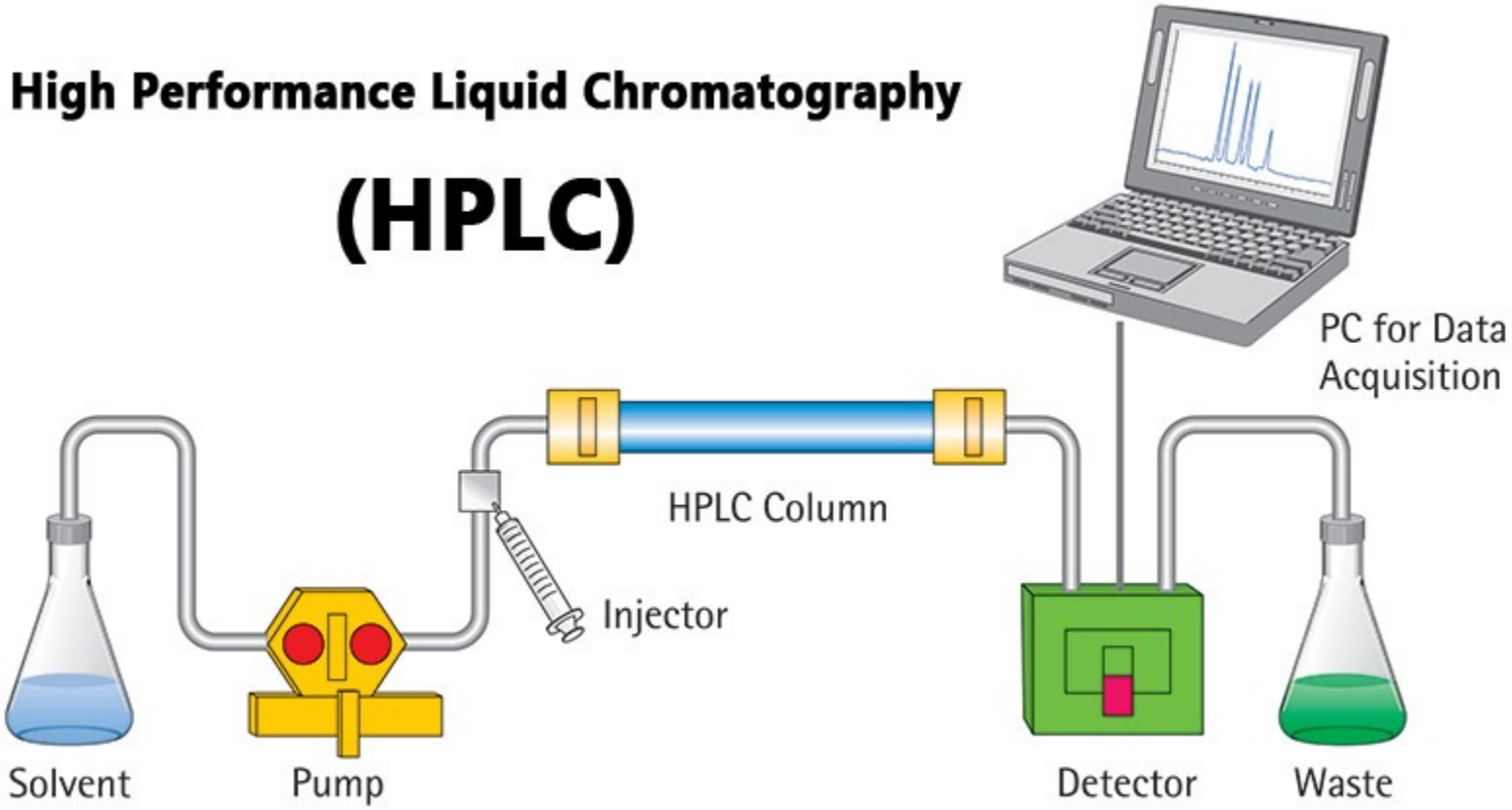
logP=1,24



6. Phenacetin

logP=1,41

LC Conditions



Column : NUCLEODUR Gravity **C8** ; L=12,5 cm ; i.d.=4,0 mm ; dp = 5 µm
Eluent: **methanol / 0,1% phosphoric acid (40/60 v/v)**
Flow rate : 1 mL/min ;
Detection UV : 240 nm ;
Temperature 25°C. Pressure 80,4 bar.

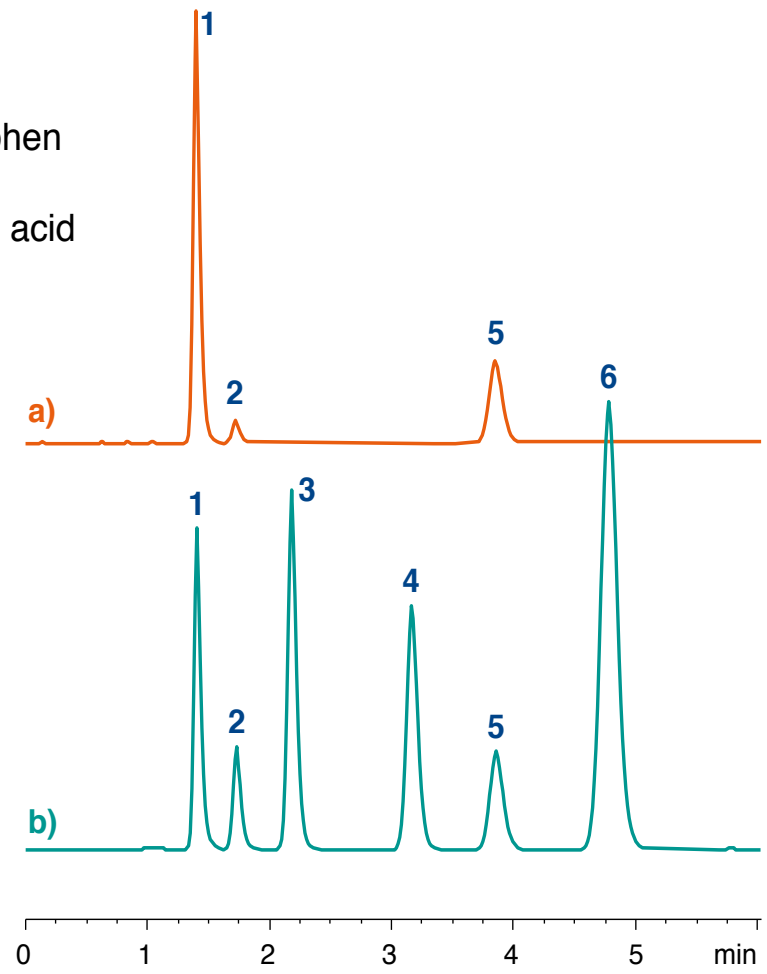
Chromatograms

a) Thomapyrin® tablet; b) standard

Thomapyrin® is a trademark of Boehringer Ingelheim Pharma KG

Peaks:

1. Paracetamol
2. Caffeine
3. 2-Acetamidophen
4. Acetanilide
5. Acetylsalicylic acid
6. Phenactin



Chromatograms

	t_R (min)	$w_{0.5}$ (min)
1. Paracetamol	1,40	0,057
2. Caffeine	1,75	0,071
3. 2-Acetamidophen	2,20	0,072
4. Acetanilide	3,17	0,086
5. Acetylsalicylic acid	3,83	0,116
6. Phenactin	4,77	0,143

Mixture of standards: chromatogram data

1. Paracetamol
logP=0,91

2. Caffeine
logP=-0,55

3. 2-acetamidophenol
logP=0,91

4. Acetanilide
logP=1,21

5. Acetylsalicylic acid
logP=1,24

6. Phenacetin
logP=1,41

Compound polarity

Question 1: What is the type of HPLC used ? (stationary phase, mobile phase, retention process).

Type of chromatography

* *Normal phase* → Polar stationary phase
  → Nonpolar mobile phase

* *Reversed phase* → Nonpolar stationary phase
  → Polar mobile phase

* *Hydrophilic Interaction* → Polar stationary phase
  → Hydro-organic mobile phase

Question 1: What is the type of HPLC used ? (stationary phase, mobile phase, retention process).

Type of chromatography

* *Reversed phase* → Nonpolar stationary phase
→ Polar mobile phase

* *Our conditions* → Stationary phase: C8 → NONPOLAR
→ Mobile phase: MeOH / 0,1% H_3PO_4 → POLAR

→ REVERSED PHASE LIQUID CHROMATOGRAPHY

There is no unique and widely accepted retention model in RP HPLC (*see "retention mechanisms in LC", p. 34*)

Question 2: Explain why phosphoric acid is added in the mobile phase

Table 1. pK_a Values of Common Mobile Phase Additives[1]

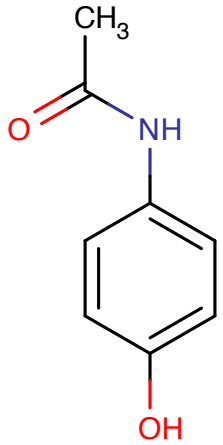
pK_a (25°C)	compound
0.3	trifluoroacetic acid[2]
2.15	phosphoric acid (pK_1)
3.13	citric acid (pK_1)
3.75	formic acid
4.76	acetic acid
4.76	citric acid (pK_2)
4.86	propionic acid
6.35	carbonic acid (pK_1)
6.40	citric acid (pK_3)
7.20	phosphoric acid (pK_2)
8.06	tris
9.23	boric acid
9.25	ammonia
9.78	glycine (pK_2)
10.33	carbonic acid (pK_2)
10.72	triethylamine
11.27	pyrrolidine[3]
12.33	phosphoric acid (pK_3)

[1] data of [1]; [2] Merck Index; [3] CRC Handbook of Chemistry and Physics

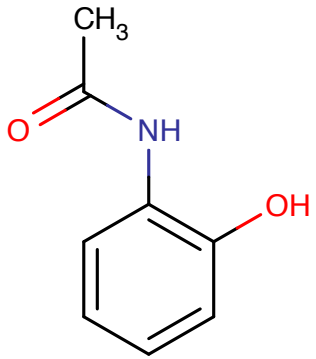
If a low-pH mobile phase is important to render all target analytes protonated, 0.1% v/v phosphoric acid provides reasonable buffering at pH 2 for LC-UV applications.

Question 2: Explain why phosphoric acid is added in the mobile phase

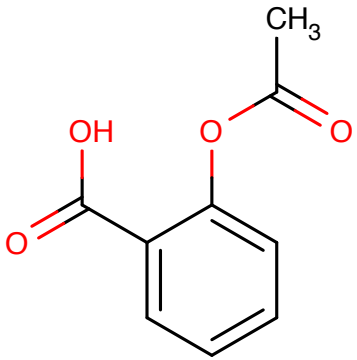
Acids



1. Paracetamol
pKa=9,5



3. 2-acetamidophenol
pKa=8,8



5. Acetylsalicylic acid
pKa=3,4

Mobile phase pH=2: inferior of pKa for all compounds

→ Analytes non ionized, less polar

→ more retained

Question 3: Explain the order of elution of the studied drugs

The **distribution coefficient**, ***P***, is defined as the ratio of concentrations of a solute between the two immiscible solvents (a biphasic system).

$$\log P_{\mathrm{oct/wat}} = \log \left(\frac{[\mathrm{solute}]^{\text{un-ionized}}_{\text{octanol}}}{[\mathrm{solute}]^{\text{un-ionized}}_{\text{water}}} \right)$$

Log *P* value is a measure of **hydrophobic character**

Hydrophobicity in increasing order:
Caffeine < 2-acetamidophenol = Paracetamol < Acetanilide < Acetylsalicylic acid < Phenacetin

Stationary phase C8: Bonded hydrophobic phase

Chromatographic Retention time Order:
Paracetamol< Caffeine <2-acetamidophenol < Acetanilide < Acetylsalicylic acid < Phenacetin

1. Paracetamol
logP=0,91

2. Caffeine
logP=-0,55

3. 2-acetamidophenol
logP=0,91

4. Acetanilide
logP=1,21

5. Acetylsalicylic acid
logP=1,24

6. Phenacetin
logP=1,41

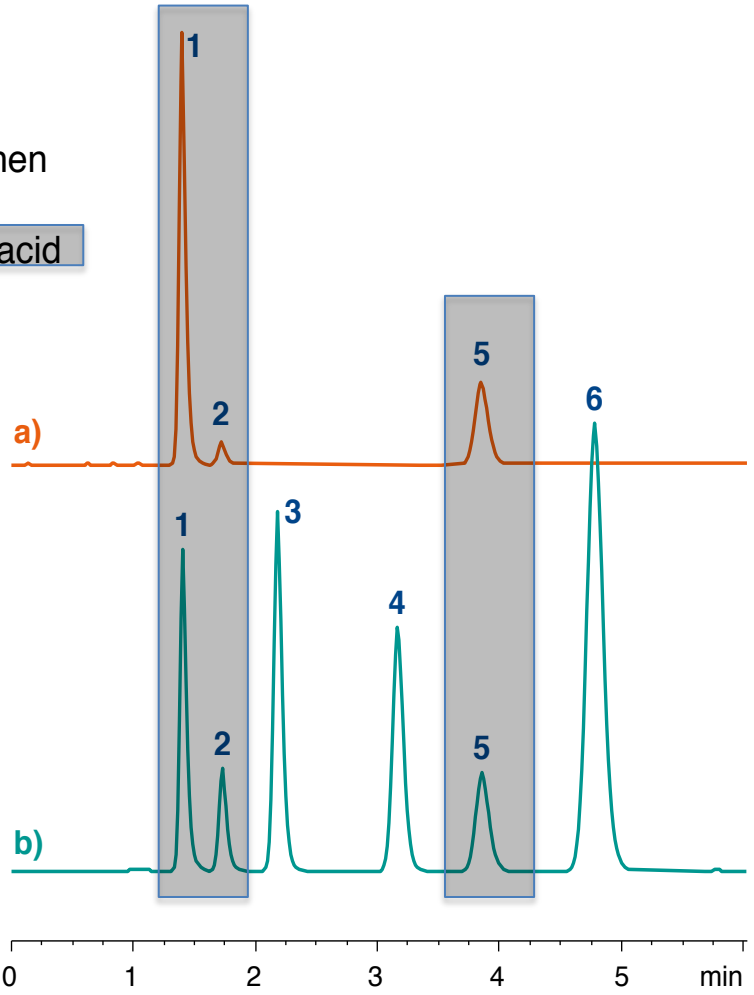
Question 4: Indicate which drugs are present in Thomapyrin® tablets

a) Thomapyrin® tablet; b) standard

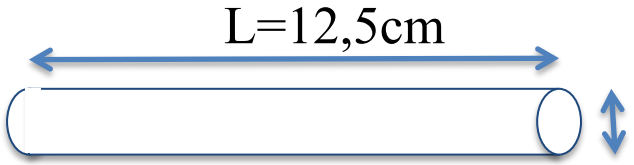
Thomapyrin® is a trademark of Boehringer Ingelheim Pharma KG

Peaks:

1. Paracetamol
2. Caffeine
3. 2-Acetamidophen
4. Acetanilide
5. Acetylsalicylic acid
6. Phenactin



Question 5: Calculate column dead volume (V_M) and dead (void) time (t_M), column porosity is Ɛ=0,73



Flow rate : 1mL/min

$$V_C = L\,A = L\,\pi\left(\frac{id}{2}\right)^2$$

$$\text{Dead Volume } (V_M) = V_C\,\varepsilon = L\,A\,\varepsilon = L\,\pi\left(\frac{id}{2}\right)^2\varepsilon$$

$$V_M = 12{,}5\text{ cm}\times 3{,}14\left(\frac{0{,}4\text{cm}}{2}\right)^2\times 0{,}73 = 1{,}146\text{ cm}^3 = 1{,}146\text{ mL}$$

$$V_M = t_M F \Rightarrow t_M = \frac{V_M}{F} \Longrightarrow t_M = \frac{1{,}146\text{ mL}}{1\text{ mL/min}} \Longrightarrow t_M = 1{,}146\text{ min}$$

Question 6a: Calculate the retention factors (k) for the 6 drugs

Dead (void) time t_M = 1,146 min

	t_R (min)	$w_{0.5}$ (min)	k
1. Paracetamol	1,40	0,057	
2. Caffeine	1,75	0,071	
3. 2-Acetamidophenol	2,20	0,072	
4. Acetanilide	3,17	0,086	
5. Acetylsalicylic acid	3,83	0,116	
6. Phenacetin	4,77	0,143	

$$k = \frac{t_R - t_M}{t_M} \Longrightarrow k_{paracetamol} = \frac{1{,}40\ \text{min} - 1{,}146\ \text{min}}{1{,}146\ \text{min}} = 0{,}22$$

$$k = \frac{t_R - t_M}{t_M} \Longrightarrow k_{Caffeine} = \frac{1{,}75\ \text{min} - 1{,}146\ \text{min}}{1{,}146\ \text{min}} = 0{,}53$$

Question 6a: Calculate the retention factors (k) for the 6 drugs

Dead (void) time t_M = 1,146 min

	t_R (min)	$w_{0.5}$ (min)	k
1. Paracetamol	1,40	0,057	0,22
2. Caffeine	1,75	0,071	0,53
3. 2-Acetamidophenol	2,20	0,072	0,92
4. Acetanilide	3,17	0,086	1,77
5. Acetylsalicylic acid	3,83	0,116	2,34
6. Phenacetin	4,77	0,143	3,16

$$k = \frac{t_R - t_M}{t_M} \Longrightarrow k_{paracetamol} = \frac{1{,}40\ \text{min} - 1{,}146\ \text{min}}{1{,}146\ \text{min}} = 0{,}22$$

$$k = \frac{t_R - t_M}{t_M} \Longrightarrow k_{Caffeine} = \frac{1{,}75\ \text{min} - 1{,}146\ \text{min}}{1{,}146\ \text{min}} = 0{,}53$$

Question 6b: Calculate the selectivity (α) between consecutive peaks.

	t_R (min)	$w_{0.5}$ (min)	k	α
1. Paracetamol	1,40	0,057	0,22	
2. Caffeine	1,75	0,071	0,53	
3. 2-Acetamidophenol	2,20	0,072	0,92	
4. Acetanilide	3,17	0,086	1,77	
5. Acetylsalicylic acid	3,83	0,116	2,34	
6. Phenacetin	4,77	0,143	3,16	

Selectivity $\alpha = \frac{k_2}{k_1} > 1$

$$\alpha_{Caffeine/paracetamol} = \frac{k_{Caffeine}}{k_{Paracetamol}} = \frac{0,53}{0,22} = 2,38$$

$$\alpha_{2Acetamidophen/caffeine} = \frac{k_{2Acetamidophen}}{k_{Caffeinel}} = \frac{0,92}{0,53} = 1,75$$

$$\alpha_{Acetanilide/2Acetamidophen} = \frac{k_{Acetanilide}}{k_{2Acetamidophen}} = \frac{1,77}{0,92} = 1,92$$

Question 6b: Calculate the selectivity (α) between consecutive peaks.

	t_R (min)	$w_{0.5}$ (min)	k	α
1. Paracetamol	1,40	0,057	0,22	
2. Caffeine	1,75	0,071	0,53	2,38
3. 2-Acetamidophenol	2,20	0,072	0,92	1,75
4. Acetanilide	3,17	0,086	1,77	1,92
5. Acetylsalicylic acid	3,83	0,116	2,34	1,33
6. Phenacetin	4,77	0,143	3,16	1,35

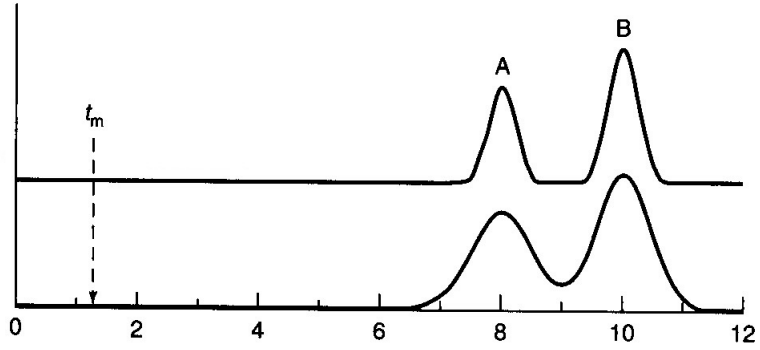
Selectivity $\alpha = \frac{k_2}{k_1} > 1$

$$\alpha_{Caffeine/paracetamol} = \frac{k_{Caffeine}}{k_{Paracetamol}} = \frac{0,53}{0,22} = 2,38$$

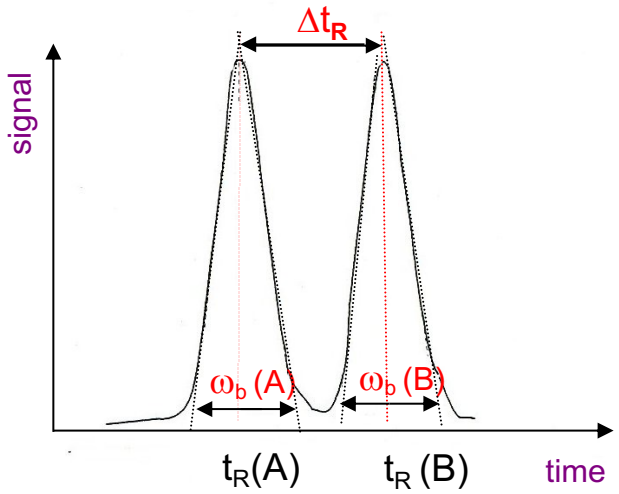
$$\alpha_{2Acetamidophen/caffeine} = \frac{k_{2Acetamidophen}}{k_{Caffeinel}} = \frac{0,92}{0,53} = 1,75$$

$$\alpha_{Acetanilide/2Acetamidophen} = \frac{k_{Acetanilide}}{k_{2Acetamidophen}} = \frac{1,77}{0,92} = 1,92$$

Question 6c: Calculate the resolution (R) between consecutive peaks.

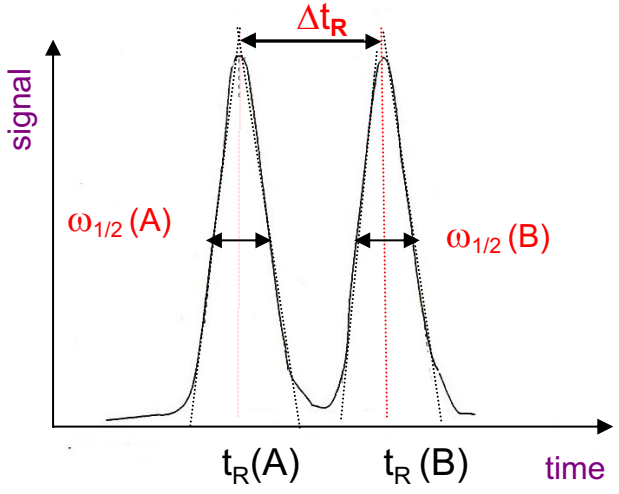


Same selectivity
Different resolution



Resolution Rs

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



Resolution Rs

$$\omega = 1{,}7\ \omega_{1/2}$$

$$\frac{2}{1{,}7} = 1{,}18$$

$$R_S = 1{,}18\,\frac{t_{R(B)} - t_{R(A)}}{\omega_{1/2(A)} + \omega_{1/2(B)}}$$

Question 6c: Calculate the resolution (R) between consecutive peaks.

	t_R (min)	$w_{0.5}$ (min)	R
1. Paracetamol	1,40	0,057	
2. Caffeine	1,75	0,071	
3. 2-Acetamidophenol	2,20	0,072	
4. Acetanilide	3,17	0,086	
5. Acetylsalicylic acid	3,83	0,116	
6. Phenacetin	4,77	0,143	

$$R_{SCaffeine/Paracetamol} = 1{,}18\frac{1{,}75-1{,}40}{0{,}057+0{,}071} = 3{,}23$$

$$R_{S\ 2Acetamidophen/Caffeine} = 1{,}18\frac{2{,}20-1{,}75}{0{,}071+0{,}072} = 3{,}71$$

$$R_{SAcetanilide/2Acetamidophen} = 1{,}18\frac{3{,}17-2{,}20}{0{,}072+0{,}086} = 7{,}24$$

Question 6c: Calculate the resolution (R) between consecutive peaks.

	t_R (min)	$w_{0.5}$ (min)	R
1. Paracetamol	1,40	0,057	
2. Caffeine	1,75	0,071	3,23
3. 2-Acetamidophenol	2,20	0,072	3,71
4. Acetanilide	3,17	0,086	7,24
5. Acetylsalicylic acid	3,83	0,116	3,86
6. Phenacetin	4,77	0,143	4,28

$$R_{SCaffeine/Paracetamol} = 1{,}18\frac{1{,}75-1{,}40}{0{,}057+0{,}071} = 3{,}23$$

$$R_{S\ 2Acetamidophen/Caffeine} = 1{,}18\frac{2{,}20-1{,}75}{0{,}071+0{,}072} = 3{,}71$$

$$R_{SAcetanilide/2Acetamidophen} = 1{,}18\frac{3{,}17-2{,}20}{0{,}072+0{,}086} = 7{,}24$$

Baseline separation at Rs ≥ 1,5

Question 7: Calculate from the peak (5) the number of theoretical plates (N) and the plate height (H) for the column.

	t_R (min)	$w_{0.5}$ (min)
5. Acetylsalicylic acid	3,83	0,116

$$N = 5{,}54\left(\frac{t_R}{\omega_{1/2}}\right)^2 \Longrightarrow N_{Acetylsalicylic\ acid} = 5{,}54\left(\frac{3{,}83}{0{,}116}\right)^2 = 6039$$

$$H = \frac{L}{N} \Longrightarrow H_{Acetylsalicylic\ acid} = \frac{12{,}5\ \text{cm}}{6039} = \frac{125\times10^3\ \mu\text{m}}{6039} = 20{,}7\ \mu\text{m}$$

Question 8: Flow rate is increased to 1,5 mL/min. How will this increase will affect the retention times, retention factors, resolution and back pressure values?

$t_M = L / u$ → Void and retention times decrease

$$\frac{\mathbf{t_{Mnew}}}{t_M} = \frac{1\ \mathrm{mL/min}}{1{,}5\ \mathrm{mL/min}} \Rightarrow t_{Mnew} = \frac{t_M}{1{,}5}$$

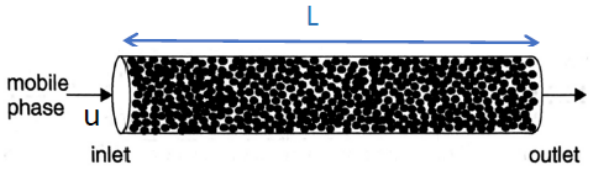
$$\frac{\mathbf{t_{Rnew}}}{t_R} = \frac{1\ \mathrm{mL/min}}{1{,}5\ \mathrm{mL/min}} \Rightarrow t_{Rnew} = \frac{t_R}{1{,}5}$$

t_R and t_M divided by 1,5

$k = (t_R - t_M)/t_M$ → **k did not change**

Question 8: Flow rate is increased to 1,5 mL/min. How will this increase will affect the retention times, retention factors, resolution and back pressure values?

- Column backpressure (ΔP)



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

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

u increased from 1 to 1,5 mL/min

Resolution slightly decreases or does not change

Darcy → ΔP increases

$$\frac{\Delta P_{new}}{\Delta P} = \frac{u_{new}}{u} = \frac{F_{new}}{F}$$

$$\Longrightarrow \frac{\Delta P_N}{\Delta P} = \frac{1{,}5\ \mathrm{mL/min}}{1\ \mathrm{mL/min}} \Longrightarrow$$

$$\Delta P_N = 80{,}4\ \mathrm{bar} \times 1{,}5 = 120{,}6\ \mathrm{bar}$$

ΔP increases by a factor of 1,5

Question 9: We change the solvent strength by decreasing methanol content to 30%. Calculate the effect on retention factors (k)?

$$\phi = 0{,}4 \qquad \phi_{new} = 0{,}3$$

ϕ: *volume fraction of organic solvent*

$$\left.\begin{aligned} logk_{new} &= logk_w - S\phi_{new} \\ logk &= logk_w - S\phi \end{aligned}\right\} \; log\left(\frac{k_{new}}{k}\right) = -S(\phi_{new} - \phi) \Longrightarrow$$

S=3

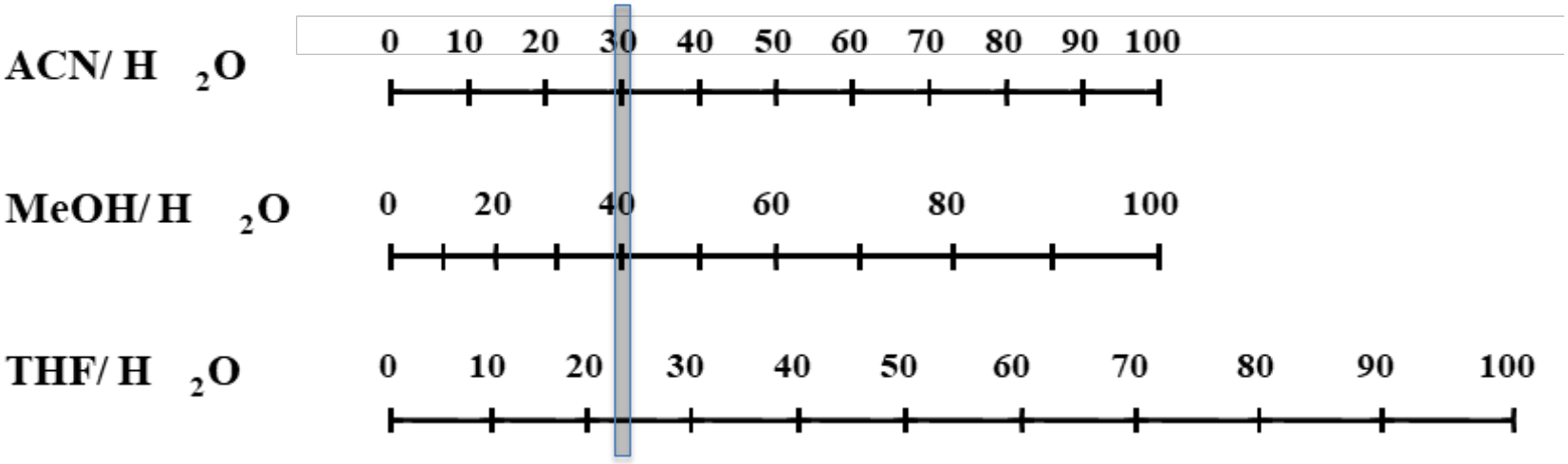
$$log\left(\frac{k_{new}}{k}\right) = -3(0{,}3 - 0{,}4) = 0{,}3 \Longrightarrow log\left(\frac{k_{new}}{k}\right) = 0{,}3$$

$$\Longrightarrow \frac{k_{new}}{k} = 10^{0{,}3} \Longrightarrow k_{new} = 2k$$

Retention factors and retention times double

Question 10: We want to change solvent selectivity (without changing solvent strength). List two mobile phase compositions that can be used.

Solvent-strength nomograph for reversed phase HPLC



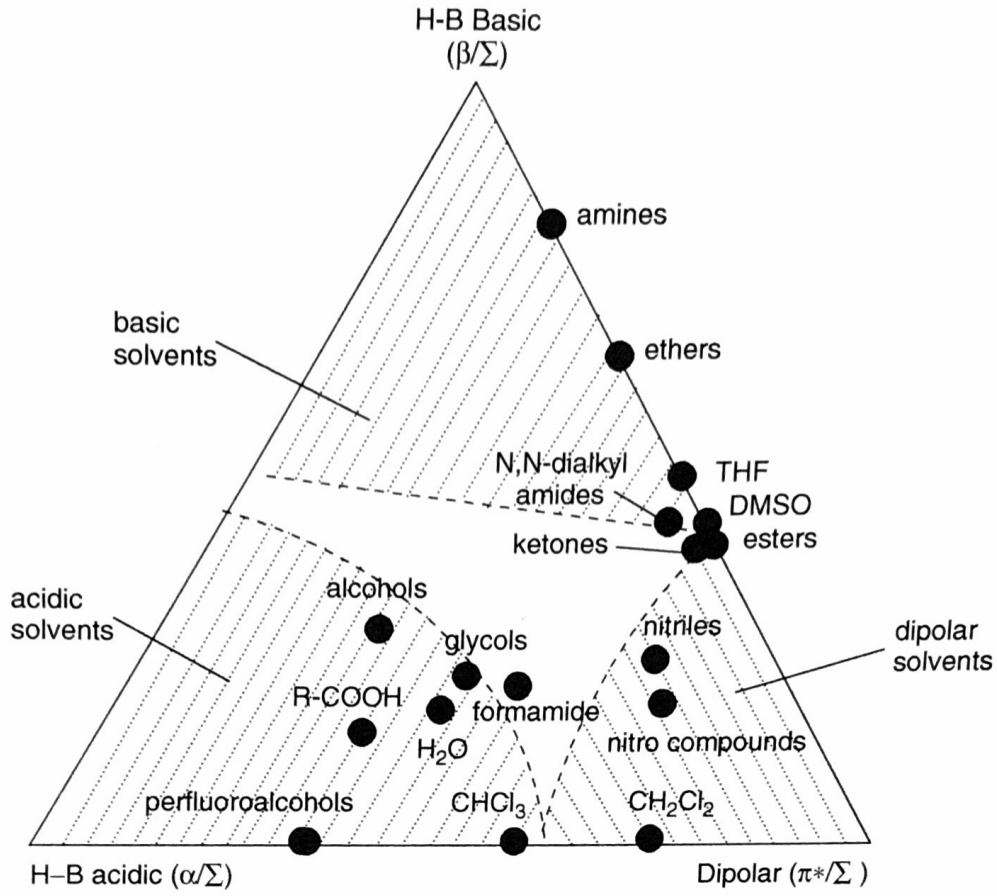
Eluent: **methanol / 0,1% phosphoric acid (40/60 v/v)**

>> Two other mobile phase compositions:

1) ACN / 0,1% phosphoric acid (30/70 v/v)

2) THF / 0,1% phosphoric acid (23/77 v/v)

Question 10: We want to change solvent selectivity (without changing solvent strength). List two mobile phase compositions that can be used.



Solvent selectivity characteristics

Question 11: Quantitative analysis

[Acetylsalicylic acid]$_s$ = 60 mg/L

Peak area (PA) of Acetylsalicylic acid standard (**PA_s**) = 689

[Thomapyrin® sample] = (20 mg/ 20 mL) x (1mL / 10 mL) = **0,1 g powder/ L**

Peak area of Acetylsalicylic acid in Thomapyrin® (**PA_T**) = 591

$$\frac{[\text{Acetylsalicylic acid}]_T}{[\text{Acetylsalicylic acid}]_S} = \frac{PA_T}{PA_S}$$

$$[\text{Acetylsalicylic acid}]_T = [\text{Acetylsalicylic acid}]_S \times \frac{PA_T}{PA_S} = 60_{mg/L} \times \frac{591}{689} = 51{,}5\ _{mg/L} = \mathbf{51{,}5\ _{mg/L}}$$

For 100 mg Thomapyrin® we have 51,5 mg Acetylsalicylic acid

>> For a tablet of 500 mg Thomapyrin®, the quantity of Acetylsalicylic acid is:

51,5 x 500 / 100 = 257,5 mg

The following equations will be given at the beginning of the exam:

$$k = \frac{t_R - t_M}{t_M}$$

$$N = 16\left(\frac{t_R}{\omega}\right)^2 \quad or \quad N = 5{,}54\left(\frac{t_R}{\omega_{1/2}}\right)^2$$

$$R_S = 2\frac{\left(t_{R(2)} - t_{R(1)}\right)}{\left(\omega_{(1)} + \omega_{(2)}\right)} \quad or \quad R_S = 1{,}18\frac{\left(t_{R(2)} - t_{R(1)}\right)}{\left(\omega_{1/2(1)} + \omega_{1/2(2)}\right)}$$

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

$$logk = logk_w - S\varphi$$ S=3 (for methanol and acetonitrile)